REVIEW

Confusing Quantitative Descriptions of *Brønsted–Lowry* Acid–Base Equilibria in Chemistry Textbooks – A Critical Review and Clarifications for Chemical Educators

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In chemistry textbooks, the pK_{a,H_2O} value of water in the solvent water at 25 °C is sometimes given as 14.0, sometimes as 15.7. This is confusing. The particular chemical reaction considered is the one in which water as *Brønsted–Lowry* acid reacts with water as *Brønsted–Lowry* base in water as solvent to yield equal concentrations of hydrated oxonium and hydroxide ions, H_3O^+(aq) and HO^-(aq), respectively. This reaction is also known as the 'self-ionization' of water for which the equilibrium constant is abbreviated as K_w with its known value of $10^{-14.0}$ at 25 °C, *i.e.*, pK_w(25 °C) = 14.0. Identical values for pK_{a,H_2O} and pK_w at a fixed temperature appear reasonable, since K_{a,H_2O} and K_w refer to one and the same reaction. Therefore, reasons for the apparent disagreement between the 'thermodynamically correct' pK_a value for water (14.0 at 25 °C) and the value reported in most organic chemistry textbooks (15.7) should be discussed when teaching acid–base chemistry. There are good arguments for introducing, from the very beginning, the concepts of activity and thermodynamic standard states when teaching quantitative aspects of chemical equilibria. This also explains in a straightforward way why all thermodynamic equilibrium constants, including K_w, are dimensionless, and why p$K_{H_3O^+}$(25 °C) = 0.

1. Introduction. – The treatment of chemical reaction equilibria – in particular the equilibrium that exists if a *Brønsted–Lowry* acid reacts with water as a base, or if a *Brønsted–Lowry* base reacts with water as an acid – is part of *all* general chemistry textbooks that are used, *e.g.*, at universities for first-year students of chemistry, biology, and other related disciplines (*Fig. 1*). The reasons for this are obvious: *i*) the description and understanding of chemical reaction equilibria is an essential part of the fundament on which chemistry or biology as scientific disciplines are built; and *ii*) the treatment of *Brønsted–Lowry* acid–base reactions in aqueous solution is a particularly important application of the general concepts of chemical equilibria which are usually first outlined in a chemistry course for reactions taking place in the gas phase, *i.e.*, in the absence of any solvent.

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Fig. 1. *According to* Johannes Nicolaus Brønsted (1879–1947; *left*) *and* Thomas Martin Lowry (1874–1936; *right*), *an acid is a molecule that is able to donate* (release) *a proton, and a base is a molecule that is able to accept* (bind) *a proton* [1][2]. This is the definition of a *Brønsted–Lowry* acid and a *Brønsted–Lowry* base (see [3]). Photographs from Encyclopedia Britannica Online, accessed February 22, 2013, http://www.britannica.com (*Brønsted*); and from *Trans. Faraday Soc.* **1936**, *32*, 1657 (*Lowry*).

Since many years, there exists an apparent inconsistency between the pK_a value of H_2O as *Brønsted–Lowry* acid in water as solvent, if tabulated values reported in some of the general-chemistry textbooks are compared with the values given in organic-chemistry textbooks. Arbitrarily chosen examples of general-chemistry textbooks report that $pK_{a,H_2O}(25\,^\circ C) = 14.0$ (see [4–11]). Apart from notable exceptions [12], organic-chemistry textbooks generally report a pK_a value for H_2O at 25 °C of 15.7 or 15.74, (see, *e.g.*, [13–22]). This is confusing, and even more so, if in one and the same textbook both values are given, a value of 14.0 in the chapter on '*Acid and Base Strength*' and a value of 15.7 in the chapter '*Structure and Reactivity: Organic Acids and Bases*' [23].

The aim of this review is to clarify this dissatisfying apparent discrepancy which was pointed out and discussed previously in a number of articles and personal statements published in chemical education journals [3][24–39]. In only a few textbooks, *e.g.*, [40][41], the authors make the critical readers aware of the '*existence of different p*K*a values*' for water: a thermodynamically meaningful value ($pK_{a,H_2O}(25\,^\circ C) = 14.0$) which is fully compatible with the standard *Gibbs* free energy for the reaction (see below in *Sects. 3* and *4* (*Fig. 2*), and a value (15.7 or 15.74) which originally was calculated by taking into account the value of $K_w(25\,^\circ C)$ and the molar concentration of water; see *Appendix*). This latter approach has its roots in those years when the *Brønsted–Lowry* acid–base concepts were applied to '*carbon acids*' and to other weakly acidic organic molecules with low water solubility, on the estimation of their acidity, and the concomitant development of relative acidity scales [43–46].

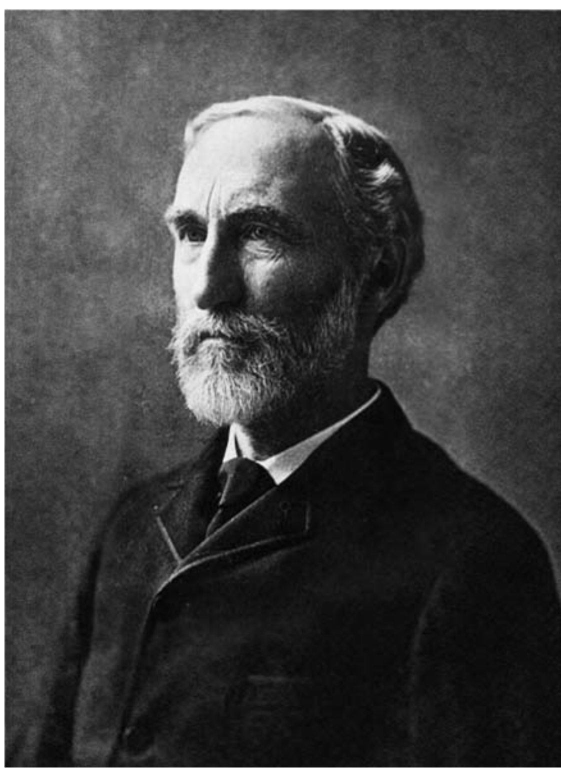

Fig. 2. *The work of* Josiah Willard Gibbs (1839–1903) *was fundamental for the development of chemical thermodynamics and for a large part of physical chemistry.* One of the most important publications of *Gibbs* was [42]. Photograph from C. S. Hastings, '*Biographical memoir of Josiah Willard Gibbs 1839–1903*', *Natl. Acad. Sci. U.S.A., Biogr. Mem.* **1909**, *6*, 373–393.

One of the reasons for the different reported pK_a values are the different conventions used when defining K_a, and the different standard states used for the solvent water in its liquid state, $H_2O(l)$. They are either *i*) pure water ('thermodynamically correct', *i.e.*, compatible with tabulated thermodynamic data), which is consistent with $pK_{a,H_2O}(25\,^\circ C) = 14.0$, or *ii*) one mole water per liter total volume (1M). Similarly, a straightforward 'thermodynamically correct' treatment of $H_3O^+(aq)$ as *Brønsted–Lowry* acid in water yields $pK_{a,H_3O^+} = 0$, while in many organic chemistry textbooks a value of -1.7 or -1.74 is given, which again is not compatible with tabulated thermodynamic data.

An additional inconsistency and confusion currently exists about the dimension of the equilibrium constant K_w as reported in chemistry textbooks, irrespective whether the textbook is on general or organic chemistry. The 'thermodynamically correct' constant $K_w(25\,^\circ C) = 10^{-14.0} = K_{a,H_2O}(25\,^\circ C)$ is dimensionless, as all thermodynamic equilibrium constants, including K_a for any type of *Brønsted–Lowry* acid. This is immediately obvious if one applies the straightforward thermodynamic conventions. It is difficult to understand why these well-elaborated thermodynamic conventions are

not used in contemporary chemistry textbooks, or why reference to the thermodynamic conventions is only made in side remarks, often in such a way that they appear dubious and more irritating than useful (see *Appendix*).

In this contribution, we describe different conventions used in chemistry textbooks for the quantitative thermodynamic description of *Brønsted–Lowry* acid–base reaction equilibria. Particularly, we outline the clear and convincing advantages of introducing and applying 'thermodynamically correct' conventions from the very beginning when teaching this important topic of general chemistry.

For some of the readers, we may be occasionally a bit too trivial, for which we apologize. However, we always try to be clear, dealing with arguments that are straightforward, always based on scientifically reasonable grounds, and hopefully easy to understand. With this, we try to contribute to an improved and consistent teaching of *Brønsted–Lowry* acid–base equilibria. We are convinced that clarifications in textbooks would help avoiding all the on-going confusion and unnecessary discussions that exist in this area of chemistry education since several decades. It is time to reconsider and to rewrite and improve certain chapters in chemistry textbooks, as it would be for the benefit of those students who are interested in chemistry and for the benefit of chemistry as scientific discipline as a whole.

2. The Classical Example: Acetic Acid Dissolved in Water as Solvent. – Before discussing the particular case in which water reacts as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base, we will discuss in detail the behaviour of acetic acid in water, the classical example which is frequently used in chemistry textbooks to outline the *Brønsted–Lowry* acid–base concepts. This example refers to the reaction that occurs if a small amount of acetic acid is added to water, typically 3.0 g (50 mmol) acetic acid dissolved in a total volume of 1 l at an assumed – and usually not explicitly mentioned – pressure of 1 bar or 1 atm. If the acetic acid molecules (CH_3COOH) come into contact with the water molecules (H_2O), a reaction between the acetic acid molecules (a *Brønsted–Lowry* acid) and the water molecules takes place in such a way that the water molecules act as *Brønsted–Lowry* base. This results in a net transfer of a proton (H^+) from a small part of the CH_3COOH molecules to some of the H_2O molecules, so that acetate ions (CH_3COO^-) and an equal amount of oxonium ions form (H_3O^+; also called hydronium ions). The net proton transfer process is very fast and often described with *Lewis* formula (*Figs. 3* and *4*)[1]).

[1]) Curved arrows are often used to indicate the formal 'electron flow', the movement of an electron pair bound to a H_2O molecule to a H-atom bound to a CH_3COOH molecule, as shown in *Fig. 4,a*, on the left hand side. With this 'curved arrow' convention [13][18][19][52–56], also called 'arrow pushing' [55][57] or 'pushing electrons' convention [46], the formal attack of the acid by the base is illustrated. The convention is that the curved arrow begins where the electrons are originally localized, at the nucleophilic, electron-rich part of the base (H_2O for the forward reaction), and points towards the electrophilic center of the acid (the H-atom of the carboxy group for the forward reaction). While a new O–H bond is formed to yield H_3O^+, the existing O–H bond in CH_3COOH is cleaved, again indicated with a curved arrow. The curved arrows on the right hand side of the reaction in *Fig. 4,a*, show the formal electron flux for the backward reaction (from right to left). Using arrows in *opposite direction*, *i.e.*, from the proton of the *Brønsted–Lowry* acid to the *Brønsted–Lowry* base (see for example [9][58–60]), is not recommended as it does not agree with

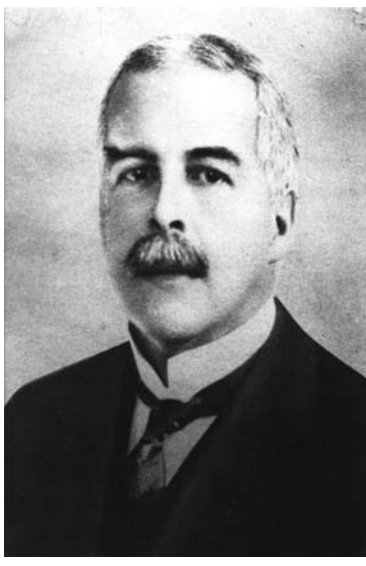

Fig. 3. *In addition to his contributions to 'chemical thermodynamics' and the general concept of 'activities'* [47][48], Gilbert Newton Lewis (1875–1946) *introduced a very useful convention for representing chemical structures, in particular organic molecules, whereby pairs of dots indicate electron pairs.* Usually pairs of electrons forming a covalent bond are drawn as line, and lone pairs (non-bonding) are either drawn as pairs of dots or as lines [49–51]. See also [50][51]. Photograph from http://www.msu.edu, accessed February 22, 2013.

In the reaction considered in *Fig. 4,a*, the H_2O molecules are assumed to be present in large excess with respect to the CH_3COOH molecules and with respect to the formed CH_3COO^- and H_3O^+ ions, *i.e.*, water is at the same time *Brønsted–Lowry* base as well as solvent. In the role of solvent, the H_2O molecules hydrate all the dissolved molecules, CH_3COOH, CH_3COO^-, and H_3O^+, forming an aqueous shell around them. This is usually emphasized by writing $CH_3COOH(aq)$, $CH_3COO^-(aq)$, and $H_3O^+(aq)$, indicating that the species actually are dissolved in water and do not form a separate phase. If we consider a closed system without any material exchange with the environment, at any time at a temperature between 0 and 100 °C, the vast majority of the H_2O molecules in this dilute solution is present as liquid water. This means that, at 25 °C, water is a liquid, indicated as $H_2O(l)$, *i.e.*, the standard state of water is liquid. The molar concentration of $H_2O(l)$ is $c_{H_2O} = 55.33\,\text{M}$ at 25 °C (with density $\varrho_{H_2O} = 0.9970\,\text{g/cm}^3$ and molar mass $M_{H_2O} = 18.02\,\text{g/mol}$). Since the concentrations of the

the mentioned curved arrow formalism which is usually used for describing organic reaction mechanisms. Another generally accepted convention is that the reaction equilibrium is represented with two half arrows (*Fig. 4,a*). In contrast, a single arrow with two heads is used to indicate existence of mesomeric structures (also called 'resonance structures'; *Fig. 4,b*). The two reasonable mesomeric structures of the CH_3COO^- ion shown in *Fig. 4,b*, are of equal energy, and, therefore, contribute equally to the 'real' situation. The curved arrow convention is sometimes also used to indicate how one obtains from one mesomeric structure another one [41][55][61], as indicated in *Fig. 4,b*. This may help students to understand how the different mesomeric structures can be obtained formally.

Fig. 4. a) *Chemical formula of the relevant chemical species present at equilibrium upon dissolving CH_3COOH in H_2O.* In the forward reaction, CH_3COOH molecules react with H_2O to yield CH_3COO^- and H_3O^+ ions; the formal electron flow for the forward reaction is shown with curved arrows on the left hand side, and for the backward reaction on the right hand side. The two straight arrows with half heads indicate that the system is a chemical equilibrium, *i.e.*, the forward as well as the backward reactions take place. b) *Illustration of the two mesomeric structures of the CH_3COO^- ion.* The curved arrows indicate here how one can arrive from one mesomeric structure to the other, by formally delocalizing electron pairs.

dissolved species are negligibly small, the concentration of $H_2O(l)$ in the reaction mixture is always almost the same as the concentration of water in pure water[2]).

The chemical reaction occurring in water between $CH_3COOH(aq)$ and $H_2O(l)$ and the resulting equilibrium situation is usually represented as shown in *Eqn. 1*:

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons CH_3COO^-(aq) + H_3O^+(aq) \quad (1)$$

Another way of describing exactly the same reaction is given in *Eqn. 1'*:

$$CH_3COOH(aq) \rightleftharpoons CH_3COO^-(aq) + H^+(aq) \quad (1')$$

In *Eqn. 1'*, the solvent, $H_2O(l)$, is not explicitly mentioned. The specification '(aq)' indicates that the solvent is water. Note that *Eqns. 1* and *1'* indicate that the concentrations of $H_3O^+(aq)$ and $H^+(aq)$ are the same, although the two species $H_3O^+(aq)$ and $H^+(aq)$ are, from a molecular point of view, not identical and not even the only ones present (*e.g.*, $H_5O_2^+(aq)$ or $H_7O_3^+(aq)$ and so on). Therefore, they all have different standard *Gibbs* energies of formation (see below).

Note that, in principle, the equilibrium of any chemical reaction can be quantitatively 'specified' by using any type of 'rules' to yield an equilibrium constant which has a defined numerical value for the particular stoichiometric reaction

[2]) For the sake of simplicity, in introductory chemistry textbooks, discussions of the reactions between *Brønsted–Lowry* acids and *Brønsted–Lowry* bases, and their quantitative treatment are often limited to room temperature, *i.e.*, to 25 °C. Furthermore, it is assumed that the pressure p is constant, $p = 1$ bar, the standard pressure, although this is not always specifically emphasized.

considered at the particular temperature and pressure. The numerical values of such equilibrium constants then not only depend on the stoichiometry of the reaction, but also on how the equilibrium constant is actually defined, *i.e.*, on the conventions used. Therefore, the numerical value of the equilibrium constant depends on the used measure of the composition (*i.e.*, molar concentrations, mass fractions, *etc.*), and whether relation to a particular reference state of the different species is made. If the conventions applied are not clearly communicated, a correct interpretation and comparison of the equilibrium constants is not possible. With different conventions different equilibrium constants are obtained, with different numerical values and different meanings. Depending on what one intends to express and to compare with a certain equilibrium constant, some of the conventions used are more useful than others. Confusion arises if equilibrium constants obtained using different conventions are compared. This is actually the main reason for the apparent 'water pK_a value problem' which is mentioned in *Sect. 1*, and which led to the writing of this review.

3. The Thermodynamic Acidity Constant of Acetic Acid in Water, K_{a,CH_3COOH}, and Its Negative Logarithm, pK_{a,CH_3COOH}. – On the basis of the definitions and conventions of classical equilibrium thermodynamics developed for ideal gases and ideal solutions (*Raoult*'s law and *Henry*'s law; see [62–64]), for the particular reaction in *Eqns. 1* and *1′*, the relevant quantities which define the thermodynamic equilibrium constant are the *activities*, $a_{i,c}$ of CH_3COOH(aq), CH_3COO^-(aq), H_3O^+(aq) or H^+(aq), and $a_{i,x}$ of H_2O(l), The activity is a quantity which is dependent on the concentrations of all species $A_{1...n}$ in the reaction mixture. For a given reaction between species listed in the stoichiometric equation, the thermodynamic equilibrium constant K is defined by taking the activities of these species into account. The numerical value for the particular equilibrium constant at fixed pressure only depends on temperature.

Before proceeding with the definition of K for the reaction in *Eqns. 1* and *1′*, an alternative and useful formalism is first mentioned, *i.e.*, *Eqns. 2* and *2′*:

$$-\mathrm{CH_3COOH(aq)} - \mathrm{H_2O(l)} + \mathrm{CH_3COO^-(aq)} + \mathrm{H_3O^+(aq)} = 0 \qquad (2)$$

$$-\mathrm{CH_3COOH(aq)} + \mathrm{CH_3COO^-(aq)} + \mathrm{H^+(aq)} = 0 \qquad (2')$$

In such type of equations, the sign of the stoichiometric coefficients ν_i is emphasized. It is negative for all species on the left hand side in *Eqns. 1* and *1′* and positive for all species on the right hand side of *Eqns. 1* and *1′*, *i.e.*, ν_i is -1 for CH_3COOH(aq), -1 for H_2O(l), $+1$ for CH_3COO^-(aq), and $+1$ for H_3O^+(aq).

A generalized formalism for any type of chemical reaction, including the discussed reaction in *Eqns. 2* and *2′*, is given in *Eqn. 3* (see *e.g.*, [63]):

$$\sum_{i=1}^{n} \nu_i \mathrm{A}_i = 0 \qquad (3)$$

Here, A_i (A_1, A_2,... A_n) represents the n various chemical species appearing in the stoichiometric reaction equation. The stoichiometric coefficient ν_i of the species A_i is

defined as above, with $\nu_i < 0$ for species on the left hand side of the reaction equation (reactants) and $\nu_i > 0$ for species on the right hand side (products), respectively. *Eqns. 2*, *2'*, and *3* are simple and useful for a straightforward mathematical treatment of equilibrium equations.

The thermodynamic equilibrium constant K for the general reaction described in *Eqn. 3* follows from the general equilibrium condition for a closed system and is defined as given in [63][3]):

$$K = \prod_{i=1}^{n} a_i^{\nu_i} \tag{4}$$

whereby a_i is the equilibrium activity of species A_i, as mentioned above.

Applying the definition for K in *Eqn. 4* to the reaction formulated with *Eqns. 1* and *2*, one obtains for K the following expression:

$$\begin{aligned} K_{Eqn.1} = K_{Eqn.2} &= a^{-1}_{\mathrm{CH_3COOH(aq)},c} \cdot a^{-1}_{\mathrm{H_2O(l)},x} \cdot a^{+1}_{\mathrm{CH_3COO^-(aq)},c} \cdot a^{+1}_{\mathrm{H_3O^+(aq)},c} \\ &= \frac{a_{\mathrm{CH_3COO^-(aq)},c} \cdot a_{\mathrm{H_3O^+(aq)},c}}{a_{\mathrm{CH_3COOH(aq)},c} \cdot a_{\mathrm{H_2O(l)},x}} \end{aligned} \tag{5}$$

This is the correct form of the thermodynamic acidity constant for CH_3COOH in water, abbreviated as ${}^{\mathrm{H_2O}}K_{\mathrm{a,CH_3COOH}}$ or simply $K_{\mathrm{a,CH_3COOH}}$, without explicit indication that the solvent is water[4]). If not stated otherwise, the solvent is always water, and the pressure is assumed to be $p = 1$ bar. Note, that the activity of the solvent is referred to its standard state which is the pure liquid (see below).

Applying the definition for K in *Eqn. 4* to the reaction in *Eqns. 1'* and *2'* leads to *Eqn. 5'*:

$$K_{Eqn.1'} = K_{Eqn.2'} = a^{-1}_{\mathrm{CH_3COOH(aq)},c} \cdot a^{+1}_{\mathrm{CH_3COO^-(aq)},c} \cdot a^{+1}_{\mathrm{H^+(aq)},c} = \frac{a_{\mathrm{CH_3COO^-(aq)},c} \cdot a_{\mathrm{H^+(aq)},c}}{a_{\mathrm{CH_3COOH(aq)},c}} \tag{5'}$$

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[3]) In a closed system, the equilibrium condition requires that the stoichiometric sum of the chemical potentials of the species vanishes, *i.e.*,

$$\sum_{i=1}^{n} \nu_i \mu_i = 0$$

The chemical potentials are given by $\mu_i = \mu_i^\circ + RT \ln a_i$, with the standard chemical potentials μ_i° and the activities a_i. Note that these latter quantities depend on the choice of the standard state. Upon substitution, we arrive at *Eqn. 4*.

[4]) If water as solvent is replaced with another solvent, *i.e.*, dimethyl sulfoxide (DMSO, $(CH_3)_2SO$), the chemical reaction considered is different as well as the equilibrium constant, ${}^{\mathrm{DMSO}}K_{\mathrm{a,CH_3COOH}}$. In this case, CH_3COOH as *Brønsted–Lowry* acid reacts with DMSO as *Brønsted–Lowry* base in DMSO as solvent according to the chemical reaction: $-$ CH_3COOH(dmso) $-$ $(CH_3)_2SO$(l) $+$ CH_3COO^-(dmso) $+$ $(CH_3)_2SOH^+$(dmso) $= 0$, whereby '(dmso)' indicates that the dissolved species are solvated by DMSO, in analogy to '(aq)' in the case of aqueous solutions.

Now, let us turn to the *definition of the activities*. Within the thermodynamic convention that is compatible with tabulated thermodynamic data for chemical compounds (see below), the activities of the *dissolved species*, *i.e.*, the activities of $CH_3COOH(aq)$, $CH_3COO^-(aq)$, and $H_3O^+(aq)$ or $H^+(aq)$, are related to the molar concentrations of the dissolved species c_i and the standard concentration c° (1 mol/l = 1M) according to *Eqn. 6* [62–64]:

$$a_{i,c} = \gamma_{i,c} \cdot \frac{c_i}{c^\circ} \tag{6}$$

whereby $\gamma_{i,c}$ is the dimensionless activity coefficient of A_i on the basis of molar concentration. The activity coefficient can be interpreted as an adjustment factor that relates the actual, real behavior of a species to the ideal behavior. In most general-chemistry textbooks, the molar concentration c_i of species A_i is denoted with square brackets, *i.e.*, $c_{CH_3COOH(aq)} = [CH_3COOH(aq)]$. Note, however, that chemistry-textbook authors, when dealing with *Brønsted–Lowry* acid–base equilibria, often use the brackets for both molar concentrations as well as for molar concentrations divided by 1 mol/l. Therefore, in one and the same textbook, even within the same chapter, $[CH_3COOH(aq)]$ may stand for a quantity with the unit mol/l, or it may stand for a dimensionless quantity – which is another point of potential confusion.

In this treatise, c_i stands for the molar concentration of species A_i and it has the unit mol/l = M, while c_i/c° is dimensionless. For strongly diluted, ideal solutions, $\gamma_{i,c} = 1$ is valid and, therefore, $a_{i,c} = c_i/c^\circ$. Here, and in most of the general-chemistry textbooks, this ideal, diluted state is assumed to prevail, since it simplifies all further discussions and calculations ($\gamma_{i,c} = 1$). There is, however, no conceptual problem at all to consider for all equations and calculations values of $\gamma_{i,c} \neq 1$, although the situation becomes more complicated. For example, in electrolyte solutions one has to deal with average, and not individual, activity coefficients due to the interdependence of oppositely charged dissolved species. This is usually outlined in detail in physical-chemistry textbooks and is highly relevant in chemistry courses dedicated to students of environmental sciences, since the concentrations of acids and bases, and other dissolved species in 'natural waters', *e.g.*, sea water, may be so high that considering them as 'diluted solutions' would be inappropriate [12]. Again, for the sake of simplicity, as it is done in most general-chemistry textbooks, we assume here that $\gamma_{i,c} = 1$.

It is very important to note that the thermodynamic standard states of dissolved species and of the solvent *are different*, and this has to be taken into account whenever activities and equilibrium constants are used. Furthermore, since these quantities are related to other thermodynamic properties such as, *e.g.*, the *Gibbs* energies of formation of the solvated species and of the solvent, which are tabulated according to defined standard states, the thermodynamic conventions have to be applied accordingly. Arbitrariness and sloppiness in this subject are the key points for all the confusing discussions in the literature.

To be compatible with tabulated thermodynamic data (*Table*), the activity of the *liquid solvent*, *i.e.*, the activity of $H_2O(l)$ for the reaction considered here, corresponds to the convention given in *Eqn. 7*.

$$a_{\text{solvent},x} = \gamma_{\text{solvent},x} \cdot x_{\text{solvent}} \tag{7}$$

whereby $\gamma_{\mathrm{solvent},x}$ is the dimensionless activity coefficient of the solvent on the basis of mole fraction (for ideal solutions, $\gamma_{\mathrm{solvent},x} = 1$ for all concentrations); and x_{solvent} is the mole fraction of the solvent ($x_{\mathrm{solvent}} = n_{\mathrm{solvent}}/n_{\mathrm{total,solution}}$; n_{solvent} being the amount of solvent and $n_{\mathrm{total,solution}}$ the total amount of species in the solution). For highly diluted solutions, $x_{\mathrm{solvent}} = 1$ is an appropriate assumption. Therefore, with this convention and assuming diluted solutions, the activity of the solvent is $a_{\mathrm{H_2O(l)},x} = 1$. This is what should be kept in mind.

Table. *Values for the Standard* Gibbs *Energy of Formation, $\Delta_f G_i^\circ(25\,°C)$, for Selected Species A_i in Their Standard States, as Used in This Treatise, from* [5][11][62]

Species A_i	Standard state	$\Delta_f G_i^\circ(25\,°C)$ [kJ/mol]
$CH_3COOH(aq)$	solution, $c = 1$M	-396.46
$CH_3COO^-(aq)$	solution, $c = 1$M	-369.31
$H_2O(l)$	pure solvent, $x = 1$	-237.13
$H^+(aq)$	solution, $c = 1$M	0
$H_3O^+(aq)$	solution, $c = 1$M	-237.13
$HO^-(aq)$	solution, $c = 1$M	-157.24

Taking into account the two definitions in *Eqns. 6* and *7*, and assuming that $\gamma_{i,c} = 1$ for all dissolved species and $\gamma_{\mathrm{H_2O(l)},x} = 1$ for the solvent, one obtains with *Eqns. 5* or *5'* the expression given in *Eqn. 8* for the thermodynamic equilibrium constant $K = K_{\mathrm{a,CH_3COOH}}$ for the *Brønsted–Lowry* acid–base reaction formulated with *Eqns. 1* or *1'*:

$$K_{\mathrm{a,CH_3COOH}} = \frac{\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c^\circ} \cdot \frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}}{\frac{c_{\mathrm{CH_3COOH(aq)}}}{c^\circ}} = \frac{\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c^\circ} \cdot \frac{c_{\mathrm{H^+(aq)}}}{c^\circ}}{\frac{c_{\mathrm{CH_3COOH(aq)}}}{c^\circ}} \tag{8}$$

Obviously, $K_{\mathrm{a,CH_3COOH}}$ is dimensionless. Furthermore, both reactions in *Eqns. 1* and *1'* yield the same equilibrium constant, since $c_{\mathrm{H_3O^+(aq)}} = c_{\mathrm{H^+(aq)}}$. This is reasonable since both chemical reactions describe the same equilibrium reaction and are formulated with the same stoichiometry.

Note that the numerical value of the molar concentration of the solvent water, $c_{\mathrm{H_2O(l)}} = 55.33$M at 25 °C, must not be considered in the equilibrium expression (*Eqn. 8*), since the thermodynamic standard state of the solvent is the pure solvent, *i.e.*, $x_{\mathrm{H_2O(l)}} = 1$ rather than $c_{\mathrm{H_2O(l)}} = 1$M.

With the definition

$$\mathrm{p}K = -\log_{10} K = -\log K \tag{9}$$

and, accordingly, $\mathrm{p}K_{\mathrm{a,CH_3COOH}} = -\log K_{\mathrm{a,CH_3COOH}}$, *Eqn. 8* can be transformed to *Eqn. 10:*

$$
\begin{aligned}
\log K_{\mathrm{a,CH_3COOH}} = -\mathrm{p}K_{\mathrm{a,CH_3COOH}} &= \log\left(\frac{\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c^\circ}\cdot\frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}}{\frac{c_{\mathrm{CH_3COOH(aq)}}}{c^\circ}}\right) \\
&= \log\left(\frac{\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c^\circ}}{\frac{c_{\mathrm{CH_3COOH(aq)}}}{c^\circ}}\right) + \log\left(\frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}\right) \\
&= \log\left(\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c_{\mathrm{CH_3COOH(aq)}}}\right) + \log\left(\frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}\right)
\end{aligned}
\qquad (10)
$$

Again, it is assumed that all activity coefficients are $\gamma_i = 1$, as mentioned above.

The pH value is *defined* as the negative logarithm to base 10 of the activity of H_3O^+(aq) or H^+(aq) [62–64] (see *Eqn. 11* and [65]).

$$
\mathrm{pH} = -\log\left(a_{\mathrm{H_3O^+(aq)},c}\right) = -\log\left(\gamma_{\mathrm{H_3O^+(aq)},c}\cdot\frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}\right) = -\log\left(\gamma_{\mathrm{H^+(aq)},c}\cdot\frac{c_{\mathrm{H^+(aq)}}}{c^\circ}\right) \qquad (11)
$$

For $\gamma_{\mathrm{H_3O^+(aq)},c} = 1$ and $\gamma_{\mathrm{H^+(aq)},c} = 1$ (see above), one obtains *Eqn. 12*:

$$
\mathrm{pH} = -\log\left(\frac{c_{\mathrm{H_3O^+(aq)}}}{c^\circ}\right) = -\log\left(\frac{c_{\mathrm{H^+(aq)}}}{c^\circ}\right) \qquad (12)
$$

With the definition of pH in *Eqn. 12*, *Eqn. 10* yields the well-known equation of *Henderson* and *Hasselbalch* (*Fig. 5*), which relates the acidity constant and the equilibrium concentrations to the pH value:

$$
\mathrm{pH} = \mathrm{p}K_{\mathrm{a,CH_3COOH}} + \log\left(\frac{c_{\mathrm{CH_3COO^-(aq)}}}{c_{\mathrm{CH_3COOH(aq)}}}\right) \qquad (13)
$$

with $c_{\mathrm{CH_3COO^-(aq)}} + c_{\mathrm{CH_3COOH(aq)}} = c_{\mathrm{CH_3COOH,total}}$, the constant $\mathrm{p}K_{\mathrm{a,CH_3COOH}}$ can be determined experimentally through simple titration experiments, in which an aqueous acetic acid solution is titrated with a solution of a strong base, typically a solution of hydroxide ions, HO^-(aq), obtained by previously dissolving NaOH(s) in water (see *Fig. 6*). The pH value is measured as a function of added amount of NaOH. The $\mathrm{p}K_{\mathrm{a,CH_3COOH}}$ value is the pH value of the solution at which the equilibrium concentration of CH_3COOH(aq) and CH_3COO^-(aq) are equal, *i.e.*, $c_{\mathrm{CH_3COO^-(aq)}}/c_{\mathrm{CH_3COOH(aq)}} = 1$, which means that $\log(c_{\mathrm{CH_3COO^-(aq)}}/c_{\mathrm{CH_3COOH(aq)}}) = 0$. Since the entire experimental titration curve in *Fig. 6* can be fitted with *Eqn. 13*, experiments and theory are in full agreement with each other, confirming that the assumptions made are reasonable, *i.e.*, $\gamma_i = 1$. An

Fig. 5. Lawrence Joseph Henderson (1878–1942; *left*) *and* Karl Albert Hasselbalch (1874–1962; *right*). See also [66–68]. Photographs from J. W. Severinghaus, P. Astrup, J. F. Murray, '*Blood gas analysis and critical care medicine*', *Am. J. Respir. Crit. Care Med.* **1998**, *157*, S114–S122 (*Henderson*); and F. Sgambato, S. Prozzo, E. Sgambato, R. Sgambato, L. Milano, '*Il centenario del pH (1909–2009) – parte seconda. Ma era proprio necessario sostituire l'equazione di Henderson con quella di Henderson-Hasselbalch?*', *Ital. J. Med.* **2011**, *5*, 215–226 (*Hasselbalch*).

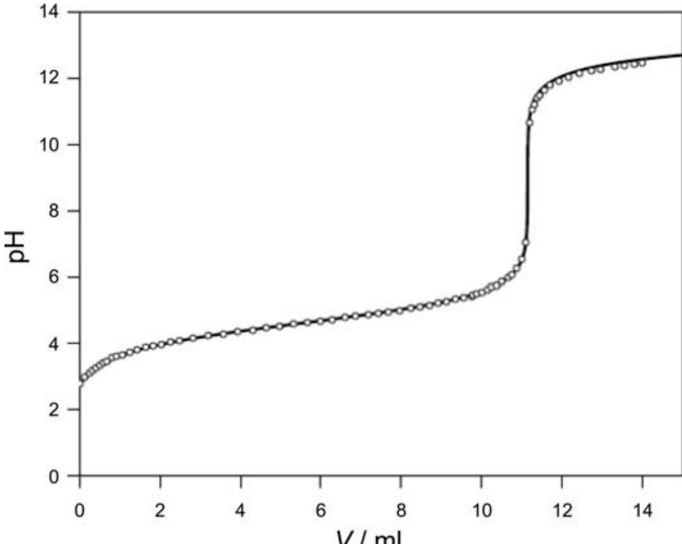

Fig. 6. *Titration of 0.1 l of a* $c_{CH_3COOH,total} = 0.1$M *aqueous CH_3COOH solution with a 1.0*M *solution of aqueous NaOH.* The pH value of the solution was measured (*open circles*) at 20°C with a *Metrohm 691* pH meter equipped with a *Metrohm* pH glass electrode calibrated at pH 4.0 and pH 7.0. The *solid line* results from a simulation on the basis of the *Henderson–Hasselbalch* equation (*Eqn. 13*), and the self-ionization of water (*Eqn. 21*), with $pK_{a,CH_3COOH} = 4.63$ and $K_w = 6.8 \cdot 10^{-15}$ (at 20°C). There is excellent agreement with the ideal model.

extrapolation of measured pK_a values obtained with different acetic acid concentrations to an infinitely diluted solution yields pK_{a,CH_3COOH}(25 °C) = 4.76, which means that $K_{a,CH_3COOH}(25\,°C) = 10^{-4.76} = 1.74 \cdot 10^{-5}$, a value which was obtained in the 1920s and 1930s with a series of very careful experiments [69–71].

In all textbooks which tabulate the pK_a value for acetic acid in water, there is a rather good agreement about its numerical value; it varies between pK_{a,CH_3COOH} = 4.76 (*e.g.*, [8][72]) and pK_{a,CH_3COOH} = 4.74 (*e.g.*, [5][73]) at 25 °C.

As already mentioned above, the conventions used so far are thermodynamically meaningful and fully compatible not only with titration experiments, but also with tabulated thermodynamic data. To demonstrate this latter compatibility, K_{a,CH_3COOH} is calculated from the standard reaction *Gibbs* energy, $\Delta_r G^\circ(T)$, for the reactions in *Eqn. 1* and *1′*, by using *Eqn. 14* [62–64].

$$K(T) = \mathrm{e}^{\frac{-\Delta_r G^\circ(T)}{RT}} \tag{14}$$

which is equivalent to *Eqn. 14′*:

$$\Delta_r G^\circ(T) = -RT\,\ln K(T) \tag{14′}$$

R is the gas constant (8.3145 J K^{-1} mol^{-1}) and T the absolute temperature (in Kelvin, K).

The magnitude of $\Delta_r G^\circ(25\,°C)$ can be calculated from tabulated values of the standard *Gibbs* energy of formation for each species A_i of the reaction, $\Delta_f G_i^\circ(25\,°C)$, (see the *Table*), as shown in *Eqn. 15*:

$$\Delta_r G^\circ(T) = \sum_{i=1}^{n} \nu_i \cdot \Delta_f G_i^\circ(T) \tag{15}$$

For the reaction of *Eqn. 1*

$$\begin{aligned}\Delta_r G^\circ(25\,°C)_{Eqn.1} = &-\Delta_f G^\circ_{CH_3COOH(aq)}(25\,°C) - \Delta_f G^\circ_{H_2O(l)}(25\,°C) + \\ &\Delta_f G^\circ_{CH_3COO^-(aq)}(25\,°C) + \Delta_f G^\circ_{H_3O^+(aq)}(25\,°C) \\ &= -(-396.46\text{ kJ/mol}) - (-237.13\text{ kJ/mol}) + \\ &(-369.31\text{ kJ/mol}) + (-237.13\text{ kJ/mol}) = 27.15\text{ kJ/mol}\end{aligned} \tag{16}$$

Note, that the tabulated values for the solvated species and for the solvent again refer to their respective standard states, *i.e.*, $c = 1$M (solvated species) and $x = 1$ (solvent).

For the reaction of *Eqn. 1′*

$$\begin{aligned}\Delta_r G^\circ(25\,°C)_{Eqn.1'} = &-\Delta_f G^\circ_{CH_3COOH(aq)}(25\,°C) + \Delta_f G^\circ_{CH_3COO^-(aq)}(25\,°C) \\ &+ \Delta_f G^\circ_{H^+(aq)}(25\,°C) = -(-396.46\text{ kJ/mol}) + (-369.31\text{ kJ/mol}) \\ &+ 0\text{ kJ/mol} = 27.15\text{ kJ/mol}\end{aligned} \tag{16′}$$

Both calculations yield exactly the same value for $\Delta_r G^\circ(25\,^\circ C) = 27.15$ kJ/mol. This is reasonable since *Eqns. 1* and *1′* are stoichiometrically equivalent representations of one and the same reaction, as discussed above.

With this calculated value for $\Delta_r G^\circ(25\,^\circ C)$, the thermodynamic equilibrium constant $K(25\,^\circ C)$ for the reactions of *Eqns. 1* and *1′* can be calculated with *Eqn. 14:*

$$K(25\,^\circ C)_{Eqn.1} = K(25\,^\circ C)_{Eqn.1'} = e^{\frac{-\Delta_r G^\circ(25\,^\circ C)}{RT}}$$

$$= e^{\frac{-27150\ J\,mol^{-1}}{8.3145\ J\,K^{-1}\,mol^{-1}\cdot 298\ K}} = e^{-10.966} = 1.74 \cdot 10^{-5} = 10^{-4.76} \qquad (17)$$

Since $K(25\,^\circ C)_{Eqn.1} = K(25\,^\circ C)_{Eqn.1'} = K_{a,CH_3COOH}(25\,^\circ C)$, the acidity constant for acetic acid in water at 25 °C, as calculated from the standard reaction *Gibbs* energy $\Delta_r G^\circ(25\,^\circ C)$, is $1.74 \cdot 10^{-5}$, and accordingly $pK_{a,CH_3COOH}(25\,^\circ C) = 4.76$. This value is in very good agreement with the value used for simulating experimental data (*Fig. 6*), confirming the statement made above that the conventions used in this *Sect.* and the definition of K are fully compatible with tabulated thermodynamic data. Any type of *Brønsted–Lowry* acid–base reaction can be treated in exactly the same way, including the particular case of the reaction of water as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base in water as solvent, as outlined in the following *Sect.*

4. The Thermodynamic Acidity Constant of Water. – Following exactly the same conventions as described in *Sect. 3* for the reaction of acetic acid with water, the thermodynamic equilibrium constant for the reaction of water as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base in water as solvent, as formulated in *Eqn. 18* can easily be obtained.

$$H_2O(l) + H_2O(l) \rightleftharpoons HO^-(aq) + H_3O^+(aq) \qquad (18)$$

Eqn. 18 can also be expressed as in *Eqn. 19*

$$-2\ H_2O(l) + HO^-(aq) + H_3O^+(aq) = 0 \qquad (19)$$

For reasons discussed above, *Eqns. 18* and *19* can also be formulated as in *Eqns. 18′* and *19′*:

$$H_2O(l) \rightleftharpoons HO^-(aq) + H^+(aq) \qquad (18')$$

$$-H_2O(l) + HO^-(aq) + H^+(aq) = 0 \qquad (19')$$

Eqns. 18, *18′*, *19*, and *19′* are 'equivalent' in the sense that they describe in a stoichiometrically equivalent way the same reaction, known as '*self-ionization of water*', '*autodissociation of water*', or '*autoprotolysis of water*'. The thermodynamic equilibrium constant for the reaction as formulated in *Eqns. 18* and *19* is given in *Eqn. 20:*

$$K_{Eqn.18} = a^{-2}_{H_2O(l),x} \cdot a^{+1}_{HO^-(aq),c} \cdot a^{+1}_{H_3O^+(aq),c} = \frac{a_{HO^-(aq),c} \cdot a_{H_3O^+(aq),c}}{a^2_{H_2O(l),x}} \quad (20)$$

With the conventions outlined above, in particular $a_{solvent,x} = a_{H_2O(l),x} = 1$, and assuming that all activity coefficients of the dissolved species are 1 ($\gamma_{i,c} = 1$), one obtains *Eqn. 21:*

$$K_{Eqn.18} = \frac{c_{HO^-(aq)}}{c^\circ} \cdot \frac{c_{H_3O^+(aq)}}{c^\circ} \quad (21)$$

Accordingly, the thermodynamic equilibrium constant for the reaction in *Eqns. 18′* and *19′* yields

$$K_{Eqn.18'} = a^{-1}_{H_2O(l),x} \cdot a^{+1}_{HO^-(aq),c} \cdot a^{+1}_{H^+(aq),c} = \frac{a_{HO^-(aq),c} \cdot a_{H^+(aq),c}}{a_{H_2O(l),x}} \quad (20')$$

and by again taking into account that $a_{H_2O(l),x} = 1$ and assuming $\gamma_{i,c} = 1$, one obtains

$$K_{Eqn.18'} = \frac{c_{HO^-(aq)}}{c^\circ} \cdot \frac{c_{H^+(aq)}}{c^\circ} \quad (21')$$

Since $c_{H_3O^+(aq)} = c_{H^+(aq)}$, K must have the same value for the reactions formulated in *Eqns. 18* and *19*, or *Eqns. 18′* and *19′*. This particular thermodynamic equilibrium constant is usually abbreviated as K_w.

Experimentally, it was found by electrochemical measurements that at 25 °C $c_{H_3O^+(aq)} = 10^{-7.0}$ M [74] (*Fig. 7*), *i.e.*, $c_{H_3O^+(aq)}/c^\circ = 10^{-7.0}$. This means that $c_{H^+(aq)}/c^\circ = 10^{-7.0}$, as well as $c_{HO^-(aq)}/c^\circ = 10^{-7.0}$. This latter relation is due to the fact that for each H_3O^+(aq) formed from H_2O though the reaction shown in *Eqns. 18* or *18′*, one HO^-(aq) is obtained. This is the actual meaning of *Eqns. 18* and *18′*. With these experimental data, one can easily calculate that $K_w(25\,°C) = 10^{-7.0} \cdot 10^{-7.0} = 10^{-14.0}$ [75], as mentioned in basically all general-chemistry textbooks, although there is no general consensus on whether the 'water self-ionization constant' has dimensions or not. The constant may have dimensions only if the conventions used for defining the constant are *different* from the thermodynamic conventions outlined here (see below and *Appendix*).

$K_w(25\,°C)$ can be calculated in the same way as outlined above for $K_{a,CH_3COOH}(25\,°C)$ by taking into account tabulated values for $\Delta_f G_i^\circ(25\,°C)$ for the relevant chemical species of the reaction, as given in the *Table* (see *Eqns. 22* and *22′* for the reactions of *Eqns. 18* and *18′*).

$$\begin{aligned}\Delta_r G^\circ(25\,°C)_{Eqn.18} &= -2\ \Delta_f G^\circ_{H_2O(l)}(25\,°C) + \Delta_f G^\circ_{HO^-(aq)}(25\,°C) + \Delta_f G^\circ_{H_3O^+(aq)}(25\,°C) \\ &= -2\ (-237.13\ \text{kJ/mol}) + (-157.24\ \text{kJ/mol}) + (-237.13\ \text{kJ/mol}) \\ &= 79.89\ \text{kJ/mol}\end{aligned} \quad (22)$$

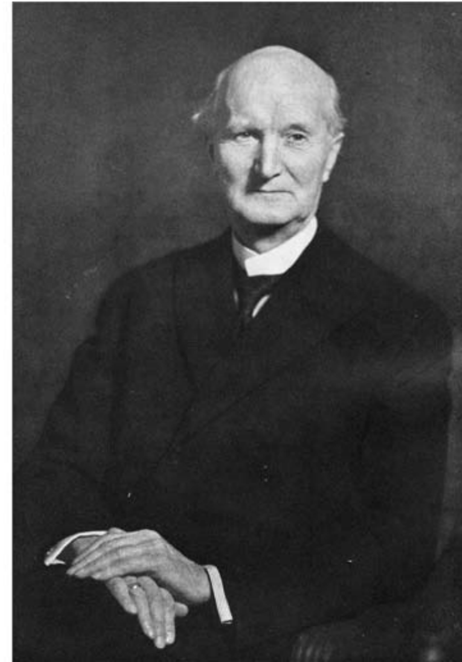

Fig. 7. Augusta Marie Unmack (1896–1990; *left*), *assistant of* Niels Janniksen Bjerrum (1879–1958; *right*) – *one of the pioneers of physical chemistry* –, *analyzed aqueous solutions with a hydrogen electrode and determined from these measurements the ionization constant of water* [74]. See [76–78]. Photographs from the Royal Library, Copenhagen, Denmark (*Unmack*, © Royal Library Copenhagen, reprinted with permission); and N. Bohr, J. A. Christiansen, K. J. Pedersen, *et al.* (Eds.), 'Niels Bjerrum. Selected Papers, edited by friends and co-workers on the occasion of his 70th birthday the 11th of March, 1949', Einar Munksgaard, Copenhagen, 1949 (*Bjerrum*).

$$\begin{aligned}\Delta_r G^\circ(25\,^\circ\mathrm{C})_{Eqn.18'} &= -\Delta_f G^\circ_{\mathrm{H_2O(l)}}(25\,^\circ\mathrm{C}) + \Delta_f G^\circ_{\mathrm{HO^-(aq)}}(25\,^\circ\mathrm{C}) + \Delta_f G^\circ_{\mathrm{H^+(aq)}}(25\,^\circ\mathrm{C}) \\ &= -(-237.13\ \mathrm{kJ/mol}) + (-157.24\ \mathrm{kJ/mol}) + 0\ \mathrm{kJ/mol} \\ &= 79.89\ \mathrm{kJ/mol}\end{aligned} \tag{22'}$$

Independent of whether the autoprotolysis of water is formulated with *Eqns. 18*, *18'*, or *Eqns. 19*, *19'*, one obtains $\Delta_r G^\circ(25\,^\circ\mathrm{C}) = 79.89$ kJ/mol. With this and applying *Eqn. 14*, one obtains $K_w(25\,^\circ\mathrm{C}) = 10^{-14.0}$.

$$K_w(25\,^\circ\mathrm{C}) = \mathrm{e}^{\frac{-\Delta_r G^\circ(25\,^\circ\mathrm{C})}{RT}} = \mathrm{e}^{\frac{-79890\ \mathrm{J\,mol^{-1}}}{8.3145\ \mathrm{J\,K^{-1}\,mol^{-1}}\cdot 298\ \mathrm{K}}} = \mathrm{e}^{-32.243} = 9.93\cdot 10^{-15} \approx 10^{-14.0} \tag{23}$$

Therefore, $\mathrm{p}K_w(25\,^\circ\mathrm{C}) = 14.0$.

Since *Eqns. 18*, *19*, and *Eqns. 18'*, *19'* represent a chemical reaction in which water reacts as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base, K_w is also the acidity constant for water. Therefore, $K_w(25\,^\circ\mathrm{C}) = 10^{-14.0}$ means that $K_{\mathrm{a,H_2O}}(25\,^\circ\mathrm{C}) =$

$10^{-14.0}$ and $pK_{a,H_2O}(25\,^\circ C) = -\log K_{a,H_2O}(25\,^\circ C) = 14.0 = pK_w(25\,^\circ C) = -\log K_w(25\,^\circ C)$. Therefore, *the thermodynamically correct* pK_a *value for the dissociation of water in the solvent water at a temperature 25 °C and an assumed pressure of* p = *1 bar is 14.0.* This value can be found in a number of general-chemistry textbooks [4–11]. Often, however, pK_a tables in introductory-chemistry textbooks do not have an entry for H_2O (or H_3O^+), probably to avoid discussing the kind of question we address in this treatise. As will be also demonstrated in *Sect. 6*, the thermodynamically correct value for the $pK_{a,H_3O^+}(25\,^\circ C)$ is 0.

Full compatibility of $pK_w(25\,^\circ C) = pK_{a,H_2O}(25\,^\circ C) = 14.0$ with tabulated thermodynamic data is further illustrated by considering the autodissociation of water as the sum of two redox half-cell reactions under standard conditions, the reduction of water to hydrogen gas and to two hydrated hydroxide ions, and the oxidation of hydrogen gas to two hydrated protons, as shown in *Eqns. 24* and *25*.

$$2\ H_2O(l) + 2\ e^- \rightleftharpoons H_2(g) + 2\ HO^-(aq) \tag{24}$$

$$H_2(g) \rightleftharpoons 2\ H^+(aq) + 2\ e^- \tag{25}$$

The sum of *Eqns. 24* and *25* yields

$$2\ H_2O(l) \rightleftharpoons 2\ HO^-(aq) + 2\ H^+(aq) \tag{26}$$

Divisions on both sides of *Eqn. 26* by 2 gives

$$H_2O(l) \rightleftharpoons HO^-(aq) + H^+(aq) \tag{27}$$

At 25 °C and standard pressure $p = 1$ bar, the tabulated standard reduction potential E°_{red} for the half-cell reaction of *Eqn. 24* is -0.8277 V [75]. The standard oxidation potential E°_{ox} for the half-cell reaction of *Eqn. 25* is 0.0000 V by definition [75], since all tabulated standard reduction potentials are related to this reaction, the standard hydrogen electrode. Therefore, the standard potential ΔE° for the reaction of *Eqn. 26* or *27* is $\Delta E^\circ_{Eqn.26} = \Delta E^\circ_{Eqn.27} = E^\circ_{red,Eqn.24} + E^\circ_{ox,Eqn.25} = -0.8277\ V + 0.0000\ V = -0.8277$ V.

The relationship between ΔE° and $\Delta_r G^\circ$ is given as [62–64]:

$$\Delta_r G^\circ = -\ n_e \cdot F \cdot \Delta E^\circ \tag{28}$$

F being the *Faraday* constant (96485 C mol^{-1} = 96485 J V^{-1} mol^{-1}), and n_e the number of electrons involved in the redox half-cell reactions leading to the net reaction. For the reaction in *Eqn. 27*, $n_e = 1$.

For the reaction of *Eqn. 27*, one obtains *Eqn. 29*

$$\begin{aligned}\Delta_r G^\circ(25\,^\circ C)_{Eqn.27} &= -1 \cdot 96485\ J\ V^{-1}\ mol^{-1} \cdot (-0.8277\ V)\\ &= 79860\ J/mol = 79.86\ kJ/mol\end{aligned} \tag{29}$$

$\Delta_r G^\circ(25\,^\circ\mathrm{C})_{Eqn.27}$ is identical with the value $\Delta_r G^\circ(25\,^\circ\mathrm{C})_{Eqn.18'}$ calculated on the basis of the tabulated $\Delta_f G_i^\circ(25\,^\circ\mathrm{C})$ values for the individual chemical species. This yields $K_w(25\,^\circ\mathrm{C}) = K_{a,H_2O}(25\,^\circ\mathrm{C}) = 10^{-14.0}$ (see *Eqn. 23*), and $\mathrm{p}K_w(25\,^\circ\mathrm{C}) = \mathrm{p}K_{a,H_2O}(25\,^\circ\mathrm{C}) = 14.0$.)

As already mentioned repeatedly above, like all other thermodynamic equilibrium constants, K_w is a dimensionless constant. However, in several textbooks, this constant is given with units ($\mathrm{mol^2\,l^{-2} = M^2}$) [9][16][46][60][79–82] (see *Appendix*). Interestingly, somewhat strange appears the translation of an American general-chemistry textbook [83a] into German [83b] which resulted in a change of the units of K_w from dimensionless in the original edition to unit $\mathrm{mol^2\,l^{-2}}$ in the German version (similar changes were made for other equilibrium constants, *e.g.*, for the solubility product, K_{sp}). This change was apparently done on purpose and not by mistake, 'in order to adapt to local customs', as quoted in the introduction to the German edition. Chemistry as scientific discipline should be language- and country-independent. Uncertainty about the proper dimensions is also evident if, in one particular case, various editions of one and the same textbook are compared: K_w was dimensionless in an early edition [84], while in a later edition [20], K_w is given in units $\mathrm{mol^2\,l^{-2}}$.

5. Calculation of Equilibrium Constants for *Brønsted–Lowry* Acid–Base Reactions in Which the Base Is not Water. – Knowing the $\mathrm{p}K_a$ values for *Brønsted–Lowry* acids in water, *i.e.*, knowing the equilibrium constants for reactions between a *Brønsted–Lowry* acid and water as *Brønsted–Lowry* base, allows calculation of equilibrium constants for aqueous *Brønsted–Lowry* acid–base equilibria in which the base is *different* from water. The equilibrium constant for such reactions can be calculated from the individual acidity constants K_a of the two acids involved in the equilibrium. To illustrate this, let us consider the titration experiment discussed above and shown in *Fig. 6* (see *Eqn. 30*). Hydrated acetic acid, $CH_3COOH(aq)$, and hydrated hydroxide ions, $HO^-(aq)$, react to yield hydrated acetate ions, $CH_3COO^-(aq)$, and $H_2O(l)$. As indicated with '(aq)', the reaction takes place in $H_2O(l)$ as solvent.

$$\mathrm{CH_3COOH(aq) + HO^-(aq) \rightleftharpoons CH_3COO^-(aq) + H_2O(l)} \tag{30}$$

The acid on the left hand side of *Eqn. 30* is $CH_3COOH(aq)$, its corresponding base on the right hand side is $CH_3COO^-(aq)$; the acid on the right hand side is $H_2O(l)$, its corresponding base on the left hand side $HO^-(aq)$. The chemical reaction represented with *Eqn. 30* can be considered as the *sum of two separate* Brønsted–Lowry *acid–base reactions*, both being reactions of *Brønsted–Lowry* acids with $H_2O(l)$ as *Brønsted–Lowry* base; the equilibrium constant, $K(25\,^\circ\mathrm{C})_{Eqn.30}$ for the reaction of *Eqn. 30* can then be obtained as the *product* of the reaction constants of the two separate reactions. The two separate reactions are given in *Eqns. 1* and *31:*

$$\mathrm{CH_3COOH(aq) + H_2O(l) \rightleftharpoons CH_3COO^-(aq) + H_3O^+(aq)} \tag{1}$$

$$\mathrm{H_3O^+(aq) + HO^-(aq) \rightleftharpoons H_2O(l) + H_2O(l)} \tag{31}$$

The *sum* of the two reactions yields *Eqn. 30*. Note that the reaction of *Eqn. 31* is the 'inverse' of the reaction of *Eqn. 18*.

In *Eqn. 1*, $CH_3COOH(aq)$ is the *Brønsted–Lowry* acid and $H_2O(l)$ the *Brønsted–Lowry* base, in *Eqn. 31* $H_3O^+(aq)$ is the *Brønsted–Lowry* acid and $HO^-(aq)$ the *Brønsted–Lowry* base. Since, at 25 °C, $K_{Eqn.1} = K_{a,CH_3COOH} = 10^{-4.76}$ and $K_{Eqn.31} = 1/K_{a,H_2O} = 1/K_w = 1/10^{-14.0} = 10^{14.0}$, one obtains $K_{Eqn.30} = K_{Eqn.1} \cdot K_{Eqn.31} = 10^{-4.76} \cdot 10^{14.0} = 10^{9.24}$.

The same value results from the tabulated thermodynamic data for the standard *Gibbs* energy of formation, $\Delta_f G_i^\circ(25\,^\circ C)$, for the different species of *Eqn. 30* (see the *Table*):

$$\begin{aligned}\Delta_r G^\circ(25\,^\circ C)_{Eqn.30} &= -\Delta_f G^\circ_{CH_3COOH(aq)}(25\,^\circ C) - \Delta_f G^\circ_{HO^-(aq)}(25\,^\circ C) + \Delta_f G^\circ_{CH_3COO^-(aq)}(25\,^\circ C) + \Delta_f G^\circ_{H_2O(l)}(25\,^\circ C) \\ &= -(-396.46\ \mathrm{kJ/mol}) - (-157.24\ \mathrm{kJ/mol}) + (-369.31\ \mathrm{kJ/mol}) + (-237.13\ \mathrm{kJ/mol}) \\ &= -52.74\ \mathrm{kJ/mol}\end{aligned} \quad (32)$$

$$\begin{aligned}K(25\,^\circ C)_{Eqn.30} &= e^{\frac{-\Delta_r G^\circ(25\,^\circ C)_{Eqn.30}}{RT}} = e^{\frac{52740\ \mathrm{J\,mol^{-1}}}{8.3145\ \mathrm{J\,K^{-1}\,mol^{-1}} \cdot 298\ \mathrm{K}}} \\ &= e^{21.286} = 1.76 \cdot 10^9 = 10^{9.24}\end{aligned} \quad (33)$$

Note that the numerical value of the equilibrium constant of $1.76 \cdot 10^9$ is a large number, since a weak acid, $CH_3COOH(aq)$, reacts with a strong base, $HO^-(aq)$. This is the basis for any type of weak acid–strong base titration experiment.

6. The Thermodynamic Acidity Constant of the Oxonium Ion (H_3O^+) in Water. – To conclude the straightforward application of the thermodynamic conventions for the quantitative treatment of *Brønsted–Lowry* acid–base equilibria, let us ask the question: 'What is the thermodynamic pK_a value of H_3O^+ in water as solvent?' The answer must be clear: $pK_{a,H_3O^+} = 0$.

The reaction considered is

$$H_3O^+(aq) + H_2O(l) \rightleftharpoons H_2O(l) + H_3O^+(aq) \quad (34)$$

with

$$K_{a,H_3O^+} = \frac{a_{H_2O(l),x} \cdot a_{H_3O^+(aq),c}}{a_{H_3O^+(aq),c} \cdot a_{H_2O(l),x}} = 1 \quad (35)$$

$K_{a,H_3O^+} = 1$ means $\Delta_r G^\circ = 0$ (see *Eqns. 14* and *14'*). Therefore, $pK_{a,H_3O^+} = -\log K_{a,H_3O^+} = -\log 1 = 0$, *independent of temperature.* This means that, whenever a H_2O molecule 'attacks' an oxonium ion, an oxonium ion and a molecule of H_2O result, irrespective of whether this process takes place at 25 °C, 50 °C, or any other temperature.

In summary, a description of *Brønsted–Lowry* acid–base reactions within the framework of the thermodynamic conventions yields $pK_{a,H_2O}(25\,^{\circ}C) = 14.0 = pK_w(25\,^{\circ}C)$ and $pK_{a,H_3O^+} = 0$.

7. Final Remarks and a Suggestion. – As outlined repeatedly above, apparent inconsistency in chemistry textbooks not only exists about the value of the pK_a of H_2O or H_3O^+ in water at 25 °C, but there is also an apparent inconsistency about the dimension of equilibrium constants, since comparison is made between constants that are defined differently. Therefore, a clear communication of the conventions applied is of outmost importance. A further observation that actually adds to the confusion in chemistry textbooks is the *change in conventions* on going from one chapter to the other, even if the topics of the two chapters may be directly related. This is particularly evident when moving from the treatment of reactions occurring in the gas phase between gaseous molecules to the treatment of reactions occurring in solution; either *i*) between dissolved species (the solvent being an 'inert' medium not directly taking part in the chemical reactions); or *ii*) between dissolved species and the solvent molecules (*e.g.*, the *Brønsted–Lowry* acid–base reactions discussed extensively in this treatise). Such a change in conventions is unnecessary, although it persists in chemistry textbooks since decades. It appears difficult to alter past customs to a uniform treatment of chemical reaction equilibria.

We are convinced that the concepts of activities and standard states – with all the consequences – could be introduced from the very beginning when teaching general chemistry. Certainly, changing the way the students get introduced into the quantitative treatment of chemical reaction equilibria – in particular *Brønsted–Lowry* acid–base reactions – would mean a considerable effort to modify certain chapters in general-chemistry textbooks. However, this can certainly be accomplished and should be accomplished. We feel that specific improvements in textbooks and in the teaching are indeed necessary. The fundamental concepts of chemistry should be independent of the textbook used, independent of the country in which chemistry is taught, and independent of the written and spoken language.

Improvements as we suggest here would be mainly for the benefit of chemistry students and for a better general reputation of chemistry as central, scientific discipline. The proposed changes are based on well-established physicochemical considerations, as outlined extensively in *Sect. 3–6*. There is no doubt that such changes can be made, but they clearly have to go beyond 'cosmetic modifications'.

The *thermodynamically correct* treatment of chemical reaction equilibria as outlined in *Sects. 3* to *6* is straightforward, useful, and transparent, and should be easy to understand. Even without a previous detailed education in chemical thermodynamics, a student should be able to follow the arguments presented. There are at the end only a few concepts and conventions to consider and apply. The main 'rules' are the following.

1) First, a chemically and stoichiometrically correct equation describing a particular chemical reaction in which one is interested in should be formulated. In this equation, the states of the chemical species involved have to be clearly indicated. The states are either gaseous (g), liquid (l), solid (s), or dissolved. If

the solvent is water, the dissolved species are indicated by adding (aq). The solvent used has to be clear from the equation.

2) Any chemical equilibrium can be represented with the generalized *Eqn. 3* in which the stoichiometric coefficients and the species involved are ν_i and A_i

$$\sum_{i=1}^{n} \nu_i A_i = 0 \tag{3}$$

Examples are *Eqns. 2* and *2′* in *Sect. 3*, and *Eqns. 19* and *19′* in *Sect. 4*.

3) The equilibrium constant K is then defined for the as formulated reaction according to *Eqn. 4*.

$$K = \prod_{i=1}^{n} a_i^{\nu_i} \tag{4}$$

whereby a_i is the activity of species A_i and ν_i is the stoichiometric coefficient of A_i. Examples are *Eqns. 5* and *5′* in *Sect. 3*, and *Eqn. 20* and *20′* in *Sect. 4*.

4) The activities of the different types of species involved in the equilibrium are *defined depending on their states*, as follows.
For *dissolved species* A_i as shown in *Eqn. 6*

$$a_{i,c} = \gamma_{i,c} \cdot \frac{c_i}{c^\circ} \tag{6}$$

$\gamma_{i,c}$ being the activity coefficient of the dissolved species A_i on the basis of molar concentration, c_i being the molar concentration of the dissolved species A_i, and c° being the standard concentration which is 1 mol l^{-1} = 1M.
For *liquid solvents* as in *Eqn. 7*

$$a_{\mathrm{solvent},x} = \gamma_{\mathrm{solvent},x} \cdot x_{\mathrm{solvent}} \tag{7}$$

$\gamma_{\mathrm{solvent},x}$ being the activity coefficient of the solvent on the basis of mole fraction, x_{solvent} being the mole fraction of the solvent ($x_{\mathrm{solvent}} = n_{\mathrm{solvent}}/n_{\mathrm{total,solution}}$, n_{solvent} being the amount of solvent and $n_{\mathrm{total,solution}}$ being the total amount of substances in the solution). In dilute solutions, $x_{\mathrm{solvent}} = 1$.
For *gaseous species* A_i, as in *Eqn. 36*

$$a_{i,p} = \gamma_{i,p} \cdot \frac{p_i}{p^\circ} \tag{36}$$

$\gamma_{i,p}$ being the activity coefficient of gaseous species A_i on the basis of pressure in units of bar, p_i being the partial pressure of the gas A_i ($p_i = x_i \cdot p_{\text{total}}$, with x_i being the mole fraction of gas A_i), and p° being the standard pressure which is 1 bar. For *solids* as in *Eqn. 37*

$$a_{\text{solid},x} = \gamma_{\text{solid},x} \cdot x_{\text{solid}} \tag{37}$$

$\gamma_{\text{solid},x}$ being the activity coefficient of the solid on the basis of mole fraction $x_{\text{solid}} = n_{\text{solid}}/n_{\text{total,solid}}$. For a pure solid phase, $x_{\text{solid}} = 1$. Note that the standard state of the solid is its most stable polymorphic form at the temperature considered.

5) Since in introductory-chemistry textbooks, the discussion of chemical equilibria of any type usually is limited to ideal systems in which, for example, the concentrations of all dissolved species are low, all activity coefficients take a value of 1, *i.e.*, $\gamma_{i,c} = 1$, $\gamma_{\text{solvent},x} = 1$, $\gamma_{i,p} = 1$, $\gamma_{\text{solid},x} = 1$.

As a general summary of our rather detailed argumentations in this treatise, we propose to introduce straightforward thermodynamic conventions and the concept of activities at the very beginning when teaching reaction equilibria in a general-chemistry course. This is independent of whether reactions occurring in the gas phase are discussed, *i.e.*, reactions occurring without any solvent, or whether the reactions take place in a solvent with its participation in the reaction. When using tabulated 'pK_a values' for organic molecules taken from organic-chemistry textbooks, students should be made aware of the fact that the listed values often are based on conventions which are different from the thermodynamic ones. Differently defined constants should not be mixed-up.

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Appendix

Aim and Content. This *Appendix* contains two *Notes* which describe in detail how quantitative aspects of *Brønsted–Lowry* acid–base reaction equilibria often are treated in chemistry textbooks, and why these treatments frequently are confusing, and why they may lead to equilibrium constants which are not compatible with tabulated thermodynamic data. The main reason for the confusion is the apparent refusal of defining equilibrium constants on the basis of activities and the appropriate thermodynamic standard states. *We show that there are clear advantages of applying the concept of activities and thermodynamic standard states* when discussing quantitative aspects of chemical reaction equilibria (see the main text).

The first *Note* is on *molar-concentration-based reaction constants* (*Note A1*) and the second one on *the molar-concentration-based 'acidity constant' of water in water at 25 °C* (*Note A2*). In this second *Note*, we analyze the arguments which were brought up in literature for obtaining for the acidity constant of water in water at 25 °C a value of $10^{-15.7}$ (or $10^{-15.74}$) *vs.* the thermodynamically meaningful value of $10^{-14.0}$ (*Sect. 4* of the main text).

General Remarks. All equations in this *Appendix* which do not appear in the main text are labelled accordingly, *e.g.*, *Eqn. A1*.

As mentioned in *Sect. 3*, in the *strictly thermodynamic treatment* of *Brønsted–Lowry* acid–base reaction equilibria, there are clear conventions about the standard states which *vary depending on the nature of the species* involved in the equilibrium. They are $c^\circ = 1\text{M}$ for dissolved species, $p^\circ = 1$ bar for gaseous reactants, and for the solvent the standard state is the pure liquid solvent. With the assumption of strongly diluted solutions, *i.e.*, $\gamma_{i,c} = 1$ and $\gamma_{\text{solvent},x} = 1$, the activity of dissolved species A_i is $a_{i,c} = c_i/c^\circ$, and the activity of the solvent is always $a_{\text{solvent},x} = 1$.

In principle, there are *various ways to define an equilibrium constant* (*Notes 1* and *2*), although such arbitrarily defined constants may not necessarily be compatible with tabulated thermodynamic data, or they may not be useful for a quantitative, comparative treatment of the equilibrium state. In any case, it is important and absolutely neccessary that the rules used are clearly specified. If such specifications are not made, it is likely that unnecessary confusion arises. This may lead to the assumption that there is an inconsistency with other values of equilibrium constants for one and the same reaction, formulated with the same stoichiometric equation, although, in fact, there is only an *apparent inconsistency* due to the different rules and conventions used. All what follows originates from a disregard of the strictly thermodynamic conventions, although this is often not explicitly made clear.

We hope that our explanations here are useful for chemical educators and help understanding how *Brønsted–Lowry* acid–base reaction equilibria are actually treated *in most chemistry textbooks.*

Note A1. On the Molar-Concentration-Based Reaction Constants, K^c *and* K'^c. In this *Note*, we explain definitions of equilibrium constants of *Brønsted–Lowry* acid–base reactions for the following two cases:

Case i, in which the equilibrium constants are expressed in terms of molar concentrations of *all species* involved (including the solvent water) *without* relation to a standard state; and

Case ii, in which the equilibrium constants are expressed in terms of dimensionless molar concentrations of *all species* involved (including the solvent, *e.g.*, water).

These cases are actually applied in many general-chemistry textbooks, and in (virtually) all organic-chemistry textbooks we consulted. Furthermore, if the textbook authors consider activity coefficients at all, they are assumed to be 1, as we did in the strictly thermodynamic treatment outlined in *Sects. 3* to *6*. With this, one can say that for *Case ii*, the equilibrium constant is defined by taking into account the 'numerical value of molarity of *all* species' [A1]. Note, that this procedure does not rely on a true consideration of thermodynamic standard states but rather on the need to have 'dimensionless concentrations'.

Having clarified this point, we will first discuss *Case ii*. The equilibrium constant, $K^c_{CH_3COOH}$ for the reaction in *Eqn. 1* following the rules of *Case ii* can then be defined as in *Eqn. A1*:

$$K^c_{CH_3COOH} = \frac{\frac{c_{CH_3COO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{CH_3COOH(aq)}}{1\text{M}} \cdot \frac{c_{H_2O(l)}}{1\text{M}}} \tag{A1}$$

In chemistry textbooks, *Eqn. A1* often appears with the different chemical species put within square brackets, as shown in *Eqn. A1'*:

$$K^c_{CH_3COOH} = \frac{[CH_3COO^-(aq)] \cdot [H_3O^+(aq)]}{[CH_3COOH(aq)] \cdot [H_2O(l)]} \tag{A1'}$$

whereby the 'bracket nomenclature' in this particular type of equation should mean 'dimensionless molar concentration', *i.e.*, the molar concentration divided by the concentration unit, 1M, *i.e.*, $[CH_3COO^-(aq)]$ actually stands for $c_{CH_3COO^-(aq)}/1\text{M}$, $[H_3O^+(aq)]$ for $c_{H_3O^+(aq)}/1\text{M}$, $[CH_3COOH(aq)]$ for $c_{CH_3COOH(aq)}/1\text{M}$, and $[H_2O(l)]$ for $c_{H_2O(l)}/1\text{M}$. As already mentioned in *Sect. 3*, such change in nomenclature within the same book is confusing; the widely accepted nomenclature is that square brackets indicate molar concentrations only, *i.e.*, $[CH_3COO^-(aq)] = c_{CH_3COO^-(aq)}$, $[H_3O^+(aq)] = c_{H_3O^+(aq)}$, *etc.*

To avoid misunderstandings, it would be much better to use square brackets ([...]) for molar concentrations only. If one likes to stick on the use of brackets, then one could use, for example, square brackets with a dot as superscript ($[...]^\bullet$) for molar concentrations divided by 1M, to emphasize the difference. With this, *Eqn. A1* can then be written as

$$K^c_{CH_3COOH} = \frac{[CH_3COO^-(aq)]^\bullet \cdot [H_3O^+(aq)]^\bullet}{[CH_3COOH(aq)]^\bullet \cdot [H_2O(l)]^\bullet} \tag{A1''}$$

Note that $K^c_{CH_3COOH}$ as defined in *Eqn. A1* or *A1''* is different from K_{a,CH_3COOH} defined in *Eqn. 8*, since $K^c_{CH_3COOH}$ contains in the denominator an additional $[H_2O(l)]^\bullet = c_{H_2O(l)}/1\text{M}$.

With the certainly correct argument that, in this type of dilute aqueous solutions, the concentration of water as solvent is constant, *Eqn. A1* or *A1''* can be multiplied on both sides with $c_{H_2O(l)}/1\text{M} = [H_2O(l)]^\bullet$, and one obtains

$$K^c_{CH_3COOH} \cdot \frac{c_{H_2O(l)}}{1\text{M}} = \frac{\frac{c_{CH_3COO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{CH_3COOH(aq)}}{1\text{M}}} = K'^c_{CH_3COOH} \tag{A2}$$

$$K^c_{CH_3COOH} \cdot [H_2O(l)]^\bullet = \frac{[CH_3COO^-(aq)]^\bullet \cdot [H_3O^+(aq)]^\bullet}{[CH_3COOH(aq)]^\bullet} = K'^c_{CH_3COOH} \tag{A2''}$$

$K'^c_{CH_3COOH}$ is here identical with the thermodynamic constant K_{a,CH_3COOH}, as elaborated for ideal solutions ($\gamma_i = 1$) (see *Eqn. 8* in *Sect. 3*). Therefore, for 25°C, at which the concentration of water is 55.33M, *i.e.*, $c_{H_2O(l)}/1\text{M} = [H_2O(l)]^\bullet = 55.33$, one obtains *Eqns. A3* and *A3''*.

$$K^c_{CH_3COOH}(25\,^\circ\text{C}) \cdot 55.33 = \frac{\frac{c_{CH_3COO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{CH_3COOH(aq)}}{1\text{M}}} = K'^c_{CH_3COOH}(25\,^\circ\text{C}) = K_{a,CH_3COOH}(25\,^\circ\text{C}) \tag{A3}$$

$$K^c_{CH_3COOH}(25\,^\circ\text{C}) \cdot 55.33 = \frac{[CH_3COO^-(aq)]^\bullet \cdot [H_3O^+(aq)]^\bullet}{[CH_3COOH(aq)]^\bullet} = K'^c_{CH_3COOH}(25\,^\circ\text{C}) = K_{a,CH_3COOH}(25\,^\circ\text{C}) \tag{A3''}$$

Note that both constants, $K^c_{CH_3COOH}$ and $K'^c_{CH_3COOH}$, are dimensionless.

If as in *Case i*, no division of c_i by 1M would be considered at all, then the constant defined in analogy to $K^c_{CH_3COOH}$, *i.e.*, $(c_{CH_3COO^-(aq)}) \cdot (c_{H_3O^+(aq)}) \cdot (c_{CH_3COOH(aq)})^{-1} \cdot (c_{H_2O(l)})^{-1}$, would also be dimensionless, while the constant defined in analogy to $K'^c_{CH_3COOH}$, *i.e.*, $(c_{CH_3COO^-aq)}) \cdot (c_{H_3O^+(aq)}) \cdot (c_{CH_3COOH(aq)})^{-1}$, would have the unit mol $l^{-1} = M$ (and another numerical value). In some general-chemistry textbooks, equilibrium constants indeed are defined according to *Case i*, *i.e.*, the 'acidity constants' of *Brønsted–Lowry* acids have dimensions [A2–A7].

The reason for defining equilibrium constants by using only molar concentrations for all chemical species involved, as in *Case i* is the early version of the 'mass action law', as originally proposed in 1864 by *Guldberg* and *Waage* (*Fig. A1* [A8–A10]). This law was formulated before *Gibbs* published his famous work on chemical thermodynamics [A11], and well before *Lewis* introduced the concept of activities [A12][A13].

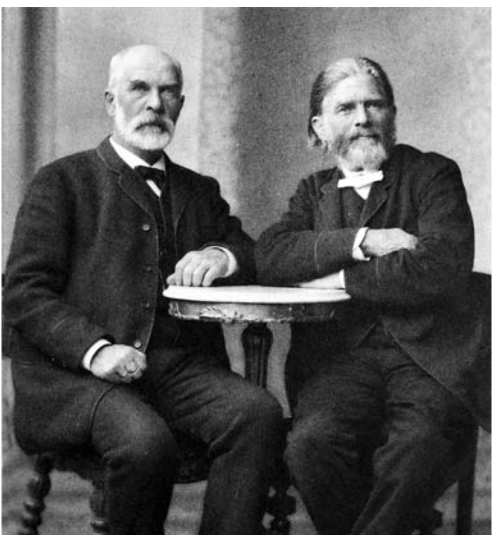

Fig. A1. Carlo Maximilian Guldberg (1836–1902; *left*) *and* Peter Waage (1833–1900; *right*). Photograph from http://de.wikipedia.org/wiki/Cato_Maximilian_Guldberg, accessed February 28, 2013.

It has to be pointed out that the equilibrium constant defined by the *Guldberg* and *Waage* version of the mass action law is not necessarily compatible with the thermodynamic equilibrium constant, since it does not make reference to the different standard states of solvated species and of the solvent (see the main text).

Let us return to the equilibrium of the reaction in *Eqn. 1.* If this equilibrium is considered but as formulated with *Eqn. 1'*, the same treatment corresponding to *Case ii* yields a reaction constant, abbreviated as $K''^c_{CH_3COOH}$, which is *different* from $K^c_{CH_3COOH}$, as shown in *Eqn. A4*, although *Eqns. 1* and *1'* represent one and the same reaction with one and the same stoichiometry.

$$K''^c_{CH_3COOH} = \frac{\frac{c_{CH_3COO^-(aq)}}{1M} \cdot \frac{c_{H^+(aq)}}{1M}}{\frac{c_{CH_3COOH(aq)}}{1M}} \tag{A4}$$

In this expression, the molar concentration of the solvent water is not considered at all, since it does not appear explicitly in *Eqn. 1′*. Since $c_{H^+(aq)}/1M = c_{H_3O^+(aq)}/1M$, one obtains *Eqn. A5*, which indicates that $K''^c_{CH_3COOH} = K'^c_{CH_3COOH} = K_{a,CH_3COOH}$.

$$K''^c_{CH_3COOH} = \frac{\frac{c_{CH_3COO^-(aq)}}{1M} \cdot \frac{c_{H^+(aq)}}{1M}}{\frac{c_{CH_3COOH(aq)}}{1M}} = \frac{\frac{c_{CH_3COO^-(aq)}}{1M} \cdot \frac{c_{H_3O^+(aq)}}{1M}}{\frac{c_{CH_3COOH(aq)}}{1M}} = K'^c_{CH_3COOH} = K_{a,CH_3COOH} \quad (A5)$$

The equilibrium constants $K'^c_{CH_3COOH}$ and $K''^c_{CH_3COOH}$ obtained as outlined in this *Note* have the same numerical values as the thermodynamical equilibrium constant K_{a,CH_3COOH} elaborated in *Sect. 3*, and all constants are dimensionless. Therefore, one may argue that it does not matter whether chemical reaction equilibria are treated in the strictly thermodynamic way outlined in *Sect. 3*, or in the way illustrated here, *Case ii*. The situation changes, however, if water is a *Brønsted–Lowry* acid and reacts with water as a *Brønsted–Lowry* base in water as solvent, *i.e.*, if the '*self-ionization of water*' is considered. The arguments then become a bit 'dubious', as outlined in *Note A2*.

Note A2. On the Molar-Concentration-Based 'Acidity Constant' of Water in Water, $K'^{c*}_{H_2O}$*(25 °C) and Its Negative Logarithm,* $pK'^{c*}_{H_2O}$*(25 °C)*. Considering the reaction in *Eqn. 18* and taking for all species, *i.e.*, the dissolved ions HO^-(aq) and H_3O^+(aq) and the solvent H_2O(l), molar dimensionless concentrations, *Case ii* mentioned in *Note A1*, one obtains – again by assuming that all activity coefficients are 1 – *Eqn. A6*

$$K^c_{H_2O} = \frac{\frac{c_{HO^-(aq)}}{1M} \cdot \frac{c_{H_3O^+(aq)}}{1M}}{\frac{c_{H_2O(l)}}{1M} \cdot \frac{c_{H_2O(l)}}{1M}} = \frac{\frac{c_{HO^-(aq)}}{1M} \cdot \frac{c_{H_3O^+(aq)}}{1M}}{\left(\frac{c_{H_2O(l)}}{1M}\right)^2} \quad (A6)$$

Multiplying both sides of *Eqn. A6* with the constant term $(c_{H_2O(l)}/1M)^2$ gives

$$K^c_{H_2O} \cdot \left(\frac{c_{H_2O(l)}}{1M}\right)^2 = \frac{c_{HO^-(aq)}}{1M} \cdot \frac{c_{H_3O^+(aq)}}{1M} = K'^c_{H_2O} = K_w \quad (A7)$$

At 25 °C, $c_{HO^-(aq)}/1M = c_{H_3O^+(aq)}/1M = 10^{-7.0}$ [A14]. Therefore, one obtains $K^c_{H_2O}(25\,°C) = 3.27 \cdot 10^{-18}$ and $K'^c_{H_2O} = 10^{-14.0}$ (see *Eqns. A8* and *A9.*)

$$K^c_{H_2O}(25\,°C) = \frac{K_w(25\,°C)}{\left(\frac{c_{H_2O(l)}}{1M}\right)^2} = \frac{10^{-14.0}}{\left(\frac{55.33\,mol\,l^{-1}}{1M}\right)^2} = \frac{10^{-14.0}}{3061.4} = 3.27 \cdot 10^{-18} \quad (A8)$$

$$K'^c_{H_2O}(25\,°C) = K_w(25\,°C) = \frac{c_{HO^-(aq)}}{1M} \cdot \frac{c_{H_3O^+(aq)}}{1M} = \frac{10^{-7.0}\,M}{1M} \cdot \frac{10^{-7.0}\,M}{1M} = 10^{-14.0} \quad (A9)$$

Again, all constants $K^c_{H_2O}$ and $K'^c_{H_2O} = K_w$ are dimensionless.

The constant $K^c_{H_2O}(25\,°C) = 3.27 \cdot 10^{-18}$ and its negative logarithm $pK^c_{H_2O}(25\,°C) = 17.5$ are not of any great direct use, since they are *not* compatible with tabulated thermodynamic data, and, therefore can, for example, not be used for a comparison of acid strengths. On the other hand, $K'^c_{H_2O}$ (25 °C) = K_w (25 °C) = $10^{-14.0}$ [A15] and its negative logarithm, $pK_w(25\,°C) = 14.0$, are fully compatible with thermodynamic data (see *Sect. 4*). $K'^c_{H_2O}(25\,°C) = K_w(25\,°C) = 10^{-14.0}$ is the self-ionization constant for water at 25 °C, which is identical with the thermodynamic acidity constant of water in water at 25 °C, $K_{a,H_2O}(25\,°C)$, *i.e.*, $pK_{a,H_2O}(25\,°C) = 14.0$ (see *Sect. 4*).

The question we address now is how a 'pK_a value' for water at 25 °C of 15.7 was attained, as mentioned in essentially all organic-chemistry textbooks. Obviously, $pK_a = 15.7$ means $K_a = 10^{-15.7}$, a value which is obtained if $K_w(25\,°C) = 10^{-14.0}$ is divided by $55.33 = c_{H_2O}/1M$. This simple mathematical

operation is hard to rationalize from a chemical point of view if one takes into account the *Brønsted–Lowry* acid–base reactivity of water as formulated the way it is done with *Eqn. 18*, unless one argues on the basis of rather 'dubious' considerations; to make it clear, *Eqn. 18* is not really compatible with an equilibrium constant of $10^{-15.7}$, as outlined in the following.

The forward reaction of the chemical equilibrium in *Eqn. 18* is the description of the reaction of water as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base in water as solvent, whereby H_2O simultaneously plays three roles, as *Brønsted–Lowry* acid, as *Brønsted–Lowry* base, and as solvent. If comparison is made with the forward reaction of *Eqn. 1*, one realizes one fundamental difference. In the forward reaction of *Eqn. 1*, water plays only two roles, as *Brønsted–Lowry* base and as solvent, while $CH_3COOH(aq)$ is the *Brønsted–Lowry* acid. One may consider a kind of 'unified view' of the two reactions shown in *Eqns. 18* and *1* by replacing *Eqn. 2* with *Eqn. A10:*

$$H_2O(aq) + H_2O(l) \rightleftharpoons HO^-(aq) + H_3O^+(aq) \tag{A10}$$

Eqn. A10 certainly is strange since it implies something which is more hairsplitting than reasonable. In any case, for *Eqn. A10* one obtains – with all the assumptions and conventions mentioned above in this chapter – for the 'acidity constant' of H_2O first *Eqn. A11*

$$K^{c*}_{H_2O} = \frac{\frac{c_{HO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{H_2O(aq)}}{1\text{M}} \cdot \frac{c_{H_2O(l)}}{1\text{M}}} \tag{A11}$$

Multiplying on both sides of *Eqn. A11* with $c_{H_2O(l)}/1\text{M}$ yields *Eqn. A12*

$$K^{c*}_{H_2O} \cdot \left(\frac{c_{H_2O(l)}}{1\text{M}}\right) = \frac{\frac{c_{HO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{H_2O(aq)}}{1\text{M}} \cdot \frac{c_{H_2O(l)}}{1\text{M}}} \cdot \frac{c_{H_2O(l)}}{1\text{M}} = \frac{\frac{c_{HO^-(aq)}}{1\text{M}} \cdot \frac{c_{H_3O^+(aq)}}{1\text{M}}}{\frac{c_{H_2O(aq)}}{1\text{M}}} = K'^{c*}_{H_2O} \tag{A12}$$

For 25 °C, $c_{HO^-(aq)} = c_{H_3O^+(aq)} = 10^{-7.0}$ M, and if one uses for $c_{H_2O(aq)}/1\text{M}$ a value of 55.33, the numerical value of $10^{-15.7}$ is obtained for $K'^{c*}_{H_2O}$, *i.e.*, as negative logarithm of this constant, $pK'^{c*}_{H_2O}(25\,°C)$, a value of 15.7. Obviously, however, it cannot be that $c_{H_2O(aq)} = c_{H_2O(l)} = 55.33\text{M}$ in one and the same solution, if $H_2O(aq)$ and $H_2O(l)$ are considered to be different species as it appears from *Eqn. A10*. At 25 °C, the *total* amount of water in 1 l is 55.33 mol, not 110.66 mol (see *Sect. 3*). Therefore, all the above arguments are 'dubious' and should not be used at all since they are wrong. In contrary, the considerations made here are useful to explain the students in a hopefully convincing way why the $pK_a(25\,°C)$ value of water in water *cannot* be 15.7, if $pK_w(25\,°C) = 14.0$.

Two questions remain. *Question 1:* 'Why should a division of $K_w(25\,°C)$ by 55.33 be appropriate for expressing the acidity of water?' *Question 2:* 'Who was, or who were, the first to define the equilibrium constant for the reaction of water as *Brønsted–Lowry* acid with water as *Brønsted–Lowry* base such that a value of $10^{-15.7}$ at 25 °C is obtained?'

The answer to *Question 1* can be given by considering instead of *Eqn. 18 Eqn. 18'*, or even – to be somehow consistent with *Eqn. 1'* – the following equation:

$$H_2O(aq) \rightleftharpoons HO^-(aq) + H^+(aq) \tag{A10'}$$

Eqn. A10' certainly is unusual but it is written here to emphasize the lines of thinking and the ideas of having a similar formalism as in *Eqn. 1'*. $H_2O(aq)$ is the same as $H_2O(l)$, *i.e.*, liquid water.

If one considers the concentration of $H_2O(aq)$ at 25 °C as the same as the concentration of $H_2O(l)$ at 25 °C, although with *Eqn. A10* it is assumed that they represent two different species, one obtains *Eqn. A13*

$$K^{c*}_{H_2O}(25\,°C) = \frac{K_w(25\,°C)}{\frac{c_{H_2O(aq)}}{1\text{M}}} = \frac{10^{-14}}{\frac{55.33\text{M}}{1\text{M}}} = \frac{10^{-14}}{55.33} = 1.81 \cdot 10^{-16} = 10^{-15.7} \tag{A13}$$

and with this value, one obtains *Eqn. A14*

$$pK^{c*}_{H_2O}(25\,^\circ C) = -\log\left(K^{c*}_{H_2O}(25\,^\circ C)\right) = -\log\left(1.81 \cdot 10^{-16}\right) = 15.7 \quad (A14)$$

Note that for the sake of distinguishing the water acidity constant, as obtained with the conventions just outlined, from the thermodynamic constant $K_{a,H_2O} = K_w$ (see *Sect. 4*), we use a different abbreviation that is $K^{c*}_{H_2O}$ (see *Eqn. A13*).

The true significance of the *thermodynamically incorrect equilibrium constant*, $K^{c*}_{H_2O}$, and its negative logarithm, $pK^{c*}_{H_2O}$, is questionable. It is very doubtful whether $pK^{c*}_{H_2O}$ is meaningful for a comparison with the acidity of organic acids which are almost insoluble in water [A16]. In this latter case, the 'pK_a values' reported for organic acids in organic-chemistry textbooks, *e.g.*, the 'pK_a values' of CH_4 or benzene are relative values and *cannot* be compared with the thermodynamic values determined for 'conventional acids' that are soluble in aqueous solution. Therefore, these 'pK_a values' should *not* be included in tables together with thermodynamic pK_a values. Alternatively, corrections of listed 'pK_a values' for organic acids should be made, as already mentioned previously [A17]. In any case, the estimated acidity of very weakly acidic organic molecules are approximate values anyway, although these approximate, relative acidity values are extremely useful when discussing the reactivity of organic molecules [A18][A19].

If two differently defined 'pK_a values' of two acids are compared, then one may draw wrong conclusions. This is what seems to be the case if the acidity of water is compared with the acidity of methanol [A20–A23]. Using for both *Brønsted–Lowry* acids the same thermodynamic definitions, then – at 25 °C – water in water ($pK_{a,H_2O}(25\,^\circ C) = 14.0$) is more acidic than methanol in water ($pK_{a,CH_3OH}(25\,^\circ C) = 15.6$).

In any case, to avoid confusion, it would be better to use different abbreviations for differently defined reaction constants, for example K_{a,H_2O} and K^{*}_{a,H_2O}, as proposed here.

An answer to *Question 2* – who was, or who were, the first stating that $pK_{a,H_2O}(25\,^\circ C) = 15.7$ – cannot be given with full certainty. They were the ones who did the pioneering work on the quantitative determination of the *relative acidity* of weakly acidic organic molecules by '*non-aqueous competition experiments*' [A24–A27]. They thought – without any experimental data – that the 'pK_a value of water' at 25 °C has to be 15.7 and the 'pK_a value of H_3O^+' −1.8, although the arguments put forward at that time [A24] were not very convincing. For example, for the reaction of H_3O^+(aq) with water, the equilibrium considered was formulated as in *Eqn. A15* [A24]:

$$H_3O^+ + H_2O(\text{solvent}) \rightleftharpoons H_3O^+ + H_2O \quad (A15)$$

and the argument was that 'H_2O(solvent)' is not included in the equilibrium constant, while the concentration of 'H_2O' is taken as 55.33M (or 55.5M as in the original report [A24]). Therefore, an equilibrium constant K is obtained with a value of 55.33M (or 55.5M, [A24]). The following text is taken from an organic textbook [A28]: '*Note that the* K$_a$ *for water is obtained by dividing* K$_w$ *by the concentration of water, 55.5 moles* L^{-1}*. This change is necessary to put all of the ionizations on the same scale and in the same units. Recall that the ion product of water,* K$_w$*, has units of moles*^{2} L^{-2} *or* M^2*, whereas* K$_a$ *values are given in units of moles* L^{-1} *or* M.' Obviously, these arguments of the necessity of having equilibrium constants with uniform units are not compatible at all with the thermodynamic conventions outlined in *Sect. 3–6*. As repeatedly emphasized in the main text, thermodynamic equilibrium constants are dimensionless, including K_w; they all have no units.

Final Remarks. There are convincing arguments for introducing and using activities and appropriate thermodynamic standard states when teaching quantitative aspects of chemical equilibria (main text). If properly and systematically performed, existing confusions in chemistry textbooks can be eliminated, and with this, full compatibility of thermodynamic equilibrium constants and tabulated thermodynamic data can be attained. This is the case not only for the *Brønsted–Lowry* acid–base equilibria discussed in this review, but also for other chemical equilibria, including the water solubility of sparingly soluble salts with their characteristic solubility products $K_{sp}(T)$.

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Irigermanone, a Noriridal with Unprecedented Methylketone Function, from *Iris germanica*

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A new iridal, irigermanone (**1**) and nine known congeners, **2**–**10**, respectively, have been isolated from the dried rhizomes of German iris (*Iris germanica*). The structure of **1** was established by spectroscopic methods including HR-ESI-MS, 1D- and 2D-NMR, and electronic circular dichroism (ECD) spectroscopy. Compound **1** is a structurally unique noriridal, and it possesses an unprecedented methylcarbonyl group instead of the α,β-unsaturated aldehyde function typical for this group of triterpenes.

Introduction. – As part of our studies on indigenous traditional medicinal plants, we investigated the lipophilic constituents of the rhizome of the German iris (*Iris germanica* L., Iridaceae), a widely distributed ornamental plant. Traditionally, its rhizomes have been used for different topical and oral applications, such as treatment of respiratory diseases, as pain relief for teething children [1], in cosmetic preparations, and in perfumery. Typical constituents of *I. germanica* are isoflavones [2] and iridals, a group of C_{31}-triterpenoids derived from squalene by an unique biosynthetic pathway. These compounds have a seco-ring-A moiety in common and are subdivided in monocyclic, bicyclic, and spirocyclic derivatives [3]. The oxidative degradation of bicyclic iridals during drying and storage of the iris rhizomes leads to formation of irones which are responsible for the violet-like scent of orris oil [3]. We report here on the isolation and structure elucidation of a structurally unique noriridal, irigermanone (**1**). This compound represents the first example within this group of triterpenes in which the characteristic α,β-unsaturated aldehyde function is replaced by a methylcarbonyl group.

Results and Discussion. – Irigermanone (**1**) and nine known congeners, **2**–**10**, were obtained in pure form, or as mixtures of inseparable *cis*/*trans* isomers, by fractionation of the CH_2Cl_2 extract by a combination of *Sephadex LH20* column chromatography, and semi-preparative and preparative HPLC.

Compound **1** showed a *quasi*-molecular-ion peak at *m*/*z* 495.3445 ($[M+Na]^+$) in the HR-ESI-MS, indicating the molecular formula $C_{30}H_{48}O_4$. The ^{1}H-NMR spectrum showed the signals of four olefinic H-atoms at δ(H) 5.26 (*dd*, $J = 6.9, 6.9$), 5.35 (*dd*, $J =$

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15.4, 10.3), 5.41 (br. *s*), and 5.98 (*d*, $J = 15.4$), and of seven O-bearing CH groups (*Table*). In addition, seven Me signals, six *singlet*s (including a deshielded signal (δ(H) 2.25)) and one *doublet*, were observed. In the ^{13}C-NMR spectrum, 30 signals were detected which were assigned to seven Me, nine CH_2, and seven CH groups consisting of three sp^3 and four sp^2 C-atoms, and seven quaternary C-atoms, including a CO group (δ(C) 210.0) and two olefinic C-atoms (*Table*). These data, together with chemotaxonomic considerations, strongly suggested that compound **1** was a triterpene belonging to the iridal series. However, a striking difference compared to this group of compounds was the absence of the typical aldehyde function which was replaced by a methylcarbonyl group. Close inspection of the ^{1}H- and ^{13}C-NMR data revealed a strong

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**. Recorded in $CDCl_3$ at 500 and 125 MHz, respectively.

Position[a])	δ(H)[b]) (*J* in Hz)	δ(C)	HMBC (H → C)[c])
2		210.0	
3	3.52–3.57 (*m*)	62.7	4 (*w*), 5
4	1.53	33.3	
5	1.79, 1.51	23.8	10, 11
6	1.92	58.6	8
7		93.6	
8	1.89, 1.79	36.6	2, 9, 11
9	2.12 (*ddd*, *J* = 13.3, 9.3, 4.6), 1.46	32.6	7, 11
10		90.1	
11		53.3	
12	1.48, 1.39	36.4	11, 13
13	2.05	24.4	12
14	5.26 (*t*, *J* = 6.9)	129.4	16, 28
15		134.3	
16	5.98 (*d*, *J* = 15.4)	137.4	14, 15, 18, 28
17	5.35 (*dd*, *J* = 15.4, 10.3)	128.8	15, 18
18	2.35 (br. *d*, *J* = 11.4)	56.5	
19		134.7	
20	5.41 (br. *s*)	121.9	
21	1.84, 1.70	32.1	
22	1.44	38.4	
23		35.8	
24	0.63	14.9	18, 22, 23, 30
25	2.25 (*s*)	29.1	2, 7
26	3.76 (br. *s*)	64.0	6, 10, 11, 12
27	1.54 (*s*)	19.0	9, 10, 11
28	1.70 (*s*)	12.9	14, 15, 16
29	1.49 (*s*)	23.4	18, 19, 20
30	0.82 (*s*)	26.7	18, 19 (*w*), 23, 24
31	0.83 (*d*, *J* = 7.1)	15.8	21, 23

[a]) According to the usual numbering of iridals. [b]) Multiplicity of overlapped signals is omitted. [c]) w: Weak.

structural similarity with the bicyclic iridal iripallidal (**6**) [4]. The signals assigned to the irone moiety, resulting from the methylation and cyclization of the homofarnesyl chain, were almost identical in both compounds. The connectivity and the relative configuration of this part of the molecule were confirmed by HMBCs and NOESY correlations (*Fig. 1*). Regarding the substitution of the *B*-ring, compound **1** possessed a HO–CH_2 group (δ(H) 3.76 (br. *s*); δ(C) 64.0), a hydroxypropyl chain (δ(C) 23.8, 33.3, 62.7) at C(6), and a Me group (δ(H) 1.54 (*s*); δ(C) 19.0) at C(10). These features were similar to the ones present in iripallidal. However, the typical α,β-unsaturated aldehyde function at C(7) was lacking, and was replaced by a Ac group (δ(H) 2.25; δ(C) 29.1, 210.0). The location of the different substituents was confirmed by HMBCs (*Fig. 1*). In particular, correlations from Me(25) to the C(2)O and C(7), from CH_2(8) to C(2), and from CH_2(9) to C(7) confirmed the position of the Ac moiety. The remaining degree of unsaturation had to be accounted for by an epoxy bridge connecting C(7) and C(10).

Its position was deduced from the strong deshielding of C(7) and C(10). A similar deshielding effect was observed before in a rare example of an iridal with an epoxy bridge between C(7) (δ(C) 87.5 [5]) and CH_2(26) [5]. Further HMBCs corroborated the constitutional formula of **1** as shown in *Fig. 1*.

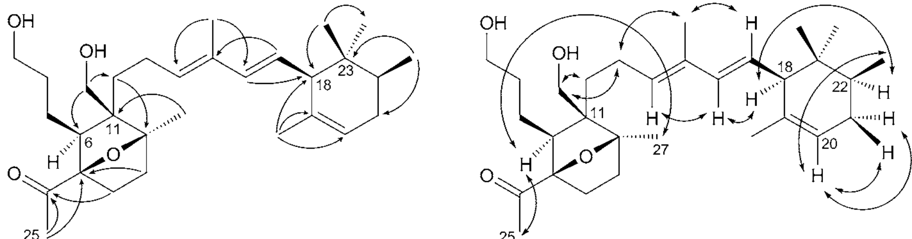

Fig. 1. *Key HMBCs* (H → C) *and NOESY* (H ↔ H) *correlations of* **1**

The relative configuration within ring *B* was deduced from NOESY correlations (*Fig. 1*) and biogenetic considerations. NOE Correlations between H–C(6) and Me(27) and COMe(25) indicated a *cis*-relationship between these substituents. The configuration at C(11) was assumed to be (*S*) as in all other iridals reported so far [6]. Moreover, *Marner* and *Jaenicke* could show, by ozonolysis of iridals and subsequent analysis of their degradation products, that the (6*R*,10*S*,11*S*) absolute configuration of the *B* ring is found in all iridals [6]. In contrast, methylation and cyclization of the homofarnesyl side chain can lead to diastereoisomeric and enantiomeric configurations. Differences in the stereospecificity of the cyclization explain that irones which are formed by degradation of iridals can have opposite absolute configurations in different *Iris* species or varieties [3][6][7]. The relative configuration of the irone moiety was deduced from the NOESY experiment. A cross-peak observed between H–C(18) and H–C(22) implied that these H-atoms were in a cofacial orientation. The absolute configuration of the irone moiety was established by comparison of the experimental electronic circular dichroism (ECD) spectrum with calculated ECD data, using time-dependent density functional theory (TDDFT) at the CAM-B3LYP/6-31G** level and MeOH as solvent. The CD spectrum of **1** showed a negative *Cotton* effect around 231 nm which is associated with the $\pi \rightarrow \pi^*$ transition of the conjugated diene. A comparison of the experimental spectrum with the calculated ECD spectra of both possible diastereoisomers is presented in *Fig. 2*. The averaged ECD spectrum for the (18*R*,22*S*)-stereoisomer and the experimental data were in good agreement. In particular, the negative *Cotton* effect at 232 nm matched well with the experimental data (231 nm, $\Delta\varepsilon = -16.7$), while the *Cotton* effect of the (18*S*,22*R*)-stereoisomer showed the opposite sign. Thus, the absolute configuration of the irone moiety was established as (18*R*,22*S*).

Known iridals were identified, on the basis of their ESI-MS data, and comprehensive 1D- and 2D-NMR analysis, as iridobelamal A (**2**) [8], a mixture of the *cis*/*trans* isomers isoiridogermanal (**3**) [8] and iridobelamal A (**2**), iriflorental (**4**) [9][10], irisgermanical C (**5**) [4], iripallidal (**6**) [4], γ-irigermanal (**7**) [11], α-dehydroirigermanal (**8**) [10][11], and a mixture of α-irigermanal (**9**) [11], and its *trans*-isomer **10**.

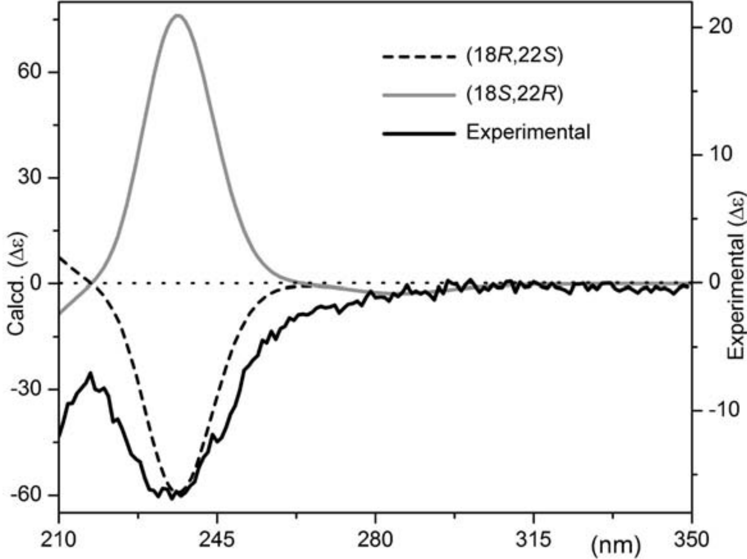

Fig. 2. *Comparison of experimental and calculated ECD spectra of* **1**. The calculations were performed with TDDFT at the CAM-B3LYP/6-31G** level with MeOH as solvent.

Iridals represent a group of more than 40 triterpenes derived from squalene through an uncommon biosynthetic pathway. These compounds have a restricted distribution in plants and have been so far only found in *Iris* spp. and *Belamcanda chinensis* (Iridaceae). Their common features are a seco-ring-*A* and typical α,β-unsaturated aldehyde moieties. To our knowledge, compound **1** is the first iridal derivative in which the aldehyde function is replaced by a ketone. As to the biogenesis of irigermanone (**1**), it is reasonable to assume that the compound is derived from iripallidal. In a first step, the formation of the epoxy bridge by the nucleophilic attack of HO–C(10) at C(7) would lead to a saturated aldehyde function. The existence of this precursor is supported by the presence of such a moiety in a previously reported iridal with a C(7)/C(26) epoxy bridge [5]. Subsequent oxidation by a dioxygenase or a peroxidase, followed by elimination of HCOOH, would afford compound **1**, possibly *via* a dioxetane intermediate [12][13].

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Amersham Biosciences*), *C-605* pump, *C-660* collector (*Büchi Labortechnik*). TLC: precoated SiO_2 Al sheets (F_{254}, 0.22-μm thickness; *Merck*). Optical rotation: *Jasco P-2000* polarimeter. CD: *Chirascan* spectropolarimeter; λ ($\Delta\varepsilon$) in nm. UV: *Perkin-Elmer Lambda 35* spectrometer; λ_{max} (log ε) in nm. Prep. HPLC: *Shimadzu LC-8A* liquid chromatograph equipped with a DAD detector, *SunFire* C_{18} column (30 × 150 mm i.d., 5 μm;

Waters) equipped with a precolumn (20 × 10 mm i.d.); flow rate, 20 ml/min; UV detection at 254 nm. Semi-prep. HPLC: *Agilent 1100 series* instrument with a DAD detector; *SunFire* C_{18} column (10 × 150 mm i.d., 5 μm; *Waters*) and *Atlantis* dC_{18} column (10 × 150 mm i.d., 5 μm; *Waters*), both equipped with a precolumn (10 × 10 mm); flow rate, 4 ml/min; UV detection at 230, 254, and 344 nm. NMR: *Avance III* spectrometer (*Bruker*) equipped with a 5-mm BBO probe (^{13}C-NMR) or a 1 mm TXI microprobe (^{1}H- and 2D-NMR); at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm with the solvent signal as internal reference, J in Hz. ESI-MS: *Esquire 3000 plus* ion-trap mass spectrometer (*Bruker*). HR-ESI-MS: *MicrOTOF* (*Bruker*).

Plant Material. The rhizomes of *Iris germanica* were purchased from *Dixa AG* (Switzerland). A voucher specimen (No. 591) is kept at the Division of Pharmaceutical Biology, University of Basel.

Extraction and Isolation. The dried rhizomes (884 g) were powdered under liquid N_2 and extracted three times overnight with 4, 3.5, and 3 l of CH_2Cl_2, resp. The combined extracts were evaporated to dryness under reduced pressure to yield 31.6 g of dry extract. The CH_2Cl_2 extract was separated in two portions over a *Sephadex LH20* column (5 × 85 cm i.d.) eluted with MeOH/$CHCl_3$ 4 : 1. After TLC (SiO_2; $CHCl_3$/MeOH 97 : 3, detection with vanillin–sulfuric acid), the fractions containing iridals were combined to provide a crude mixture (5.4 g). This mixture was further separated by prep. HPLC (28 injections) with a gradient of 80 – 95% MeOH in H_2O over 45 min, followed by 95% MeOH for 10 min. Nine peaks were collected, from which ten compounds were finally purified, or obtained as mixtures of inseparable geometric isomers, by semi-prep. HPLC with optimized gradients of MeCN or MeOH in H_2O: **1** (6.9 mg), **2** (6.6 mg), a mixture of the *cis*/*trans* isomers **2**/**3** (32.7 mg), **4** (2.7 mg), **5** (6.4 mg), **6** (35.1 mg), **7** (5.3 mg), **8** (5.1 mg), and a mixture **9**/**10** (43.6 mg). The *cis*/*trans* isomeric mixtures **2**/**3** and **9**/**10**, resp., could not be separated, possibly due to isomerization during the isolation and/or characterization process.

Computational Methods. Conformation analysis of **1** was performed with *Schrödinger* MacroModel 9.1 (*Schrödinger*, LLC, New York) employing the OPLS2005 (optimized potential for liquid simulations) force field in H_2O. Conformers within a 1 kcal/mol energy window from the global minimum were selected for geometrical optimization and energy calculation applying DFT with the *Becke*'s nonlocal three parameter exchange and correlation functional, and the *Lee*–*Yang*–*Parr* correlation functional level (B3LYP) using the 6-31 G** basis set in the gas phase with the Gaussian 09 program package [14]. Vibrational evaluation was performed at the same level to confirm minima. Excitation energy (denoted by wavelength in nm), rotator strength dipole velocity (R*vel*), and dipole length (R*len*) were calculated in MeOH by TD-DFT/CAM-B3LYP/6-31 G**, using the SCRF method, with the CPCM model. The ECD curves were obtained on the basis of rotator strengths with a half-band of 0.25 eV using SpecDis v1.53 [15]. The spectra were combined after *Boltzmann* weighting according to their population contribution.

Irigermanone (*=1-[(1*R,*2*S,*3*S,*4*S*)-3-(Hydroxymethyl)-2-(3-hydroxypropyl)-4-methyl-3-{(3*E,*5*E*)-4-methyl-6-[(1*R,*5*S*)-2,5,6,6-tetramethylcyclohex-2-en-1-yl]hexa-3,5-dien-1-yl}-7-oxabicyclo[2.2.1]hept-1-yl]ethanone*; **1**). Amorphous white powder. $[\alpha]_D^{26} = 0$ ($c = 0.1$, $CHCl_3$). UV (MeOH): 231 (4.8), CD (MeOH): 231 (−16.7). ^{1}H- and ^{13}C-NMR: see the *Table.* ESI-MS (pos.): 495.5 ($[M + Na]^+$), 473.4 ($[M + H]^+$), 455.4 ($[(M - H_2O) + H]^+$), 437.4 ($[(M - 2\,H_2O) + H]^+$). HR-ESI-MS (pos.): 495.3445 ($[M + Na]^+$, $C_{30}H_{48}NaO_4^+$; calc. 495.3450).

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Insights into the Synthesis of Steroidal *A*-Ring Olefins

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The classical synthesis, followed by purification of the steroidal *A*-ring Δ^1-olefin, 5α-androst-1-en-17-one (**5**), from the Δ^1-3-keto enone, (5α,17β)-3-oxo-5-androst-1-en-17-yl acetate (**1**), through a strategy involving the reaction of Δ^1-3-hydroxy allylic alcohol, 3β-hydroxy-5α-androst-1-en-17β-yl acetate (**2**), with $SOCl_2$, was revisited in order to prepare and biologically evaluate **5** as aromatase inhibitor for breast cancer treatment. Surprisingly, the followed strategy also afforded the isomeric Δ^2-olefin **6** as a by-product, which could only be detected on the basis of NMR analysis. Optimization of the purification and detection procedures allowed us to reach 96% purity required for biological assays of compound **5**. The same synthetic strategy was applied, using the Δ^4-3-keto enone, 3-oxoandrost-4-en-17β-yl acetate (**8**), as starting material, to prepare the potent aromatase inhibitor Δ^4-olefin, androst-4-en-17-one (**15**). Unexpectedly, a different aromatase inhibitor, the $\Delta^{3,5}$-diene, androst-3,5-dien-17-one (**12**), was formed. To overcome this drawback, another strategy was developed for the preparation of **15** from **8**. The data now presented show the unequal reactivity of the two steroidal *A*-ring Δ^1- and Δ^4-3-hydroxy allylic alcohol intermediates, 3β-hydroxy-5α-androst-1-en-17β-yl acetate (**2**) and 3β-hydroxyandrost-4-en-17β-yl acetate (**9**), towards $SOCl_2$, and provides a new strategy for the preparation of the aromatase inhibitor **12**. Additionally, a new pathway to prepare compound **15** was achieved, which avoids the formation of undesirable by-products.

Introduction. – Among several biological activities, steroidal *A*-ring olefins, with a C(17)=O group, particularly Δ^3- and Δ^4-olefins (*Fig.*) [1][2], were shown to be interesting aromatase inhibitors (AIs). AIs block the biosynthesis of estrogens and offer a therapeutic alternative for the treatment of estrogen-dependent cancers, namely breast cancer [3–5]. This has been attributed to two different factors: the planarity conferred by the C=C bonds, which allows better fitting the enzyme receptor core, and the H-bonding capacity of the C(17)=O O-atom to a receptor residue [1][6][7].

As part of a project on new structure–activity relationships (SAR) of steroidal AIs [1][6][7], we are now interested in synthesizing Δ^1- and Δ^4-olefins **5** and **15**, respectively (*Schemes 1*, *2*, and *3*). Concerning **5**, there are very few references describing its preparation, and the most complete one is a 50 years old reporting the

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Figure *Steroidal Δ³- and Δ⁴-olefin aromatase inhibitors*

synthesis using the Δ^1-3-keto enone **1** as starting material, and the allylic alcohol **2** as intermediate [8]. In a recent work, we revisited this synthesis [7] and, in the present work, we further explored new insights into the referred strategy. In this study, along with the previously reported Δ^1-olefin **5**, the Δ^2-isomer **6** was identified as a by-product (*Scheme 1*).

Scheme 1. *Synthesis of (5α)-Androst-1-en-17-one* (**5**) *from (5α,17β)-3-Oxoandrost-1-en-17-yl Acetate* (**1**)

i) Li(*t*-BuO)$_3$AlH, anh. THF, reflux, 3 h; 94%. *ii*) $SOCl_2$, benzene, 5–8°, 1–2 h. *iii*) $LiAlH_4$, Et_2O, reflux, 11 h. *iv*) CrO_3, H_2SO_4, acetone, 0°: 73% of **5**/**6**.

Furthermore, to achieve an alternative synthetic strategy for the preparation of the Δ^4-olefin **15** [2][7], that avoids the formation of the Δ^4-17β-hydroxylated derivative as well as the Δ^3-olefin as by-products, we applied the same methodology using the Δ^4-3-keto enone **8** as starting material. Unexpectedly, in this case, instead of the desired **15**, another AI, the $\Delta^{3,5}$-diene **12** [9–16], was obtained as the only product (*Scheme 2*). To overcome this problem, another synthetic strategy, using also **8** as starting material, was developed for the preparation of **15** (*Scheme 3*).

Scheme 2. *Formation of Androsta-3,5-dien-17-one* (**12**) *from Testosterone* (**7**)

i) Ac_2O, dry pyridine, r.t., 21 h 25 min; 84%. *ii*) $Li(t\text{-}BuO)_3AlH$, anh. THF, reflux, 3 h 30 min. *iii*) $SOCl_2$, benzene, 5–8°, 5 h 30 min; 56%. *iv*) $LiAlH_4$, Et_2O, reflux, 8 h; 91%. *v*) CrO_3, pyridine, r.t., 19 h; 38%.

Scheme 3. *Preparation of Androst-4-en-17-one* (**15**) *from (17β)-3-Oxoandrost-4-en-17-yl Acetate* (**8**)

i) $NaBH_4$, CF_3COOH, AcOH, MeCN, CH_2Cl_2, r.t., 3 h 30 min; 99%. *ii*) KOH, H_2O, dioxane, r.t., 2–3 d; 99%. *iii*) CrO_3, H_2SO_4, H_2O, acetone, 0°, 5 min; 75%.

^{1}H-NMR Spectroscopy turned out to be the most adequate technique for the detection of the Δ^2-olefin **6**, as well as of its precursors, and the 2D-COSY experiment allowed us to unequivocally identify the precursor **10** of the $\Delta^{3,5}$-diene **12**.

Results and Discussion. – The Δ^1-olefin **5** was prepared as described in [7][8] (*Scheme 1*). Briefly, reduction of enone **1** gave allylic alcohol **2**, which afforded an

untractable crude (TLC, NMR, and LC/MS control) after treatment with $SOCl_2$. Reaction of this crude with $LiAlH_4$ and conventional workup furnished a mixture (one TLC spot) of the isomers **3** and **4** in similar amounts (NMR analysis). To isolate the desired compound **3**, the isomeric mixture was subjected to column chromatography using neutral alumina and hexane/CH_2Cl_2. However, the isolated fractions, subjected to ^{1}H-NMR analysis, always turned out to be mixtures **3/4** with variable compositions (from 1:3 to 9:1). Given the impossibility of obtaining the pure compound **3**, the next oxidative step was performed with the the 9:1 mixture of **3/4** using *Jones* reagent, which allowed, after a laborious column chromatography purification process of the obtained crude, isolation of the Δ^1-olefin **5** in the required purity for further biological studies (96% by LC/MS control) (*Scheme 1*).

Although the formation of the Δ^2-isomer **6** has not been reported before, our results revealed that migration of the C=C bond from the C(1) to the more stable C(2) occurred to a considerable extent. In fact, the Δ^1- and Δ^2-isomers **5** and **6**, respectively, possessed similar physico-chemical properties, exhibiting the same R_f values with several chromatography solvents and similar crystallization conditions. Accordingly, it is very difficult to distinguish the two isomers. Facing these difficulties, the complete diagnosis of the C=C bond position in this kind of compounds could only be achieved by ^{1}H-NMR spectroscopy. The Δ^1-isomer **5** presents two signals at 5.52 ppm (H–C(2)) and 5.83 ppm (H–C(1)) for the olefinic H-atoms, whereas the Δ^2-isomer **6** displays only one common typical *multiplet* around 5.9 ppm for both olefinic H-atoms, allowing the accurate identification of both compounds **5** and **6**. Therefore, the NMR analysis appears to be the most adequate technique to disclose the nature of the compounds isolated after column chromatography.

To obtain the Δ^4-olefin **15**, the above-mentioned synthetic strategy was further applied to the Δ^4-3-keto enone **8** (prepared from **7** [7]) as starting material (*Scheme 2*). In this case, instead of **15** we obtained the $\Delta^{3,5}$-diene **12**. Treatment of **8** with Li(*t*-BuO)$_3$AlH, under the conditions previously described, led to Δ^4-3β-hydroxy allylic alcohol **9** with traces of its 3α-isomer. Treatment of this compound with $SOCl_2$ in benzene, contrarily to what was expected, gave the $\Delta^{3,5}$-diene precursor **10**. Then, **10** was treated with $LiAlH_4$ to afford the $\Delta^{3,5}$-diene derivative **11**, which, after oxidation with CrO_3 in pyridine [10], led to **12**. This approach opens a new way to prepare compound **12**, which is also an AI [9].

As the ^{1}H-NMR did not allow unequivocal elucidation of the position of the diene C=C bonds in the *A*/*B*-ring system, a 2D-COSY analysis of **10** was performed. The most relevant signals in the COSY spectrum were those of the three olefinic H-atoms at 5.9, 5.6, and 5.4 ppm, of the H_α–C(17) at 4.5 ppm, and of the Me(18) and Me(19) groups at 0.82 and 0.95 ppm, respectively. The H_α–C(17) correlates with Me(18) (^3J) which resonates at 0.82 ppm; therefore, the resonance at 0.95 ppm is due to Me(19). Focusing on the three olefinic H-atoms, of the $\Delta^{3,5}$-diene isomer **10**, the H–C(4) will correlate strongly with the H–C(3) and weakly with the H–C(6). In fact, we observed that an olefinic H-atom absorbing at 5.9 ppm strongly correlates with an olefinic H-atom at 5.6 ppm (^3J); the same olefinic H-atom (at 5.9 ppm) has a very weak correlation with an olefinic H-atom absorbing at 5.4 ppm (^4J), and there is no observable correlation between the olefinic H-atom signals at 5.6 ppm and 5.4 ppm (^5J). Therefore, the signal

at 5.6 ppm corresponds to H–C(3), the signal at 5.9 ppm to H–C(4), and the signal at 5.4 ppm to H–C(6), which is in agreement with the structure of compound **10**.

To obtain the Δ^4-olefin **15**, an alternative synthetic strategy was developed from the same starting material **8** (*Scheme 3*). In this case, reaction of **8** with a mixture of $NaBH_4$ in CF_3COOH (TFA), glacial AcOH, and MeCN was performed in a controlled environment [17], to yield compound **13** in 99% yield. This compound was then submitted to a base-catalyzed hydrolysis to give quantitatively compound **14**, which was then subjected to *Jones* oxidation to furnish the desired **15** in 75% yield. The above-mentioned strategy avoided the formation of the Δ^4-17β-hydroxylated derivative as well as the Δ^3-olefin isomer as by-products.

In summary, the preparation of Δ^1-olefin **5** from the Δ^1-3-keto enone **1** according to the revisited protocol takes place with the formation of the Δ^2-isomer **6** as a by-product, which can only be detected by NMR analysis. However, an adequate sequential purification procedure by column chromatography, assisted by ^{1}H-NMR control of the separated fractions, allows the isolation of the AI **5** in 96% purity, which is an adequate purity for compounds to proceed to biochemical assays.

The potent AI Δ^4-olefin **15** cannot be obtained from the Δ^4-3-keto enone **8** by the reported strategy. Instead, another important aromatase inhibitor, the $\Delta^{3,5}$-diene **12**, is formed. This achievement offers a new synthetic way to compound **12**. From these data, presented for the first time, to the best of our knowledge, it is possible to establish the unequal reactivity of steroidal Δ^1- and Δ^4-3-hydroxy allylic alcohol intermediates **2** and **9**, respectively, towards the elimination reactions by treatment with $SOCl_2$ (*Schemes 1* and *2*). Indeed, while the Δ^1-isomer **2** renders the mixture of olefins **3/4** by the expected elimination process, followed by isomerization of the C=C bond, the Δ^4-isomer **9** embarks in an additional elimination of a H–C(6) H-atom, followed by the migration of the C=C bond with the formation of the $\Delta^{3,5}$-diene derivative **10**.

The preparation of the AI **15** can be achieved by a new method, using also the Δ^4-3-keto enone **8** as starting material, by a sequence involving the reduction at C(3), followed by the hydrolysis of the AcO group at C(17) of **13**, and subsequent oxidation of the resulting OH group, which precludes the formation of undesirable by-products.

Experimental Part

General. Testosterone (**7**) was purchased from *Pharmacia & Upjohn Company*, Kalamazoo, Michigan (USA), and *(5α,17β)-3-oxoandrost-1-en-17-yl acetate* (**1**) and *(5α)-androst-2-en-17-one* (**6**) were purchased from *Steraloids, Inc.* (Newport RI, USA). Other reagents and solvents were used as obtained from the suppliers without further purification, with the exception of CH_2Cl_2, which was dried through reflux and distilled from CaH_2 [18]. M.p.: *Reichert Thermopan* hot-block apparatus; uncorrected. IR Spectra: *Jasco 420FT/IR* spectrometer. ^{1}H- and ^{13}C-NMR spectra: *Varian* 600 MHz spectrometer, using a 3-mm broadband NMR probe; chemical shifts in ppm downfield from TMS used as an internal standard; all *J* values in Hz. ESI- and LC-MS: mass spectrometer *QIT-MS Thermo Finningan*, model *LCQ Advantage MAX*, coupled to a liquid chromatograph of high performance *Thermo Finningan* (column: *C18*; reversed phase (RP); H_2O/MeCN 40:60).

(3β,5α,17β)-3-Hydroxyandrost-1-en-17-yl Acetate (**2**). See [7].

(5α,17β)-Androst-1-en-17-ol (**3**) *and (5α,17β)-Androst-2-en-17-ol* (**4**). Prepared as described in [7]. ^{1}H-NMR Analysis of the obtained crude product revealed a mixture **3/4** 1:1. The crude product was purified by column chromatography (CC) (neutral Al_2O_3; hexane/CH_2Cl_2 80:20) to give a white solid (one TLC spot). ^{1}H-NMR Analysis of this solid indicated an enriched mixture of **3** (90%) with **4** (10%).

Data of **3**. ^{1}H-NMR ((D_6)DMSO; selected signals): see [7].

Data of **4**. ^{1}H-NMR ((D_6)DMSO; selected signals): 0.63 (*s*, Me(18)); 0.71 (*s*, Me(19)); 3.42 (*ddd*, $J(17\alpha,16\alpha) = 9.0$, $J(17\alpha,16\beta) = 9.0$, $J(17\alpha,\text{OH}) = 5.0$, H_α–C(17)); 4.42 (*d*, $J(\text{OH},17\alpha) = 5.0$, HO_β–C(17)); 5.53 – 5.59 (*m*, H–C(2), H–C(3)).

(5α)-Androst-1-en-17-one (**5**) *and (5α)-Androst-2-en-17-one* (**6**). Prepared as described in [7]. The obtained crude product was crystallized from MeOH/H_2O to give white crystals (one TLC spot). ^{1}H-NMR Analysis of these crystals revealed the presence of a mixture **5** (83%)/**6** (17%). Purification by CC (hexane/Et_2O), followed by consecutive recrystallizations from MeOH, gave **5** in 96% purity (LC/MS analysis) with a small amount of isomer **6**.

Data of **5**. ^{1}H-NMR ($CDCl_3$): see [7].

Data of **6**. ^{1}H-NMR ($CDCl_3$; selected signals): 0.78 (*s*, Me(19)); 0.87 (*s*, Me(18)); 5.55 – 5.62 (*m*, H–C(2), H–C(3)).

(17β)-3-Oxoandrost-4-en-17-yl Acetate (**8**). To a soln. of **7** (2.0 g, 6.93 mmol) in dry pyridine (48 ml), Ac_2O (7.9 ml, 83.9 mmol) was added, and the mixture was stirred for 21 h 25 min at r.t. (20°), until all the starting material was consumed (TLC control). Then, CH_2Cl_2 (250 ml) was added, and the org. layer was washed with 10% $NaHCO_3$ (3 × 150 ml), 10% HCl (3 × 150 ml), and H_2O (3 × 150 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness. Crystallization of the obtained residue from AcOEt gave pure **8** (1.92 g, 84%). M.p.: 141 – 142° ([19]: 139 – 140°). IR ($CHCl_3$): 3018 (=CH), 1736 (C=O), 1675 (C=C), 1248 (C–O). ^{1}H-NMR ($CDCl_3$; selected signals): 0.82 (*s*, Me(18)); 1.18 (*s*, Me(19)); 2.03 (*s*, MeCOO); 4.58 (*dd*, $J(17\alpha,16\alpha) = 9.0$, $J(17\alpha,16\beta) = 8.0$, H_α–C(17)); 5.71 (*s*, H–C(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 12.0 (C(18)); 17.4 (C(19)); 20.5; 21.1; 23.4; 27.4; 31.4; 32.7; 33.9; 35.4; 35.7; 36.6; 38.6; 42.4; 50.2; 53.7; 82.4 (C(17)); 123.9 (C(4)); 170.9 (C(5)); 171.1 (OC=O); 199.4 (C(3)).

(3β,17β)-3-Hydroxyandrost-4-en-17-yl Acetate (**9**). To a soln. of **8** (2.0 g, 6.05 mmol) in anh. THF (75 ml) under N_2, Li(*t*-BuO)$_3$AlH (2.0 g, 7.86 mmol) was added, and the mixture was heated under reflux for 2 h, then an excess of Li(*t*-BuO)$_3$AlH (500.1 mg, 1.97 mmol) was added. The reaction proceeded until complete transformation of the starting material (3½ h). After removal of the solvent under vacuum, H_2O (200 ml) was added, and the aq. layer was extracted with CH_2Cl_2 (3 × 200 ml). The org. layer was then washed with H_2O (200 ml), dried (anh. Na_2SO_4), filtered, and concentrated to dryness to give 1.97 g of a crude material mainly composed of **9**. ^{1}H-NMR ((D_6)DMSO; selected signals): 0.76 (*s*, Me(18)); 0.99 (*s*, Me(19)); 1.98 (*s*, MeCOO); 3.88 – 3.92 (*m*, H_α–C(3)); 4.49 (*dd*, $J(17\alpha,16\alpha) = 9.0$, $J(17\alpha,16\beta) = 8.0$, H_α–C(17)); 4.54 (*d*, $J(\text{OH},3\alpha) = 5.5$, HO_β–C(3)); 5.19 (br. *s*, H–C(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 11.8 (C(18)); 18.4 (C(19)); 20.0; 20.8; 22.9; 27.0; 28.9; 31.4; 32.2; 35.1; 35.2; 36.2; 36.7; 41.9; 49.7; 53.8; 65.8 (C(3)); 81.8 (C(17)); 125.5 (C(4)); 143.9 (C(5)); 170.2 (OC=O).

(17β)-Androsta-3,5-dien-17-yl Acetate (**10**). A soln. of crude **9** (500.4 mg) in benzene (10 ml) was kept at 5 – 8° under N_2, treated with $SOCl_2$ (0.5 ml, 6.72 mmol), and the mixture was stirred for 2 h 25 min. Then, an excess of $SOCl_2$ (0.1 ml, 1.38 mmol) was added. The reaction did not proceed to the complete transformation of the starting material (5½ h). Benzene was evaporated under vacuum at r.t. giving an oily residue, to which solid $NaHCO_3$ (500 mg) was added, followed by 10% $NaHCO_3$ (100 ml). The aq. layer was extracted with CH_2Cl_2 (3 × 100 ml), and the resulting org. layer was washed with H_2O (3 × 100 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness to give a white solid residue. This residue was purified by CC (silica gel *60*; PE (60 – 80°)/AcOEt) to afford 263.4 mg of **10** as a white crystalline residue in an overall yield of 56% from **8**. Recrystallization from PE 60 – 80°/AcOEt. M.p. 116 – 119°. IR ($CHCl_3$): 3018 (=CH), 1736 (C=O ester), 1648 (C=C), 1244 (C–O). ^{1}H-NMR ($CDCl_3$; selected signals): 0.83 (*s*, Me(18)); 0.96 (*s*, Me(19)); 2.04 (*s*, MeCOO); 4.61 (*dd*, $J(17\alpha,16\alpha) = 9.0$, $J(17\alpha,16\beta) = 8.0$, H_α–C(17)); 5.37 – 5.38 (*m*, H–C(6)); 5.58 – 5.60 (*m*, H–C(3)); 5.91 – 5.93 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 12.0 (C(18)); 18.8 (C(19)); 20.4; 21.2; 22.9; 23.5; 27.5; 31.3; 31.6; 33.7; 35.2; 36.8; 42.5; 48.3; 51.2; 82.8 (C(17)); 122.6 (C(6)); 125.1 (C(3)); 128.8 (C(4)); 141.5 (C(5)); 171.2 (OC=O). ESI-MS: 315.1 (76, $[M + H]^+$).

(17β)-Androsta-3,5-dien-17-ol (**11**). To a soln. of **10** (100.8 mg, 0.32 mmol) in Et_2O (15 ml), $LiAlH_4$ (76.2 mg, 2.01 mmol) was added cautiously under N_2, and the mixture was heated under reflux for 8 h. Then, a sat. soln. of sodium potassium tartrate (150 ml) was added, and the mixture was extracted with Et_2O (4 × 100 ml). The org. layer was then washed with H_2O (4 × 100 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness to give pure **11** (79.4 mg, 91%). White solid. Recrystallization from AcOEt/

hexane. M.p. 140–142°. IR ($CHCl_3$): 3302 (OH), 3021 (=CH), 1646 (C=C), 1054 (C–O). ^{1}H-NMR ((D_6)DMSO; selected signals): 0.67 (*s*, Me(18)); 0.89 (*s*, Me(19)); 3.45 (*ddd*, J(17α,OH) = 5.0, J(17α,16α) = 9.0, J(17α,16β) = 9.0, H_α–C(17)); 4.45 (*d*, J(OH,17α) = 5.0, HO_β–C(17)); 5.33–5.35 (*m*, H–C(6)); 5.56–5.58 (*m*, H–C(3)); 5.87–5.89 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 11.2 (C(18)); 18.5 (C(9)); 20.1; 22.4; 22.9; 29.8; 30.8; 31.4; 33.2; 34.6; 36.3; 42.3; 48.0; 50.9; 79.9 (C(17)); 122.6 (C(6)); 124.5 (C(3)); 128.8 (C(4)); 140.8 (C(5)). ESI-MS: 271.2 (100, $[M-H]^+$).

Androsta-3,5-dien-17-one (**12**). To a soln. of **11** (62.0 mg, 0.23 mmol) in pyridine (3 ml), a pyridine soln. (2.3 ml) of CrO_3 (98.0 mg, 0.98 mmol) was added at 0°. The mixture was stirred at r.t. for 19 h until total transformation of the starting material (TLC control). The mixture was then diluted with Et_2O (150 ml) and poured into H_2O (50 ml). The org. phase was washed with brine (6 × 150 ml) and H_2O (3 × 200 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness giving a yellow residue which was purified by CC (neutral Al_2O_3; PE (40–60°)) to furnish pure **12** (9.0 mg, 38%). M.p. 81–83° ([20]: 80–82°). IR ($CHCl_3$): 3018 (=CH), 1739 (C=O), 1652 (C=C). ^{1}H-NMR ($CDCl_3$; selected signals): 0.91 (*s*, Me(19)); 0.97 (*s*, Me(18)); 5.39–5.41 (*m*, H–C(6)); 5.59–5.62 (*m*, H–C(3)); 5.92–5.94 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 13.7 (C(19)); 18.8 (C(18)); 20.2; 21.8; 22.9; 30.6; 31.3; 31.4; 33.7; 35.3; 35.8; 47.7; 48.5; 51.9; 122.1 (C(6)); 125.3 (C(3)); 128.7 (C(4)); 141.6 (C(5)); 221.0 (C(17)). ESI-MS: 269.1 (99, $[M-H]^+$).

(17β)-Androst-4-en-17-yl Acetate (**13**). $NaBH_4$ (566.2 mg, 14.97 mmol) was added in small portions under stirring and cooling to a previously cooled mixture of CF_3COOH (3.5 ml), glacial AcOH (3.5 ml), and MeCN (3.5 ml). A soln. of **8** (1.0 g, 3.03 mmol) in dry CH_2Cl_2 (18 ml) was added to the mixture. Then, the mixture was let to react at r.t. under magnetic stirring and N_2, until consumption of all the starting material (3½ h; TLC control). The mixture was then neutralized with a soln. of 10% $NaHCO_3$ and extracted with CH_2Cl_2 (4 × 100 ml). The org. layer was washed with H_2O (4 × 100 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness to give **13** (945.0 mg, 99%). White solid. Recrystallization from CH_2Cl_2/hexane/EtOH. M.p. 95–99°. IR ($CHCl_3$): 3024 (=CH), 1737 (C=O), 1663 (C=C), 1043 (C–O). ^{1}H-NMR ($CDCl_3$; selected signals): 0.80 (*s*, Me(18)); 1.01 (*s*, Me(19)); 2.03 (*s*, MeCOO); 4.58 (*dd*, J(17α,16α) = 9, J(17α,16β) = 8, H_α–C(17)); 5.29–5.30 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 12.0 (C(18)); 19.2 (C(19)); 19.4; 20.9; 21.2; 23.5; 25.7; 32.4; 32.8; 35.8; 36.9; 37.1; 37.8; 42.5; 50.5; 54.4; 82.8 (C(17)); 119.3 (C(4)); 144.7 (C(5)); 171.2 (OC=O).

(17β)-Androst-4-en-17-ol (**14**). Compound **13** (945.0 mg, 2.99 mmol) was added to a mixture dioxane/H_2O 85:15 (90 ml) with 2% NaOH (18 ml), at r.t. The mixture was let to react until total transformation of the starting material (52 h; TLC control) and then neutralized with an aq. soln. of 5% HCl. The dioxane was evaporated under vacuum leading to a white solid residue that was diluted with H_2O (200 ml) and extracted with AcOEt (4 × 100 ml). The org. layer was then washed with H_2O (4 × 100 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness to afford **14** (819.0 mg, 99%). White solid. Recrystallization from hexane/AcOEt. M.p. 143–146°. IR ($CHCl_3$): 3278 (OH), 2959 (=CH), 1651 (C=C). ^{1}H-NMR ((D_6)DMSO): 0.65 (*s*, Me(18)); 0.97 (*s*, Me(19)); 3.43 (*ddd*, J(17α,16α) = 9.0, J(17α,16β) = 9.0, J(17α,OH) = 5.0, H_α–C(17)); 4.43 (*d*, J(OH,17α) = 5.0, HO_β–C(17)); 5.23–5.25 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 11.2 (C(18)); 18.8 (C(19)); 19.1; 20.6; 23.0; 25.1; 29.8; 31.9; 32.5; 35.5; 36.4; 36.5; 37.2; 42.4; 50.3; 54.1; 79.9 (C(17)); 118.7 (C(4)); 144.2 (C(5)).

Androst-4-en-17-one (**15**). *Jones* reagent (2.7 ml) was added dropwise to a soln. of **14** (839.8 mg, 3.06 mmol) in acetone/dioxane 60:10 (190 ml), at 0° under magnetic stirring, until a persistent brown coloration was obtained. Then, the excess of the oxidant was destroyed with the addition of ^{i}PrOH until the soln. turned greenish. The dioxane and acetone were evaporated under vacuum. To the remaining residue, H_2O (200 ml) was added, and the aq. phase was extracted with AcOEt (4 × 100 ml). The org. layer was then washed with 10% $NaHCO_3$ (3 × 100 ml) and H_2O (3 × 100 ml), dried (anh. $MgSO_4$), filtered, and concentrated to dryness to give a white solid residue (129.3 mg) after the addition of some drops of Et_2O. This residue was then purified by CC (SiO_2; hexane/AcOEt 97:03) to afford pure **15** (624.2 mg, 75%). White crystalline solid. Recrystallization from hexane/AcOEt. M.p. 74–76°. IR ($CHCl_3$): 3018 (=CH), 1738 (C=O), 1657 (C=C). ^{1}H-NMR ($CDCl_3$; selected signals): 0.88 (*s*, Me(18)); 1.03 (*s*, Me(19)); 5.31–5.33 (*m*, H–C(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 13.7 (C(18)); 19.2 (C(19)); 19.4; 20.6; 21.8; 25.7; 31.5; 32.1; 32.3; 35.5; 35.8; 37.1; 37.8; 47.7; 51.2; 54.5; 119.6 (C(4)); 144.3 (C(5)); 221.3 (C(17)). ESI-MS: 271.0 ($[M-H]^+$, 50%).

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Perturbations of the *Dushman* Reaction with Piroxicam: Experimental and Model Calculations

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Perturbation of the *Bray–Liebhafsky* non-oscillating subsystem (mixture of KIO_3 and H_2SO_4), *i.e.*, *Dushman* reaction (*DR*), by piroxicam (PX), was observed in an open reactor, *i.e.*, in the continuously fed well-stirred tank reactor (CSTR). Monitoring the response of *DR* to perturbations by different concentrations of PX allows developing a simple procedure for quantitative determination of this analyte in both bulk drug and pharmaceutical preparation (injection). A tentative perturbation mechanism of PX action on the *DR* matrix, based on a kinetic scheme that was suggested by *Agreda et al.*, is proposed. The PX reactivity in *DR* has been generally related to the reaction of PX with hypoiodous acid (HIO) present in the matrix.

1. Introduction. – Oscillatory reactions are among the most fascinating chemical reactions, and represent typical examples of complex dynamic systems. The investigation of the mentioned non-linear chemical systems with their self-organization and temporal dynamic structures have numerous aims, from theoretical examinations and modeling [1–3] of the considered system or related ones, through their application in the study of antioxidant behavior of different antioxidants [4–6], to their analytical applications to depict kinetic characterizations of catalysts [7], or to measure concentrations below current detection limits [8–10].

The present work represents a continuation of our systematic studies with the aim to apply nonlinear chemical reactions for analytical determinations. So far, for quantitative determination of different species [9][11][12], the kinetic method based on employing the analyte pulse perturbation technique (APP) [13] to the *Bray–Liebhafsky* (*BL*) oscillatory reaction [14][15] as very nonlinear systems, has been used.

For developing kinetic analytical methods [16], besides the mentioned *BL* oscillating system, the *BL* non-oscillating subsystem, *i.e.*, the reaction of reduction of iodate by iodide in acid solution, known as the *Dushman* reaction (*DR*; *Eqn. 1*) [17] was used [12].

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$$IO_3^- + 5\,I^- + 6\,H^+ \rightarrow 3\,I_2 + 3\,H_2O \quad (DR) \tag{1}$$

Moreover, *DR* itself is a very important reaction in analytical chemistry, and has a crucial role in many interesting systems such as the mentioned *BL* oscillating reaction system [18–21], as well as the iodate–arsenous system [22]. Hence, the *DR* is a part of the highly sensitive *BL* oscillator, but it is also a potential medium for analytical procedures. Therefore, there is a need to investigate advantages of both *BL* and *DR* reaction systems in analytical evaluation of PX.

In this work, we analyzed the steady state of the *DR* by applying pulsed perturbations with different amounts of piroxicam (=4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-benzothiazine-3-carboxamide 1,1-dioxide; PX), a non-steroidal anti-inflammatory drug (NSAID) from the oxicam family (*Fig. 1*). This effective analgesic and anti-inflammatory agent is used in the symptom management of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute pain in musculoskeletal disorder and acute gout [23]. The steady states of *DR* were analyzed to quantitatively determine the PX concentration. To achieve this, different amounts of PX were added to the mixture. In other words, the strength of the applied perturbation varied, and subsequent response of the *DR* was analyzed. In particular, the objective was to develop an analytical method which should be capable of detecting PX contained in pharmaceutical preparation (injection).

Finally, to clarify the obtained perturbation effect of PX on the *DR* matrix, we discuss a possible interaction between PX and *DR* matrix, as well as perform numerical simulations based on an already published variant of the model *Dushman* reaction [24] in its initial form, in order to test the validity of our interpretations.

OH O N H N S N O O

Fig. 1. *Structure of piroxicam* (PX)

2. Experimental. – 2.1. *Aparatus.* The *DR*, used as the matrix system, was conducted in an open reactor, *i.e.*, in the continuously fed well-stirred tank reactor (CSTR) [25]. It consists of *ca.* 52-ml glass CSTR vessel (*Metrohm* model *876-20*) wrapped in the water recirculation jacked connected to a thermostat (*Julabo*, series *ED*) and equipped with the magnetic stirrer (*Ingenieurbüro, M. Zipperrer GmbH, Cat-ECM5*). Temporal evolution of the system was recorded by means of a Pt electrode (*Metrohm*, model *6.0301.100*) and double junction Ag/AgCl electrode (*Methrom*, model *6.0726.100*) interfaced to a PC-AT 12-MHz compatible computer *via* a *PC-MultiLab EH4 16-bit ADC.* It is known [26] that the Pt electrode may be used for determination of I^- in low acidic iodide solns., which is supported and confirmed in our research [9]. The flows (inflow and outflow) of chemical species through CSTR were driven by peristaltic pumps (*Ismatec*). *Tygon* tubes (*Ismatec*, *OLE DICH*) were used to transport the reactants from their reservoirs to the reaction vessel. These tubes were connected to *Teflon* tubes (*Varian*), and reagents were introduced to the reaction vessel through them. In all experiments, the feed substances were kept in reservoirs at r.t., and were introduced into the reaction vessel separately and without being previously thermostated. The volume, *V*, of the mixture was kept constant at 22.2 ± 0.2 ml

by removing the surplus volume of the mixture through the U-shaped glass tube, ending on free surface above the mixture.

The analyte was introduced with micropipettes (*Brand*, DE-Wertheim). A 50-μl shot is estimated to last *ca.* 0.5 s. The intensity of the perturbation corresponds to the injected amount (in M) of PX standard samples.

The simulations were performed using the MATLAB program package. The differential equations derived from the model were integrated using the ode15s solver. All numerical simulations were performed with numerical precision of 10^{-16}.

2.2. *Chemicals.* The used chemicals were of anal. grade, and deionized water was used for preparing the solns. of KIO_3 (*Merck*) and H_2SO_4 (*Fluka*). Standard stock soln. of PX (*Sigma*) was prepared at a concentration of 4.8×10^{-3} M in MeOH (*Merck*) and stored in a refrigerator in the dark. Dosage form of PX (*i.e.*, *Feldene* injection; 20 mg/ml) was supplied by *Pfizer*. Besides PX, this pharmaceutical formulation contains benzyl alcohol, EtOH, HCl, nicotinamide, propylene glycol, monobasic sodium phosphate, NaOH, and H_2O for injection, as excipients. Samples of injections were analyzed by the proposed procedure. For such purposes, the contents of five ampoules were mixed. Then, 1 ml of the injection soln. equivalent to 20 mg of PX was transferred in a 50-ml volumetric flask, and made up to volume with MeOH.

2.3. *Procedure.* This procedure is based on experiments carried out in acidic iodate solns. when the inflow concentration of H_2O_2 is zero. Thermostated at $55.0 \pm 0.1°$ and shielded from light, the reaction vessel was filled up by three separate inflows of 5.90×10^{-2} M KIO_3, 7.60×10^{-2} M H_2SO_4, and H_2O, at a maximum flow rate of 5 ml/min. Under these conditions, within 3 min, about twice the volume of the mixture became charged. Then, the inflows were stopped, the stirrer was turned on (900 rpm), and the excess of the mixture was sucked out through the U-shaped glass tube, to achieve the actual mixture volume of 22.2 ± 0.2 ml. After *ca.* 20 min, the inflows were turned on at the required specific flow rate, 2.95×10^{-2} min^{-1}, and they attained steady state perturbed with different concentration of PX. The preparatory procedure took *ca.* 30 min.

3. Results and Discussion. – 3.1. *Sensitivity of* DR *to External Perturbation*. Previously, we have used almost similar conditions ($T = 55.0°$, $[H_2SO_4]_0 = 7.6 \times 10^{-2}$ M, $[KIO_3]_0 = 5.9 \times 10^{-2}$ M, $[H_2O_2]_0 = 1.5 \times 10^{-1}$ M, and $j_0 = 2.95 \times 10^{-2}$ min^{-1}) of the procedure for the PX determination [11]. It is worth noting that the experimental setup reported here is different from those reported in [11]. The PX determination was based on a sensitivity of steady state obtained in *DR* matrix. Therefore, under the CSTR conditions characterized by constant parameters ($T = 55.0°$, $[H_2SO_4]_0 = 7.6 \times 10^{-2}$ M, $[KIO_3]_0 = 5.9 \times 10^{-2}$ M, and $j_0 = 2.95 \times 10^{-2}$ min^{-1}), the effect of concentration of PX was studied; it was found that a variation of its concentration resulted in a variation in potential of the matrix.

The sensitivity of the *DR* to the perturbations with PX is illustrated in *Fig. 2,a*. As can be seen, before the perturbation, the system is in a steady state, while its corresponding potential denoted as E_s is constant. When a trace amount of PX was injected into *DR*, an abrupt decay of the potential to the value denoted E_p was observed. This is the minimal value of the potential. After achieving this value, the potential relaxes back to the value denoted E_s that is equal or slightly different from the potential of the initial steady state. Here, as in the *BL* matrix system [11], analytical signal is defined as the maximal potential displacement, *i.e.*, $\Delta E_m = E_p - E_s$ (*Fig. 2,a*).

A plot of ΔE_m *vs.* the injected PX concentrations provided a calibration graph that was fitted by the least-squares method (*Fig. 3*). This calibration curve obeys the following linear regression equation (*Eqn. 2*):

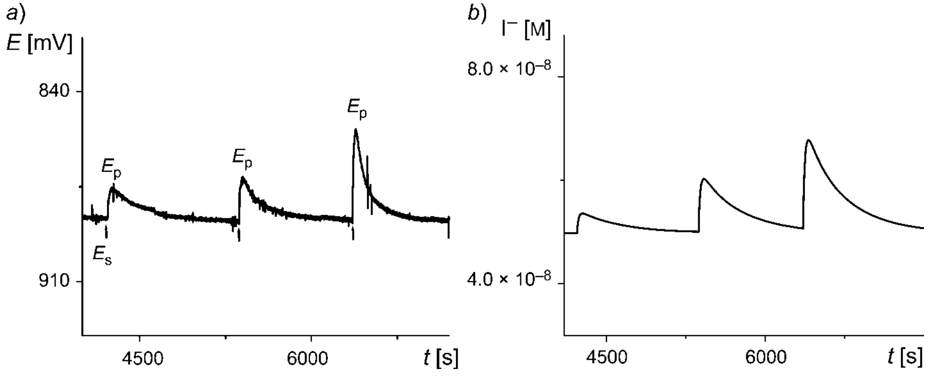

Fig. 2. a) *The responses of* DR *matrix obtained experimentally and* b) *by numerical simulations after its perturbations with different concentrations of PX.* The inflow concentration of H_2SO_4 was 7.6×10^{-2} M. Arrows indicate the moments at which (from left to right) 2.0×10^{-5}, 3.1×10^{-5}, and 5.0×10^{-5} M PX was injected.

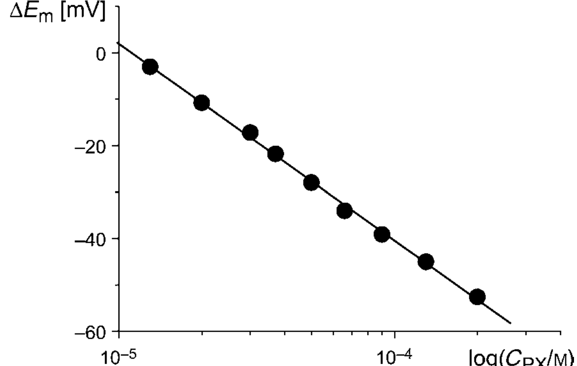

Fig. 3. *Plot of potential shift* vs. *concentration of piroxicam in* Dushman *reaction*

$$\Delta E_m = -210.7\ (\pm 2.9) - 42.5\ (\pm 0.6)\ \log\ (C_{PX}/M) \quad (r = 0.999) \tag{2}$$

The response of potential shift *vs.* logarithm of the PX concentrations was linear over the range 1.3×10^{-5} M $\leq C_{PX} \leq 3.0 \times 10^{-4}$ M. For higher PX concentrations, the analytical signal increases, but for these perturbation strengths the calibration curve was no longer linear, and the matrix relaxation rate is too slow ($0.048\ min^{-1}$). The limit of detection (*LOD*) [27] and instrumental sensitivity are 1.3×10^{-5} M and 42.5 mV/decade. The uncertainty of the estimated value of the PX concentration arises from uncertainties in the estimated values of ΔE_m, and is propagated through all of the analysis using the technique described in [28]. The average relative standard deviation (*RSD*) is 3.1% which provides the accepted reproducibility for analysis of a real sample. The sample throughput was 4 samples/h.

As mentioned above, recently the *BL* reaction in a stable steady state near a bifurcation point has been used as matrix for quantitative determination of PX [11]. If the developed method is compared with the method based on perturbations of the *BL* matrix [11], the proposed method is characterized by both higher analytical sensitivity (assessed as *LOD*) and method precision (assessed as *RSD*), although this method has lower sample throughput as well as narrower linear concentration range. As we pointed out in [11], the main advantage of perturbing *Dushman* reaction as matrix is that it will be well appreciated by investigators who have little or no experience with oscillatory reaction systems. Here, it is not necessary to determine the bifurcation point, and any change in the dynamic pattern following perturbation is easily observable and unmistakably recognized. On the other hand, the estimated time for a full analysis depends on the matrix system's relaxation rate to the same or slightly different initial steady state, after perturbation with PX. The time required may be shortened by using *BL* oscillator as matrix.

3.2. *Determination of PX in a Pharmaceutical.* As an application for the proposed method described here, the PX content in a pharmaceutical (injection) was determined under the above experimental conditions. The respond curve obtained by perturbation of the *DR* with sample solution (injection) is shown in *Fig. 4.* As already mentioned, the analyzed pharmaceutical, besides PX as active component, contains several other species (see *Sect. 2.2.*); some of them can probably change the form of signal profile of the examined analyte. However, as we have seen, the form of signal profiles in real sample (*Fig. 4.*) is similar to the obtained one in the cases of PX standard samples analyzed by the proposed method (*Fig. 2,a*).

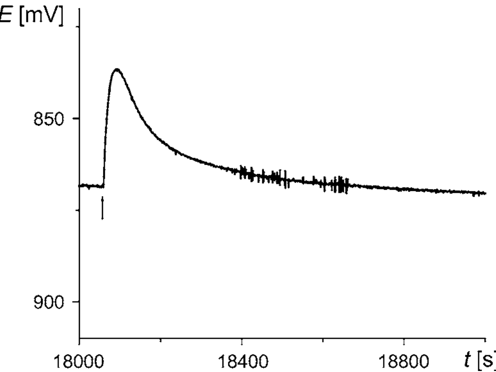

Fig. 4. *Typical response curve obtained after perturbing* DR *matrix by addition of sample.* Arrow indicates the moments at which steady state was perturbed.

Analyzing five samples in parallel without pretreatment, the average PX concentration in the pharmaceutical was 20.3 mg (*RSD* 3.9%) which was in good agreement with the concentrations of 19.8 and 20.8 mg of PX determined by spectrophotometric method [29], as well as by the kinetic method based on perturbations of the *BL* matrix [11]. The average recovery value (*RCV*) of 101.5% indicates that the developed method is free from any interference and provides accurate results; this method can be considered to be applicable to real analysis of PX contents in ampoules.

3.3. *Mechanism of Action of PX on* DR *Matrix*. A possible mechanism of PX interaction with non-oscillating *DR* as matrix was tested on a kinetic scheme that was suggested by *Agreda et al.* [24], and that expressed the kinetic complexity of the *DR* [30][31]. According to this model, the kinetic scheme of the *DR* consists of ten reactions for several independent species among which $H_2I_2O_3$ (*i.e.*, I_2O_2) was postulated as the kinetically and stoichiometrically significant intermediate species [24].

For investigation of the perturbation mechanism, in preliminary experimental examinations, we tested whether PX reacted with the reactants of *DR*, which were present in large excess. With this aim, we tested PX interactions with individual reactants: *1*) H_2SO_4 and *2*) KIO_3. Potential *vs.* time curves (*Fig. 5,a* and *b*) were recorded by using the same experimental setup as described above. Significant effects were observed by injecting the PX into CSTR containing H_2SO_4 alone, as well as KIO_3 alone, that can be ascribed to its electrochemical behavior [32][33]. On the other hand, the forms of signal profiles (*Fig. 5,a* and *b*) in those cases do not correspond to the one obtained in the case of PX injections in the mixture H_2SO_4/KIO_3 (*Fig. 2,a*).

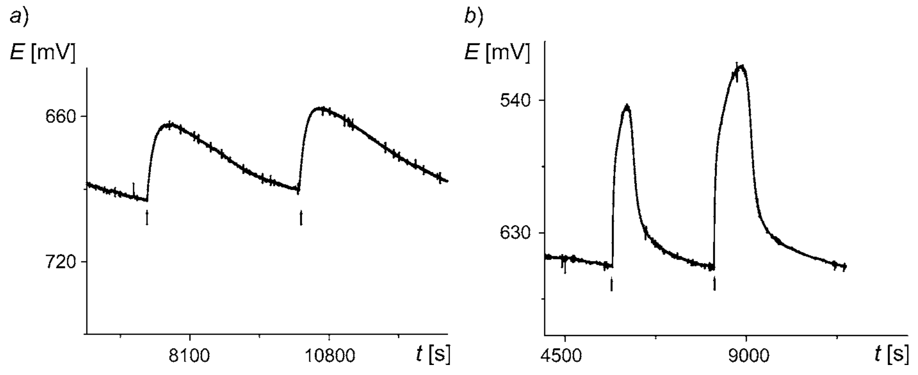

Fig. 5. *Typical response curves obtained after PX injection in* a) H_2SO_4 *and* b) KIO_3. Arrows indicate the moments at which (from left to right) 5.0×10^{-5} M and 9.0×10^{-5} M PX was injected.

According to the obtained experimental results, and taking in account that PX can be oxidized with HOBr [34], we suggest, as a first approximation, that PX oxidation through interactions with HIO is crucial, and the rate-determining step through which the ratio between iodine intermediates ($[HIO]_{ss}$ and $[I^-]_{ss}$), established in the stationary state before perturbations is altered (*Scheme*).

Scheme

PX + HIO → $PXOH^+$ + I^-

Table. *Model of the* Dushman *Reaction* (*DR*)

Step	Reaction
1	$IO_3^- + H^+ \rightleftharpoons HIO_3$
2	$HIO_3 + H^+ \rightleftharpoons H_2IO_3^+$
3	$H_2IO_3^+ + I^- \rightleftharpoons H_2I_2O_3$
4	$H_2I_2O_3 + I^- + H^+ \rightleftharpoons H_3IO_3 + I_2$
5	$H_3IO_3 \rightarrow HIO_2 + H_2O$
6	$HIO_2 + H^+ \rightleftharpoons IO_2\,H_2^+$
7	$IO_2\,H_2^+ + I^- \rightleftharpoons H_2I_2O_2$
8	$H_2I_2O_2 + I^- + H^+ \rightarrow HIO + I_2 + H_2O$
9	$HIO + H^+ \rightleftharpoons IOH_2^+$
10	$IOH_2^+ + I^- \rightleftharpoons I_2 + H_2O$

Thus, the basic kinetic scheme of the *Dushman* reaction (*Reactions 1–10*, *Table*) is extended with this above reaction, and this extended model is tested in numerical simulations.

A perturbation in the numerical simulation was performed by instantaneously changing the concentration of PX during the course of integration of the kinetic equations. The initial concentrations of the external species and flow rate were the same as in the experimental procedure. In the simulations, concentrations of all feed species ([IO_3^-] and [H^+]) were treated as dynamic variables. Rate constants for *DR* model reactions were taken from the literature [24]. The rate constant for the additional reaction, *R*, that was estimated through a computer simulation procedure was comparable with the rate of reaction involving HBrO [34].

Numerically simulated temporal traces upon PX injections in case when the model of *DR* is extended with reaction *R* ($k_R = 6.0 \times 10^4$ M^{-1}s^{-1}) is shown in *Fig. 2,b*. The numerically simulated forms of signal profiles, obtained before and after perturbation of the *DR* by PX, are in excellent agreement with experimentally obtained results. Under the examined experimental conditions, a plot of the ΔE_m against injected PX concentrations is linear. The obtained linear concentration range is $3.0 \times 10^{-5} – 2.0 \times 10^{-4}$ M, and regression equation is $\Delta E_m = -235.5 - 50.8 \log (C_{PX}/M)$ ($r = 0.991$). The obtained results resemble experimental ones at a most satisfying level.

In summary, for the investigated interactions of PX with *DR* as matrix, good qualitative and quantitative agreements between experiments and simulated results were obtained: the form of the signal profile and the relaxation times are virtually identical, and the linearity ranges of the obtained regression equation are very well comparable with experimentally determined ones. Considering that the simulated results obtained by including the reaction *R* in the reaction mechanism for *Dushman* non-oscillatory reaction successfully reproduce the experimental ones, we suggest, as a first approximation, that PX oxidation through interaction with HIO is crucial, and the rate-determining step in a possible model of the mechanism of the interaction between PX and *DR* matrix reaction system.

4. Conclusion. – The *DR* is a useful matrix for quantitative determination of piroxicam in both bulk drug and pharmaceutical (injection). For analytical determi-

nation of PX, *DR* in a steady state is perturbed with different PX concentrations, and the obtained matrix responses are recorded potentiometrically by a Pt electrode. The proposed kinetic method has rather good analytical attributes (limit of detection, precision, and accuracy are 1.3×10^{-5} M, 3.1%, and 97.9%, resp.). Also, excellent qualitative and quantitative agreements between the experimental and simulated results indicates that the proposed model mechanism, though extremely simplified, reflects well the dynamics of PX interaction with *DR*, and can be used as a very good starting point for further optimization of the method.

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Two New Cytotoxic Spirostane-Steroidal Saponins from the Roots of *Bletilla striata*

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Two new spirostane-steroidal saponins, bletilnoside A (**1**) and bletilnoside B (**2**), together with five known compounds, **3**–**7**, were isolated from the roots of *Bletilla striata* (THUNB.) REICHB. F. The structures of the new compounds were determined based on their 1D- and 2D-NMR spectral data. The isolated compounds **1**–**7** were tested for cytotoxicity against four human tumor cells (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using a sulforhodamin B bioassay, and compounds **1**, **2**, and **5** showed significant cytotoxicities against all tested tumor cell lines with IC_{50} values ranging from 3.98 ± 0.16 to 12.10 ± 0.40 μM.

1. Introduction. – *Bletilla striata* (THUNB.) REICHB. F. (Orchidaceae) is a perennial herb that is widely distributed throughout Northeast Asia. The roots of *B. striata* have been used as a hemostatic agent in Korean traditional medicine [1]. Phenanthrenes, stilbenes, triterpenoids, and anthocyanins have been isolated from this source [2–9]. Its MeOH extract shows antimitotic [10], and antimicrobial activities [11]. In the course of our continuing search for potential lead compounds in Korean traditional medicinal plants, we studied the MeOH extract of *B. striata* roots and isolated two new spirostane steroidal saponins, bletilnoside A (**1**) and bletilnoside B (**2**), together with five known compounds, **3**–**7** (*Fig. 1*). The structures of the new compounds were elucidated by spectroscopic and chemical methods. Here, we report the isolation and structure determination of the isolated spirostane-steroidal saponins. The isolated compounds, **1**–**7**, were evaluated for their cytotoxicities against four human tumor cells *in vitro* using a sulforhodamin B bioassay.

2. Results and Discussion. – 2.1. *Structure Elucidations of New Compounds.* Compound **1** was obtained as a white amorphous powder. The molecular formula was deduced as $C_{38}H_{62}O_{12}$ from the *quasi*-molecular ion ($[M+H]^+$) peak at *m/z* 711.9083 in its positive-ion mode HR-FAB-MS spectrum. The ^{1}H-NMR spectrum (*Table 1*) of **1** showed signals of two tertiary Me groups at δ(H) 1.58 (*s*, Me(19)) and 1.03 (*s*, Me(18)), two secondary Me groups at δ(H) 1.41 (*d*, $J = 7.0$, Me(21)) and 1.35 (*d*, $J = 7.0$, Me(27)), three O-bearing CH groups at δ(H) 4.80–4.90 (*m*, H–C(16)), 4.66 (br. *s*, H–C(1)), and 4.45 (br. *s*, H–C(3)), and two O-bearing CH_2 groups at δ(H) 4.34 (*dd*, $J = 10.5, 2.0$, H_a–C(26)) and 3.64 (*dd*, $J = 10.5, 2.0$, H_b–C(26)). The ^{13}C-NMR and DEPT experiments displayed 27 C-atom signals including those of four Me groups at δ(C) 19.4

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Fig. 1. *Structure of compounds* **1**–**7**, *isolated from* B. striata

(C(19)), 16.7 (C(18)), 16.3 (C(27)), and 15.0 (C(21)); three O-bearing CH groups at δ(C) 81.3 (C(16)), 76.0 (C(1)), and 67.4 (C(3)); one O-bearing CH_2 group at δ(C) 65.2 (C(26)), seven CH groups at δ(C) 63.0 (C(17)), 56.4 (C(14)), 42.0 (C(9)), 35.6 (C(8)), 31.8 (C(5)), and 27.6 (C(25)); nine CH_2 groups at δ(C) 40.3 (C(12)), 34.2 (C(15)), 32.2 (C(11)), 27.3 (C(2)), 26.4 (C(4)), 26.3 (C(24)), 26.2 (C(7) and C(23)), and 21.5 (C(6)), and three quaternary C-atoms at δ(C) 109.6 (C(22)), 40.6 (C(13)), and 39.3 (C(10)), suggesting that **1** was a spirostane-type steroid derivative [12]. The ^{1}H- and ^{13}C-NMR (including DEPT) and 2D-NMR (^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY) spectra of **1** revealed the presence of a xylose unit [13] (^{1}H-NMR: δ(H) 5.30 (*d*, $J = 7.5$, H–C(1′)), 4.56–4.66 (*dd*, $J = 12.0, 10.5$, H_a–C(5′)), 4.46–4.57 (*m*, H–C(2′)), 4.42–4.54 (*m*, H–C(3′)), 4.36–4.47 (*m*, H–C(4′)), and 3.94 (*t*, $J = 10.5$, H_b–C(5′)); ^{13}C-NMR: δ(C) 98.2 (C(1′)), 79.2 (C(3′)), 76.9 (C(2′)), 71.6 (C(4′)), and 67.4 (C(5′))) and a rhamnose unit [14] (^{1}H-NMR: δ(H) 6.38 (br. *s*, H–C(1″)), 5.04 (overlap, H–C(2″,3″)), 4.95–5.05 (*m*, H–C(5″)), 4.47–4.57 (*m*, H–C(4″)), and 2.01 (*d*, $J = 6.5$, H–C(6″)); ^{13}C-NMR: δ(C) 102.0 (C(1″)), 74.5 (C(4″)), 72.2 (C(2″)), 72.0 (C(3″)), 69.9 (C(5″)), and 18.8 (C(6″))). The coupling constant ($J = 7.5$) of the xylose anomeric H-atom suggested a β-form [13]. The configuration of rhamnose was determined to be α by comparing the ^{13}C-NMR data of C(3″) (δ(C) 72.0) and C(5″) (δ(C) 69.9) (α-form: δ(C) 72.5, 69.0; β-form: δ(C) 73.8, 73.1 [14]). The position of the sugar was confirmed by the HMBC spectrum by correlations between Xyl H–C(1′)/C(1) and Rha H–C(1″)/C(2′) (*Fig. 2*). The connectivities of the sugars were also confirmed by the ROESY correlations between xyl H–C(1′)/H–C(1) and rha H–C(1″)/H–C(2′) [14]. The broad *singlet* at δ(H) 4.66 in the ^{1}H-NMR spectrum revealed the β-orientation for H–C(1) [15], since an α-oriented H-atom at C(1) reportedly appears as a double *doublet* ($J = 12.0, 2.0$) [16]. This was further confirmed by the cross-peak between H–C(1) and

Table 1. *^{1}H- and ^{13}C-NMR* ((D$_5$)pyridine) *Data for* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.66 (br. *s*)	76.0	4.35 (br. *s*)	75.9
2	2.56–2.59 (*m*), 2.51–2.53 (*m*)	27.3	2.18–2.30 (*m*), 1.99–2.08 (*m*)	27.4
3	4.45 (br. *s*)	67.4	4.15 (br. *s*)	67.3
4	1.72–1.77 (*m*), 1.63–1.67 (*m*)	26.4	1.78–1.89 (*m*), 1.27–1.36 (*m*)	26.6
5	2.74–2.82 (*m*)	31.8	1.92–2.07 (*m*)	31.7
6	1.58[a])	21.5	1.21[a])	21.5
7	2.41[a]), 2.21[a])	26.2	1.20–1.35 (*m*), 0.97–1.08 (*m*)	26.3
8	1.88–1.98 (*m*)	35.6	1.53–1.68 (*m*)	35.3
9	1.51–1.57 (*m*)	42.0	1.43[a])	41.8
10		39.3		39.4
11	2.26–2.35 (*m*), 1.64–1.75 (*m*)	32.2	2.52–2.61 (*m*), 1.89–2.03 (*m*)	32.0
12	1.92–2.21 (*m*), 1.38–1.42 (*m*)	40.3	1.59–1.72 (*m*), 1.07–1.25 (*m*)	40.1
13		40.6		40.7
14	1.36–1.45 (*m*)	56.4	1.21–1.34 (*m*)	56.3
15	2.30–2.37 (*m*), 1.90–1.99 (*m*)	34.2	1.97–2.03 (*m*), 0.99–1.08 (*m*)	33.2
16	4.80–4.90 (*m*)	81.3	4.49–4.60 (*m*)	81.4
17	2.08–2.19 (*m*)	63.0	1.76–1.86 (*m*)	63.1
18	1.03 (*s*)	16.7	0.78 (*s*)	16.6
19	1.58 (*s*)	19.4	1.05 (*s*)	19.3
20	2.12–2.23 (*m*)	42.5	1.81[a])	41.9
21	1.41 (*d*, J = 7.0)	15.0	1.06 (*d*, J = 7.0)	14.9
22		109.6		109.3
23	1.72[a]), 1.58[a])	26.2	2.24[a]), 1.53[a])	26.2
24	1.64[a]), 1.54[a])	26.3	1.05[a]), 1.03[a])	28.9
25	1.80–1.90 (*m*)	27.6		144.4
26	4.34 (*dd*, J = 10.5, 2.0), 3.64 (*dd*, J = 10.5, 2.0)	65.2	4.42 (*d*, J = 10.5), 3.64 (*d*, J = 10.5)	65.3
27	1.35 (*d*, J = 7.0)	16.3	4.77 (br. *s*), 4.72 (br. *s*)	108.6
Xyl				
1′	5.30 (*d*, J = 7.5)	98.2	4.42 (*d*, J = 7.5)	98.1
2′	4.46–4.57 (*m*)	76.9	4.18–4.29 (*m*)	76.8
3′	4.42–4.54 (*m*)	79.2	4.11–4.22 (*m*)	79.1
4′	4.36–4.47 (*m*)	71.6	4.16–4.28 (*m*)	71.5
5′	4.56–4.66 (*m*), 3.94 (*t*, J = 10.5)	67.4	4.30 (*dd*, J = 10.5, 2.0), 3.61 (*t*, J = 10.5)	67.5
Rha				
1″	6.38 (br. *s*)	102.0	6.56 (br. *s*)	101.9
2″	5.04[a])	72.2	4.68–4.79 (*m*)	72.1
3″	5.04[a])	72.0	4.66–4.74 (*m*)	71.9
4″	4.47–4.57 (*m*)	74.5	4.38–4.50 (*m*)	74.4
5″	4.95–5.05 (*m*)	69.9	4.48–4.53 (*m*)	69.8
6″	2.01 (*d*, J = 6.5)	18.8	1.70 (*d*, J = 6.5)	18.7

[a]) Overlapped with other signals.

H–C(19) in the ROESY spectrum (*Fig. 3*). The α-orientation of the OH group at C(3) was assigned based on the chemical shift of C(3) (δ(C) 67.4) in the ^{13}C-NMR spectrum

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

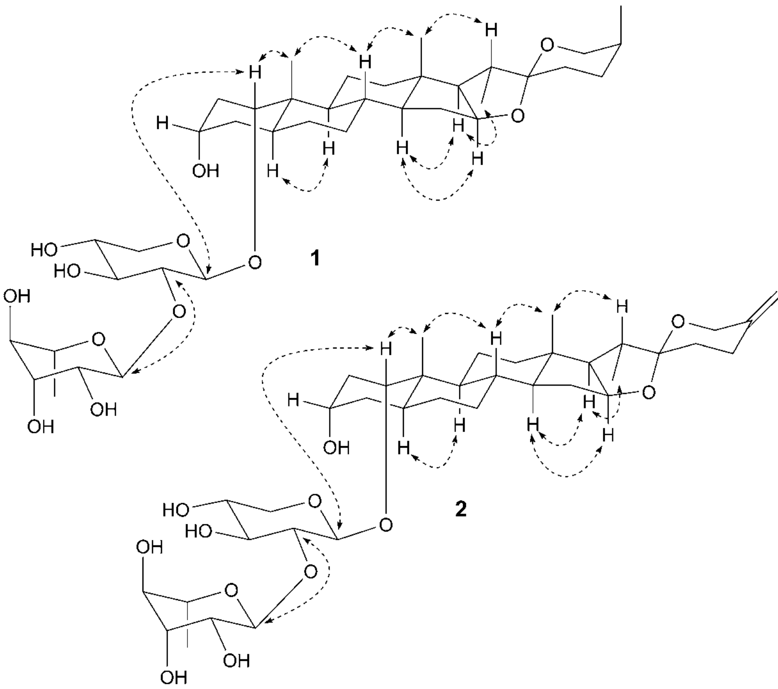

Fig. 3. *Key ROESY correlations* (H ↔ H) *of compounds* **1** *and* **2**

(δ(C) 66.0 for the α-orientation of HO–C(3); δ(C) 71.1 for the β-orientation of HO–C(3) [16][17]). The configurations of all other ring junctions were identified as *trans* by ROESY correlations (*Fig. 3*) [16]. The configuration at C(25) was confirmed to be (*S*), based on the comparison of the ^{1}H-NMR data of the Me group at C(25) (δ(H) 1.35 (δ(H) 1.10 for the (*S*)-form [18]) and the ^{13}C-NMR spectral data of C(23), C(24), C(25), C(26), and C(27) [19][20]. Acid hydrolysis of **1** with 1N HCl yielded an aglycone **1a** and two sugars. The aglycone (25*S*)-5α-spirostan-1α,3α-diol (**1a**) was identified by comprehensive ^{1}H- and ^{13}C-NMR, 2D-NMR, and HR-EI-MS analyses. Two sugars were confirmed by GC analysis after derivatization (D-xylose, t_R: 6.16 min, L-rhamnose, t_R: 6.11 min) [21]. Thus, compound **1** was identified as (1α,3α,25*S*)-1-[(β-D-xylopyranosyl-(2 → 1)-α-L-rhamnopyranosyl)oxy]-5α-spirostan and named bletilnoside A.

Compound **2** was obtained as a white amorphous powder. The molecular formula was deduced as $C_{38}H_{60}O_{12}$ from the $[M+Na]^+$ peak at *m*/*z* 731.8744 (calc. for $C_{38}H_{60}NaO_{12}^+$: 731.8746) in the HR-FAB-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**. The major difference was the presence of two olefinic H-atom signals (δ(H) 4.77 (br. *s*, H_a–C(27)) and 4.72 (br. *s*, H_b–C(27)); δ(C) 144.4 (C(25)) and 108.6 (C(27))) in **2**. The position of the exocyclic CH_2 group was confirmed by the HMBC spectrum, in which a correlation was observed between the CH_2(27) (δ(H) 4.77 and 4.72), and C(26) and C(24) (δ(C) 65.3, 28.9, resp.; *Fig. 2*). The configuration of **2** was assumed to be same as **1** by comparson of their NMR data, which were confirmed by ROESY correlations (*Fig. 3*). Acid hydrolysis of **2** yielded aglycone (**2a**) and two sugars. The aglycone (5α)-spirost-25(27)-ene-1α,3α-diol (**2a**) was identified by ^{1}H-NMR and HR-EI-MS analyses, and two sugars were identified as D-xylose and L-rhamnose by GC analysis [21]. Thus, compound **2** was identified as (1α,3α)-1-[(β-D-xylopyranosyl-(2 → 1)-α-L-rhamnopyranosyl)oxy]-25(27)-ene-5α-spirostan and named bletilnoside B.

The known compounds were identified as 3-epiruscogenin (**3**), 3-epineoruscogenin (**4**) [22], 3-*O*-β-D-glucopyranosyl-3-epiruscogenin (**5**), 3-*O*-β-D-glucopyranosyl-3-epineoruscogenin (**6**) [23], (20*S*,22*R*)-1β,2β,3β,4β,5β,7α-hexahydroxyspirost-25(27)-en-6-one (**7**) [24], by comparing their spectroscopic data with those in previous reports. To the best of our knowledge, all compounds were isolated for the first time from this plant source and the genus *Bletilla*. In addition, this is the first report on the occurrence of steroidal saponins in the genus *Bletilla*.

2.2. *Biological Evaluation of Compounds.* The cytotoxic activities of the isolated compounds **1**–**7** were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay [25]. The compounds **1**, **2**, and **5** showed significant cytotoxicities against all tested tumor cell lines with IC_{50} values ranging from 3.98 ± 0.16 to 12.10 ± 0.40 μM (*Table 2*). Especially, the new compounds **1** and **2** exhibited potent cytotoxicities against all of the cell lines tested with IC_{50} values in the range of 3.98 ± 0.16–9.29 ± 1.23 μM. Compounds **3**, **4**, and **6** showed low cytotoxicities against tested cell lines ($IC_{50} > 30$ μM).

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Table 2. *Cytotoxic Activities of Compounds* **1**, **2**, *and* **5** *against Four Cultured Human Tumor Cell Lines in the SRB Bioassay*

Compound	IC_{50} [μM][a])			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	4.56 ± 0.29	4.00 ± 0.06	3.98 ± 0.16	5.08 ± 0.51
2	8.79 ± 1.01	8.08 ± 0.83	5.29 ± 0.34	9.29 ± 1.23
5	12.10 ± 0.40	11.80 ± 0.28	11.55 ± 0.27	11.00 ± 0.23
Doxorubicin[b])	0.0035 ± 0.0025	0.0037 ± 0.0022	0.0009 ± 0.0001	0.1574 ± 0.0569

[a]) The concentration of the compound that caused a 50% inhibition of cell growth. The data are presented as the mean ± SEM of at least three distinct experiments. [b]) Positive control.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*, Germany) and *Lichroprep RP$_{18}$* gel (40–60 μM, *Merck*, DE-Darmstadt). TLC: Silica gel *60 F$_{254}$* and *RP-18 F$_{254s}$* silica gel plates (*Merck*); detection under UV light and by spraying with 10% aq. H_2SO_4 soln., followed by heating at 120° for 1 min. HPLC: Prep. HPLC *Gilson 306 pump*, *Gilson-101 RI* detector, *Phenomenex-Luna-C$_{18}$-(2)* column (250 mm × 10.00 mm (i.d.), 5 μM); 85, 90% MeOH as mobile phase (2 ml/min); t_R in min. M.p.: *Gallenkamp* apparatus; uncorrected. IR Spectra: *Bruker IFS-66/S* FT-IR spectrometer; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian UNITY INOVA 500* FT-NMR instrument; δ in ppm rel. to Me_4Si as internal standard, J in Hz. FAB-, HR-FAB-, and EI-MS: *JEOL JMS-700* (*Jeol*, Japan); in m/z.

Plant Material. The roots of *B. striata* (3.6 kg) were purchased from Kyungdong herbal market, Seoul, Korea, in June 2011, and were identified by one of the authors (*K. R. L.*). A voucher specimen (SKKU-NPL 1106) was deposited with the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. Dried roots of *B. striata* (Orchidaceae) (3.6 kg) were extracted three times with 80% MeOH under reflux. The resulting MeOH extracts (568 g) were suspended in dist. H_2O (4 × 800 ml), and then successively partitioned with hexane, $CHCl_3$, AcOEt, and BuOH, yielding residues of 3, 42, 51, and 159 g, resp. The $CHCl_3$-soluble extract (40 g) was fractionated by CC (*RP-C$_{18}$* (1 kg); $CHCl_3$/MeOH 50:1 → 1:1) to give seven fractions, *Frs. A–G*. *Fr. D* (4.0 g) was subjected to CC (*RP-C$_{18}$* (200 g); MeOH/H_2O 3:2 → 1:0): *Frs. D$_1$–D$_{10}$*. *Fr. D$_{10}$* (119 mg) was further separated by CC (SiO_2; $CHCl_3$/MeOH 20:1): *Frs. D$_{10-1}$–D$_{10-4}$*. *Frs. D$_{10-2}$* (10 mg) and *D$_{10-4}$* (15 mg) were purified by prep. HPLC (*RP-C$_{18}$*; 85% MeOH) to yield **3** (t_R 12.5; 5 mg) and **4** (t_R 14.3; 7 mg). *Fr. F* (7.2 g) was subjected to CC (*RP-C$_{18}$* (400 g); MeOH/H_2O 7:3 → 1:0) to furnish six fractions, *Frs. F$_1$–F$_6$*. *Fr. F$_4$* (88 mg) in MeOH was filtered using filter paper. Then, the precipitate was washed with MeOH and subsequently dried to yield compound **7** (26 mg). *Fr. F$_5$* (2.9 g) was separated by CC (SiO_2 (150 g); $CHCl_3$/MeOH 30:1 → 1:1): *Frs. F$_{5-1}$–F$_{5-2}$*. *Fr. F$_{5-1}$* (1.5 g) was purified by prep. HPLC (*RP-C$_{18}$*; 85% MeOH) to give **5** (t_R 15.7; 76 mg) and **6** (t_R 19.2; 53 mg). *Fr. F$_6$* (378 mg) was separated by CC (SiO_2 (50 g); $CHCl_3$/MeOH 20:1) and further purified by prep. HPLC (*RP-C$_{18}$*; 90% MeOH) to afford **1** (t_R 11.5; 30 mg) and **2** (t_R 13.8; 5 mg).

Bletilnoside A (*=(1α,3α,25S)-1-[(β-D-Xylopyranosyl-(2 → 1)-α-L-rhamnopyranosyl)oxy]-5α-spirostan = (2*S*,3*R*,4*R*,5*R*,6*S*)-2-{[(2*S*,4*S*,5*R*)-Tetrahydro-4,5-dihydroxy-2-{[(1α,3α,5α,25*S*)-3-hydroxyspirostan-1-yl]oxy}-2*H*-pyran-3-yl]oxy}-6-methyltetrahydro-2*H*-pyran-3,4,5-triol*; **1**). White amorphous powder. M.p. 210–215°. $[\alpha]_D^{25} = -4.7$ ($c = 0.235$, MeOH). IR (KBr) 3407, 2951, 1054, 1032, 1017, 698. ^1H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 711.9083 ($[M+H]^+$, $C_{38}H_{63}O_{12}^+$; calc. 711.4320).

Bletilnoside B (= *(1α,3α)-1-[(β-*D*-Xylopyranosyl-(2 → 1)-α-*L*-rhamnopyranosyl)oxy]-25(27)-ene-5α-spirostan = (2*S*,3*R*,4*R*,5*R*,6*S*)-2-{[(2*S*,4*S*,5*R*)-Tetrahydro-4,5-dihydroxy-2-{[(1α,3α,5α)-3-hydroxy-spirost-25(27)-en-1-yl]oxy}-2*H*-pyran-3-yl]oxy}-6-methyltetrahydro-2*H*-pyran-3,4,5-triol*; **1**). White amorphous powder. M.p. 209–213°. $[\alpha]_D^{25} = -3.5$ ($c = 0.175$, MeOH). IR (KBr): 3423, 2949, 2843, 1656, 1450, 1032, 1018, 694. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 731.8744 ($[M + Na]^+$, $C_{38}H_{60}NaO_{12}^+$; calc. 731.3982).

Acid Hydrolysis of **1** *and* **2**. A soln. of **1** (5.1 mg) in 1N HCl (dioxane/H_2O 1:1, 3 ml) was heated for 4 h at 100°. The hydrolysate was extracted with $CHCl_3$ and evaporated under reduced pressure to yield aglycone **1a** (0.9 mg). In the same way, **2** (4.7 mg) was treated with 1N HCl soln. to furnish aglycone **2a** (0.7 mg).

Data of **1a**. White amorphous powder. ^{1}H-NMR (500 MHz, (D_5)pyridine): 4.39 (*q*, $J = 7.0$, H–C(16)); 4.17 (br. *s*, H–C(4)); 3.95 (*dd*, $J = 10.5$, 2.0, H_a–C(26)); 3.84 (*s*, H–C(1)); 3.29 (*dd*, $J = 10.5$, 2.0, H_b–C(26)); 2.72–2.81 (*m*, H_a–C(2)); 2.49–2.55 (*m*, H–C(8)); 2.20–2.29 (*m*, H_b–C(2)); 2.19–2.28 (*m*, H–C(6)); 2.15–2.26 (*m*, H_a–C(11)); 2.13–2.23 (*m*, H_a–C(15)); 2.12–2.20 (*m*, H_a–C(7)); 2.10–2.19 (*m*, H_a–C(24)); 2.09–2.17 (*m*, H_b–C(7)); 2.01–2.08 (*m*, H–C(5)); 1.95–2.08 (*m*, H–C(20)); 1.88–1.90 (*m*, H_a–C(23)); 1.85–1.96 (*m*, H_a–C(4)); 1.79–1.84 (*m*, H_b–C(11)); 1.78–1.88 (*m*, H–C(17)); 1.75–1.85 (*m*, H_b–C(15)); 1.70–1.76 (*m*, H–C(9)); 1.63–1.73 (*m*, H_a–C(12)); 1.60–1.69 (*m*, H_b–C(4)); 1.39–1.48 (*m*, H_b–C(23)); 1.30–1.40 (*m*, H_b–C(24)); 1.20–1.29 (*m*, H–C(25)); 1.13 (*s*, Me(19)); 1.08–1.19 (*m*, H–C(14)); 1.08 (*d*, $J = 7.0$, H–C(21)); 1.05–1.14 (*m*, H_b–C(12)); 0.98 (*d*, $J = 7.0$, H–C(27)); 0.77 (*s*, Me(18)). ^{13}C-NMR (125 MHz, (D_5)pyridine): 109.7 (C(22)); 81.2 (C(16)); 73.3 (C(1)); 68.1 (C(3)); 65.1 (C(26)); 63.1 (C(17)); 56.4 (C(14)); 42.5 (C(20)); 42.1 (C(9)); 40.7 (C(13)); 40.6 (C(10)); 40.3 (C(12)); 35.8 (C(8)); 34.2 (C(15)); 32.9 (C(5)); 32.2 (C(11)); 29.9 (C(2)); 27.5 (C(25)); 26.7 (C(4)); 26.4 (C(23)); 26.2 (C(7,24)); 21.0 (C(6)); 19.3 (C(19)); 16.6 (C(18)); 16.2 (C(27)); 14.8 (C(21)). HR-EI-MS: 432.3239 (M^+, $C_{27}H_{44}O_4^+$; calc. 432.3240).

Data of **2a**. White amorphous powder. ^{1}H-NMR (500 MHz, ((D_5)pyridine): 4.78 (br. *s*, H_a–C(27)); 4.70 (br. *s*, H_b–C(27)); 4.41 (*d*, $J = 10.5$, H_a–C(26)); 4.36 (*q*, $J = 7.0$, H–C(16)); 4.15 (br. *s*, H–C(3)); 3.80 (br. *s*, H–C(1)); 3.62 (*d*, $J = 10.5$, H_b–C(26)); 2.70–2.79 (*m*, H_a–C(2)); 2.50–2.59 (*m*, H_a–C(11)); 2.21–2.30 (*m*, H_b–C(2)); 2.21–2.24 (*m*, H_a–C(23)); 2.20–2.27 (*m*, H–C(6)); 1.96–2.07 (*m*, H–C(5)); 1.93–2.00 (*m*, H_a–C(15)); 1.90–1.97 (*m*, H_b–C(11)); 1.89–1.94 (*m*, H_a–C(4)); 1.80–1.84 (*m*, H–C(17)); 1.79–1.87 (*m*, H–C(20)); 1.62–1.71 (*m*, H_a–C(12)); 1.60–1.67 (*m*, H_b–C(4)); 1.50–1.55 (*m*, H_b–C(23)); 1.49–1.53 (*m*, H–C(8)); 1.38–1.47 (*m*, H–C(9)); 1.30–1.40 (*m*, H_a–C(7)); 1.20–1.30 (*m*, H–C(14)); 1.10 (*s*, Me(19)); 1.08–1.20 (*m*, H_b–C(12)); 1.06 (*d*, $J = 7.0$, H–C(21)); 1.00–1.07 (*m*, H_a–C(24)); 0.99–1.05 (*m*, H_b–C(15)); 0.98–1.07 (*m*, H_b–C(7)); 0.98–1.04 (*m*, H_b–C(24)); 0.78 (*s*, Me(18)). HR-EI-MS: 430.3287 (M^+, $C_{27}H_{42}O_4^+$; calc. 430.3083).

Determination of Absolute Configuration of the Monosaccharide Units. The sugars obtained from the hydrolysis were dissolved in anh. pyridine (0.5 ml), and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60° for 1.5 h and trimethylsilylated with 1-(trimethylsilyl)-1*H*-imidazole (0.1 ml) for 2 h. The mixture was partitioned between hexane and H_2O (1 ml each), and org. layer (1 µl) was analyzed by GC-MS [21]. D-Xylose and L-rhamnose were identified in the hydrolysates of **1** and **2** by co-injection with derivatized authentic samples, giving single peaks at 6.16 (D-xylose) and 6.11 min (L-rhamnose). Retention times of authentic samples treated in the same way were 6.16 (D-xylose) and 6.11 min (L-rhamnose).

Cytotoxicity Assay. A sulforhodamine B (SRB) bioassay was used to evaluate the cytotoxicity of each compound isolated against four cultured human tumor cell lines [25]. The assays were performed at the *Korea Research Institute of Chemical Technology*. The cell lines used were A549 (non-small-cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin was used as a positive control.

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Three New 24-Nortriterpenoids from the Roots of *Ilex asprella*

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Asprellols A – C (**1** – **3**, resp.), three new 24-nortriterpenoids, were isolated from the $CHCl_3$-soluble fraction of 95% EtOH extract of the roots of *Ilex asprella*, together with a known nortriterpenoid. The structures of the new compounds were elucidated as 2,6β,20β-trihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4-dien-28,13β-olide (**1**), 2,6β-dihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4,20(30)-trien-28,13β-olide (**2**), and 2,6β-dihydroxy-3-oxo-11α,12α-epoxy-24-noroleana-1,4-dien-28,13β-olide (**3**) on the basis of spectroscopic analyses.

Introduction. – *Ilex asprella* (HOOK. et ARN.) CHAMP. ex BENTH. (Aquifoliaceae) is a deciduous shrub growing in southern China and Southeast Asia. The roots of *I. asprella*, known as '*Gangmei*' in Guangdong Province of China, have been used as a folk medicine for the treatment of viral and bacterial infectious diseases, such as influenza, tonsillitis, sphagitis, tracheitis, and pertussis [1]. Furthermore, it was also an important ingredient of many herbal beverages against viral and bacterial infections. Several constituents, such as steroids, triterpenoids, lignans, and flavonoids, as well as their glycosides, were isolated from the roots or leaves of *I. asprella* [2 – 8]. A continuing interest in antiviral plants [9 – 12] prompted us to investigate the chemical constituents of the title plant, leading to the isolation of asprellols A – C (**1** – **3**, resp.), and 2,6β-dihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4-dien-28,13β-olide (**4**) [13]. The present work represents the first report on the occurrence of 24-norursane-type

	R^1	R^2	R^3
1	Me	Me	OH
2	Me	=CH_2	
3	H	Me	Me
4	Me	Me	H

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and 24-noroleanane-type triterpenoids in the title plant, describing the isolation and structure elucidation of these new nortriterpenoids.

Results and Discussion. – Asprellol A (**1**) was obtained as an amorphous powder and its molecular formula was determined as $C_{29}H_{38}O_7$ by HR-ESI-MS. The IR spectrum showed absorptions for OH groups (3498 and 3430 cm^{-1}), a lactone C=O group (1752 cm^{-1}), and a cross-conjugated cyclohexadienone moiety (1625 cm^{-1}). The analysis of 1D- and 2D-NMR data allowed us to elucidate the structure of **1** as 2,6β,20β-trihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4-dien-28,13β-olide.

The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited signals for an olefinic H-atom at δ(H) 6.50 (*s*), three O-bearing CH H-atoms at δ(H) 5.20 (br. *d*, $J = 3.0$), 3.62 (*dd*, $J = 3.7, 2.0$), and 3.09 (*d*, $J = 3.7$), five tertiary Me groups at δ(H) 2.03, 1.68, 1.61, 1.22, and 0.98, and a secondary Me group at δ(H) 1.20 ($J = 6.5$). The ^{13}C-NMR and HSQC (*Table 2*) spectra of **1** displayed 29 C-atom signals (for 6 Me, 5 CH_2, 7 CH groups, and 11 quaternary C-atoms), including those of two CO groups at δ(C) 183.9 and 181.3, four olefinic C-atoms at δ(C) 160.5, 146.5, 132.0, and 127.3, two O-bearing quaternary C-atoms at δ(C) 90.7 and 72.1, an O-bearing CH group at δ(C) 69.0, and an epoxy group at δ(C) 57.9 and 55.9. The similarities of the ^{1}H- and ^{13}C-NMR data of **1** and **4** [13], combined with an additional O-atom in the molecule of **1**, suggested **1** to be a hydroxylated analog of **4**. Comparing the ^{13}C-NMR data of **1** with those of **4**, the most important difference turned out to be the signal of an O-bearing quaternary C-atom at δ(C) 72.1 in **1** instead of a CH group at δ(C) 40.2 (C(20) of **4**). Furthermore, the signals of C(18), C(22), and C(29), three C-atoms in γ-position relative to HO–C(20), were observed to be shifted upfield ($\Delta\delta$ (**1**–**4**) -4.3, -3.5, and -4.3 ppm, resp.) due to γ-gauche effects (*Fig. 1*) [14], indicating both HO–C(20) and H–C(18) of **1** to be in axial orientations in a chair-form *E*-ring. The above evidences indicated **1** to be 20β-OH derivative of **4**, which was also confirmed by HMBC and ROESY experiments (*Fig. 2*).

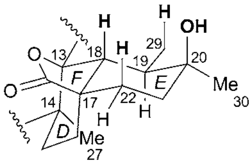

Fig. 1. *Steric γ-gauche effect caused by HO–C(20) of* **1**

Asprellol B (**2**), obtained as a white amorphous powder, had the molecular formula $C_{29}H_{36}O_6$ as deduced from the HR-ESI-MS *pseudo*-molecular-ion peak at *m/z* 503.2405 ($[M + Na]^+$), indicating two H-atoms less than that of **4**. The IR spectrum showed similar bands at 3432, 1778, 1631, 1432, 1388, 933, and 879 cm^{-1}, as observed for **4** and **1**. The structure of **2** was finally determined to be 2,6β-dihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4,20(30)-trien-28,13β-olide by means of NMR spectra.

The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*) spectra of **2** were similar to those of **4**. Some diagnostic signals were observed, such as those attributed to a conjugated ring-*A* at δ(C) 182.1, 159.0, 144.5, 130.5, and 124.9, and δ(H) 6.54 (*s*, H–C(1)) and 6.29 (*s*,

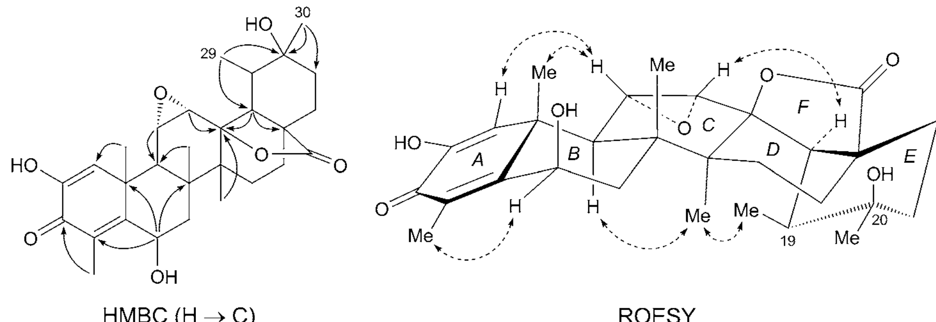

Fig. 2. *Significant HMBCs and ROESY correlations of* **1**

Table 1. *¹H-NMR Data of Compounds* **1**–**3** (500 MHz, δ in ppm, *J* in Hz)

H-Atom	**1** (CD_3OD)	**2** ($CDCl_3$)	**3** ($CDCl_3$)
H–C(1)	6.50 (*s*)	6.54 (*s*)	6.54 (*s*)
HO–C(2)	–	6.29 (*s*)	6.29 (*s*)
H–C(6)	5.20 (br. *d*, *J* = 3.0)	5.28 (br. *s*)	5.27 (br. *d*, *J* = 4.2)
$H_α$–C(7)	1.61 (br. *d*, *J* = 13.7)	1.55–1.63 (*m*)	1.50–1.58 (*m*)
$H_β$–C(7)	1.74 (br. *d*, *J* = 13.7)	1.75–1.82 (*m*)	1.13–1.22 (*m*)
H–C(9)	1.83 (*d*, *J* = 2.0)	1.91 (*d*, *J* = 2.3)	1.89 (*d*, 2.3)
H–C(11)	3.62 (*dd*, *J* = 3.7, 2.0)	3.46 (*dd*, *J* = 3.8, 2.5)	3.36 (*dd*, *J* = 3.9, 2.1)
H–C(12)	3.09 (*d*, *J* = 3.7)	3.09 (*d*, *J* = 3.8)	3.19 (*d*, *J* = 3.8)
$H_α$–C(15)	1.21 (br. *d*, *J* = 13.8)	1.17–1.22 (*m*)	1.69–1.81 (*m*)
$H_β$–C(15)	1.84 (*td*, *J* = 13.8, 4.2)	1.80–1.91 (*m*)	1.30–1.40 (*m*)
$H_α$–C(16)	2.31 (*td*, *J* = 13.8, 5.8)	2.29–2.37 (*m*)	2.10–2.19 (*m*)
$H_β$–C(16)	1.31 (br. *dd*, *J* = 13.8, 5.9)	1.52 (br. *dd*, *J* = 14.1, 5.7)	1.02–1.12 (*m*)
H–C(18)	2.28 (*d*, *J* = 12.1)	1.86 (*d*, *J* = 11.9)	2.36 (*dd*, *J* = 14.3, 3.6)
$H_α$–C(19)	2.05 (*dq*, *J* = 12.1, 6.5)	1.27 (*dq*, *J* = 11.9, 6.5)	1.81 (*t*, *J* = 13.6)
$H_β$–C(19)	–	–	1.58–1.68 (*m*)
$H_α$–C(21)	1.71 (*dd*, *J* = 14.1, 10.2)	2.25 (*td*, *J* = 13.9, 4.8)	1.30–1.38 (*m*)
$H_β$–C(21)	1.60 (br. *d*, *J* = 14.1)	2.29–2.39 (*m*)	1.30–1.38 (*m*)
$H_α$–C(22)	1.54 (*dt*, *J* = 12.6, 3.0	1.51–1.60 (*m*)	1.62–1.70 (*m*)
$H_β$–C(22)	1.84 (*td*, *J* = 13.8, 4.1)	1.93 (*ddd*, *J* = 13.3, 4.6, 2.4)	1.62–1.70 (*m*)
Me(23)	2.03 (*s*)	2.08 (*s*)	2.08 (*s*)
Me(25)	1.68 (*s*)	1.68 (*s*)	1.67 (*s*)
Me(26)	1.61 (*s*)	1.61 (*s*)	1.61 (*s*)
Me(27)	0.98 (*s*)	1.04 (*s*)	0.93 (*s*)
Me(29)	1.20 (*d*, *J* = 6.5)	1.33 (*d*, *J* = 6.2)	0.93 (*s*)
Me(30) or CH_2(30)	1.22 (*s*)	4.78 (br. *s*), 4.84 (br. *s*)	0.99 (*s*)

HO–C(2)), a lactone ring-*F* at δ(C) 178.1 (*s*) and 88.1 (*s*), a OH-bearing CH group at δ(C) 68.5 and δ(H) 5.28 (br. *s*) due to deshielding by a neighboring conjugated system, one 11,12-epoxy group at δ(C) 56.5 and 54.5, as well as δ(H) 3.46 (*dd*, *J* = 3.8, 2.5) and 3.09 (*d*, *J* = 3.8), four tertiary Me groups at δ(H) 2.08, 1.68, 1.61 and 1.04, and one secondary Me group at δ(H) 1.33 (*d*, *J* = 6.2). Furthermore, signals of an additional exocyclic C=C bond at δ(C) 150.9 and 108.7, as well as at δ(H) 4.84 and 4.78 (each br. *s*)

Table 2. *^{13}C-NMR Data of* **1**–**4** (125 MHz)[a])

C-Atom	**1**	**2**	**3**	**4**	C-Atom	**1**	**2**	**3**	**4**
C(1)	127.3	124.9	124.8	124.9	C(16)	23.1	22.7	21.2	22.6
C(2)	146.5	144.5	144.5	144.4	C(17)	46.1	45.0	43.8	45.1
C(3)	183.9	182.1	182.1	182.1	C(18)	56.2	61.1	49.5	60.5
C(4)	132.0	130.5	130.5	130.5	C(19)	40.3	36.0	37.7	37.5
C(5)	160.5	159.0	159.1	159.1	C(20)	72.1	150.9	31.5	40.2
C(6)	69.0	68.5	68.5	68.5	C(21)	36.3	31.6	34.2	30.5
C(7)	38.1	37.2	37.1	37.3	C(22)	27.8	32.7	26.9	31.3
C(8)	42.6	41.2	41.1	41.2	C(23)	11.0	11.1	11.2	11.1
C(9)	48.2	46.2	45.5	46.2	C(25)	24.0	23.7	23.8	23.8
C(10)	42.8	41.6	41.4	41.5	C(26)	21.5	20.9	20.7	20.9
C(11)	55.9	54.5	52.5	54.7	C(27)	16.5	16.4	19.0	16.5
C(12)	57.9	56.5	57.4	56.6	C(28)	181.3	178.1	179.0	178.9
C(13)	90.7	88.1	87.1	88.6	C(29)	12.9	16.1	33.2	17.2
C(14)	43.2	42.2	41.6	42.1	C(30)	28.3	108.7	23.8	19.5
C(15)	28.0	27.0	26.9	27.0					

[a]) Solution in CD_3OD (for **1**) or $CDCl_3$ (for **2**–**4**)

were also detected, replacing a Me *doublet* and one CH signal of **4**. These findings indicated **2** to be a derivative of **4**, dehydrogenated between C(20)–C(30) or C(19)–C(29). The exocyclic C=C bond was finally positioned between C(20) and C(30) on the basis of HMBCs of Me–C(19) (δ(H) 1.33)/C(18) (δ(C) 61.1) and C(13) (δ(C) 88.1).

Asprellol C (**3**), a white amorphous powder, had the molecular formula $C_{29}H_{38}O_6$ based on HR-ESI-MS *pseudo*-molecular-ion peak $[M+Na]^+$ at *m/z* 505.2568. The structure of **3** was established as 2,6β-dihydroxy-3-oxo-11α,12α-epoxy-24-noroleana-1,4-dien-28,13β-olide by detailed analysis of NMR data.

The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) of **3** showed nearly identical H- and C-atom signals within rings *A*–*D*, and *F* of **4**. The main differences in the *E*-ring were two Me *singlet*s at δ(H) 0.99 and 0.93 in **3** instead of two Me *doublet*s at δ(H) 1.18 and 0.93 of **4**, suggesting **3** to be a 24-noroleanane-type isomer of **4**. The ^{1}H- and ^{13}C-NMR data of ring *E* of **3**, along with those of rings *C*, *D*, and *F*, were almost coincident with those of 6β-hydroxy-3-oxo-11α,12α-epoxyolean-28,13β-olide [15], indicating that the two compounds have the same rings *C*–*F*. The above evidence is consistent with **3** to be a 19-demethylated and 20-methylated derivative of **4**.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 400 mesh; *Qingdao Haiyang Co., Ltd.*, Qingdao, P. R. China). TLC: Silica gel *$HSGF_{254}$* (*Yantai Jiangyou Guijiao Kaifa Co., Ltd.*, Yantai, P. R. China). Semi-prep. HPLC: *Waters* HPLC system, *Waters-2545-HPLC* pump, *Waters-2489* detector, column: *xbridge-C18*, 5 μm, i.d. 10 × 250 mm. Optical rotation: *PerkinElmer 341* polarimeter. UV Spectra: *Shimadzu UV-2550* spectrophotometer. IR Spectra: *Nicolet-Magna-750-FTIR* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AV-500* instrument at 500 (^{1}H) and 125 MHz (^{13}C); in $CDCl_3$ or CD_3OD soln.; δ in ppm rel. to Me_4Si; *J* in Hz. ESI- and HR-ESI-MS: *Bruker Esquire 3000 plus* and *Finnigan LC QDECA* mass spectrometers, in *m/z* (rel. int.).

Plant Material. The roots of *Ilex asprella* were collected from Zijin County, Guangong Province, P. R. China, in April 2011, and identified by Prof. *Da-Yuan Zhu*. A voucher specimen (No. 2011-83) was deposited with the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. The air-dried roots of *I. asprella* (10 kg) were extracted with 95% EtOH at r.t. three times, and the extract was concentrated under reduced pressure to remove alcohol and then partitioned successively with petroleum ether (PE), $CHCl_3$, and BuOH. The $CHCl_3$-soluble fraction (50 g) was subjected to CC (SiO_2 (2 kg), column i.d. 10×80 cm; $CHCl_3/CH_3OH$ $50:1 \rightarrow 3:1$ (*v/v*)): *Frs. A – K*. *Fr. C* (7.9 g) was separated by CC (SiO_2 (400 g); PE/acetone $50:1 \rightarrow 1:1$ (*v/v*)): *Frs. C1 – C16*. *Fr. C10* (730 mg) was purified by CC (SiO_2; PE/acetone 5:1): *Frs. C8.1 – C8.8*. *Fr C8.5* (104 mg) yielded solid **4** (17 mg), the remaining mother liquor afforded **1** (8 mg), **2** (3 mg), and **3** (6 mg) using semi-prep. HPLC (MeOH/H_2O 48:52).

Asprellol A (*=2,6β,20β-Trihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4-dien-28,13β-olide = (1*R*,2*S*,4a*R*,6a*S*,6b*R*,8*R*,12a*S*,12b*S*,12c*S*,13a*S*,13b*S*,13c*R*)-1,3,4,5,6,6a,6b,7,8,12a,12b,12c,13a,13c-Tetradecahydro-2,8,11-trihydroxy-1,2,6a,6b,9,12a-hexamethyl-2*H*,10*H*-13b,4a-(epoxymethano)piceno[13,14-*b*]oxirene-10,15-dione*; **1**). White amorphous powders. UV (MeOH): 205 (2338), 258 (2311). $[\alpha]_D^{20} = 14.7$ ($c = 0.085$, MeOH). IR: 3498, 3430, 2933, 1752, 1625, 1425, 1328, 1236, 1010, 931, 877. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 481 ($[M + H - H_2O]^+$), 1019 ($[2M + Na]^+$). ESI-MS (neg.): 543.5 ($[M + HCOO]^-$). HR-ESI-MS: 521.2519 ($[M + Na]^+$, $C_{29}H_{38}NaO_7^+$; calc. 521.2515).

Asprellol B (*=2,6β-Dihydroxy-3-oxo-11α,12α-epoxy-24-norursa-1,4,20-trien-28,13β-olide = (1*R*,4a*S*,6a*S*,6b*R*,8*R*,12a*S*,12b*S*,12c*S*,13a*S*,13b*S*,13c*R*)-1,3,4,5,6,6a,6b,7,8,12a,12b,12c,13a,13c-Tetradecahydro-8,11-dihydroxy-1,6a,6b,9,12a-pentamethyl-2-methylidene-2*H*,10*H*-13b,4a-(epoxymethano)piceno[13,14-*b*]oxirene-10,15-dione*; **2**). White amorphous powders. UV (MeOH): 210 (3050), 257 (1162). $[\alpha]_D^{20} = 27.7$ ($c = 0.130$, MeOH). IR: 3432, 2929, 2861, 1778, 1631, 1461, 1432, 1388, 1238, 1133, 1068, 1035, 933, 879. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 463 ($[M + H - H_2O]^+$), 983 ($[2M + Na]^+$). ESI-MS (neg.): 525 ($[M + HCOO]^-$). HR-ESI-MS: 503.2405 ($[M + Na]^+$, $C_{29}H_{36}NaO_6^+$; calc. 503.2410).

Asprellol C (*=2,6β-Dihydroxy-3-oxo-11α,12α-epoxy-24-noroleana-1,4-dien-28,13β-olide = (4a*S*,6a*S*,6b*R*,8*R*,12a*S*,12b*S*,12c*S*,13a*S*,13b*S*,13c*R*)-1,3,4,5,6,6a,6b,7,8,12a,12b,12c,13a,13c-Tetradecahydro-8,11-dihydroxy-2,2,6a,6b,9,12a-hexamethyl-2*H*,10*H*-13b,4a-(epoxymethano)piceno[13,14-*b*]oxirene-10,15-dione*; **3**). White amorphous powders. UV (MeOH): 210 (2972), 252 (631). $[\alpha]_D^{20} = 15$ ($c = 0.120$, MeOH). IR: 3423, 2929, 1778, 1631, 1455, 1436, 1388, 1361, 1321, 1238, 1137, 1039, 931, 871, 802. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 465 ($[M + H - H_2O]^+$), 987 ($[2M + Na]^+$). ESI-MS (neg.): 527 ($[M + HCOO]^-$). HR-ESI-MS: 505.2568 ($[M + Na]^+$, $C_{29}H_{38}NaO_6^+$; calc. 505.2566).

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Palladium- and Solvent-Free Synthesis of Ynones by Copper(I)-Catalyzed Acylation of Terminal Alkynes with Acyl Chlorides under Aerobic Conditions

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Air-stable Cu^I/cryptand-22 complex was found to be a highly active catalyst for the solvent-free cross-coupling reaction of terminal alkynes with different acyl chlorides in the presence of Et_3N as base to give the corresponding ynones in quantitative yields.

Introduction. – Among several protocols for the synthesis of ynones [1–3], the reaction of acyl chlorides with alkynyl organometallic reagents, based on copper [4], zinc [5], tin [6], silver [7], lithium [8], and silicon [9], is arguably one of the most versatile and efficient methods. Recently, the coupling reaction of terminal alkynes and acyl chlorides catalyzed by Pd catalysts has received much attention [10]; but, on the other hand, efficient Pd-free systems would obviously be much more interesting for economic reasons, regarding both the higher cost of Pd as well as the difficulties in removing the metal (and its ligands) from the products. Indeed, it is a common industrial practice to avoid, whenever possible, the use of Pd catalysts in the last steps of the synthesis of complex molecules. Nevertheless, there are several other reports in which coupling of acyl chlorides with terminal alkynes catalyzed by $Pd(PPh_3)_2Cl_2$/CuI provided ynones [11]. However, most of the reported methods require anhydrous solvents, inert gas, long reaction times, and expensive and toxic reagents. Moreover, the use of CuI has been avoided in many cases, because the *in situ* formed copper(I) acetylides derived from CuI undergo homocoupling upon exposure to air, yielding side products (*Glaser* coupling) [12]; moreover, the copper(I) acetylides formed show an extremely high reactivity and may explode in open air [13].

In practice, from an ecological point of view, 'the best solvent is without doubt no solvent'. There are, of course, many reactions which can already be carried out without solvent. Reports on solvent-free reactions have, indeed, become increasingly frequent and specialized over recent years. There are also reactions in which at least one reactant is liquid under the conditions employed, implying that the solvent that would normally be used can simply be left out.

Results and Discussion. – The traditional Cu^I-catalyzed cross-coupling between terminal alkynes and acyl chlorides require long reaction times and an inert atmosphere [14]. In an effort to find an efficient Cu^I complex that could promote the mentioned coupling, we investigated a class of Cu^I catalyst system based on a commercially available azacrown ether, 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (*Kryptofix®22* or cryptand-22), which was first reported by *Mühle* and *Sheldrick* [15]. This catalyst,

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CuI/cryptand-22 (or CuI/C22), was prepared in our laboratory by an easier method than the previously reported one [15]. This catalytic system is an air-stable solid powder and remains so for at least one month with no change in its catalytic activity. We assumed that the flexible macrocyclic and chelating effect of such N- and O-containing ligands may assist the stabilization of the reactive copper intermediates. To study the coordination of the ligand, cryptand-22, to CuI, resulting in complex CuI/C22, the IR spectrum of free ligand was compared with that of the complex. A medium intensity $\nu_{(N-H)}$ band was observed at 3327 cm^{-1} in the IR spectrum of the ligand, whereas the same band was observed at 3264 cm^{-1} of the complex, supporting the assumption that the ligand coordinates to the metal ion through the N-atoms of the ligand.

Herein, we report a new homogeneous copper catalyst system, CuI/C22, that suppresses the Cu^{I}-mediated homocoupling formation even in the presence of air. It is an air-stable and highly efficient catalyst system for the ynone cross-coupling reaction of terminal alkynes **2** with acyl chlorides **1**. To optimize the reaction conditions with respect to solvent, temperature, base, and the amount of the catalyst, the coupling reaction between benzoyl chloride (**1a**; 1.4 equiv.), phenylacetylene (**2a**; 1.0 equiv.), and a base (1.2 equiv.) was performed as a model reaction under aerial condition (*Table 1*). The best result was obtained when 2.6 mol-% of the catalytic system and Et_3N, as base, were used under solvent-free conditions at 60° (*Entry 5*, *Table 1*). After

Table 1. *Optimization of the CuI/C22-Catalyzed Reaction*[a])

Entry	Base	Temp. [°]	Solvent	Catalyst [mol-%]	Yield [%][b])
1	Et_3N	25	–	2	73
2	Et_3N	25	–	2.6	81
3	Et_3N	25	–	3	64
4	Et_3N	40	–	2.6	85
5	Et_3N	60	–	2.6	93
6	Et_3N	70	–	2.6	94
7	$EtN^{i}Pr_2$	60	–	2.6	32
8	Piperidine	60	–	2.6	N.R.
9	K_2CO_3	60	–	2.6	14
10	Et_3N	60	THF	2.6	12
11	Et_3N	60	DMF	2.6	N.R.
12	Et_3N	60	Toluene	2.6	37
13	Et_3N	60	NMP[c])	2.6	N.R.
14	Et_3N	60	MeCN	2.6	25

[a]) Reaction conditions: benzoyl chloride (**1a**; 1.4 equiv.), phenylacetylene (**2a**; 1.0 equiv.), base (1.2 equiv.), 30 min, aerial conditions. [b]) Yields of isolated products; N.R., no reaction. [c]) 1-Methylpyrrolidin-2-one.

optimization, the generality of this cross-coupling was demonstrated with a variety of acyl chlorides **1** (aromatic, heteroaromatic, and aliphatic) and terminal alkynes **2**. Representative results are listed in *Table 2*. All products were fully characterized by spectroscopic methods and compared with authentic spectra. As seen in *Table 2*, generally, aliphatic terminal alkynes needed longer reaction times and afforded lower yields (*Entries 11–14*, *Table 2*) than phenylacetylene (**2a**). Both aryl and alkyl acyl chlorides coupled with alkynes in air.

The reaction was almost equally facile with both electron-withdrawing and electron-donating substituents in the aroyl chlorides. Besides, no significant steric effect was observed due to Me–C(2) of 2-methylbenzoyl chloride. Heteroaromatic acyl chlorides, *e.g.*, thiophene-2-carbonyl chloride (**1g**), also reacted with terminal alkynes to give the corresponding ynones in good-to-high yields (*Entries 7* and *12*, *Table 2*).

Finally, we compared the performance of our catalyst with other catalytic systems reported in the literature for the cross-coupling of benzoyl chloride (**1a**) with phenylacetylene (**2a**; *Table 3*). The results established the superior catalytic activity of CuI/C22 in this reaction.

Conclusions. – We have developed an easy, very efficient, and fast solvent-, phosphorous-, and Pd-free protocol for the cross-coupling reaction of acyl chlorides with terminal alkynes catalyzed by the air-stable CuI/C22 complex, affording almost quantitative yields of the corresponding ynones. This reaction is very useful both from economical and environmental point of view, displaying the advantages of *i*)

Table 2. *CuI/C22-Catalyzed Cross-Coupling of Acyl Chlorides* **1** *with Terminal Alkynes* **2**[a])

$R^1C(O)Cl$ (**1**) + R^2–C≡CH (**2**) —[CuI/C22 (2.6 mol-%), Et_3N, 60°]→ $R^1C(O)C≡CR^2$ (**3**)

Entry	Acyl chloride	R^1	Alkyne	R^2	Product	Time [h]	Yield [%][b])
1	**1a**	Ph	**2a**	Ph	**3a**	0.5	93
2	**1b**	4-Me–C_6H_4	**2a**	Ph	**3b**	0.75	91
3	**1c**	2-Me–C_6H_4	**2a**	Ph	**3c**	1	92
4	**1d**	4-Cl–C_6H_4	**2a**	Ph	**3d**	0.75	92
5	**1e**	4-NO_2–C_6H_4	**2a**	Ph	**3e**	0.25	90
6	**1f**	4-CN–C_6H_4	**2a**	Ph	**3f**	0.25	91
7	**1g**	Thiophen-2-yl	**2a**	Ph	**3g**	5	87
8	**1h**	Cyclohexyl	**2a**	Ph	**3h**	1	88
9	**1i**	^{i}Pr	**2a**	Ph	**3i**	1.25	81
10	**1j**	Cyclopropyl	**2a**	Ph	**3j**	1.5	82
11	**1a**	Ph	**2b**	Bu	**3k**	2.5	68
12	**1g**	Thiophen-2-yl	**2b**	Bu	**3l**	3	63
13	**1k**	4-MeO–C_6H_4	**2b**	Bu	**3m**	3	35
14	**1a**	Ph	**2c**	Hexyl	**3n**	5.3	52

[a]) Reaction conditions: 1.0 mmol of **2**, 1.4 mmol of **1**, 1.2 mmol of Et_3N, 60°, aerobic condition. [b]) Yields of isolated products.

Table 3. *Comparison of Different Catalytic Systems for the Coupling Reaction of Benzoyl Chloride* (**1a**) *with Phenylacetylene* (**2a**)

Entry	Catalyst	Conditions	Time [h]	Yield [a]) [%]	Ref.
1	$Pd(OAc)_2$	Neat, r.t., Ar	0.17	93	[10c]
2	$Pd(OAc)_2$	Toluene, 110°	1	70	[16]
	Palladacycle	Toluene, 110°	1	75	[16]
3	$PdCl_2(PPh_3)$/CuI	H_2O, 65°	4	98	[11a]
4	$PdCl_2(PPh_3)$/CuI	THF, r.t.	0.17	96	[11b]
5	NS-MCM-41-Pd/CuI	Et_3N, 50°, N_2	36	93	[17]
6	CuI	Neat, r.t., Ar	30	78	[14a]
7	CuI/C22	Neat, 60°	0.5	93	[b])

[a]) Yield of isolated product. [b]) This work.

operational simplicity, *ii*) mild reaction conditions, *iii*) avoidance of organic solvents, and toxic and expensive reagents, *iv*) short reaction times, and *v*) high product yields.

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Experimental Part

Preparation of the Catalyst. Copper(I) iodide (76 mg, 0.4 mmol) was added into a soln. of cryptand-22 (106 mg, 0.4 mmol) in MeCN (8 ml) under stirring. The mixture was refluxed for 45 min, during which time the color of the mixture was turned from colorless to brown, and all the materials dissolved thoroughly. Then, while the volume of the soln. was reduced to half, a cream-colored precipitate formed. The precipitate was washed with MeCN and finally air-dried to give the complex CuI/C22 (142 mg, 78%). M.p. 169–170°. The Cu content of the complex was determined by atomic absorption spectroscopy (14.52%), which was in good agreement with the calculated value (14.03%).

General Procedure for Preparation of Ynones. A test tube was charged with acyl chloride **1** (1.4 mmol), the terminal alkyne **2** (1.0 mmol), CuI/C22 (12.0 mg, 2.6 mol%), and Et_3N (1.2 mmol). The mixure was then stirred for 30 min at 60° under aerobic conditions. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the mixture was extracted with $CHCl_3$ (2×5 ml). The combined org. layers were washed with H_2O (2×10 ml). The org. layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the crude product, which was further purified by prep. TLC (silica gel; hexane/AcOEt 9:1) to afford the desired pure product **3**.

Large-Scale Cross-Coupling Reaction of Phenylacetylene (**2a**) *with Benzoyl Chloride* (**1a**) *Catalyzed by CuI/C22.* In an oven-dried flask, a mixture of *phenylacetylene* (**2a**; 0.51 g, 5.0 mmol), Et_3N (0.61 g, 6.0 mmol), *benzoyl chloride* (**1a**; 0.98 g, 7.0 mmol), and CuI/C22 (0.06 g, 2.6 mol%) was stirred for 40 min at 60° under aerobic conditions. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the mixture was extracted with $CHCl_3$ (2×15 ml). The combined org. layers were washed with H_2O (2×15 ml). The org. layer was separated, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product which was further purified by prep. TLC (silica gel; hexane/AcOEt 9:1) to afford 1.0 g (98%) of pure *1,3-diphenylprop-2-yn-1-one* (**3a**).

1-(4-Methylphenyl)-3-phenylprop-2-yn-1-one (**3b**). Brown solid. M.p. 88–89°. IR (neat): 2199 (C≡C), 1637 (C=O). ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.13 (*d*, *J* = 8.2, 2 H); 7.69 (*dd*, *J* = 7.9, 1.6, 2 H); 7.39–7.52 (*m*, 3 H); 7.32 (*d*, *J* = 7.9, 2 H); 2.45 (*s*, 3 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 177.7; 145.2; 134.6; 133.0; 130.6; 129.7; 129.3; 128.7; 120.2; 92.6; 86.9; 21.8.

1-(2-Methylphenyl)-3-phenylprop-2-yn-1-one (**3c**). Yellow oil. IR (neat): 2196 (C≡C), 1638 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.35 (*d*, *J* = 7.6, 1 H); 7.69 (*d*, *J* = 6.8, 2 H); 7.38 – 7.52 (*m*, 5 H); 7.29 – 7.32 (*m*, 1 H); 2.72 (*s*, 3 H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 179.8; 140.5; 135.7; 133.3; 133.0; 132.9; 130.7; 128.7; 126.0; 120.4; 91.9; 88.4; 22.0.

3-Phenyl-1-(thiophen-2-yl)prop-2-yn-1-one (**3g**). Brown solid. M.p. 56 – 58°. IR (KBr): 2197 (C≡C), 1641 (C=O). ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.02 (*d*, *J* = 3.8, 1 H); 7.73 (*d*, *J* = 5.4, 1 H); 7.67 (*d*, *J* = 6.1, 2 H); 7.39 – 7.50 (*m*, 3 H); 7.19 (*t*, *J* = 4.4, 1 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 169.8; 144.9; 135.3; 135.1; 132.8; 130.9; 128.7; 128.2; 119.9; 91.8; 86.5.

1-Cyclohexyl-3-phenylprop-2-yn-1-one (**3h**). Yellow oil. IR (neat): 2197 (C≡C), 1658 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.61 (*d*, *J* = 7.5, 2 H); 7.48 (*t*, *J* = 7.6, 1 H); 7.41 (*t*, *J* = 7.6, 2 H); 2.54 (*tt*, *J* = 11.8, 2.9, 1 H); 2.09 (*d*, *J* = 10.7, 2 H); 1.83 – 1.87 (*m*, 2 H); 1.71 – 1.73 (*m*, 1 H); 1.50 – 1.55 (*m*, 2 H); 1.27 – 1.40 (*m*, 3 H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 191.6; 133.0; 130.6; 128.6; 120.2; 91.4; 87.2; 52.3; 28.3; 25.8; 25.4.

4-Methyl-1-phenylpent-1-yn-3-one (**3i**). Yellow oil. IR (neat): 2197 (C≡C), 1668 (C=O). ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.58 (*d*, *J* = 8.0, 2 H); 7.35 – 7.48 (*m*, 3 H); 2.78 (*sept.*, *J* = 7.0, 1 H); 1.27 (*d*, *J* = 7.0, 6 H).^{13}C-NMR ($CDCl_3$, 75 MHz): 192.2; 133.0; 131.5; 128.6; 128.1; 91.6; 85.8; 43.1; 18.1.

1-Cyclopropyl-3-phenylprop-2-yn-1-one (**3j**). Yellow oil. IR (neat): 2197 (C≡C), 1643 (C=O). ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.55 – 7.58 (*m*, 2 H); 7.35 – 7.49 (*m*, 3 H); 2.13 – 2.20 (*m*, 1 H); 1.31 – 1.39 (*m*, 2 H); 1.09 – 1.14 (*m*, 2 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 188.4; 133.0; 130.6; 128.6; 120.0; 90.4; 86.2; 24.6; 11.2. EI-MS: 170 (5, M^+), 167 (19), 149 (26), 129 (89), 105 (24), 75 (22), 69 (100), 57 (29). Anal. calc. for $C_{12}H_{10}O$: C 84.68, H 5.92; found: C 84.90, H 5.87.

1-(Thiophen-2-yl)hept-2-yn-1-one (**3l**). Yellow oil. IR (neat): 2228 (C≡C), 1623 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.92 (*d*, *J* = 3.6, 1 H); 7.70 (*d*, *J* = 4.8, 1 H); 7.17 (*t*, *J* = 4.8, 1 H); 2.51 (*t*, *J* = 7.2, 2 H); 1.67 (*quint.*, *J* = 7.2, 2 H); 1.52 (*sext.*, *J* = 7.2, 2 H); 0.98 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 170.1; 145.1; 134.9 (2C); 128.2; 95.4; 79.3; 29.8; 22.1; 18.8; 13.5.

1-(4-Methoxyphenyl)hept-2-yn-1-one (**3m**). Yellow oil. IR (neat): 2202 (C≡C), 1632 (C=O). ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.08 – 8.13 (*m*, 2 H); 6.91 – 6.97 (*m*, 2 H); 3.88 (*s*, 3 H); 2.49 (*t*, *J* = 7.1, 2 H); 1.66 (*quint.*, *J* = 7.0, 2 H); 1.51 (*sext.*, *J* = 7.1, 2 H); 0.96 (*t*, *J* = 7.3, 3 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 177.1; 164.3; 131.9; 113.7; 113.6; 95.9; 79.6; 55.6; 29.9; 22.1; 18.9; 13.5.

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Unexpected Ring Enlargement of 2-Hydrazono-2,3-dihydro-1,3-thiazoles to 1,3,4-Thiadiazines

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In memoriam Prof. Dr. *Ehrenfried Bulka*

The cyclization of thiosemicarbazide with α-bromoacetophenone can result in the formation of isomeric 1,3,4-thiadiazines and two different thiazoles. We studied the use of 4-methyl- and 4-ethylthiosemicarbazide as dinucleophilic building blocks. In this context, we observed an unprecedented rearrangement of a 2-hydrazono-2,3-dihydrothiazole to a 1,3,4-thiadiazine. While ring contractions of 1,3,4-thiadiazines to thiazoles are quite common, ring enlargements are new. The course of the reaction depends on the substitution pattern of the substrate.

Introduction. – 1,3,4-Thiadiazines are of considerable biological and pharmacological relevance. Many 1,3,4-thiadiazin-2-amine derivatives are important matrix metalloproteinase inhibitors [1]. 1,3,4-Thiadiazines exhibit cardiotonic [2] and antithrombotic [3] activities. In continuation of our previous work on 1,3,4-thiadiazines [4–16], we studied the use of 4-methyl- and 4-ethylthiosemicarbazide as dinucleophilic building blocks in their reaction with α-bromoacetophenone. In this context, we observed an unprecedented rearrangement of a 2-hydrazono-2,3-dihydrothiazole to a 1,3,4-thiadiazine. While ring contractions of 1,3,4-thiadiazines to thiazoles are quite common, ring enlargements are new. The course of the reaction depends on the substitution pattern of the substrate.

Results and Discussion. – The reaction of 4-methyl- and 4-ethylthiosemicarbazide, **2a** and **2b**, respectively, with α-bromoacetophenone (**1a**) resulted, as reported by *Bose* [17], in the formation of 1,3,4-thiadiazines **3a** and **3b** (*Scheme 1* and *Table*). The cyclization involved the (more nucleophilic) NH_2 group and the S-atom of the thiosemicarbazide. We observed the formation of 2-hydrazono derivatives **8a** and **8b** as side products. These compounds, which were formed by cyclization *via* the substituted N- and the S-atom, were isolated in form of their benzylidenehydrazono derivatives **7a**

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and **7b**, respectively. This result prompted us to study the cyclization of α-bromoacetophenones **1a** and **1b** with thiosemicarbazones **4a**–**4e** which can be regarded as protected thiosemicarbazides. These reactions afforded the 2,3-dihydro-2-(isopropylidenehydrazono)-1,3-thiazoles **5a**–**5d** and the 2,3-dihydro-2-(4-nitrobenzylidenehydrazono)-1,3-thiazoles **6a**–**6c**, respectively. The structure of **6c** was independently established by X-ray crystal-structure analysis (*Fig.*). Hydrolysis of 2-hydrazono-2,3-dihydro-5-methylthiazoles **5c** and **5d** with 2M HCl afforded the deprotected 2-hydrazono-2,3-dihydrothiazoles **8c** and **8d**, respectively, by cleavage of the hydrazone moiety.

Scheme 1. *Cyclization of* **1a** *with* **2a** *and* **2b**

1a; **2a** R^1 = Me; **2b** R^1 = Et → (5M HCl or conc. HCl or EtOH) **3a** R^1 = Me; **3b** R^1 = Et

(EtOH) → [**8a** R^1 = Me; **8b** R^1 = Et] → **7a** R^1 = Me; **7b** R^1 = Et

1a R^4 = H; **1b** R^4 = Me

	R^1	R^2	R^3
4a	Me	Me	Me
4b	Et	Me	Me
4c	^{i}Pr	Me	Me
4d	Me	4-NO_2–C_6H_4	H
4e	Et	4-NO_2–C_6H_4	H

	R^1	R^2	R^3	R^4
5a	Me	Me	Me	H
5b	Et	Me	Me	H
5c	Me	Me	Me	Me
5d	^{i}Pr	Me	Me	Me
6a	Me	4-NO_2–C_6H_4	H	H
6b	Et	4-NO_2–C_6H_4	H	H
6c	^{i}Pr	4-NO_2–C_6H_4	H	Me

5c, **5d** → (2M HCl) **8c** R^1 = Me; **8d** R^1 = ^{i}Pr

In contrast to the hydrolysis of **5c** and **5d**, all attempts to isolate **8a** and **8b** (R^1 = Me, Et) by hydrolysis of 5-unsubstitituted 2-hydrazono-2,3-dihydrothiazoles **5a** and **5b** failed. Interestingly, the 2-(alkylamino)-5-phenyl-6*H*-1,3,4-thiadiazines **3a** and **3b**,

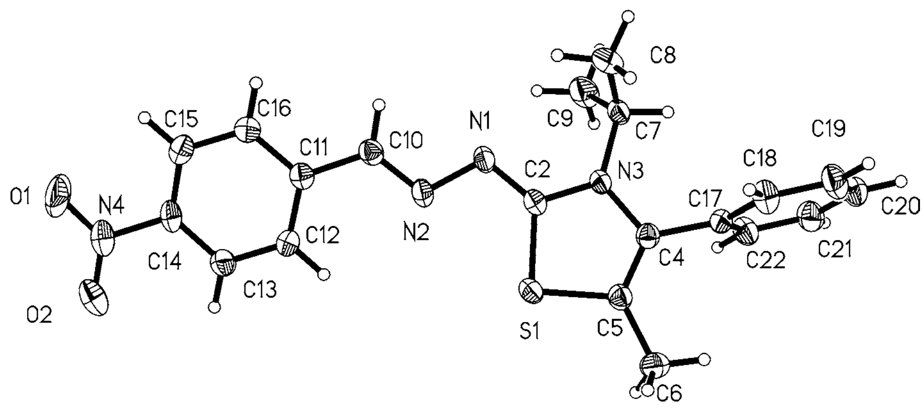

Fig. 1. *ORTEP Plot of* **6c**

Table 1. *Yields of 1,3,4-Thiadiazines, Thiosemicarbazones, and 2,3-Dihydro-1,3-thiazoles*

	R^1	R^2	R^3	R^4	Yield [%][a])
3a	Me				73 (*A*), 95 (*B*), 58 (*C*) 88 (*D*), 70 (*E*)
3b	Et				50 (*A*), 95 (*B*), 50 (*C*) 85 (*D*), 66 (*E*)
4a	Me	Me	Me		98
4b	Et	Me	Me		97
4c	^{i}Pr	Me	Me		81
4d	Me	4-$NO_2C_6H_4$	H		57
4e	Et	4-$NO_2C_6H_4$	H		97
4f	Me	Ph	H		97
4g	Et	Ph	H		94
5a	Me	Me	Me	H	96
5b	Et	Me	Me	H	95
5c	Me	Me	Me	Me	82
5d	^{i}Pr	Me	Me	Me	84
6a	Me	4-$NO_2C_6H_4$	H	H	30 (*A*), 71 (*B*)
6b	Et	4-$NO_2C_6H_4$	H	H	39 (*A*), 91 (*B*), 1.1 (*C*)
6c	^{i}Pr	4-$NO_2C_6H_4$	H	Me	83 (*A*), 71 (*B*)
7a	Me	Ph	H	H	86
7b	Et	Ph	H	H	94
8c	Me	–	–	Me	44
8d	^{i}Pr	–	–	Me	82

[a]) Yields of isolated products (in brackets, method of preparation, see the *Exper. Part*).

respectively, were formed when 2M HCl was employed (*Scheme 2*). 2-(Alkylimino)-3-amino-2,3-dihydro-5-phenyl-1,3-thiazoles **9a** and **9b** were isolated as side-products. Isomers **3** and **9** could be separated by extraction with Et_2O. In addition, traces of **8b** were formed in case of **9b**. Products **9a** and **9b** could be converted into thiadiazines **3a** and **3b**, respectively, by reaction with concentrated HCl. The reaction of **5a** and **5b** with concentrated HCl resulted in selective formation of thiadiazines **3a** and **3b**, respectively, in high yields.

Scheme 2. *Ring Enlargements of* **5a** *and* **5b**

The structures of **9a** and **9b** were elucidated as follows: the reaction of **9a** and **9b** with 4-nitrobenzaldehyde afforded the 3-(4-nitrobenzylidene) derivatives **10a** and **10b**, respectively, which are not identical with compounds **6a** and **6b**. In addition, **9a** and **9b** were transformed into 2-(alkylamino)-4-phenyl-5-benzylideneamino-1,3-thiazoles **12a** and **12b** by deamination, nitroslyation, reduction, and subsequent reaction with benzaldehyde (*Scheme 3*). Products **12a** and **12b**, respectively, can also be prepared by nitrosylation of 1,3-thiazoles **11a** and **11b**, respectively (*Scheme 3*). The formation of benzaldehyde [*N*-alkyl-*N*-(1,3-thiazol-2-yl)]hydrazones **14a** and **14b**, which are isomers of **12a** and **12b**, respectively, could not be detected. In contrast, products **14a** and **14b** are available by condensation of benzaldehyde-2-alkyl-thiosemicarbazones **13a** and **14b**, respectively, with α-bromoacetophenone (*Scheme 3*).

In conclusion, we reported the cyclization of 4-methyl- and 4-ethylthiosemicarbazide with α-bromoacetophenone. In this context, we observed an unprecedented rearrangement of a 2-hydrazono-2,3-dihydrothiazole to a 1,3,4-thiadiazine. While ring contractions of 1,3,4-thiadiazines to thiazoles are quite common, ring enlargements are new. The course of the reaction depends on the substitution pattern of the substrate.

Experimental Part

General. All reactions were carried out in oven-dried reaction pressure tubes under Ar. The chemicals were purchased from *Aldrich*. Dry solvents (DMF, CH_2Cl_2) were purged with Ar before use. TLC: *Merck* precoated aluminium plates (Si *60 F_{254}*). Column chromatography (CC): *Merck* silica gel *60* (SiO_2; 0.043–0.06 mm). UV/VIS: *Lambda 5* (*PerkinElmer*) spectrophotometer with a soln. concentration of $c = 10^{-6}$ mol/l of the compounds. Fluorescence spectra: *Hitachi F-4010* fluorescence spectrophotometer using similar soln. concentrations in various solvents. IR Spectra: *Nicolet 205* FT-IR and *Nicolet Protege 460* FT-IR instrument; as KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR data: *Bruker ARX 300* and *Bruker ARX 400* spectrometers. ^{1}H- and ^{13}C-NMR chemical shifts referenced to signals of deuterated solvents and residual protonated solvents, resp. GC/MS: *Agilent HP-5890* instrument with an *Agilent HP-5973* mass-selective detector (EI) and *HP-5* cap. column with He as carrier gas. HR-ESI-MS: *Agilent 1969A* TOF mass-spectrometer.

Scheme 3. *Chemical Establishment of the Structures of* **9a** *and* **9b**

N-*Methyl-5-phenyl-6*H-*1,3,4-thiadiazin-2-amine* (**3a**). *Method A. 2,3-Dihydro-3-methyl-4-phenyl-2-(propan-2-ylidenehydrazinylidene)-1,3-thiazole* (**5a**; 24.5 g, 100.0 mmol) in 2M HCl (120 ml) was subjected to steam distillation, until acetone was no longer detectable in the distillate. The distillation residue was filtered to remove impurities and then placed in a separatory funnel with Et_2O. The pH was adjusted to 4–5 with aq. NH_3 and then alkaline with aq. Na_2CO_3 under vigorous shaking. The Et_2O phase was worked up (distillation of Et_2O) to give **9a**. Yield: 4.1 g (20%). M.p. 110°. The precipitate formed in the aq. phase was filtered under suction, dissolved in benzene, and reprecipitated by addition petroleum ether (PE) to afford **3a**. Yield: 15 g (73%). Pale-yellow rods (acetone). M.p. 145°. IR: 3217*s*, 3010*s*, 1542*s*, 1512*s*, 1440*m*, 1400*s*, 1326*m*, 1217*s*, 1167*s*, 1059*m*, 982*m*, 916*w*, 694*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.12 (*s*, Me); 3.56 (*s*, CH_2(6)); 5.05 (br. *s*, NH); 7.34–7.88 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.5 (Me); 30.6 (C(6)); 126.5 (arom. CH); 128.6 (arom. CH); 129.5 (arom. CH); 135.8 (arom. C); 147.5 (C(5)); 149.6 (C(2)). EI-MS (70 eV): 205 (19, M^+), 164 (21), 103 (86), 91 (15), 75 (100), 51 (29), 28 (16). Anal. calc. for $C_{10}H_{11}N_3S$ (205.28): C 58.51, H 5.40, N 20.47; found: C 58.62, H 5.51, N 20.61.

Method B. Hydrolysis of **5a** (24.5 g, 100.0 mmol) in conc. HCl (120 ml) was performed as described under *Method A*; the pH of the mixture was then adjusted to 4–5 with aq. NH_3, and then alkaline with aq. Na_2CO_3, and the precipitate was filtered under suction. Yield: 19.5 g (95%). Pale-yellow rods (acetone). M.p. 145°.

*Method C. 2-(Methylimino)-4-phenyl-1,3-thiazol-3(2*H*)-amine* (**9a**; 0.5 g, 2.5 mmol) was refluxed in conc. HCl (30 ml) for 30 min. The mixture was made alkaline with aq. Na_2CO_3, and the precipitate was filtered under suction. Yield: 0.3 g (58%). Pale-yellow rods (acetone). M.p. 145°.

Method D. A mixture of *4-methylthiosemicarbazide* (= N-*methylhydrazinecarbothioamide*; **2a**; 2.1 g, 20.0 mmol) and phenacyl bromide (=*2-bromo-1-phenylethanone*; **1a**; 3.98 g, 20.0 mmol) in 5M HCl

(30 ml) was refluxed briefly. The hot soln. was filtered. After cooling, aq. NH_3 was added. A crystalline precipitate was formed. Yield: 3.6 g (88%). Pale-yellow rods (acetone). M.p. 145°.

Method E. A mixture of *4-methylthiosemicarbazide* (**2a**; 2.1 g, 20.0 mmol) and **1a** (3.98 g, 20.0 mmol) in EtOH (30 ml) was refluxed 15 min. The hot soln. was filtered. After cooling, aq. NH_3 was added. A crystalline precipitate formed. Yield: 2.87 g (70%). Pale-yellow rods (acetone). M.p. 145°.

N-*Ethyl-5-phenyl-6*H-*1,3,4-thiadiazin-2-amine* (**3b**). *Method A*. The product was obtained by hydrolysis of **5b** (13 g, 50.0 mmol) in 2M HCl (80 ml) as described for **3a** under *Method A*. Yield: 5.5 g (50%). Yellow needles (benzene). M.p. 156°. The workup of the Et_2O soln. gave **3b**. IR: 3172*s*, 2973*s*, 1555*s*, 1517*s*, 1468*s*, 1434*m*, 1410*m*, 1340*m*, 1266*m*, 1214*m*, 1152*m*, 1055*w*, 920*w*, 985*w*, 758*m*, 689*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.27 (*t*, *J* = 8.0, Me); 3.54 – 3.57 (*m*, CH_2); 3.61 (*s*, CH_2(6)); 5.47 (br. *s*, NH); 7.38 – 7.86 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.4 (Me); 22.8 (CH_2); 39.1 (C(6)); 126.8 (arom. CH); 128.8 (arom. CH); 129.3 (arom. CH); 129.8 (arom.CH); 136 (arom. C); 147.7 (C(5)); 149.4 (C(2)). EI-MS (70 eV): 219 (48, M^+), 164 (55), 119 (7), 104 (49), 103 (100), 88 (96), 77 (49), 61 (46), 51 (17), 43 (5), 28 (14). Anal. calc. for $C_{11}H_{13}N_3S$ (219.31): C 60.24, H 5.97, N 19.16; found: C 59.85, H 6.22, N 18.98.

Method B. Hydrolysis of **5b** (13 g, 50.0 mmol) in conc. HCl (80 ml) was performed as described for **3a** under *Method A*. The mixture was made alkaline with aq. Na_2CO_3, and the precipitate formed under suction. Yield: 10.4 g (95%). M.p. 156°.

*Method C. 2-(Ethylimino)-5-phenyl-1,3-thiazol-3(2*H*)-amine* (**9b**; 0.5 g, 2.5 mmol) was refluxed for 30 min in conc. HCl (10 ml). The mixture was made alkaline with aq. Na_2CO_3, and the precipitate formed was filtered under suction. Yield: 0.25 g (50%). M.p. 156°.

Method D. 4-Ethylthiosemicarbazide (= N-*ethylhydrazinecarbothioamide*; **2b**; 2.4 g, 20.0 mmol) and **1a** (3.98 g, 20.0 mmol) in 5M HCl (30 ml) were refluxed briefly. The hot soln. was filtered to remove impurities. The mixture was made alkaline with aq. NH_3, and the precipitate formed was filtered under suction. Yield: 3.7 g (85%). M.p. 156°.

Method E. A mixture of **2b** (2.4 g, 20.0 mmol) and **1a** (3.98 g, 20.0 mmol) in EtOH (30 ml) was refluxed for 15 min. EtOH was evaporated by distillation to give an oil. The product and PhCHO (0.5 g) was refluxed briefly in a water bath. Subsequently, acetone (50 ml) was added. A yellow precipitate formed, which was filtered off. The product was dissolved in MeOH/H_2O 1:1 and then placed in a separatory funnel with Et_2O. The pH was then adjusted to alkaline with aq. Na_2CO_3 under vigorous shaking. The Et_2O phase was worked up to give **9b**. The precipitate formed in the aq. phase was filtered under suction, dissolved in benzene, and reprecipitated by addition of PE to afford **3b**. Yield: 2.9 g (66%). Yellow needles (benzene). M.p. 156°.

N-*Methyl-2-(propan-2-ylidene)hydrazinecarbothioamide* (**4a**). Compound **2a** (2.1 g, 20.0 mmol) was refluxed in acetone (25 ml). Acetone was distilled off *in vacuo*. Yield: 2.85 g (98 %). Colorless lamella (acetone). M.p. 116 – 117°. IR: 3390*s*, 3250*s*, 1556*s*, 1455*s*, 1479*s*, 1408*s*, 1343*m*, 1268*s*, 1226*s*, 1105*m*, 1059*s*, 1017*s*, 768*m*, 714*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.89 (*s*, Me); 2.0 (*s*, Me); 3.20 (*s*, Me); 7.52 (br. *s*, NH); 8.46 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 16.5 (Me); 25.0 (Me); 30.8 (Me); 148.9 (C=N); 178.6 (C=S). EI-MS (70 eV): 145 (96, M^+), 130 (54), 89 (23), 75 (69), 62 (14), 58 (75), 42 (52), 28 (100). Anal. calc. for $C_5H_{11}N_3S$ (145.23): C 41.35, H 7.63, N 28.93; found: C 41.35, H 7.69, N 28.87.

N-*Ethyl-2-(propan-2-ylidene)hydrazinecarbothioamide* (**4b**). Compound **2b** (2.4 g, 20.0 mmol) was refluxed in acetone (25 ml) for 10 min, and acetone was distilled off *in vacuo*. Yield: 3.1 g (97%). Colorless lamella (EtOH/H_2O). M.p. 80°. IR: 3273*m*, 3193*s*, 2978*m*, 1534*s*, 1490*s*, 1436*m*, 1363*m*, 1332*m*, 1363*m*, 1332*m*, 1263*s*, 1218*s*, 1218*s*, 1104*s*, 1062*s*, 796*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.27 (*t*, *J* = 8.0, Me); 1.88 (*s*, Me); 2.00 (*s*, Me); 3.67 – 3.76 (*m*, CH_2); 7.46 (br. *s*, NH); 8.33 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.3 (Me); 16.5 (Me); 25.0 (Me); 39.1 (CH_2); 148.9 (C=N)); 177.4 (C=S). EI-MS (70 eV): 159 (50, M^+), 144 (11), 103 (11), 73 (11), 72 (10), 61 (38), 59 (41), 28 (100). Anal. calc. for $C_6H_{13}N_3S$ (159.25): C 45.25, H 8.23, N 26.39; found: C 45.30, H 8.29, N 26.64.

N-*(Propan-2-yl)-2-(propan-2-ylidene)hydrazinecarbothioamide* (**4c**). *4-Isopropylthiosemicarbazide* (= N-*(propan-2-yl)hydrazinecarbothioamide*; **2c**; 1.33 g, 10 mmol) was refluxed in acetone (20 ml) for 15 min, and acetone was distilled off *in vacuo*.Yield: 1.4 g (81%). Colorless lamella (EtOH). M.p. 112.5°. Anal. calc. for $C_7H_{15}N_3S$ (173.28): C 48.52, H 8.73, N 24.25; found: C 48.50, H 8.79, N, 24.25.

N-*Methyl-2-(4-nitrobenzylidene)hydrazinecarbothioamide* (**4d**). A mixture of **2a** (4.2 g, 40.0 mmol) and 4-nitrobenzaldehyde (6 g, 40 mmol) in EtOH (70 ml) was refluxed for 30 min. Yellow crystals was

separated by boiling and recrystallized (BuOH). Yield: 5.5 g, (57%). Yellow needles. M.p. 229°. IR: 3376*s*, 3149*s*, 2994*s*, 1553*s*, 1523*s*, 1342*s*, 1219*s*, 1161*m*, 1100*s*, 1044*s*, 922*m*, 848*s*, 784*m*, 749*m*, 691*m*, 625*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.29 (*s*, Me); 7.79–8.28 (*m*, 4 arom. H); 7.50 (br. *s*, NH); 9.42 (*s*, CH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 30.9 (Me); 123.8 (arom. CH); 128.0 (arom. CH); 139.0 (arom. C); 140.8 (arom. C); 147.5 (C=N); 178.0 (C=S). EI-MS (70 eV): 238 (13, M^+), 165 (3), 112 (5), 103 (3), 91 (3), 90 (10), 75 (28), 57 (40), 28 (100). Anal. calc. for $C_9H_{10}N_4O_2S$ (238.27): C 45.37, H 4.23, N 23.51; found: C 45.47, H 4.51, N 23.81.

N-*Ethyl-2-(4-nitrobenzylidene)hydrazinecarbothioamide* (**4e**). Compound **2b** (1.2 g, 10.0 mmol) and 4-nitrobenzaldehyde (1.5 g; 10 mmol) in EtOH (15 ml) were refluxed for 15 min. Yield: 2.4 g (97%). Lemon yellow needles (EtOH). M.p. 259–260°. IR: 3365*s*, 3147*s*, 2984*s*, 1544*s*, 1510*s*, 1340*s*, 1240*s*, 1218*s*, 1102*s*, 1050*m*, 922*m*, 845*m*, 689*m*, 587*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.32 (*t*, $J = 8.0$, Me); 3.78–3.81 (*m*, CH_2); 7.4 (br. *s*, NH); 7.79–8.30 (*m*, 4 arom. H); 9.27 (*s*, CH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.4 (Me); 38.36–40.26 (CH_2); 123.7 (arom. CH); 127.9 (arom. CH); 139.0 (arom C); 140.7 (arom. C); 147.4 (C=N); 176.9 (C=S). EI-MS (70 eV): 252 (19, M^+), 150 (27), 118 (7), 103 (20), 88 (13), 77 (16), 61 (50), 44 (78), 28 (100). Anal. calc. for $C_{10}H_{12}N_4O_2S$ (252.29): C 47.61, H 4.79, N 22.21; found: C 47.65, H 4.83, N 22.25.

*2-Benzylidene-*N*-methylhydrazinecarbothioamide* (**4f**). Compound **2a** (4.7 g, 40.0 mmol) and benzaldehyde (4.2 g, 40.0 mmol) in EtOH (30 ml) were refluxed briefly. Yield: 7.5 g (97%). Colorless needles (EtOH). M.p. 157°. IR: 3366*s*, 3182*m*, 1553*s*, 1520*s*, 1450*m*, 1261*s*, 1097*m*, 1037*m*, 758*m*, 695*m*, 569*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.27 (*d*, $J = 8.0$, Me); 7.38–7.89 (*m*, 5 arom. H); 9.97 (*s*, HC=). ^{13}C-NMR (75 MHz, $CDCl_3$): 31.0 (Me); 127.2 (arom. CH); 128.7 (arom. CH); 130.3 (arom. C); 133.3 (arom. C); 142.7 (HC=N); 178.0 (C=S). EI-MS (70 eV): 193 (46, M^+), 119 (25), 104 (17), 93 (17), 92 (15), 90 (36), 89 (20), 77 (37), 75 (67), 57 (100), 51 (38), 30 (30), 28 (27). Anal. calc. for $C_9H_{11}N_3S$ (193.27): C 55.93, H 5.74, N 21.74; found: C 55.81, H 5.87, N 21.58.

*2-Benzylidene-*N*-ethylhydrazinecarbothioamide* (**4g**). Compound **2b** (4.8 g, 40.0 mmol) and benzaldehyde (4.3 g, 40.0 mmol) in EtOH (30 ml) were refluxed briefly. After cooling, the precipitate was filtered. Yield: 7.8 g (94%). Colorless lamella (EtOH/H_2O). M.p. 140°. IR: 3327*m*, 3184*m*, 2975*m*, 1545*s*, 1308*s*, 1236*s*, 1098*s*, 942*m*, 757*m*, 691*m*, 624*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.32 (*t*, $J = 8.0$, Me); 3.73–3.82 (*m*, CH_2); 7.38–7.96 (*m*, 5 arom. H); 10.39 (*s*, HC=). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.5 (Me); 39.2 (CH_2); 127.2 (arom. CH); 128.7 (arom. CH); 130.2 (arom. C); 133.3 (arom. C); 142.7 (HC=N); 176.8 (C=S). EI-MS (70 eV): 207 (57, M^+), 120 (13), 119 (23), 106 (51), 104 (30), 103 (28), 93 (13), 88 (13), 77 (37), 72 (26), 66 (13), 61 (51), 51 (26), 44 (100), 28 (29). Anal. calc. for $C_{10}H_{13}N_3S$ (207.30): C 57.94, H 6.32, N 20.27; found C 57.97, H 6.51, N 20.10.

Compound **5a**. A mixture of **4a** (2.9 g, 20.0 mmol) and α-bromoacetophenone (**1a**; 3.98 g, 20.0 mmol) in acetone (30 ml) was refluxed for 15 min. Acetone was removed by distillation, and an oil was formed. The product was dissolved in hot MeOH and neutralized with aq. NH_3. A crystalline product formed, which was filtered off and recrystallized (EtOH/H_2O). Yield: 4.7 g (96%). Yellow needles (EtOH/H_2O). M.p. 88°. IR: 2941*m*, 2909*m*, 1623*s*, 1551*s*, 1440*m*, 1418*s*, 1360*s*, 767*s*, 703*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.05 (*s*, Me); 2.08 (*s*, Me); 3.29 (*s*, Me); 5.84 (*s*, H–C(5)); 7.33–7.43 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 18.1 (Me); 24.9 (Me); 33.3 (Me); 98.4 (C(5)); 128.5 (arom. CH); 128.6 (arom. CH); 128.8 (arom. CH); 131.3 (arom. C); 140 (C=N);158.7 (C(4)); 167.1 (C(2)). EI-MS (70 eV): 246 (100, M^+), 212 (7), 189 (39), 189 (39), 176 (22), 162 (34), 134 (42), 118 (79), 102 (78), 91 (24), 89 (15), 76 (30), 56 (19), 42 (50), 28 (36). Anal. calc. for $C_{13}H_{15}N_3S$ (245.34): C 63.64, H 6.16, N 17.13; found: C 63.75, H 6.18, N 17.34.

3-Ethyl-2,3-dihydro-4-phenyl-2-(propan-2-ylidenehydrazinylidene)-1,3-thiazole (**5b**). A mixture of **4b** (3.18 g, 20.0 mmol) and **1a** (3.98 g, 20.0 mmol) in acetone (30 ml) was refluxed for 15 min. Further workup as described for **2a** afforded **5b** (5 g, 95%). Yellow needles (EtOH/H_2O). M.p. 96°. IR: 2932*m*, 1625*s*, 1557*s*, 1488*m*, 1441*s*, 1377*s*, 1330*s*, 1255*s*, 1167*m*, 1071*s*, 986*m*, 819*m*, 768*s*, 702*s*, 576*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.18 (*t*, $J = 8.0$, Me); 2.05 (*s*, Me); 2.07 (*s*, Me); 3.79 (*q*, $J = 8.0$, CH_2); 5.80 (*s*, H–C(5)); 7.34–7.43 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.1 (Me); 18.2 (Me); 24.9 (Me); 40.8 (CH_2); 98.6 (C(5)); 128.5 (arom. CH); 128.7 (arom. CH); 128.8 (arom. CH); 131.8 (arom. C); 140.3 (C=N); 158.7 (C(4)); 166.1 (C(2)). EI-MS (70 eV): 259 (100, M^+), 216 (29), 203 (42), 176 (28), 147 (59),

134 (37),103 (13), 91 (10), 56 (15), 29 (14), 28 (7). Anal. calc. for $C_{14}H_{17}N_3S$ (259.38): C 64.83, H 6.60, N 16.20; found: C 64.93, H 6.78, N 16.25.

2,3-Dihydro-3,5-dimethyl-4-phenyl-2-(propan-2-ylidenehydrazinylidene)-1,3-thiazole (**5c**). A acetone soln. (20 ml) of **4a** (3.04 g, 20 mmol) and *α-bromopropiophenone* (**1b**; 4.26 g, 20 mmol) was refluxed for 2 h. After cooling to r.t. and addition of NH_3, a crystalline precipitate formed. The product was filtered off and recrystallized from EtOH to give **5c** (4.30 g, 82%). Slightly-yellow needles. M.p. 112°. IR: 2911*m*, 1626*s*, 1566*s*, 1413*s*, 1346*s*, 1259*m*, 1067*m*, 982*m*, 786*m*, 702*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.98 (*s*, Me(5)); 2.05, 2.07 (2*s*, N=CMe_2); 3.15 (*s*, MeN); 5.94 (*s*, H–C(5)); 7.25–7.48 (*m*, 5 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.7; 18.1; 25.0; 33.3; 109.2; 128.7; 128.7; 130.1; 130.6; 134.7; 158.0; 166.1. Anal. calc. for $C_{14}H_{17}N_3S$ (259.37): C 64.83, H 6.61, N 16.20; found: C 65.00, H 6.78, N 15.96.

2,3-Dihydro-5-methyl-4-phenyl-3-(propan-2-yl)-2-(propan-2-ylidenehydrazinylidene)-1,3-thiazole (**5d**). Reaction of **4c** (1.73 g, 10 mmol) and **1b** (2.13 g, 10 mmol) in acetone (20 ml) as described by **2c**, yielded **5d** (2.41 g, 84%). Yellow lamella. M.p. 131°. IR: 2970*m*, 2928*m*, 2917*m*, 1645*m*, 1623*m*, 1558*s*, 1445*m*, 1369*m*, 1334*s*, 1303*s*, 1253*m*, 1109*m*, 1075*m*, 812*m*, 788 *m*, 735*m*, 703*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.44, 1.46 (2*d*, J = 8.0, *Me*$_2$CH); 1.87 (*s*, Me(5)); 2.04, 2.06 (2*s*, N=CMe_2); 3.84–3.89 (*m*, NCH); 5.95 (*s*, H–C(5)); 7.23–7.44 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.7; 18.4; 18.8; 24.6; 50.5; 108.4; 128.5; 130.0; 131.8; 134.8; 157.3; 164.2. Anal. calc. for $C_{16}H_{21}N_3S$ (287.42): C 66.86, H 7.36, N 14.62; found: C 66.82, H 7.46, N 14.58.

2,3-Dihydro-3-methyl-2-[(4-nitrobenzylidene)hydrazinylidene]-4-phenyl-1,3-thiazole (**6a**). *Method A*. A mixture of **5a** (1.23 g, 5.0 mmol) and 4-nitrobenzaldehyde (0.75 g, 5.0 mmol) in EtOH (10 ml) and HCl (0.1 ml) was refluxed for 15 min. After cooling to 20°, a crystalline precipitate formed, which was filtered off and recrystallized from BuOH. Yield: 0.5 g (30%). Red lamella. M.p. 202°. IR: 2932*m*, 1589*s*, 1516*m*, 1421*m*, 1360*m*, 1333*s*, 1170*m*, 1107*m*, 1050*m*, 984*m*, 842*m*, 769*m*, 703*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.14 (*s*, Me); 6.09 (*s*, H–C(5)); 7.38–8.35 (*m*, 9 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 35.7 (Me); 100.7 (C(5)); 123.9 (arom. C); 127.3 (arom. C); 128.8 (arom. C); 129.4 (arom. C); 130.5 (arom. C); 141.1, 147; 147.5, 147.7, 172.6 (C(2)). EI-MS (70 eV): 338 (98, M^+), 307 (10), 292 (3), 244 (3), 189 (7), 177 (55), 135 (23), 118 (12), 102 (91), 89 (19), 77 (15), 64 (11), 30 (42), 28 (100). Anal. calc. for $C_{17}H_{14}N_4O_2S$ (338.38): C 60.34, H 4.17, N 16.56; found: C 60.44, H 4.18, N 16.47.

Method B. A mixture of **4d** (1.19 g, 5.0 mmol) and **1a** (1 g, 5.0 mmol) in EtOH (30 ml) was refluxed for 30 min. After cooling, a red precipitate formed. Yield: 1.2 g (71%). M.p. 202°.

3-Ethyl-2,3-dihydro-2-[(4-nitrobenzylidene)hydrazinylidene]-4-phenyl-1,3-thiazole (**6b**). *Method A*. The compound was obtained from **5b** (1.3 g, 5.0 mmol) and 4-nitrobenzaldehyde, as described for **6a** under *Method A*. Yield: 0.6 g (39%). Red needles (dioxane). M.p. 221°. IR: 1625*m*, 1587*s*, 1502*s*, 1459*m*, 1407*m*, 1374*m*, 1336*s*, 1266*w*, 1166*m*, 1111*m*, 1062*s*, 1004*m*, 845*m*, 758*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.22 (*t*, J = 8.0, Me); 3.87–3.94 (*m*, CH_2); 6.04 (*s*, H–C(5)); 7.38–8.33 (*m*, 9 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.7 (Me); 41.1 (CH_2); 101.1 (C(5)); 127.9 (arom. C); 127.3 (arom. C); 128.8 (arom. C); 129.1 (arom. C); 129.7 (arom. C); 130.9 (arom. C); 140.8 (arom. C); 142.5 (arom. C); 147.4, 147.5, 172 (C(2)). EI-MS (70 eV): 352 (100, M^+), 322 (18), 203 (20), 190 (20), 177 (30), 176 (46), 134 (50), 104 (13), 102 (22), 91 (12), 89 (16), 77 (13), 28 (30). Anal. calc. for $C_{18}H_{16}N_4O_2S$ (352.41): C 61.35, H 4.58, N 15.90; found: C 61.35, H 4.59, N 15.84.

Method B. Compounds **4e** and **1a** (1 g, 5.0 mmol) in EtOH (30 ml) was refluxed for 15 min. During boiling, a red precipitate formed. Yield: 1.6 g (91%). Red needles (dioxane). M.p. 221°.

Method C. To the EtOH soln. of recrystallized **9b**, 4-nitrobenzaldehyde (0.75 g, 5 mmol) was added, and the mixture was refluxed briefly. A red precipitate formed, which filtered off. Yield: 0.2 g (1.1%). M.p. 221°.

2,3-Dihydro-5-methyl-2-[(4-nitrobenzylidene)hydrazinylidene]-4-phenyl-3-(propan-2-yl)-1,3-thiazole (**6c**). *Method A*. A mixture of **5d** (1.44 g, 5.0 mmol) and 4-nitrobenzaldehyde (0.75 g, 5.0 mmol) in EtOH (20 ml) and HCl (0.1 ml) was refluxed for 2 h. After cooling to 20°, a crystalline precipitate formed, which was filtered off and recrystallized from BuOH. Yield: 1.8 g (83%). Green needles. M.p. 232°. Anal. calc. for $C_{20}H_{20}N_4O_2S$ (380.46): C 63.14, H 5.30, N 14.73; found: C 63.10, H 5.40, N 14.54.

Method B. A mixture of 2-(4-nitrobenzylidene)-*N*-(propan-2-yl)hydrazinecarbothioamide (2.56 g, 10 mmol) and **1b** (2.13 g, 10 mmol) in EtOH (30 ml) was refluxed for 3 h. After cooling, a green precipitate formed. Yield: 3.6 g (95%). M.p. 232°.

2-(Benzylidenehydrazinylidene)-2,3-dihydro-3-methyl-4-phenyl-1,3-thiazole (**7a**). *Hydrobromide.* Compound **4f** (3.86 g, 20.0 mmol) and **1a** (3.98 g, 20.0 mmol) in EtOH (40 ml) was refluxed for 10 min. During boiling, a precipitate formed. Yield: 6.4 g (86%). Colorless needles (MeOH). M.p. 216°. Anal. calc. for $C_{17}H_{16}BrN_3S$ (374.30): C 54.55, H 4.31, N 11.23; found: C 54.58, H 4.30, N 11.32.

Free Base: Method A. The hydrobromide of **7a** was converted into the free base by dissolation in MeOH/H_2O (3:1) and addition of aq. Na_2CO_3 soln. Yellow lamella (EtOH). M.p. 118°. IR: 3084*m*, 1602*s*, 1571*s*, 1518*s*, 1487*s*, 1441*m*, 1420 *m*, 1358*m*, 1310*m*, 1185*m*, 1053*m*, 984*s*, 877*m*, 810*m*, 760*s*, 697*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.36 (*s*, Me); 5.97 (*s*, H–C(5)); 7.32 – 7.77 (*m*, 10 arom. H); 8.35 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 33.5 (Me); 99.7 (C(5)); 127.2 (arom. CH); 128.4 (arom. CH); 128.7 (arom. CH); 129.1 (arom. CH); 130.9 (arom. C); 135.0 (arom. C); 140.8 (HC=N); 151.0 (C(4)); 170.8 (C(2). EI-MS (70 eV): 293 (100, M^+), 264 (9), 176 (80), 174 (11), 147 (10), 134 (33), 118 (13), 102 (62), 90 (52), 77 (41), 51 (26), 44 (11), 28 (38). Anal. calc. for $C_{17}H_{15}N_3S$ (293.39): C 69.59, H 5.15, N 14.32; found: C 69.59, H 5.15, N 14.32.

Method B. Benzaldehyde (0.5 g) was added to the mother liquor of **3a** (*Method E*) and refluxed briefly. The solvent was distilled off *in vacuo.* Yield: 0.5 g (8.5%). Yellow lamella (EtOH/H_2O). M.p. 118°.

2-[Benzylidenehydrazinylidene]-3-ethyl-2,3-dihydro-4-phenyl-1,3-thiazole (**7b**). *Hydrobromide.* A mixture of **4g** (4.14 g, 20.0 mmol) and **1a** in acetone (25 ml) was refluxed for 15 min. After cooling to r.t., the product was suction-filtered. Yield: 7.3 g (94%). Colorless lamella (MeOH). M.p. 170°. Anal. calc. for $C_{18}H_{18}N_3SBr$ (388.32): C 55.67, H 4.67, N 10.82; found: C 55.59, H 4.65, N 10.92.

Free Base: Method A. The EtOH soln. of the hydrobromide of **7b** (3.88 g, 10 mmol) was treated with aq. NH_3, and a yellow precipitate formed. Yield: 3.41 g (88%). Yellow lamella (EtOH/H_2O). M.p. 107°. IR: 3094*m*, 2970*m*, 1604*s*, 1572*s*, 1517*s*, 1493*s*, 1441*s*, 1375*m*, 1273*m*, 1048*m*, 1003*m*, 944*m*, 760*s*, 695*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.19 (*t*, $J = 8.0$, Me); 3.86 (*q*, $J = 8.0$, CH_2); 5.92 (*s*, H–C(5)); 7.31 – 7.77 (*m*, 10 arom. H); 8.34 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.6 (Me); 40.8 (CH_2); 100.1 (C(5)); 127.1 (arom. CH); 128.0 (arom. CH); 128.4 (arom. CH); 128.6 (arom. CH); 129.0 (arom. CH); 131.6 (arom. C); 135.0 (arom. C); 140.4 (HC=N); 150.7 (C(2)). EI-MS (70 eV): 307 (100, M^+), 203 (15), 190 (35), 176 (43), 132 (21), 118 (10), 104 (21), 102 (22), 90 (37), 77 (42), 52 (20), 28 (72). Anal. calc. for $C_{18}H_{17}N_3S$ (307.41): C 70.33, H 5.57, N 13.67; found: C 70.38, H 5.61, N 13.47.

Method B. The Et_2O soln. of **3b** (*Method E*) was dried with $CaCl_2$. The solvents was distilled *in vacuo*. Yield: 0.6 g (10%). Yellow lamella (EtOH/H_2O). M.p. 107°.

2-Hydrazinylidene-2,3-dihydro-3,5-dimethyl-4-phenyl-1,3-thiazole (**8c**). *Hydrochloride.* Compound **5c** (7.80 g, 30 mmol) was dissolved in an aq. soln, of 18% HCl (200 ml). A steam distillation of the mixture gave 5 – 6 l of an aq. soln. The hot soln. was filtered, cooled, and, subsequently, NH_3 was added. A yellow oil was separated, which was washed with H_2O. The oil was dissolved in EtOH/HCl and treated with Et_2O, until the soln. became cloudy. After a few min, a colorless precipitate formed, which was separated by filtration. The product was recrystallized from EtOH/Et_2O to give hydrochloride of **8c** as colorless rods (3.40 g, 44%). M.p. 185 – 187°. IR: 3325*m*, 3242*s*, 3132*s*, 2984*s*, 2834*s*, 1665*s*, 1588*s*, 1459*m*, 1431*m*, 1124*m*, 787*m*, 709*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.13 (*s*, Me(5)); 3.58 (*s*, MeN); 3.69 – 3.79 (br. *s*, NH_2); 5.94 (*s*, H–C(5)); 7.24 – 7.56 (*m*, 5 arom. H); 11.98 (*s*, NH_3^+). ^{13}C-NMR (75 MHz, (D_6)DMSO): 17.7, 18.5, 34.5, 55.9, 127.9, 129.0, 129.8, 130.3, 136.6. Anal. calc. for $C_{11}H_{14}ClN_3S$ (255.77): C 51.66, H 5.52, N 16.43; found: C 51.83, H 5.71, N 16.24.

2-Hydrazinylidene-5-methyl-4-phenyl-3-(propan-2-yl)-2,3-dihydro-1,3-thiazole (**8d**). *Hydrochloride.* The compound was obtained from **5d** (2.87 g, 10 mmol) as described for **8c**. Yield: 2.32 g (82%). M.p. 196 – 204°. Colorless prisms (EtOH, HCl, Et_2O). IR: 3255*m*, 3162*m*, 2946*m*, 2817*m*, 1661*s*, 1567*m*, 1326*m*, 1316*m*, 1198*s*, 1125*w*, 730*m*, 809*w*, 699*s*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.53 (*d*, *Me*$_2$CH); 2.02 (*s*, Me–C(5)); 4.39 – 4.44 (*m*, CH); 5.37 (br. *s*, NH_2); 5.95 (*s*, H–C(5)); 7.26 – 7.57 (*m*, 5 arom. H); 11.67 (*s*, NH_3^+). ^{13}C-NMR ($CDCl_3$, 75 MHz): 11.8, 19.2, 53.2, 116.5, 128.5, 129.1, 130.2, 137.5, 169.7. Anal. calc. for $C_{13}H_{18}ClN_3S$ (283.82): C 55.01, H 6.39, N 14.81; found: C 55.12, H 6.56, N 14.88.

*2-(Methylimino)-4-phenyl-1,3-thiazol-3(2*H*)-amine* (**9a**). The Et_2O soln. of **3a** (*Method A*) was dried with $CaCl_2$. The solvent was then distilled off *in vacuo*, the residue was boiled several times with EtOH, and filtered hot. Upon addition of H_2O, a precipitate formed. Yield: 4.1 g (20%). Light-yellow needles (EtOH/H_2O). M.p. 110°. IR: 3297*m*, 3149*s*, 2957*m*, 2845*m*, 1620*m*, 1567*m*, 1493*m*, 1493*m*,

1444*m*, 1399*s*, 957*w*, 850*w*, 758*m*, 688*s*, 495*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.09 (*s*, Me); 4.28 (*s*, NH_2); 5.80 (*s*, H–C(5)); 7.38 – 7.53 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 48.5 (Me); 91.8 (C(5)); 128.2 (arom. CH); 128.5 (arom. CH); 128.8 (arom. CH); 131.2 (arom. C); 141.1 (C(4)); 159.4 (C(2)). EI-MS (70 eV): 204.9 (100, M^+), 188 (88), 163 (24), 146 (41), 134 (48) 131 (16), 119 (17), 103 (30), 102 (62), 91 (34), 77 (36), 42 (12), 28 (10). Anal. calc. for $C_{10}H_{11}N_3S$ (205.28): C 58.51, H 5.40, N 20.47; found: C 58.72, H 5.58, N 20.51.

*2-(Ethylimino)-4-phenyl-1,3-thiazol-3(2*H*)-amine* (**9b**).The Et_2O soln. of **3b** (*Method A*) was dried with $CaCl_2$. Et_2O was distilled off under reduced pressure. The crude product was crystallized from EtOH. Yield: 4.1 g (37%). Colorless needles. M.p. 124°. IR: 3278*s*, 3166*m*, 2953*m*, 1607*s*, 1492*m*, 1376*m*, 1350*m*, 1274*m*, 1173*m*, 1111*m*, 967*m*, 765*s*, 647*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.31 (*t*, *J* = 8.0, Me); 3.22 (*q*, *J* = 8.0, CH_2); 4.29 (*s*, NH_2); 5.76 (*s*, H–C(5)); 7.78 – 7.52 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.9 (Me); 48.4 (CH_2); 91.6 (C(5)); 128.1 (arom. CH); 128.4 (arom. CH); 128.7 (arom. CH); 131.0 (arom. C); 141.1 (C(4)); 159.0 (C(2)). EI-MS (70 eV): 219 (100, M^+), 203 (33), 190 (18), 174 (22), 147 (11), 134 (38), 104 (11), 102 (32), 91 (33), 76 (21), 29 (23), 28 (7). Anal. calc. for $C_{11}H_{13}N_3S$ (219.31): C 60.24, H 5.97, N 19.16; found: C 59.95, H 6.21, N 18.99.

N-*Methyl-3-[(4-nitrobenzylidene)amino]-4-phenyl-1,3-thiazol-2(3*H*)-imine* (**10a**). Compound **9a** (1 g, 5.0 mmol) and 4-nitrobenzaldehyde (0.75 g, 5.0 mmol) in EtOH (10 ml) was refluxed briefly. After cooling, a orange-red precipitate formed. Yield: 0.95 g (56%). Orange-red needles (EtOH). M.p. 149°. IR: 1624*s*, 1608*m*, 1563*m*, 1512*s*, 1420*w*, 1420*m*, 1403*m*, 1341*s*, 1253*s*, 1108*m*, 695*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.19 (*s*, Me); 6.09 (*s*, H–C(5)); 7.34 – 8.35 (*m*, 9 arom. H); 10.56 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.9 (Me); 96.5 (C(5)); 123.8 (arom. CH); 127.1 (arom. CH); 127.5 (arom. CH); 128.3 (arom. CH); 129.0 (arom. CH); 131.8 (arom. C); 142.4 (arom. C); 143.3 (arom. C); 145.0 (HC=N); 147.9 (C(4)); 154.5 (C(2)). EI-MS (70 eV): 338 (79, M^+), 292 (3), 190 (19), 176 (50), 162 (23), 134 (50), 118 (13), 102 (100), 90 (23), 77 (15), 64 (11), 28 (16). Anal. calc. for $C_{17}H_{14}N_4O_2S$ (338.38): C 60.34, H 4.17, N 16.56; found: C 60.24, H 4.28, N 16.57.

N-*Ethyl-3-[(4-nitrobenzylidene)amino]-4-phenyl-1,3-thiazol-2(3*H*)-imine* (**10b**). Compound **9b** (1.1 g, 5.0 mmol) and 4-nitrobenzaldehyde (0.75 g, 5.0 mmol) in EtOH (10 ml) was refluxed briefly. After cooling, a crystalline product formed. Yield: 0.9 g (51%). Orange-red needles (EtOH). M.p. 145°. IR: 2969*m*, 1622*s*, 1603*s*, 1563*s*, 1516*s*, 1446*m*, 1384*m*, 1340*s*, 1253*s*, 1180*s*, 1108*m*, 829*m*, 692*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.40 (*t*, *J* = 8.0, Me); 3.27 (*q*, *J* = 8.0, CH_2); 6.04 (*s*, H–C(5)); 7.37 – 8.32 (*m*, 9 arom. H); 10.60 (*s*, HC=). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.9 (Me); 50.3 (CH_2); 96.4 (C(5)); 123.9 (arom. CH); 123.9 (arom. CH); 127.5 (arom. CH); 127.9 (arom. CH); 128.7 (arom. CH); 131.8 (arom. C); 140.9 (arom. C); 142.4 (arom. C); 145.0 (HC=N); 147.9 (C(4)); 154.5 (C(2)). EI-MS (70 eV): 352 (46, M^+), 322 (6), 229 (9), 204 (74), 176 (72), 134 (100), 104 (30), 102 (64), 91 (43), 89 (31), 77 (12), 28 (93). Anal. calc. for $C_{18}H_{16}N_4O_2S$ (352.42): C 61.35 H 4.58, N 15.90; found: C 61.27, H 4.73, N 15.86.

N-*Methyl-4-phenyl-1,3-thiazol-2-amine* (**11a**). N-*Methylthiourea* (=*1-(methylsulfanyl)urea*; 0.9 g, 10.0 mmol) and **1a** (1.99 g, 10.0 mmol) in EtOH (80 ml) was refluxed for 25 min. After cooling, a precipitate of the hydrobromide was isolated. Yield: 2.4 g (89%). M.p. 147°. The EtOH soln. of the hydrobromide was treated with aq. NH_3, a crystalline precipitate formed, which was filtered off. Yield: 1.6 g (84%). Colorless prisms (EtOH). M.p. 127°. IR: 3226*s*, 3118*s*, 1588*s*, 1446*w*, 1404*s*, 1332*m*, 1058*m*, 776*m*, 712*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.87 (*d*, *J* = 8.0, Me); 7.35 – 7.40 (*m*, 5 arom. H); 7.61 (br. *s*, NH); 7.85 (*s*, H–C(5)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 30.9 (Me); 100.7 (C(5)); 125.5 (arom. CH); 127.1 (arom. CH); 128.3 (arom. CH); 134.8 (arom. C); 150.0 (C(4)); 169.2 (C(2)). EI-MS (70 eV): 190 (100, M^+), 162 (28), 134 (48), 102 (21), 89 (15), 70 (4), 64 (6), 42 (10), 30 (18), 28 (51). Anal. calc. for $C_{10}H_{10}N_2S$ (190.26): C 63.13, H 5.30, N 14.72; found: C 63.38, H 5.35, N 14.66.

N-*Ethyl-4-phenyl-1,3-thiazol-2-amine* (**11b**). N-*Ethylthiourea* (=*1-(ethylsulfanyl)urea*; 4.2 g, 40.0 mmol) and **1a** (7.96 g, 40.0 mmol) in EtOH (80 ml) was refluxed for 25 min. After cooling, aq. NH_3 was added, and a crystalline product formed, which was filtered off. Yield: 7 g (86%). Colorless prisms (EtOH/H_2O). M.p. 78°. IR: 3208*m*, 2973*m*, 1586*s*, 1480*m*, 1436*m*, 1379*w*, 1154*w*, 1333*s*, 1263*w*, 1058*m*, 773*m*, 705*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.31 (*t*, *J* = 8.0, Me); 3.33 – 3.37 (*m*, CH_2); 5.20 (br. *s*, NH); 6.71 (*s*, H–C(5)); 7.29 – 7.81 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.5 (Me); 40.8 (CH_2); 100.4 (C(5)); 126.0 (arom. CH); 127.5 (arom. CH); 128.4 (arom. CH); 134.9 (arom. C); 151.5 (C(4)); 169.9 (C(2)). EI-MS (70 eV): 204 (40, M^+), 189 (32), 176 (20), 134 (32), 102 (10), 89 (11), 77 (8), 44

(13), 32 (20), 28 (100). Anal. calc. for $C_{11}H_{12}N_2S$ (204.29): C 64.67, H 5.92, N, 13.71; found: C 64.88, H 5.95, N 13.65.

*5-[Benzylideneamino]-*N*-methyl-4-phenyl-1,3-thiazol-2-amine* (**12a**). *Method A*. Compound **9a** (1 g, 5 mmol) in 2M HCl (30 ml) was suspended, and under stirring and cooling by 0° a soln. of $NaNO_2$ (from 0.69 g (10.0 mmol) $NaNO_2$ and 10 ml of H_2O) was added dropwise. A yellow precipitate formed was filtered off. The product was washed with 2M HCl and acetone. The precipitate was dissolved in AcOH (20 ml) and EtOH (5 ml). The mixture was stirred. Zn (1 g) was added to the soln. After 20 min, PhCHO (0.5 ml) was added to the soln. The mixture was allowed to stand overnight at 20° and poured into H_2O (40 ml). A precipitate formed, which was separated and recrystallized from acetone. Yield: 0.5 g (34%). Yellow needles (acetone). M.p. 182°. IR: 3202*s*, 1671*s*, 1585*s*, 1559*s*, 1515*s*, 1480*s*, 1404*m*, 1330*s*, 1235*w*, 1185*s*, 1121*m*, 1075*m*, 819*m*, 768*m*, 704*s*, 685*s*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 12.92 (*t*, *J* = 8.0, Me); 7.32 – 8.06 (*m*, 10 arom. H); 8.29 (*s*, HC=N); 11.81 (br. *s*, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 30.29 (Me); 127.8 (arom. CH); 128.4 (arom. CH); 128.9 (arom. CH); 129.2 (arom. CH); 130.2 (arom. CH); 131.8 (arom. CH); 133.0 (arom. C); 134.5 (arom. C); 151.4; 163.5; 168.4; 177.2 (C(2)). EI-MS (70 eV): 293 (100, M^+), 216 (7), 147 (3), 121 (10), 117 (29), 104 (20), 89 (13), 75 (24), 43 (6), 28 (18). Anal. calc. for $C_{17}H_{15}N_3S$ (293.39): C 69.59, H 5.15, N 14.32; found: C 69.78, H 5.29, N 14.52.

Method B. Compound **11a** (3.8 g, 20.0 mmol) was suspended in 2M HCl (60 ml). Under cooling and stirring was added dropwise $NaNO_2$ soln. (1.38 g, (20.0 mmol) in 10 ml of H_2O). Workup was conducted as described for *Method A*. Yield: 5.1 g (87%). Yellow prisms (EtOH). M.p. 182°.

*5-[Benzylideneamino]-*N*-ethyl-4-phenyl-1,3-thiazol-2-amine* (**12b**). *Method A*. The compound was obtained from **9b** (1.1 g, 5.0 mmol) as desribed for **12a**. Yield: 0.8 g (52%). Yellow prisms (EtOH). M.p. 121°. IR: 3203*s*, 2973*s*, 1584 *s*, 1548*s*, 1480*s*, 1429*m*, 1334*s*, 1334*s*, 1309*m*, 1334*s*, 1191*m*, 1154*m*, 1071*m*, 761*m*, 776*m*, 692*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.20 (*t*, *J* = 8.0, Me); 3.28 – 3.30 (*m*, CH_2); 6.19 (*s*, NH); 7.32 – 7.98 (*m*, 10 arom. H); 8.24 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.9 (Me); 40.4 (CH_2); 127.7 (arom. CH); 128.0 (arom. CH); 128.4 (arom. CH); 128.5 (arom. CH); 129.6 (arom. CH); 131.3 (arom. CH); 133.6 (arom. C); 134.5 (arom. C); 147.1; 151.2; 169.2; 175.1 (C(2)). EI-MS (70 eV): 307 (100, M^+), 147 (3), 121 (7), 117 (34), 104 (17), 77 (12), 28 (50). Anal. calc. for $C_{18}H_{17}N_3S$ (307.41): C 70.33, H 5.57, N 13.67; found: C 70.48, H 5.56, N 13.52.

Method B. Compound **11b** (4.1 g, 20.0 mmol) was suspended in 2M HCl (60 ml). Under cooling and stirring, a $NaNO_2$ soln. (1.38 g, 20.0 mmol in 10 ml of H_2O) was added dropwise. Workup was performed as described for *Method A*. Yield: 4.2 g (68%). Yellow prisms (EtOH). M.p. 121°.

2-Benzylidene-1-methylhydrazinecarbothioamide (**13a**). A mixture of *2-methylthiosemicarbazide* (=*1-methylhydrazinecarbothioamide*; 3.53 g, 30.0 mmol) and PhCHO (3.2 g, 30.0 mmol) in EtOH (20 ml) was refluxed for 15 min. During boiling, a precipitate formed. The product was filtered off. Yield: 5.21 g (90%). Colorless rods (EtOH). M.p. 167°. IR: 3261*s*, 3122*m*, 1475*s*, 1452*s*, 1426*s*, 1365*s*, 1185*m*, 1001*s*, 908*m*, 759*m*, 627*m*, 512*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 3.88 (*s*, MeN); 6.54 (*s*, NH_2); 7.40 – 7.70 (*m*, 5 arom. H); 7.80 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 32.9 (Me); 127.4 (arom. CH); 128.9 (arom. CH); 130.4 (arom. CH); 133.7 (arom. C); 140.7 (HC=N); 181.6 (C=S). EI-MS (70 eV): 193 (86, M^+), 133 (47), 118 (13), 116 (19), 106 (14) 104 (23), 90 (97), 107 (7), 77 (30), 61 (54), 57 (100), 30 (26), 28 (12). Anal. calc. for $C_9H_{11}N_3S$ (193.27): C 55.93, H 5.74, N 21.74; found: C 55.84, H 5.62, N 21.63.

2-Benzylidene-1-ethylhydrazinecarbothioamide (**13b**). A mixture of 2-ethylthiosemicarbazide (3.6 g, 30.0 mmol) and PhCHO (3.2 g, 30.0 mmol) in EtOH (20 ml) was refluxed for 15 min. During boiling, a precipitate formed. The product was filtered off. Yield: 5.6 g (90%). Colorless rods (EtOH). M.p. 175°. IR: 3251*s*, 3125*m*, 1572*s*, 1360*s*, 1237*m*, 1008*s*, 908*m*, 806*m*, 757*m*, 621*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.31 (*t*, *J* = 8.0, Me); 3.72 – 3.82 (*m*, CH_2); 6.50 (*s*, NH_2); 6.55 (*s*, HC=N); 7.40 – 7.76 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.5 (Me); 39.15 (CH_2); 127.4 (arom. CH); 128.8 (arom. CH); 130.4 (arom. CH); 133.8 (arom. C); 140.8 (HC=N); 181.5 (C=S). EI-MS (70 eV): 207 (90, M^+), 145 (48), 132 (14), 130 (23), 120(15) 104 (23), 90 (90), 107 (7), 77 (32), 61 (55), 57 (100), 30 (28), 28 (15). Anal. calc. for $C_{10}H_{13}N_3S$ (207.30): C 57.94, H 6.32, N 20.27; found: C 57.84, H 6.42, N 20.42.

2-(2-Benzylidene-1-methylhydrazinyl)-4-phenyl-1,3-thiazole (**14a**). Compound **13a** (1.93 g, 10.0 mmol) and **1a** (1.99 g, 10.0 mmol) in EtOH (40 ml) were refluxed for 10 min. After cooling, aq NH_3 was added. A precipitate formed, which was separated and recrystallized (EtOH) to give **14a**. Yield: 2.64 g (90%). Yellow prisms (EtOH). M.p. 191°. IR: 695*m*, 722*m*, 755*m*, 955*w*, 1104*s*, 1194*w*, 1254*m*,

1335*m*, 1443*m*, 1486*m*, 1516*s*, 1537*s*, 1573*m*, *1597m*, 3112*w*, ^{1}H-NMR (300 MHz, $CDCl_3$): 3.71 (*s*, MeN); 6.89 (*s*, H–C(5)); 7.30 – 7.71 (*m*, 10 arom. H); 7.88 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 32.2 (Me); 104.9 (C(5)); 125.8 (arom. C); 126.6 (arom. C); 127.5 (arom. C); 128.5 (arom. C); 128.7 (arom. C); 129 (arom. C); 134.9 (arom. C); 135.1 (arom. C); 135.8 (HC=N); 151.1 (C(4)); 170.2 (C(2)). EI-MS (70 eV): 293 (44, M^+), 215 (19), 190 (100), 162 (31), 147 (13), 134 (65), 121 (13), 104 (12), 107 (7), 77 (34), 51 (13), 28 (1). Anal. calc. for $C_{17}H_{15}N_3S$ (293.39): C 69.59, H 5.15, N 14.32; found: C 69.78, H 5.29, N 14.52.

2-(2-Benzylidene-1-ethylhydrazinyl)-4-phenyl-1,3-thiazole (**14b**). Compound **13b** (2.07 g, 10.0 mmol) and **1a** (1.99 g, 10.0 mmol) was refluxed for 15 min. Workup as described for **14a** afforded **14b** (2.55 g, 83%). Yellow lamella (EtOH). M.p. 142.5 – 143.0°. IR: 3113*w*, 1598*m*, 1538*s*, 1445*m*, 1518 *s*, 1256*m*, 1105*s*, 756*m*, 724*m*, 696*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.28 (*t*, $J = 8.0$, Me); 3.67 – 3.76 (*m*, CH_2); 6.92 (*s*, H–C(5)); 7.32 – 7.70 (*m*, 10 arom. H); 7.81 (*s*, HC=N). ^{13}C-NMR (75 MHz, $CDCl_3$): 32.2 (Me); 39.1 (CH_2); 105.1 (C(5)); 125.8 (arom. C); 126.7 (arom. C); 127.6 (arom. C); 128.5 (arom. C); 128.7 (arom. C); 129 (arom. C); 134.9 (arom. C); 135.4 (arom. C); 137.1 (HC=N); 151.5 (C(4)); 170.5 (C(2)). EI-MS (70 eV): 307 (100, M^+), 121 (34), 104 (27), 77 (42), 28 (30). Anal. calc. for $C_{18}H_{17}N_3S$ (307.41): C 70.33, H 5.57, N 13.67; found: C 70.43, H 5.66, N 13.42.

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Eudesmanolides and Guaianolides from *Carpesium triste*

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A new eudesmanolide, 1-oxo-11*αH*-eudesma-2,4(14)-dien-12,8*β*-olide (**1**), and four new guaianolides, 9*β*,10*β*-epoxy-4*α*-hydroxy-1*βH*,11*αH*-guaian-12,8*α*-olide (**2**), 9*β*,10*β*-epoxy-4*α*-hydroxy-1*βH*,11*βH*-guaian-12,8*α*-olide (**3**), 4*α*,9*α*-dihydroxy-1*βH*,11*αH*-guai-10(14)-en-12,8*α*-olide (**4**), and 4*α*,9*α*-dihydroxy-1*βH*,11*βH*-guai-10(14)-en-12,8*α*-olide (**5**), together with one known eudesmanolide and two known germacranolides, were isolated from the whole plants of *Carpesium triste*. Their structures and relative configurations were elucidated on the basis of spectroscopic methods, including 2D-NMR techniques.

Introduction. – *Carpesium triste* species are herbaceous plants from the family Compositae, genus *Carpesium* [1]. The whole plant of *C. triste* was used as a traditional Chinese medicine for the treatment of sore throat, toothache, urinary-tract infection, diarrhea, and mastitis [2]. Previously, sesquiterpenoids and diterpenoids were isolated from the seeds of *C. triste*, and some of them were reported to show cytotoxic activities [3][4]. As a part of our ongoing research on utilization of medicinal plants in the ethnical areas of China, we performed a phytochemical study on the whole plant of *C. triste* collected in Guizhou Province, southwestern China. As a result, five new sesquiterpenoids, **1**–**5** (*Fig. 1*), along with three known sesquiterpenoids, granilin [5], eriolin, and 11(13)-dehydroivaxillin [6], were isolated. Their structures were elucidated by spectroscopic methods, including IR, HR-ESI-MS, and 1D- and 2D-NMR experiments. Herein, we describe the isolation and structure elucidation of **1**–**5**.

1

2 R = *β*-Me
3 R = *α*-Me

4 R = *β*-Me
5 R = *α*-Me

Fig. 1. *Structures of compounds* **1**–**5**, *isolated from* Carpesium triste

Results and Discussion. – Compound **1** was obtained as a colorless oil and had the molecular formula $C_{15}H_{18}O_3$ on the basis of HR-ESI-MS (*m/z* 247.1341 ([*M* + H]$^+$)), indicating seven degrees of unsaturations. The presence of a *α*,*β*-saturated *γ*-lactone

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group (1742 cm^{-1}) and C=O bond (1723 cm^{-1}) were indicated by its IR spectrum. The ^{1}H- and ^{13}C-NMR (DEPT) spectra (*Table 1*) of **1** showed signals of two Me groups at δ(H) 1.29–1.31 (*d*, Me(13)) and 1.05 (*s*, Me(15)), along with those of three CH_2 and six CH groups, and four quaternary C-atoms. Furthermore, considering of the functional groups, signals of an O-bearing C-atom (δ(C) 77.0, C(8)), a pair of exocyclic olefinic C-atoms (δ(C) 119.1 (C(14)) and 142.8 (C(4))), and an olefinic *AB* system (δ(C) 125.0 (C(2)) and 146.8 (C(3))), as well as signals of a ketone (δ(C) 203.7 (C(1)=O)) and an ester (δ(C) 178.0 (C(12)=O)) were observed in the ^{13}C-NMR (DEPT) spectrum. Thus, the structure of **1** was deduced as an eudesmane sesquiterpene lactone, which was similar to that of 1-oxo-5α*H*,6,11β*H*-eudesma-2,4(14)-dien-6,13-olide [7]. However, the ^{1}H,^{1}H-COSY and HSQC features (*Fig. 2*) evidenced the following sequence: CH(5)–CH_2(6)–CH(7)–CH(8)–CH_2(9) and Me(13)–CH(11)–CH(7)–CH(8)–CH_2(9), indicating that the α,β-saturated γ-lactone in **1** should be located at C(8), instead of C(6). This assignment was further confirmed by the following HMBCs (*Fig. 2*): H–C(2) (δ(H) 5.87–5.89)/C(10) (δ(C) 44.1) and C(4); H–C(3) (δ(H) 7.10–7.29)/C(1), C(14), C(4), and C(5) (δ(C) 42.0); H–C(8) (δ(H) 4.60–4.62)/C(6) (δ(C) 20.5) and C(10); H–C(11) (δ(H) 2.88–2.91)/C(6), C(7) (δ(C) 39.1), C(12), and C(13) (δ(C) 9.4).

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** (at 600 and 150 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, *J* in Hz)

Position	**1**	
	δ(H)	δ(C)
1		203.7
2	5.87–5.89 (*d*, *J* = 9.9)	125.0
3	7.10–7.29 (*d*, *J* = 9.9)	146.8
4		142.8
5	2.54–2.57 (*m*)	42.0
6	1.87–188 (*ddd*, *J* = 13.7, 4.5), 1.37–1.40 (*q*, *J* = 12.4)	20.5
7	2.42–2.46 (*m*)	39.1
8	4.60–4.62 (br. *s*)	77.0
9	1.78–1.82 (*dd*, *J* = 16.1, 4.1), 2.58–2.61 (*dd*, *J* = 16.1, 2.0)	32.9
10		44.1
11	2.88–2.91 (*m*)	41.8
12		178.0
13	1.29–1.31 (*d*, *J* = 7.2)	9.4
14	5.36 (*s*), 5.48 (*s*)	119.1
15	1.05 (*s*)	17.9

In the ^{13}C-NMR spectrum, the signal at δ(C) 9.4 (Me(13)) is typical for the eudesmanolides with a β-Me group at C(11) by means of empirical rules [8]. Moreover, in the NOE difference spectrum of **1** (*Fig. 2*), the signals of H–C(7) and H–C(11) were enhanced upon irradiation of H–C(8), while irradiation of H–C(7) enhanced the signals of H–C(5) (δ(H) 2.54–2.57 (*m*)), H–C(8), and H–C(11). All these data suggested that H–C(5), H–C(8), H–C(7), and H–C(11) were on the same side of the plane, and Me(13) on the opposite side. As a result, the structure of **1** was elucidated as 1-oxo-11α*H*-eudesma-2,4(14)-dien-12,8β-olide.

Fig. 2. *^{1}H,^{1}H-COSY Correlations* (—), *Key HMBCs* (H → C), *and key 1D-NOE* (H ↔ H) *correlations of* **1**

Compound **2** was obtained as a colorless oil, its molecular formula, $C_{15}H_{22}O_4$, was deduced from HR-ESI-MS (*m/z* 267.1600 ([*M* + H]$^+$)), indicating five degrees of unsaturation. The IR spectrum evidenced the presence of an α,β-saturated γ-lactone moiety (1737 cm^{-1}) and a OH group (3443 cm^{-1}). The ^{1}H- and ^{13}C-NMR (DEPT) spectra (*Table 2*) indicated the presence of three Me groups, *i.e.*, Me(13) (δ(H) 1.21 – 1.23 (*d*)), Me(14) (δ(H) 1.40 (*s*)), and Me(15) (δ(H) 1.20 (*s*)), along with three CH_2 and six CH groups, and three quaternary C-atoms. The ^{1}H,^{1}H-COSY and HSQC features (*Fig. 3*) provided the following sequence: Me(13)–CH(11)–CH(7) –CH(8)–CH_2(9) and CH(7)–CH_2(6)–CH(5)–CH(1)–CH_2(2)–CH_2(3), suggesting that **2** was a guaianolide. This deduction was supported by the HMBCs (*Fig. 3*), such as H–C(8) (δ(H) 4.18 – 4.22)/C(6) (δ(C) 28.0), C(7) (δ(C) 42.2), and C(9) (δ(C) 64.9), H–C(9) (δ(H) 3.05 – 3.06)/C(10) (δ(C) 63.8) and C(14) (δ(C) 17.3), H–C(11) (δ(H) 2.70 – 2.75)/C(8) (δ(C) 83.2), C(12) (δ(C) 178.1), and C(13) (δ(C) 10.8). Indeed, all the spectral data of **2** were similar to those of 9β,10β-epoxy-4α-hydroxy-1β*H*-guai-11(13)-en-12,8α-olide [9], except that an α-methylene γ-lactone group in the known compound was replaced by the α-methyl γ-lactone group in **2**.

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2** *and* **3** (at 600 and 150 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, *J* in Hz)

Position	**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.55 – 1.61[a])	49.0	1.59 – 1.64[a])	48.8
2	1.92 – 1.94 (*m*), 1.69 – 1.76[a])	22.6	1.91 – 1.96[a]), 1.71 – 1.76[a])	22.5
3	1.69 – 1.76[a])	41.7	1.71 – 1.76[a])	41.7
4		79.5		79.4
5	1.69 – 1.76[a])	52.0	1.71 – 1.76[a])	51.8
6	2.03 – 2.06 (*m*), 1.19 – 1.23[a])	28.0	1.91 – 1.96[a]), 1.12 – 1.20 (*q*, *J* = 12.0)	31.4
7	2.40 – 2.42 (*m*)	42.2	2.25 – 2.29[a])	47.2
8	4.18 – 4.22 (*dd*, *J* = 11.2, 5.5)	83.2	4.03 – 4.06 (*dd*, *J* = 11.2, 5.5)	83.3
9	3.05 – 3.06 (*d*, *J* = 5.5)	64.9	3.03 – 3.04 (*d*, *J* = 5.5)	64.3
10		63.8		60.2
11	2.70 – 2.75 (*m*)	38.7	2.25 – 2.29[a])	41.3
12		178.1		178.1
13	1.21 – 1.23 (*d*, *J* = 8.0)	10.8	1.27 – 1.28 (*d*, *J* = 8.0)	12.8
14	1.40 (*s*)	17.3	1.39 (*s*)	17.3
15	1.20 (*s*)	22.9	1.18 (*s*)	22.9

[a]) Overlapped.

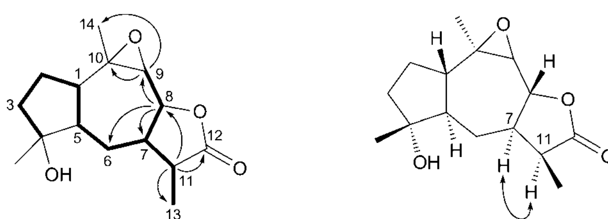

Fig. 3. *$^{1}H,^{1}H$-COSY Correlations* (—), *Key HMBCs* (H → C), *and key 1D-NOE* (H ↔ H) *correlations of* **2**

It was reported that in this type of guaianolides, H–C(1) (δ(H) 1.55 – 1.61 (overlapped)), H–C(8), and Me(15) were all β-configured, and H–C(5) (δ(H) 1.69 – 1.76 (overlapped)), H–C(7) (δ(H) 2.40 – 2.42 (*m*)), and Me(14) were all in α-configuration [9]. Furthermore, the chemical shifts of H–C(8), as well as the large coupling constants (*J*(7,8) = 11.2) suggested that H–C(8) and H–C(7) should be *trans*-oriented. In the NOE difference spectrum (*Fig. 3*), irradiation of H–C(7) led to an enhancement of the H–C(11) resonance, while irradiation of H–C(8) enhanced the signal at H–C(1). Accordingly, the structure of **2** was elucidated as 9β,10β-epoxy-4α-hydroxy-1β*H*,11α*H*-guaian-12,8α-olide.

Compound **3** was obtained as a colorless oil, with the molecular formula $C_{15}H_{22}O_4$ deduced from HR-ESI-MS (*m*/*z* 267.1595 ([*M* + H]$^+$)). The ^{1}H- and ^{13}C-NMR data of **3** were similar to those of **2**, indicating that **3** was an analog of **2** (*Table 2*). The differences in chemical shifts between these two compounds were presumably due to the opposite configuration at Me(13) (δ(H) 1.27 – 1.28 (*d*)), which was confirmed by the NOE difference spectra. The irradiation of H–C(8) (δ(H) 4.03 – 4.06 (*d*)) produced NOE enhancements of both H–C(11) (δ(H) 2.25 – 2.29 (overlapped)) and H–C(1) (δ(H) 1.59 – 1.64 (overlapped)) resonances, indicating β-configuration for H–C(11). Therefore, the structure of **3** was elucidated as 9β,10β-epoxy-4α-hydroxy-1β*H*,11β*H*-guaian-12,8α-olide.

Compound **4** was obtained as a colorless oil, with the molecular formula $C_{15}H_{22}O_4$ deduced from HR-ESI-MS (*m*/*z* 267.1599 ([*M* + H]$^+$)). The ^{1}H- and ^{13}C-NMR data of **4** (*Table 3*) were similar to those of **2**, indicating the same skeleton as **2**. However, according to the HMBCs (*Fig. 4*), such as CH_2(14) (δ(H) 5.26 and 5.32)/C(1) (δ(C)

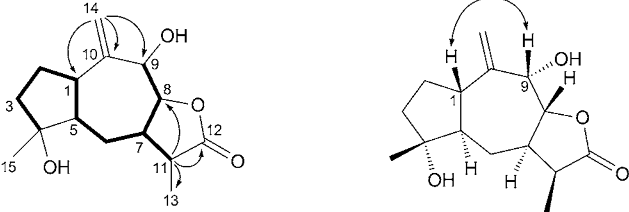

Fig. 4. *$^{1}H,^{1}H$-COSY Correlations* (—), *Key HMBCs* (H → C), *and key 1D-NOE* (H ↔ H) *correlations of* **4**

Table 3. *^{1}H- and ^{13}C-NMR Data of* **4** *and* **5** (at 600 and 150 MHz, resp., in $CDCl_3$ at 27°; δ in ppm, *J* in Hz)

Position	**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	2.31–2.35 (*m*)	43.5	2.25–2.27 (*m*)	43.6
2	1.96–2.00[a]), 1.78–1.82[a])	26.2	1.88–1.95 (*m*), 1.73–1.81[a])	26.3
3	1.87–1.91 (*m*), 1.78–1.82[a])	41.3	1.73–1.81[a])	41.4
4		80.4		80.4
5	1.54–1.59 (*ddd*, *J* = 12.1, 4.0)	60.2	1.57–1.62 (*ddd*, *J* = 12.1, 4.2)	60.4
6	1.96–2.00[a]), 1.26–1.32[a])	27.7	1.98–2.00 (*m*), 1.25–1.30[a])	31.4
7	2.20–2.27 (*m*)	39.9	2.14–2.20 (*m*)	41.9
8	4.36–4.40 (*dd*, *J* = 10.1, 7.0)	88.0	4.23–4.25 (*dd*, *J* = 10.1, 7.1)	87.7
9	4.37–4.38 (*d*, *J* = 7.2)	80.0	4.33–4.36 (*d*, *J* = 7.3)	79.5
10		150.8		150.7
11	2.70–2.75 (*m*)	39.4	2.32–2.36 (*m*)	45.4
12		179.1		177.8
13	1.26–1.27 (*d*, *J* = 7.8)	11.3	1.26–1.28 (*d*, *J* = 8.0)	13.2
14	5.26 (*s*), 5.32 (*s*)	115.7	5.26 (*s*), 5.32 (*s*)	115.8
15	1.24 (*s*)	24.0	1.24 (*s*)	24.3

[a]) Overlapped.

43.5), C(9) (δ(C) 80.0), and C(10) (δ(C) 150.8), the presence of a terminal C=C bond at C(10) and of a OH group at C(9) were evidenced in **4**, instead of an epoxy group in **2**. In the NOE difference spectrum (*Fig. 4*), irradiation of H–C(1) (δ(H) 2.31–2.35 (*m*)) led to an enhancement of the H–C(9) (δ(H) 4.37–4.38 (*d*)) resonance, suggesting β-configuration for H–C(1) and H–C(9). Therefore, the structure of **4** was elucidated as 4α,9α-dihydroxy-1β*H*,11α*H*-guai-10(14)-en-12,8α-olide.

Compound **5** was obtained as a colorless oil, with the molecular formula $C_{15}H_{22}O_4$ deduced from HR-ESI-MS (*m/z* 267.1595 ($[M+H]^+$)). Comparison of ^{1}H- and ^{13}C-NMR data of **5** (*Table 3*) and **3** indicated that they share the same skeleton. However, as the difference between **4** and **2**, a terminal C=C bond at C(10) (δ(C) 150.7) and a OH group at C(9) (δ(C) 79.5) in **5** replaced the oxygenated substituents at C(9) and C(10) in **3**. Thus, the structure of **5** was elucidated as 4α,9α-dihydroxy-1β*H*,11β*H*-guai-10(14)-en-12,8α-olide.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Co.*, P. R. China) and *Sephadex LH-20* (*Pharmacia*). TLC: SiO_2 GF_{254} (*Qingdao Marine Chemical Co.*, P. R. China); visualization under UV or by heating after spraying with 10% H_2SO_4 in EtOH. Flash-type extractor: *JHBE-50S* (*Henan Jinnai Sci-Tech Development Ltd.*, Zhengzhou, P. R. China). Optical rotations: *JASCO-P-1020* polarimeter (*JASCO Corporation*, Tokyo, Japan). UV Spectra: *Shimadzu-UV-2450* UV/VIS spectrophotometer; λ_{max} in nm. IR Spectra: *Bruker-VERTEX 70* FT-IR spectrometer; KBr

pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Bruker-AV-500* and *Bruker-AV-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *LCT Premier XE TOF* mass spectrometer; in m/z.

Plant Material. The whole plants of *Carpesium triste* were collected in Kuankuoshui National Nature Reserve, Guizhou Province, P. R. China, in July, 2009, and identified by Prof. *Wei Liang* (College of Life Sciences, Hainan Normal University). A voucher specimen (NO. 20090701) was deposited with the Herbarium of the College of Life and Environment Sciences, Minzu University of China.

Extraction and Isolation. The air-dried whole plants (337 g) of *C. triste* were extracted with MeOH at r.t by flash-type extractor (20 g of material were extracted for 1 min, each time). After filtration, the extracts were combined and evaporated under vacuum. The residue (33.5 g) was subjected to CC (SiO_2; petroleum ether (PE)/acetone 25 : 1, 20 : 1, 15 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 2 : 1, and 1 : 1) to afford nine fractions, *Frs. 1–9*. Then, *Fr. 3*, eluted with PE/acetone 15 : 1, was further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1), and then by prep. TLC (PE/AcOEt/MeOH 10 : 3 : 1; R_f 0.45) to afford **1** (3.2 mg). *Fr. 4*, eluted with PE/AcOEt 10 : 1, was further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1; then SiO_2; $CHCl_3$/acetone 12 : 1) to afford four subfractions, *Frs. 4a–4d*. *Fr. 4a* was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to furnish eriolin (12.0 mg) and 11(13)-dehydroivaxillin (4.0 mg). *Fr. 4b* was subjected to repeated CC (SiO_2; $CHCl_3$/AcOEt 1 : 1) to afford **3** (3.1 mg), *Fr. 4d* was submitted to repeated CC (SiO_2; $CHCl_3$/AcOEt 2 : 3) to afford **2** (2.3 mg). *Fr. 5*, eluted with $CHCl_3$/acetone 8 : 1, afforded four sub-fractions, *Frs. 5a–5d*, and *Fr. 5b* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to provide granilin (4.0 mg). *Fr. 7*, eluted with $CHCl_3$/acetone 2 : 1, furnished four subfractions, *Frs. 7a–7d*. *Fr. 7a* was subjected to repeated CC (SiO_2; AcOEt/MeOH 8 : 1) to afford **4** (2.6 mg); *Fr. 7d* was subjected to repeated CC (SiO_2; AcOEt/MeOH 7 : 1) to give **5** (2.1 mg).

*1-Oxo-11α*H*-eudesma-2,4(14)-dien-12,8β-olide* (*=(3*S*,3a*R*,4a*S*,8a*R*,9a*R*)-3a,4a,5,8a,9,9a-Hexahydro-3,8a-dimethyl-5-methylidenenaphtho[2,3-*b*]furan-2,8(3*H*,4*H*)-dione*; **1**): Colorless oil. $[\alpha]_D^{20} = +22$ ($c = 0.015$, $CHCl_3$). UV (MeOH): 265. IR (KBr): 1742, 1723, 1466, 1345, 1131, 939. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 247.1341 ($[M + H]^+$, $C_{15}H_{18}O_3^+$; calc. 247.1334).

*9β,10β-Epoxy-4α-hydroxy-1β*H*,11α*H*-guaian-12,8α-olide* (*=(1a*S*,1b*R*,4*S*,4a*R*,5a*R*,6*R*,8a*S*,8b*R*)-Decahydro-6-hydroxy-4,6,8b-trimethyloxireno[7,8]azuleno[6,5-*b*]furan-3(1b*H*)-one*; **2**): Colorless oil. $[\alpha]_D^{20} = -27$ ($c = 0.012$, $CHCl_3$). IR (KBr): 3443, 1737, 1685, 1230, 1030, 926. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 267.1600 ($[M + H]^+$, $C_{15}H_{22}O_4^+$; calc. 267.1596).

*9β,10β-Epoxy-4α-hydroxy-1β*H*,11β*H*-guaian-12,8α-olide* (*=(1a*S*,1b*R*,4*R*,4a*R*,5a*R*,6*R*,8a*S*,8b*R*)-Decahydro-6-hydroxy-4,6,8b-trimethyloxireno[7,8]azuleno[6,5-*b*]furan-3(1b*H*)-one*; **3**): Colorless oil. $[\alpha]_D^{20} = -10$ ($c = 0.007$, $CHCl_3$). IR (KBr): 3461, 1753, 1675, 1235, 1041, 931. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 267.1595 ($[M + H]^+$, $C_{15}H_{22}O_4^+$; 267.1596).

*4α,9α-Dihydroxy-1β*H*,11α*H*-guai-10(14)-en-12,8α-olide* (*=(3*S*,3a*R*,4a*R*,5*R*,7a*S*,9*S*,9a*R*)-Decahydro-5,9-dihydroxy-3,5-dimethyl-8-methylideneazuleno[6,5-*b*]furan-2(3*H*)-one*; **4**): Colorless oil. $[\alpha]_D^{20} = -29$ ($c = 0.014$, $CHCl_3$). IR (KBr): 3423, 3316, 1737, 1662, 1063, 929. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-MS: 267.1599 ($[M + H]^+$, $C_{15}H_{22}O_4^+$; 267.1596).

*4α,9α-Dihydroxy-1β*H*,11β*H*-guai-10(14)-en-12,8α-olide* (*=(3*R*,3a*R*,4a*R*,5*R*,7a*S*,9*S*,9a*R*)-Decahydro-5,9-dihydroxy-3,5-dimethyl-8-methylideneazuleno[6,5-*b*]furan-2(3*H*)-one*; **5**): Colorless oil. $[\alpha]_D^{20} = -12$ ($c = 0.006$, $CHCl_3$). IR (KBr):3430, 3326, 1742, 1685, 1070, 941. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-MS: 267.1595 ($[M + H]^+$, $C_{15}H_{22}O_4^+$; 267.1596).

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Rare C_{25} Steroids Produced by *Penicillium chrysogenum* P1X, a Fungal Endophyte of *Huperzia serrata*

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Three new metabolites, norcyclocitrinol A (**1**), *erythro*-11*α*-hydroxyneocyclocitrinol (**2**), and pesudocyclocitrinol A (**3**), along with six known analogs, *i.e.*, neocyclocitrinols A–D (**4**–**7**, resp.), cyclocitrinol (**8**), and 24-epicyclocitrinol (**9**), were isolated and identified from the culture broth of *Penicillium chrysogenum* P1X, a fungal endophyte of *Huperzia serrata*. Compounds **1**–**9** were identified by spectroscopic methods to share the same C_{25}-steroid skeleton featuring an unusual bicyclo[4.4.1] *A*/*B* ring system. In particular, **1** represents the first example of a C_{25} steroid with a bisnor C-atom side chain. All compounds were evaluated for their cytotoxic activities against HeLa and HepG2 cell lines. However, none of them exhibited a significant cytotoxicity at a concentration of 20 μM.

Introduction. – Microorganisms, especially those living in special biotopes and niches, have attracted much attention due to their unique habitat and great prospect in offering novel structures with pharmaceutical potential. Endophytes were regarded as such a kind of special-biotope microorganisms by residing in the tissues of living plants without causing any apparent symptoms [1] [2]. During their life cycles, endophytes had to develop special mechanisms to penetrate into the host tissue and to establish mutualistic associations with both their counterparts and the host. As a result, the endophytes may produce various metabolites to compete with the epiphytes and also with the plant pathogens to maintain a critical balance between fungal virulence and plant defense [3]. Studies on the metabolites of these less explored microorganisms would help interpret the relationship between plants and endophytes, and would also provide alternative directions for natural-product drug discovery.

As part of our ongoing search for novel bioactive compounds from plant endophytes [4], we focused on the fungal strain *Penicillium chrysogenum* P1X isolated from the healthy stems of *Huperzia serrata*, a traditional Chinese herb which earned its worldwide fame since the discovery of the potent acetylcholinesterase inhibitor, huperzine A, among its constituents. The fungus was grown in liquid potato-dextrose medium. Chemical investigation of the resulting fungal culture broth afforded three new metabolites, namely norcyclocitrinol A (**1**), *erythro*-11*α*-hydroxyneocyclocitrinol (**2**), and pesudocyclocitrinol A (**3**), along with six known analogs, neocyclocitrinols A–D (**4**–**7**, resp.), cyclocitrinol (**8**), and 24-epicyclocitrinol (**9**). Compounds **2**–**9** were determined by spectroscopic methods to share the same C_{25}-steroid backbone, while

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bearing four different side-chain types. Herein, we report the isolation, structure elucidation, and biological-activity screening of these pentanor triterpenes.

Results and Discussion. – An AcOEt extract of the culture broth of *Penicillium chrysogenum* P1X was subjected to extensive purification steps to afford three new C_{25} steroids **1**–**3**, as well as the six known analogs **4**–**9** (*Fig. 1*).

Fig. 1. *The structures of compounds* **1**–**9**

Compound **1** was obtained as a white solid, and according to the $[M+H]^+$ peak at m/z 375.2535 in HR-ESI-MS, it was assigned to have the molecular formula $C_{23}H_{34}O_4$, indicating seven degrees of unsaturation. The IR spectrum displayed absorption bands at 3400 and 1653 cm^{-1}, evidencing the presence of OH and conjugated CO groups, respectively. The ^{1}H-NMR spectrum showed signals for two olefinic H-atoms at δ(H) 5.60 (*dd*, $J = 6.5, 6.5$, H–C(1)) and 5.59 (*s*, H–C(7)), and two Me *singlet*s at δ(H) 1.34 (*s*, Me(21)) and 0.85 (*s*, Me(19)). A typical *AB* spin system comprising one pair of geminal H-atom signals at δ(H) 3.85 (*ddd*, $J = 16.5, 10.5, 6.0$) and 3.71–3.75 (*m*) could be easily recognized and ascribed to the O-bearing CH_2(23). The ^{13}C-NMR and DEPT spectra (*Table*) revealed 23 C-atom resonances including those of one CO, four quaternary C-atoms, and seven CH, nine CH_2, and two Me groups. The NMR data for **1** showed similarities with those of the known compounds **4**–**9**, unveiling a C_{23}-steroid profile of **1**. Detailed interpretation of the 2D-NMR spectra (*Fig. 2*) verified that compound **1** possessed the common tetracyclic C_{23}-steroid skeleton with a bicyclo[4.4.1] *A*/*B* ring

Table. *NMR Data of Compounds* **1**–**3**. δ in ppm, *J* in Hz.

Position	**1**[a])		**2**[b])		**3**[c])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	5.60 (*dd*, *J* = 6.5, 6.5)	123.0	5.66 (*dd*, *J* = 7.5, 7.5)	126.5	5.55 (*dd*, *J* = 7.0, 7.0)	122.0
2	2.45–2.53 (*m*), 2.20–2.28 (*m*)	36.3	2.50–2.58 (*m*), 2.20–2.29 (*m*)	37.4	2.31–2.37 (*m*), 2.06–2.10 (*m*)	35.9
3	3.39–3.46 (*m*)	64.8	3.31–3.40 (*m*)	65.5	3.09–3.16 (*m*)	63.0
4	2.84–2.88 (*m*), 1.65–1.73 (*m*)	41.8	2.85 (br. *d*, *J* = 13.0), 1.64–1.71 (*m*)	42.6	2.64 (br. *d*, *J* = 13.0), 1.47–1.57 (*m*)	41.3
5	2.75–2.80 (*m*)	50.2	2.75–2.80 (*m*)	50.2	2.67–2.70 (*m*)	48.0
6	–	207.4	–	207.5	–	203.9
7	5.59 (*s*)	125.6	5.58 (*s*)	126.7	5.64 (*s*)	124.4
8	–	159.7	–	157.3	–	156.8
9	2.81 (*dd*, *J* = 13.0, 6.5)	54.9	2.72 (*d*, *J* = 9.5)	64.3	2.72 (*dd*, *J* = 11.5, 5.0)	53.2
10	–	146.6	–	144.7	–	145.4
11	1.87–1.91 (*m*), 1.60–1.64 (*m*)	28.4	3.91 (*td*, *J* = 10.5, 5.0)	69.8	1.76 (*dd*, *J* = 13.0, 4.0), 1.49–1.54 (*m*)	27.2
12	2.19–2.24 (*m*), 1.50–1.57 (*m*)	40.2	2.27 (*dd*, *J* = 12.0, 5.0), 1.46 (*dd*, *J* = 12.0, 12.0)	48.5	1.57–1.69 (*m*), 1.46 (*dd*, *J* = 12.0, 4.5)	36.7
13	–	47.0	–	48.0	–	47.7
14	2.15–2.22 (*m*)	56.9	2.41 (br. *t*, *J* = 9.5)	56.6	2.41 (br. *t*, *J* = 9.0)	54.2
15	1.62–1.68 (*m*), 1.55–1.60 (*m*)	23.3	1.65–1.70 (*m*), 1.59–1.64 (*m*)	23.8	1.61–1.65 (*m*), 1.57–1.61 (*m*)	22.5
16	1.85–1.93 (*m*), 1.81–1.87 (*m*)	22.8	1.94–2.01 (*m*), 1.79–1.84 (*m*)	25.6	1.85–1.91 (*m*), 1.60–1.70 (*m*)	24.0
17	1.72 (*t*, *J* = 9.5)	61.0	2.40 (br. *t*, *J* = 9.5)	60.6	2.90 (br. *t*, *J* = 9.0)	50.8
18	2.60 (br. *s*), 2.59 (br. *s*)	28.2	2.63–2.68 (*m*), 2.53–2.64 (*m*)	28.7	2.45–2.51 (*m*), 2.41–2.47 (*m*)	27.1
19	0.85 (*s*)	14.6	0.63 (*s*)	15.4	0.64 (*s*)	14.6
20	–	75.8	–	139.1	–	136.6
21	1.34 (*s*)	26.3	1.78 (*s*)	19.4	1.71 (*s*)	22.0
22	1.84–1.90 (*m*), 1.61–1.69 (*m*)	43.8	5.37 (*d*, *J* = 8.5)	127.4	5.34 (*d*, *J* = 9.0)	130.5
23	3.85 (*ddd*, *J* = 16.5, 10.5, 6.0), 3.71–3.75 (*m*)	59.5	4.30 (*dd*, *J* = 8.5, 4.5)	73.7	3.99 (*ddd*, *J* = 9.0, 5.0, 5.0)	70.3
24	–	–	3.69–3.72 (*m*)	72.0	3.40–3.43 (*m*)	70.0
25	–	–	1.12 (*d*, *J* = 6.5)	18.6	1.01 (*d*, *J* = 6.0)	19.7
3-OH	–	–	–	–	4.58 (*d*, *J* = 4.5)	–
23-OH	–	–	–	–	4.28 (*d*, *J* = 5.0)	–
24-OH	–	–	–	–	4.27 (*d*, *J* = 4.5)	–

[a]) Recorded in $CD_3OD/CDCl_3$ 1 : 1. [b]) Recorded in CD_3OD. [c]) Recorded in (D_6)DMSO.

Fig. 2. *Selected ^1H,^1H-COSY* (—) *and HMBC* (H → C) *features of compounds* **1**–**3**

system, while featuring a previously unreported bisnor C-atom side chain. The presence of a bicyclo[4.4.1] system combining rings *A* and *B* was established by the key ^{1}H,^{1}H-COSY plot H–C(5)/CH_2(18) and the HMBCs from H–C(7) to C(5), from CH_2(4) to C(6), from H–C(9) to C(1), C(10), and C(18), and from CH_2(18) to C(1), C(4), C(5), C(6), C(9), and C(10). The side chain was also constructed by analysis of the 2D-NMR spectra. Specifically, ^{1}H,^{1}H-COSY plot of CH_2(22)/CH_2(23) evidenced the presence of the fragment of CH_2(22)–CH_2(23)OH, whose linkage to C(17) and C(21) *via* the O-bearing quaternary C-atom C(20) was supported by the HMBCs from Me(21) to C(17), C(20) and C(22), as well as from CH_2(23) to C(20). Based on biogenetic considerations, compound **1** is likely to be generated by the side-chain degradation of cyclocitrinol (**8**) or 24-epicyclocitrinol (**9**) without alteration at the stereogenic center C(20), indicating identical configuration at C(20) for all three compounds. The structure of **1** was thus established and named norcyclocitrinol A.

Compound **2** was also obtained as a white solid with a molecular formula $C_{25}H_{36}O_5$ according to the $[M+H]^+$ peak at *m/z* 417.2641 in the HR-ESI-MS, indicating eight degrees of unsaturation. The IR spectrum displayed absorption bands for OH groups (3389 cm^{-1}) and for a conjugated CO group (1647 cm^{-1}). In the ^{1}H-NMR spectrum of **2**, signals for three olefinic H-atoms at δ(H) 5.66 (*dd*, $J = 7.5$, 7.5, H–C(1)), 5.58 (*s*, H–C(7)) and 5.37 (*d*, $J = 8.5$, H–C(22)), and H-atoms of four O-bearing CH groups at δ(H) 4.30 (*dd*, $J = 8.5$, 4.0, H–C(23)), 3.91 (*ddd*, $J = 15.0$, 10.5, 4.0, H–C(11)), 3.69–3.72 (*m*, H–C(24)), and 3.31–3.40 (*m*, H–C(3)) were readily discerned, as well as resonances assignable to three Me groups at δ(H) 1.78 (*s*, Me(21)), 1.12 (*s*, Me(25)), and 0.63 (*s*, Me(19)). The ^{13}C-NMR and DEPT spectra (*Table*) indicated the presence of one CO group, four quaternary C-atoms, eleven CH, six CH_2, and three Me groups. The ^{1}H- and ^{13}C-NMR data of **2** greatly resemble those of neocyclocitrinols A–D (**4**–**7**, resp.), four isomers sharing the same constitutional formula. The key discrepancy was the replacement of a CH_2 group (CH_2(11)) in **4**–**7** by an O-bearing CH group in **2**. The above mentioned characteristics, in combination with the molecular formula, allowed us to propose a hydroxylated neocyclocitrinol structure for **2**. Further in-depth analysis of the 2D-NMR spectra (*Fig. 2*) enabled us to establish the detailed structure

of **2**. The ^{1}H,^{1}H-COSY plots of H–C(9)/H–C(11) and H–C(11)/CH_2(12) revealed that the additional OH group was at C(11), which was reinforced by the HMBC from H–C(11) to C(10). Thus, compound **2** was shown to possess the same skeleton as **4**–**7** [4]. The orientation of H–C(11) was established as β based on the NOE correlation between H–C(11) and Me(19). The *J*(23,24) value of 4.5 Hz in **2** indicated an *erythro*-configuration of H–C(23) and H–C(24), according to the relevance between the *J*(23,24) values and the 23,24-*erythro*- or 23,24-*threo*-configuration deduced from the data of **4**–**7** [4][5]. Hence, the structure of **2** was elucidated as *erythro*-11α-hydroxyneocyclocitrinol.

Compound **3** was determined to be an isomer of neocyclocitrinols A–D (**4**–**7**, resp.) with the same molecular formula of $C_{25}H_{36}O_4$. The ^{1}H- and ^{13}C-NMR data of **3** were almost superimposable with those of **4**–**7**, except for deviations of the chemical shifts of C(17), C(19), C(20), C(21), C(22), and C(23) (*Table*). These observations indicated that **3** is a 20,22-didehydro stereoisomer of **4**–**7**. The C-atom resonance of C(21) of **4**–**7** was shifted upfield (**4** and **6**: δ(C) 17.3; **5** and **7**: δ(C) 18.9 and 18.7, resp.), and C(17) was shifted downfield (**4** and **6**: δ(C) 58.9; **5** and **7**: δ(C) 58.6, resp.) due to γ-gauche effect compared with that of **3** [5][6]. The configuration of the C(20)=C(22) bond was determined as (*Z*) and confirmed by the NOE correlations between H–C(23) and H–C(17). A *J*(23,24) value of 5.0 Hz in **3** suggested an *erythro*-configuration around H–C(23)/H–C(24) as in the case of **2**. The structure of **3** was thus established and named pesudocyclocitrinol A.

The known compounds were identified as neocyclocitrinols A–D (**4**–**7**, resp.), cyclocitrinol (**8**), and 24-epicyclocitrinol (**9**) by comparison of their spectroscopic data with those in the literature [5].

Compounds **1**–**9** were evaluated for their cytotoxic activities against HeLa and HepG2 cell lines. However, no compound exhibited a significant cytotoxicity at a concentration of 20 μM.

The isolates belong to a class of rare steroids featuring an unusual bicyclo[4.4.1] *A*/*B* ring system which was proposed to originate from ergosterol *via* a 1,2 migration of the C(5)–C(10) bond to give a new HC(5)–CH_2(18) bond [7]. To date, only 16 natural products with this unique skeleton have been reported before our present work [6–8]. Different structures and stereoisomerism within the side chains greatly contributed to the structural diversities of the C_{25}-steroid family. As in the case of our work, nine compounds comprised four different types of side chains. In particular, norcyclocitrinol A (**1**) represented the first example of C_{25} steroids with a bisnor C-atom side chain. Interestingly, all these compounds were metabolized by fungi of the genus *penicillium*. It is, therefore, reasonable to infer that these fungi may have developed unique gene cluster(s) responsible for the biosynthesis of these steroids during the long periods of evolution.

Experimental Part

General. Solvents were of anal. grade (*Shanghai Chemical Plant*) and filtered through a microporous membrane of 0.45 μm before being used for semi-prep. HPLC separation. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), *MCI CHP20P gel* (75–150 μm; *Mitsubishi Chemical Industries Ltd.*, Japan), *ODS C-18* gel (50 μm; *YMC Co.*

Ltd., Kyoto, Japan), and *Toyopearl HW-40C* gel (50–100 μm; *Tosoh Corpora tion*, Japan). Semi-prep. HPLC: *Sepax GP C18* (5 μm, 20 mm × 250 mm), *Waters 600* pump, *Waters 2487* UV detector, and *N 2000* chromatography workstation. TLC: Pre-coated silica gel GF_{254} plates (*Qingdao Marine Chemical Inc.*, Qingdao, P. R. China); visualization with UV light and 10% H_2SO_4/EtOH. Optical rotations: *Rudolph Research Autopol III* automatic polarimeter. UV Spectra: *Shimadzu-UV-2450* spectrometer. IR Spectra: *Thermo-Nicolet-6700* FT-IR microscope instrument (FT-IR microscope transmission). NMR Spectra: *Bruker-AM-500* apparatus; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI- and HR-ESI-MS: *Agilent-6210-LC/TOF* mass spectrometer; in *m*/*z*.

Fungus and Culture Conditions. The fungus was isolated from the stems of a traditional Chinese medicinal plant *Huperzia serrata* collected in Xishuangbanna Tropical Plant Garden, Chinese Academy of Sciences, Yunnan Province, P. R. China, in September 2007. It was identified as *Penicillium chrysogenum* based on the DNA sequence analysis conducted by *Sangon Biotech* (Shanghai) *Co. Ltd.* The original culture was deposited with the Zhejiang University of Technology under the deposit code HS-ZJUT-P1X. The cultivation was carried out on shakers at 28° and 185 rpm for 6 d in liquid PD medium (potato extracts, 200 g; glucose, 20 g, dist. H_2O, 1 l), followed by static cultivation for another 24 d.

Extraction and Isolation. The cultures (40 l) were filtered through cheesecloth to separate broth and mycelium. The broth was condensed under reduced pressure at 40° to *ca.* 5 l which was partitioned with AcOEt (5 × 3 l). The AcOEt extract was evaporated under reduced pressure to yield a residue (18 g), which was subjected to CC (SiO_2; petroleum ether/Me_2CO 10 : 1 → 1 : 1 (*v*/*v*)): *Frs. A–D. Fr. B* was then successively subjected to CC (*Toyopearl HW-40C*; MeOH; then, *ODS C-18*; MeOH/H_2O 20 : 80 → 75 : 25 (*v*/*v*)): *Frs. B1A–B1C.* The three sub-fractions were all separated by semi-prep. HPLC. *Fr. B1A* (*Sepax GP C18*; MeOH/H_2O 55 : 45): **8** (2.3 mg) and **9** (1.5 mg); *Fr. B1B* (*Sepax GP C18*; MeOH/H_2O 55 : 45): **4** (2.2 mg), **5** (4.9 mg), **6** (8.7 mg), and **7** (6.5 mg); *Fr. B1C* (*Sepax GP C18*; MeOH/H_2O 60 : 40): **1** (3.2 mg) and **3** (5.6 mg). *Fr. C* was successively submitted to CC (*Toyopearl HW-40C*; MeOH; then, *ODS C-18*; MeOH/H_2O 40 : 60): **2** (2.1 mg).

Norcyclocitrinol A (= *(3*S*,3a*S*,5a*R*,9*S*,11*S*,13b*R*)-3-[(2*S*)-2,4-Dihydroxybutan-2-yl]-1,2,3,3a,4,5,5a,8,9,10,11,13b-dodecahydro-9-hydroxy-3a-methyl-12*H*-6,11-methanocyclodeca[*e*]inden-12-one*; **1**). White powder. $[\alpha]_D^{20} = +30.0$ ($c = 0.02$, $CHCl_3$). UV ($CHCl_3$): 242 (3.83). IR: 3401, 3348, 2874, 1654, 1370, 1033, 866. ^1H- and ^{13}C-NMR: see the *Table.* HR-ESI-MS: 375.2540 ($[M + H]^+$, $C_{23}H_{35}O_4^+$; calc. 375.2530).

erythro-*11α-Hydroxyneocyclocitrinol* (= *(3*R*,3a*R*,5*R*,5a*R*,9*S*,11*S*,13b*R*)-3-[(2*E*)-*erythro*-4,5-Dihydroxyhex-2-en-2-yl]-5,9-dihydroxy-3a-methyl-1,2,3,3a,4,5,5a,8,9,10,11,13b-dodecahydro-12*H*-6,11-methanocyclodeca[*e*]inden-12-one*; **2**). White powder. $[\alpha]_D^{20} = +85.0$ ($c = 0.08$, MeOH). IR: 3375, 2939, 2873, 1647, 1457, 1377, 1330, 1252, 1179, 1126, 867. UV (MeOH): 242 (4.11). ^1H- and ^{13}C-NMR: see the *Table.* HR-ESI-MS: 417.2641 ($[M + H]^+$, $C_{25}H_{37}O_5^+$; calc. 417.2636).

Pesudocyclocitrinol A (= *(3*R*,3a*R*,5a*R*,9*S*,11*S*,13b*R*)-3-[(2*Z*)-*erythro*-4,5-Dihydroxyhex-2-en-2-yl]-1,2,3,3a,4,5,5a,8,9,10,11,13b-dodecahydro-9-hydroxy-3a-methyl-12*H*-6,11-methanocyclodeca[*e*]inden-12-one*; **3**). White powder. $[\alpha]_D^{20} = +17.9$ ($c = 0.11$, $CHCl_3$). IR: 3367, 2925, 2854, 1647, 1460, 1253, 1007, 774. UV ($CHCl_3$): 242 (3.95). ^1H- and ^{13}C-NMR: see the *Table.* HR-ESI-MS: 423.2502 ($[M + Na]^+$, $C_{25}H_{36}NaO_4^+$; calc. 423.2511).

Cytotoxicity Assay. Compounds **1**–**9** were evaluated for cytotoxicity against HeLa and HepG2 cells by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay according to a standard protocol [9].

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New Dammarane-Type Saponins from the Roots of *Panax notoginseng*

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Three new dammarane-type triterpenoid saponins, **1**–**3**, were isolated and identified as (20*S*)-20-*O*-[*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]dammar-24-ene-3*β*,6*α*,12*β*,20-tetrol (**1**), (20*S*)-6-*O*-[(*E*)-but-2-enoyl-(1→6)-*β*-D-glucopyranosyl]dammar-24-ene-3*β*,6*α*,12*β*,20-tetrol (**2**), and (20*S*)-6-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-xylopyranosyl]dammar-24-ene-3*β*,6*α*,12*β*,20-tetrol (**3**) from the roots of *Panax notoginseng* (BURKILL) F.H.CHEN (Araliaceae). Their structures were elucidated on the basis of spectroscopic analyses, including 1D- and 2D-NMR techniques and HR-ESI-MS, as well as by acidic hydrolysis.

Introduction. – All saponins present in the roots of *Panax notoginseng*, as well as their derivatives, have been approved by the State Food and Drug Administration in China as clinical drugs, which are widely used in the prevention and treatment of cardiovascular diseases. Recent pharmaceutical studies have disclosed diverse bioactivities of the saponins from *Panax notoginseng*, such as anti-inflammatory [1][2], antitumor [3][4], antioxidant [5], hepatoprotective [6], immunomodulative, and immune-adjunctive activities [7]. A detailed phytochemical investigation of the root of *Panax notoginseng* was carried out in the present work. As a result, three new dammarane-type saponins, **1**–**3**, one natural compound, **7**, and other 20 known dammarane-type saponins, **4**–**6** and **8**–**24**, were isolated and identified, of which **14** and **15** were isolated for the first time.

Results and Discussion. – The 80% EtOH extract of the air-dried root of *Panax notoginseng* was chromatographed repeatedly to afford compounds **1**–**24** (*Figs. 1* and *2*). Three new minor saponins, **1**–**3**, and one new natural compound, 6′-*O*-acetylginsenoside Rh_1 (**7**) [8], along with 20 known compounds, 6-*O*-(*β*-D-glucopyranosyl)-20-*O*-(*β*-D-xylopyranosyl)-3*β*,6*α*,12*β*,20(*S*)-tetrahydroxydammar-24-ene (**4**) [9], (20*S*)-ginsenoside Rh_1 (**5**) [10], (20*R*)-ginsenoside Rh_1 (**6**) [11], (20*S*)-ginsenoside Rg_2 (**8**) [12], notoginsenoside-R_2 (**9**) [10], ginsenoside-F_1 (**10**) [13], ginsenoside Rg_1 (**11**) [14], notoginsenoside-R_1 (**12**) [10], ginsenoside Re (**13**) [15], (20*S*)-protopanaxatriol-20-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**14**) [16], ginsenoside-Rh_4 (**15**) [17], vinaginsenoside R_4 (**16**) [18], (20*R*)-ginsenoside Rg_3 (**17**) [19], ginsenoside-Rd (**18**) [18], ginsenoside Rb_1 (**19**) [14], ginsenoside Ra_3 (**20**) [20][21], notoginsenoside

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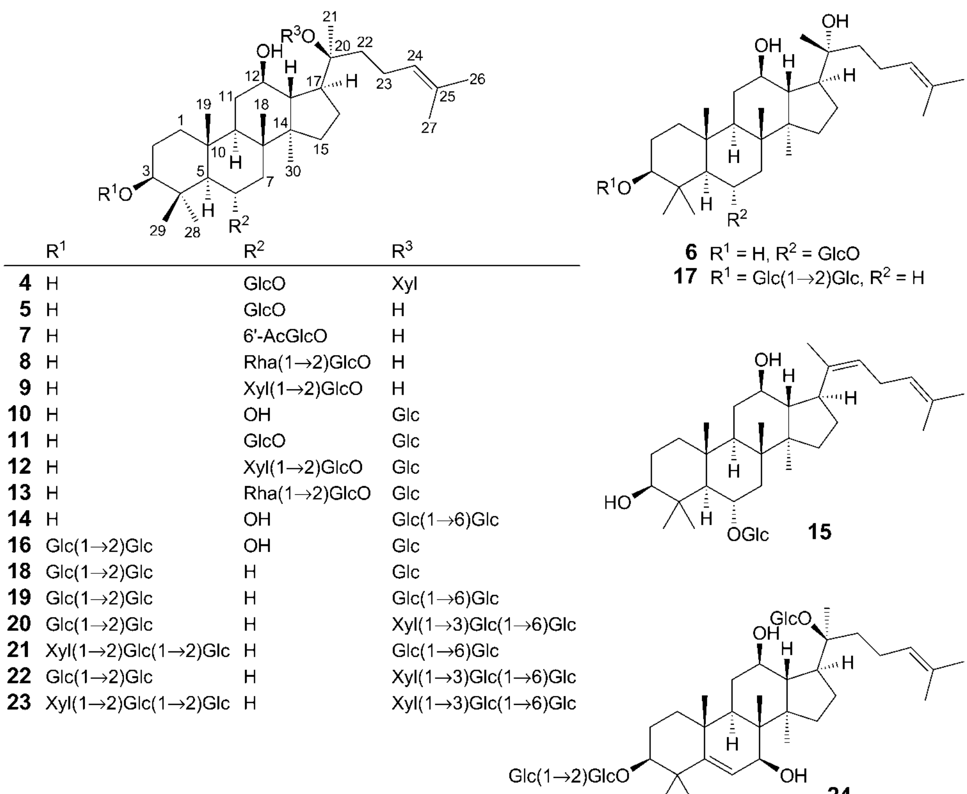
Fig. 1. *New compounds* **1**–**3** *isolated from the root of* Panax notoginseng



	R¹	R²	R³
4	H	GlcO	Xyl
5	H	GlcO	H
7	H	6'-AcGlcO	H
8	H	Rha(1→2)GlcO	H
9	H	Xyl(1→2)GlcO	H
10	H	OH	Glc
11	H	GlcO	Glc
12	H	Xyl(1→2)GlcO	Glc
13	H	Rha(1→2)GlcO	Glc
14	H	OH	Glc(1→6)Glc
16	Glc(1→2)Glc	OH	Glc
18	Glc(1→2)Glc	H	Glc
19	Glc(1→2)Glc	H	Glc(1→6)Glc
20	Glc(1→2)Glc	H	Xyl(1→3)Glc(1→6)Glc
21	Xyl(1→2)Glc(1→2)Glc	H	Glc(1→6)Glc
22	Glc(1→2)Glc	H	Xyl(1→3)Glc(1→6)Glc
23	Xyl(1→2)Glc(1→2)Glc	H	Xyl(1→3)Glc(1→6)Glc

Fig. 2. *Chemical structures of* **4**–**24** *isolated from the root of* Panax notoginseng

Fa (**21**) [19], notoginsenoside R_4 (**22**) [14], notoginsenoside D (**23**) [22], and notoginsenoside-G (**24**) [23], were also isolated and identified by comparison of their

spectroscopic data with those reported in the literature. Among them, two known compounds, **14** and **15**, were isolated for the first time from *Panax notoginseng*.

Compound **1** was obtained as a white, amorphous powder. Its molecular formula was determined as $C_{47}H_{80}O_{18}$ by HR-ESI-MS (*m/z* 955.5229 ($[M+Na]^+$, $C_{47}H_{80}NaO_{18}^+$; calc. 955.5242)). The ^{1}H- and ^{13}C-NMR data (*Table*) of **1** were very similar to those of ginsenoside F_1 (**10**), except for two sets of signals due to a glucose and a xylose units. In the ^{1}H-NMR ((D_5)pyridine) spectrum of **1**, diagnostic signals were found for a sapogenin moiety with those of eight Me groups at δ(H) 0.97, 1.01, 1.09, 1.44, 1.60, 1.64, 1.64, 1.97 (each *s*, Me(30), Me(18), Me(19), Me(29), Me(26), Me(21), Me(27), Me(28), resp.), and of an olefinic H-atom at δ(H) 5.31 (*t*, $J=6.2$, H–C(24)). In the ^{13}C-NMR spectrum of **1**, 47 C-atom signals were detected, including two olefinic C-atom signals of C(24) (δ(C) 126.0) and C(25) (δ(C) 131.0). The signals of C(5) and C(20) were shifted downfield to δ(C) 61.7 [24] and 83.4, respectively. In addition, the ^{1}H-NMR spectrum showed signals for three anomeric H-atoms at δ(H) 4.94 (*d*, $J=7.5$, H–C(1‴)), 5.01 (*d*, $J=7.7$, H–C(1″)), and 5.11 (*d*, $J=7.8$, H–C(1′)), which showed HSQCs to anomeric C-atom signals at δ(C) 105.9 (C(1‴)), 105.5 (C(1″)) and 98.0 (C(1′)), respectively. Based on the coupling constants of the anomeric H-atoms, all sugar substituents were identified as β-configured.

Acid hydrolysis of **1** revealed the presence of xylose and glucose moieties, which were in relative proportions of 1:2, as determined by GC/MS analysis [25]. All these data suggested that compound **1** was a protopanaxatriol-type ginsenoside, and that the trisaccharide unit was attached to C(20). The exact oligoglycoside structure at C(20) in **1** was determined from the HMBC spectrum, which showed HMBCs from H–C(1′) (δ(H) 5.11) to C(20) (δ(C) 83.4), from H–C(1″) (δ(H) 5.01) to C(6′) (δ(C) 70.3), and from H–C(1‴) (δ(H) 4.94) to C(6″) (δ(C) 69.9) (*Fig. 3*). The relative configurations at the ring junctions were confirmed by a NOESY spectrum, which revealed the correlations from H–C(3) (δ(H) 3.48–3.53) to H–C(5) (δ(H) 1.20) and Me(28) (δ(H) 1.97), from H–C(6) (δ(H) 4.37–4.39) to Me(18) (δ(H) 1.01) and Me(19) (δ(H) 1.09), from H–C(12) (δ(H) 4.19–4.23) to H–C(9) (δ(H) 1.52–1.56) and Me(30) (δ(H) 0.97) (*Fig. 4*). The NOE correlation from Me(17) (δ(H) 2.57) to H–C(12) (δ(H) 4.19–4.23), Me(21) (δ(H) 1.64), and Me(30) (δ(H) 0.97) suggested that the configuration at C(20) should be (*S*). Furthermore, the HMBCs from Me(26) (δ(H) 1.60) and Me(27) (δ(H) 1.64) to C(24) (δ(C) 126.0) and from H–C(24) (δ(H) 5.31) to C(26) (δ(C) 25.8) and C(27) (δ(C) 17.9) confirmed that the C=C bond was located at the side train. The ^{1}H- and ^{13}C-NMR assignments for **1** were accomplished unambiguously based on HSQC, HMBC, and TOCSY data. Thus, the structure of **1** was determined as (20*S*)-20-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol.

Compound **2** was obtained as an amorphous powder. Its molecular formula was determined as $C_{40}H_{66}O_{10}$ by HR-ESI-MS (*m/z* 1413.9362 ($[2M+H]^+$, $C_{80}H_{133}O_{20}^+$; calc. 1413.9390)) and NMR data. The ^{1}H- and ^{13}C-NMR data (*Table*) of **2** were very similar to those of ginsenoside Rh_1 (**5**), except for a set of signals arising from the presence of a butenoyl unit. In the ^{1}H-NMR ((D_5)pyridine) spectrum of **2**, signals of eight Me groups at δ(H) 0.91, 1.04, 1.23, 1.41, 1.55, 1.61, 1.65, 2.01 (each *s*, Me(30), Me(19), Me(18), Me(21), Me(29), Me(26), Me(27), Me(28), resp.) and of an olefinic H-atom at δ(H) 5.31 (*t*, $J=7.2$, H–C(24)) were displayed, and in the ^{13}C-NMR spectrum of **2**, 40 C-atom

Table. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp.) *Data of Compounds* **1**–**3**. In (D_5)pyridine; δ in ppm, *J* in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	0.99[a]), 1.70–1.74 (*m*)	39.3	1.69[a]), 1.00–1.03 (2*m*)	39.3	1.64–1.67, 0.97–1.00 (2*m*)	39.4
2	1.83[a]), 1.90–1.93 (*m*)	28.1	1.88–1.91, 1.81–1.85 (2*m*)	27.9	1.79–1.81, 1.82–1.86 (2*m*)	27.7
3	3.48–3.53 (*m*)	78.5	3.50 (br. *d*, *J* = 12.2)	78.6	3.45–3.49 (*m*)	78.6
4		40.3		40.2		40.0
5	1.20 (*d*, *J* = 10.4)	61.7	1.41–1.43 (*m*)	61.4	1.38[a])	61.2
6	4.37–4.39 (*m*)	67.7	4.38 (*td*, *J* = 10.5, 3.2)	80.0	4.32[a])	78.8
7	1.85[a]), 1.93–1.95 (*m*)	47.5	2.51 (*dd*, *J* = 12.6, 3.3), 1.93–1.97 (*m*)	45.6	1.93 (*t*, *J* = 11.6), 2.27[a])	45.3
8		41.2		41.2		41.1
9	1.52–1.56 (*m*)	49.9	1.57[a])	50.1	1.52[a])	50.0
10		39.3		39.7		39.6
11	1.59[a]), 2.04–2.09 (*m*)	30.8	2.12–2.16 (*m*), 1.55[a])	32.1	2.09–2.12 (*m*), 1.51[a])	32.0
12	4.19–4.23 (*m*)	70.1	3.90–3.94 (*m*)	71.0	3.90 (*t*, *J* = 9.8)	70.7
13	1.98–2.00 (*m*)	49.1	2.06–2.10 (*m*)	48.2	2.02[a])	48.2
14		51.3		51.7		51.6
15	0.99[a]), 1.57[a])	30.7	1.71–1.73, 1.16–1.20 (2*m*)	31.5	1.56–1.59, 1.01–1.06 (2*m*)	31.3
16	1.28–1.34 (*m*), 1.82[a])	26.6	1.80–1.86, 1.37–1.40 (2*m*)	26.9	1.78–1.82 (*m*), 1.37[a])	26.8
17	2.57[a])	51.5	2.30–2.34 (*m*)	54.8	2.29[a])	54.7
18	1.01 (*s*)	17.6	1.23 (*s*)	17.6	1.12 (*s*)	17.3
19	1.09 (*s*)	17.4	1.04 (*s*)	17.4	0.96 (*s*)	17.6
20		83.4		72.9		72.9
21	1.64 (*s*)	22.3	1.41 (*s*)	27.0	1.39 (*s*)	27.0
22	1.75–1.77, 2.33–2.37 (2*m*)	36.2	2.05[a]), 1.69[a])	35.8	2.04[a]), 1.68–1.72 (*m*)	35.8
23	2.37–2.40 (*m*), 2.58[a])	23.1	2.58–2.64, 2.25–2.30 (2*m*)	22.9	2.58–2.62 (*m*), 2.26[a])	23.0
24	5.31 (*t*, *J* = 6.2)	126.0	5.31 (*t*, *J* = 7.2)	126.2	5.30 (*t*, *J* = 7.1)	126.3
25		131.0		130.8		130.7
26	1.60 (*s*)	25.8	1.61 (*s*)	25.8	1.60 (*s*)	25.8
27	1.64 (*s*)	17.9	1.65 (*s*)	17.9	1.63 (*s*)	17.6
28	1.97 (*s*)	31.9	2.01 (*s*)	31.5	2.03 (*s*)	31.9
29	1.44 (*s*)	16.5	1.55 (*s*)	16.5	1.39 (*s*)	16.9
30	0.97 (*s*)	17.4	0.91(*s*)	16.9	0.87 (*s*)	16.9

Table (cont.)

Position	1		2		3	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	20-Glc		6-Glc		6-Xyl	
1′	5.11 (*d*, *J* = 7.8)	98.0	5.06 (*d*, *J* = 7.8)	106.2	5.09 (*d*, *J* = 6.2)	104.2
2′	3.89 (*t*, *J* = 8.4)	74.8	4.09[a])	75.3	4.40[a])	80.0
3′	4.35 – 4.37 (*m*)	79.3	4.22 (*t*, *J* = 8.9)	79.2	4.34[a])	77.6
4′	4.18 – 4.21 (*m*)	71.6	4.01 (*t*, *J* = 9.3)	71.5	4.20[a])	70.9
5′	4.03[a])	76.9	4.09[a])	75.1	4.41[a]), 3.73 (*dd*, *J* = 11.4, 8.9)	66.2
6′	4.75 (*dd*, *J* = 11.5, 1.3), 4.30[a])	70.3	5.11 (*dd*, *J* = 11.7, 1.6), 4.78 (*dd*, *J* = 11.7, 6.7)	65.1		
	6′-Glc		6′-But-2-enoyl		6′-Xyl	
1″	5.01 (*d*, *J* = 7.7)	105.5		166.5	5.65 (*d*, *J* = 7.4)	104.9
2″	3.96 (*t*, *J* = 8.1)	75.1	6.05 (*dq*, *J* = 15.6, 1.4)	123.1	4.15[a])	75.6
3″	4.14[a])	78.3	7.09 (*dq*, *J* = 15.8, 6.8)	144.8	4.15[a])	78.7
4″	4.15[a])	71.5	1.74 (*dd*, *J* = 6.9, 1.7)	17.6	4.22[a])	71.2
5″	4.03[a])	77.0			4.31[a]), 3.64 (*t*, *J* = 10.7)	67.3
6″	4.29[a]), 4.80 (*dd*, *J* = 11.5, 2.0)	69.9				
	6″-Xyl					
1‴	4.94 (*d*, *J* = 7.5)	105.9				
2‴	4.01[a])	74.8				
3‴	4.11[a])	78.1				
4‴	4.02[a])	71.1				
5‴	3.63 – 3.67, 4.30 – 4.32 (2*m*)	67.1				

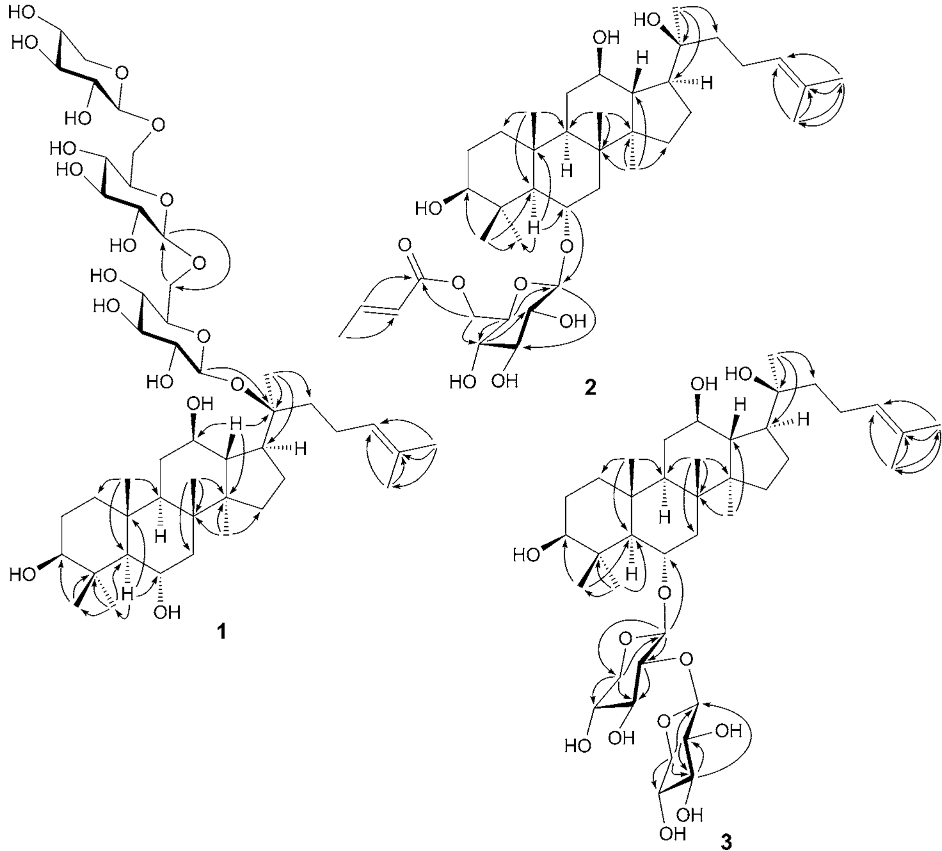


Fig. 3. *Key HMBCs of* **1**–**3**

signals were displayed, including those of two olefinic C-atom signals of C(24) (δ(C) 126.2) and C(25) (δ(C) 130.8). The signals of C(5) and C(6) were shifted downfield to δ(C) 61.4 and 80.0, respectively. In addition, the ^{1}H-NMR spectrum showed an anomeric H-atom signal at δ(H) 5.06 (*d*, *J* = 7.8, H–C(1′)), which showed HSQC to the C-atom signal at δ(C) 106.2 (C(1′)). Based on the coupling constant of the anomeric H-atom, the sugar substituent was identified as β-configurated, and acid hydrolysis of **2** revealed the presence of a glucose moiety, identified by GC/MS analysis [25]. The above data suggested that compound **2** was also a protopanaxatriol-type ginsenoside, and that the sugar unit was at C(6). The location of the sugar unit at C(6) (δ(C) 80.0) was established by the HMBC experiment (*Fig. 3*). The anomeric H-atom at δ(H) 5.06 (*d*, *J* = 7.8, H–C(1′)) was correlated through a three-bond coupling with C(6) (δ(C) 80.0), and the H–C(6) signal (δ(H) 4.38) correlated, in turn, with the anomeric C-atom signal at δ(C) 106.2 (C(1′)). The presence of a butenoyl group was confirmed on the basis of HSQC and HMBC data (HMBCs from H–C(3″) (δ(H) 7.09) to C(1″) (δ(C) 166.5), and from H–C(4″) (δ(H) 1.74) to C(2″) (δ(C) 123.1) and C(3″) (δ(C) 144.8)).

The HMBCs from H–C(6′) (δ(H) 5.11) to C(1″) (δ(C) 166.5), coupled with the C-atom signal of C(6′) downfield-shifted (δ(C) 63.0 to 65.1) by comparing it with that of ginsenoside Rh_1, revealed that the butenoyl group was at C(6′) of the glucose unit. The relative configurations at the ring junctions were confirmed by NOESY spectrum, which have revealed the correlations from H–C(3) (δ(H) 3.50) to H–C(5) (δ(H) 1.41 – 1.43) and Me(28) (δ(H) 2.01), from H–C(6) (δ(H) 4.38) to Me(18) (δ(H) 1.23) and Me(19) (δ(H) 1.04), and from H–C(12) (δ(H) 3.90 – 3.94) to H–C(9) (δ(H) 1.57) and Me(30) (δ(H) 0.91) (*Fig. 4*). The NOE correlations from H–C(17) (δ(H) 2.30 – 2.34) to H–C(12) (δ(H) 3.90 – 3.94), Me(21) (δ(H) 1.41), and Me(30) (δ(H) 0.91) suggested that the configuration at C(20) should be (*S*). Furthermore, the HMBCs from Me(26) (δ(H) 1.61) and Me(27) (δ(H) 1.65) to C(24) (δ(C) 126.2), from H–C(24) (δ(H) 5.31) to C(26) (δ(C) 25.8) and C(27) (δ(C) 17.9) confirmed that the C=C bond is located in the side chain. The ^{1}H- and ^{13}C-NMR assignments for **2** were accomplished unambiguously based on HSQC and HMBC data. Thus, the structure of **2** was determined as (20*S*)-(6-*O*-[(*E*)-but-2-enoyl-(1 → 6)-β-D-glucopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol.

Fig. 4. *Selected NOESY correlations of* **1** – **3**

Compound **3** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{40}H_{68}O_{12}$ by HR-ESI-MS (*m/z* 763.4605 $[M+Na]^+$, $C_{40}H_{68}NaO_{12}^+$; calc.763.4608) and NMR data. Comparing the ^{1}H- and ^{13}C-NMR data of **3** with those of **1** and **2**, all these three compounds turned out to possess the same aglycone moiety. In the ^{1}H-NMR ((D_5)pyridine) spectrum of **3**, signals of eight Me groups at δ(H) 0.87, 0.96, 1.12, 1.39 (2 ×), 1.60, 1.63, 2.03 (each *s*, Me(30), Me(19), Me(18), Me(21), Me(29), Me(26), Me(27), Me(28), resp.) and of an olefinic H-atom at δ(H) 5.30 (*t*, *J* = 7.1, H–C(24)) were displayed, and in the ^{13}C-NMR spectrum of **3**, 40 C-atom signals were detected, including those of two olefinic C-atom signals of C(24) (δ(C) 126.3) and C(25) (δ(C) 130.7). The signals of C(5) and C(6) were shifted downfield to δ(C) 61.2, and 78.8, respectively. In addition, the ^{1}H-NMR spectrum exhibited two anomeric H-atom signals at δ(H) 5.09 (*d*, *J* = 6.2, H–C(1′)) and 5.65 (*d*, *J* = 7.4, H–C(1″), which showed HSQCs to C-atom signal at δ(C) 104.2 (C(1′)) and 104.9 (C(1″)). Based on the coupling constant of the anomeric H-atom, the sugar substituent was identified as β-configurated, and acid hydrolysis of **3** revealed the presence of a xylcose moiety, identified by GC/MS analysis [25]. All these data suggested that compound **3** was also a protopanaxatriol-type ginsenoside, and the sugar unit was at C(6). The location of the sugar unit at C(6) (δ(C) 78.8) was established by the HMBC experiment (*Fig. 3*). The signal of the anomeric H-atom at δ(H) 5.09 (*d*, *J* = 6.2, H–C(1′)) correlated through a

three-bond coupling with that of C(6) (δ(C) 78.8), and the H–C(6) signal (δ(H) 4.32) correlated, in turn, with that of the anomeric C-atom at δ(C) 104.2 (C(1′)). The relative configurations at the ring junctions were confirmed by NOESY spectrum, which exhibited correlations from H–C(3) (δ(H) 3.45–4.49) to H–C(5) (δ(H) 1.38) and Me(28) (δ(H) 2.03), from H–C(6) (δ(H) 4.32) to Me(18) (δ(H) 1.12) and Me(19) (δ(H) 0.96), and from H–C(12) (δ(H) 3.90) to H–C(9) (δ(H) 1.52) and Me(30) (δ(H) 0.87) (*Fig. 4*). The NOE correlation from H–C(17) (δ(H) 2.29) to H–C(12) (δ(H) 3.90), Me(21) (δ(H) 1.39), and Me(30) (δ(H) 0.87), suggested that the configuration at C(20) should be (*S*). Furthermore, the HMBCs from Me(26) (δ(H) 1.60) and Me(27) (δ(H) 1.63) to C(24) (δ(C) 126.3), from H–C(24) (δ(H) 5.30) to C(26) (δ(C) 25.8) and C(27) (δ(C) 17.6) confirmed that the C=C bond was located in the side train. The ^{1}H- and ^{13}C-NMR assignments for **3** were achieved unambiguously based on HSQC and HMBC data. Thus, the structure of **3** was determined as (20*S*)-6-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-xylopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol.

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Experimental Part

General. Column chromatography (CC): silica gel *G* (SiO_2; 100–200 and 200–300 mesh, *Qingdao Sea Chemical Factory*, P. R. China), *D101* macroporous resin (*Cangzhou Bon Adsorber Technology Co., Ltd.*, P. R. China), and *Sephadex LH-20* (*Amersham Biosciences*, Germany). Medium-pressure liquid-chromatography (MPLC): *MCI* gel (*CHP20P*, 75–150 µm; *Mitsubishi Chemical Corporation*, Japan) and reversed-phase C_{18} silica gel (40–63 µm; *Merck*, Germany). TLC: precoated silica gel *G* plates (*Qingdao Sea Chemical Factory*, P. R. China); visualization with 10% H_2SO_4 in alcohol, followed by heating. MPLC: *Eyela Ceramic VSP 3050* pump, *Eyela* glass column (300 × 30 mm). Prep. HPLC: *Shimadzu LC-6AD* pump, *Shimadzu SPD-20A* UV detector, *YMC ODS-A* (20 × 250 mm, 10 µm). M.p.: *X-4* micro-melting-point apparatus (*Shanghai*, P. R. China); uncorrected. IR Spectra: *PerkinElmer Spectrum One* FT-IR spectrometer. NMR Spectra: *Bruker ARX-600* spectrometer in (D_5)pyridine with TMS as an internal standard. ESI-MS: *Agilent 1100-LC/MS DTrap SL*. HR-ESI-MS: *Waters API QSTAR Pular-1* mass spectrometer and a *Waters Synapt G2* MS mass spectrometer. GC/MS: *Agilent 7000B Triple Quad GC/MS-7890* GC system.

Plant Material. The roots of *Panax notoginseng* were purchased in *Yi Xin Pharmaceutical Co. Ltd*. (Nanning, Guangxi) in October, 2011. The sample was authenticated by TCM-Pharmacist *Jia-Fu Wei* from Guangxi Zhuang Autonomous Region Food and Drug Administration. A voucher specimen No. SQ20111019 was deposited with the Laboratory of Natural Products of the College of Pharmacy, Guangxi Medical University.

Extraction and Isolation. Dried roots (8.9 kg) of *Panax notoginseng* were extracted with tenfold 80% EtOH under reflux, and *ca.* 1,800 g of extract were obtained. The extract was suspended in H_2O and then partitioned with CH_2Cl_2 and BuOH successively.

The BuOH (1517 g) extract was subjected to CC (*D101* macroporous resin; H_2O/EtOH 100:0 → 0:100): *Frs. B1–B5*. *Fr. B3* (256 g) was subjected to CC (*D101* macroporous resin; H_2O/EtOH 90:10 → 70:30): *Frs. B31–B32*. *Fr. B32* (56 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 100:1 → 0:100): *Frs. B321–B327. Fr. B326* (10 g) was recrystallized from MeOH/H_2O to yield **11** (5536 mg). The mother soln. from *Fr. B326* was subjected to MPLC (RP C_{18} silica gel; H_2O/MeOH 100: 0 → 0:100): *Frs. B3261–B3266*. *Fr. B3264* (0.21 g) and *Fr. B3265* (0.53 g) were submitted to prep. HPLC (MeOH/

H_2O 70 : 30) to afford **1** (51 mg), **24** (141 mg), and **16** (145 mg). *Fr. B325* (37 g) was subjected to MPLC (RP C_{18} silica gel; H_2O/MeOH 100: 0 → 0 : 100): *Frs. B3251 – B3255*. *Fr. B3251* (0.2 g) was submitted to prep. HPLC (MeOH/H_2O 50 : 50) to furnish **12** (17 mg). *Fr. B3254* (1.4 g) was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH, 1 : 1) and prep. HPLC (MeOH/H_2O 53 : 47) to afford **4** (79 mg), **11** (105 mg), and **14** (53 mg). *Fr. B327* (8.8 g) was subjected to MPLC (RP C_{18} silica gel; H_2O/MeOH 100: 0 → 0 : 100): *Frs. B3271 – B3277*. *Fr. B3275* (7.7 g) was separated by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) and prep. HPLC (MeOH/H_2O 62 : 38) to give **19** (5536 mg), **20** (87 mg), **21** (422 mg), **22** (320 mg), and **23** (68 mg). *Fr. B5* (150 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 100 : 0 : 0 → 6 : 4 : 0.4): *Frs. B51 – B55*. *Fr. B55* (80 g) was purified by CC (SiO_2; $CHCl_3$/MeOH 10 : 1 → 1 : 1): *Frs. B521 – B524*. *Fr. B523* (8.1 g) was separated by MPLC (*MCI* gel; H_2O/MeOH, 30 : 70 → 0 : 100) and prep. HPLC (MeOH/H_2O 70 : 30) to yield **6** (129 mg) and **10** (30 mg). *Fr. B524* (2.1 g) was separated by MPLC (RP C_{18} silica gel; H_2O/MeOH 70: 30 → 0 : 100), MPLC (*MCI* gel; H_2O/MeOH 100 : 0 → 0 : 100), and prep. HPLC (MeOH/H_2O 50 : 50) to furnish **3** (9 mg), **5** (100 mg), **8** (100 mg), and **9** (50 mg). *Fr. B54* (100 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 8 : 2 → 7 : 3): *Frs. B541 – B543*. *Fr. B543* (92 g) was re-subjected to CC (SiO_2; AcOEt/EtOH/H_2O 5 : 1 : 0 → 6 : 3 : 0.3) to afford **17** (200 mg) and **18** (531 mg).

The CH_2Cl_2 extract (72 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 1 : 0 → 5 : 1): *Frs. C1 – C7*. *Fr. C6* (8.2 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 20 : 1 → 5 : 1): *Frs. C61 – C65*. *Fr. C64* (2.8 g) was submitted to MPLC (*MCI* gel; H_2O/MeOH, 50 : 50 → 0 : 100): *Frs. C641 – C644*. *Fr. C641* (1.1 g) was subjected to MPLC (RP C_{18} silica gel; H_2O/MeOH 100: 0 → 0 : 100), and prep. HPLC (MeOH/H_2O 60 : 40) to give **2** (15 mg), **7** (54 mg), **15** (15 mg), and **16** (135 mg).

*(20*S*)-20-*O*-[β-*D*-Xylopyranosyl-(1 → 6)-β-*D*-glucopyranosyl-(1 → 6)-β-*D*-glucopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol* (*= (3β,6α,12β)-3,6,12-Trihydroxydammar-24-en-20-yl* O*-β-*D*-Xylopyranosyl-(1 → 6)-*O*-β-*D*-glucopyranosyl-(1 → 6)-β-*D*-glucopyranoside*; **1**). White amorphous power (MeOH). M.p. 187 – 188°. IR (KBr): 3399, 2927, 1638, 1384, 1042. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 955.5229 ($[M + Na]^+$, $C_{47}H_{80}NaO_{18}^+$; calc. 955.5242).

*(20*S*)-6-*O*-[(*E*)-But-2-enoyl-(1 → 6)-β-*D*-glucopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol* (*= (3β,6α,12β)-3,12,20-Trihydroxydammar-24-en-6-yl 6-*O*-[(2*E*)-1-Oxobut-2-en-1-yl]-β-*D*-glucopyranoside*; **2**). White amorphous power (MeOH). M.p. 159 – 160°. IR (KBr): 3399, 2933, 2962, 1712, 1655, 1384, 1029. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 1413.9362 ($[2 M + H]^+$, $C_{80}H_{133}O_{20}^+$; calc. 1413.9390).

*(20*S*)-6-*O*-[β-*D*-Xylopyranosyl-(1 → 2)-β-*D*-xylopyranosyl]dammar-24-ene-3β,6α,12β,20-tetrol* (*= (3β,6α,12β)-3,12,20-Trihydroxydammar-24-en-6-yl 2-*O*-β-*D*-Xylopyranosyl-β-*D*-xylopyranoside*; **3**). White amorphous power (MeOH). M.p. 171 – 172°. IR (KBr): 3391, 2932, 1642, 1384, 1033. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 763.4605 ($[M + Na]^+$, $C_{40}H_{68}NaO_{12}^+$; calc. 763.4608).

Acid Hydrolysis of **1** – **3**. Each compound (1.5 mg) was hydrolyzed with 1.5 ml of 1M HCl at 100° for 4 h. The mixture was extracted with $CHCl_3$ (3 ×), and the aq. residue was evaporated under reduced pressure. Then, 1 ml of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the residue, and the mixture was heated at 90° for 1 h. After cooling, Ac_2O (0.5 ml) was added, and the mixture was heated at 90° for 1 h. The mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC/MS using standard aldononitrile peracetates as reference samples.

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A Stereoselective Aldol Approach for the Total Syntheses of Two 6-Alkylated 2*H*-Pyran-2-ones

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A simple and highly efficient stereoselective total synthesis of the 6-alkylated pyranones (6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one (**1**) and (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**2**) was developed using *Crimmins*' aldol reaction, SmI_2 reduction, *Grubbs-II*-catalyzed olefin cross-metathesis, and *Still*'s modified *Horner–Wadsworth–Emmons* reaction.

Introduction. – The α-pyrone (=2*H*-pyran-2-one) ring system occurs in a number of natural products and is also featured in many intermediates that are required for the synthesis of biologically important compounds [1]. 5,6-Dihydro-α-pyrone-containing natural products with a substituted arylalkyl side chain at C(6) have attracted much attention over the last decade due to the *Michael*-acceptor nature of the α,β-unsaturated α-pyrones for the amino acid residues of receptors [2]. In particular, α,β-unsaturated α-pyrones have been shown to exhibit a wide range of biological activities including inhibition of HIV protease, and antileukemic, anticancer, antifeedent, antifungal, antibacterial, and antitumor activities [3]. Most of these compounds carry a (poly)hydroxylated chain at C(6) of the α-pyrone moiety, *e.g.*, (6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one (**1**), (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**2**) and, (+)-strictifolione (**3**) (*Fig.*). Compounds **1** and **2** were isolated from *Ravensara crassifolia* by *Hostetmann* and co-workers [4a], and a structurally similar compound **3** was isolated by *Aimi* and co-workers [4b] from the stem bark of *Cryptocarya strictifolia*. The structures and absolute configurations were established through NMR spectroscopic studies. Compounds **1** and **2** exhibited antifungal activities against the phytopathogenic fungus *Cladosporium cucumarinum*. Therefore, the syntheses of both compounds **1** [5] and **2** [6] have recently become attractive targets to organic chemist. Recently, we reported the synthesis of compound **2** [6a] and evaluated its biological properties. Our continuing interest towards the total synthesis of lactone-containing natural products [7] and important biological properties of **1** and **2** (*Fig.*) prompted us to undertake the

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Figure. *Structures of (6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one* (**1**), *(6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one* (**2**), *and (+)-strictifolione* (**3**)

synthesis of these compounds starting from a common, commercially available starting material.

Herein, we report the stereoselective synthesis of (6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one (**1**) by applying *Crimmins*' aldol reaction, SmI_2 reduction of an alkoxy ketone, *Grubbs-II*-catalyzed olefin cross-metathesis from commercially available 5-phenylpentan-1-ol (**4**) and L-aspartic acid. The synthesis of (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**2**) was accomplished by *Crimmin*'s aldol reaction and *Still*'s modified *Horner–Wadsworth–Emmons* olefination reaction as key steps starting from commercially available 5-phenylpentan-1-ol (**4**). The retrosynthetic analyses of compounds **1** and **2** are depicted in *Scheme 1*.

Results and Discussion. – The synthesis of **1** and **2** started from 5-phenylpentan-1-ol (**4**), which was subjected to oxidation using 2-iodoxybenzoic acid (IBX) in DMSO to give the corresponding aldehyde in 94% yield. The latter was reacted with (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethanone [8] in the presence of EtN^iPr_2 and $TiCl_4$ according to the *Crimmins*' protocol to give the easily separable diastereoisomers of the β-hydroxy amide with the required *syn*-product **5** and *anti*-product **5a** in 82% yield (*syn*/*anti* 8.4:1.6 [9]; *Scheme 2*). The diastereoselectivity of the *Crimmins*' aldol reaction was determined by HPLC (column, *DISCOVERY C8* 250 × 4.6 mm, 5 μm; $MeCN/H_2O$ 60:40; flow rate, 1.0 ml/min, $\lambda = 210$ nm: t_R 17.01 min (minor; 16%), 17.98 min (major; 84%). The OH group in compound **5** was then protected as MOM (methoxymethyl) ether **6**, and subsequent reaction with DIBAL-H afforded aldehyde **7** [6e] (*Scheme 2*), which was subjected to the Zn-mediated allylation in aqueous medium to afford diastereoisomers of secondary alcohol **8** (1:1), which, on further oxidation with *Dess–Martin* periodinane (DMP), furnished the ketone **9**. The stereoselective reduction of the oxo group in **9** with SmI_2 [10a] (*Scheme 2*) in THF and MeOH as proton source for 12 h afforded the required *anti*-1,3-diastereoisomer **10** as the major product (*Yadav et al.* prepared this intermediate *via* a different approach using a *Prins* cyclization [6f]). The 1,3-*anti*-relationship of the two OH groups in **9** was established by analysis of the ^{13}C-NMR spectrum of the corresponding acetonide [10b].

Scheme 1. *Retrosynthetic Analysis*

The lactone **16** is a key intermediate in the syntheses of various compounds and has been prepared by several groups [11]; thus, there is still a need for a simple and efficient procedure for its synthesis. We have prepared **16** according to a known procedure [11e] from the chiral synthon **11**, which was obtained from L-aspartic acid [12] (*Scheme 3*). Opening of epoxide **11** with Me_3SI and BuLi in dry THF provided the secondary allyl alcohol **12** in 77% yield (*Scheme 3*), which was protected as its methoxymethyl (MOM) ether **13**. Deprotection of the Bn group in compound **13** was achieved with lithium naphthalenide [13] to yield the primary alcohol **14** in 81% yield. The OH group of **14** was oxidized using 2-iodoxybenzoic acid (IBX) in DMSO to give the corresponding aldehyde in 85% yield, which was subjected to *Still*'s modified *Horner–Wadsworth–Emmons* olefination reaction [14] to afford the unsaturated ester **15** in 76% yield. Compound **15** was treated with TsOH in benzene to afford 5,6-dihydro-6-vinyl-α-pyrone **16** in 80% yield (*Scheme 3*).

Finally, compounds **10** and **16** (in a 1:3 ratio) were subjected to olefin cross-metathesis using *Grubbs-II* catalyst (5 mol-%) [15] in CH_2Cl_2 under reflux conditions to yield the desired compound **17** in 74% yield (*Scheme 4*). The MOM protecting group in **17** was removed by treatment with $CeCl_3 \cdot 7\ H_2O$, MeCN/MeOH 1:1 to afford **1** in 78% yield (*Scheme 4*). The optical rotation, ^1H- and ^{13}C-NMR data of the synthetic compound **1** are in good agreement with those of the natural product [4].

After having accomplished the synthesis of **1**, we prepared compound **2**, by reacting aldehyde **7** with (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethanone in the presence of $TiCl_4$ using *Crimmins*' protocol to give the easily separable diastereoisomers of β-hydroxy amide, *i.e.*, the required *syn*-product **18** and the *anti*-product **18a** in 79% yield (*syn*/*anti* 7.8:2.8; *Scheme 5*). The OH group of **18** was protected as MOM ether **19**,

Scheme 2

a) 1. IBX (2-Iodoxybenzoic acid), dry DMSO, dry CH_2Cl_2, 0° to r.t., 2 h; 94%; 2. 1-[(4*R*)-4-benzyl-2-thioxo-1,3-thiazolidin-3-yl]ethanone, $TiCl_4$, EtN^iPr_2, dry CH_2Cl_2, – 78°, 30 min, 82%. *b*) Methoxymethyl chloride (MOMCl), EtN^iPr_2, dry CH_2Cl_2, 0° to r.t., 4 h; 87%. *c*) Diisobutylaluminium hydride (DIBAL-H), dry CH_2Cl_2, – 78°, 3 h; 90%. *d*) Zn, allyl bromide (C_3H_5Br), NH_4Cl, 0° to r.t., 4 h; 92%. *e*) *Dess–Martin* periodinane (DMP), $NaHCO_3$, dry CH_2Cl_2, 0° to r.t., 3 h; 90%. *f*) SmI_2, MeOH/THF, r.t., 12 h, 76%.

Scheme 3

a) Me_3SI (trimethylsulfonium iodide), BuLi, dry THF, – 10° to r.t., 12 h; 77%. *b*) MOMCl, EtN^iPr_2, dry CH_2Cl_2, 0° to r.t., 3 h; 85%, *c*) Li in naphthalene, – 20°, 3 h; 81%. *d*) 1. IBX, dry DMSO, dry CH_2Cl_2, 0° to r.t., 3 h; 85%; 2. $MeO_2CCH_2P(O)(OCH_2CF_3)_2$, NaH, dry THF, – 78°, 4 h; 76%. *e*) TsOH, dry benzene, reflux, 12 h; 80%.

Scheme 4

a) *Grubbs-II*-catalyst (5 mol-%), dry CH_2Cl_2, 40°, 12 h; 74%. *b*) $CeCl_3 \cdot 7\ H_2O$, MeCN/MeOH 1:1, reflux, 6 h; 85%.

which was reduced with DIBAL-H to yield the corresponding aldehyde, which was further subjected to *Still*'s modified *Horner–Wadsworth–Emmons* olefination reaction [14] to afford the unsaturated ester **20** in a (*Z*)/(*E*) ratio of 95:5 and 74% yield. Lactonization of compound **20** was carried out by treatment with 3% HCl in THF to afford a separable mixture of the natural product **2** and compound **2a** (7:3) in 78% yield. The formation of a significant amount of the bicyclic lactone **2a** was presumably due to the involvement of the OH group of **2** (*Scheme 5*) in the *Michael* addition

Scheme 5

a) 1-[(4*R*)-4-Benzyl-2-thioxo-1,3-thiazolidin-3-yl]ethanone, $TiCl_4$, EtN^iPr_2, dry CH_2Cl_2, –78°, 30 min; 79%. *b*) MOMCl, EtN^iPr_2, dry CH_2Cl_2, 0° to r.t., 5 h; 83%. *c*) 1. DIBAL-H, dry CH_2Cl_2, –78°, 3 h; 82%; 2. $MeO_2CCH_2P(O)(OCH_2CF_3)_2$, NaH, dry THF, –78°, 4 h; 74%. *d*) 3M HCl/THF 1:1, 0° to r.t., 3 h; 78%.

reaction. The optical rotation, and ^{1}H- and ^{13}C-NMR data of the synthetic compound **2** are in good agreement with those of the natural product [4].

Conclusions. – In conclusion, total syntheses of (6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one (**1**) and (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**2**) have been achieved by successful application of *Crimmins*' aldol reaction, SmI_2 reduction of alkoxy ketone, and *Grubbs-II*-catalyzed olefin cross-metathesis as key steps starting from commercially available 5-phenylpentan-1-ol (**4**) and L-aspartic acid.

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Experimental Part

General. Solvents were dried over standard drying agents and freshly distilled prior to use. The reagents were purchased from *Aldrich* and *Acros*, and were used without further purification unless otherwise stated. All moisture-sensitive reactions were carried out under N_2. Org. soln. were dried over anh. Na_2SO_4 and concentrated *in vacuo* below 40°. Column chromatographic (CC) separations: silica gel (*Acme*'s, 60–120 mesh and 100–200 mesh; SiO_2). Optical rotations: *Horiba* highly sensitive polarimeter *SEPA-300* at 25°. IR Spectra: *PerkinElmer IR-683* spectrophotometer with NaCl optics. ^{1}H- and ^{13}C-NMR (300 and 75 MHz, resp.) spectra: *Bruker Avance 300* instrument with TMS as internal standard in $CDCl_3$; *J* values in Hz. MS: *Agilent Technologies 1100 Series* (*Agilent* Chemistation Software).

*(3*R*)-1-[(4*R*)-4-Benzyl-2-thioxo-1,3-thiazolidin-3-yl]-3-hydroxy-7-phenylheptan-1-one* (**5**). To a cooled (0°) stirred soln. of IBX (5.56 g, 19.87 mmol) in dry DMSO (15 ml) was added a soln. of **4** (2.2 g, 13.25 mmol) in dry CH_2Cl_2 (25 ml) at 0°, and the mixture was stirred for 2 h at r.t. After completion of the reaction, the mixture was filtered, diluted with H_2O (15 ml), and extracted into CH_2Cl_2 (2 × 30 ml). The combined org. extract was washed with brine, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude residue was purified by CC (AcOEt/hexane 1:9) to give pure aldehyde (2.04 g, 94%) as a colorless liquid. The aldehyde was directly used for the next reaction. To a cooled (0°) soln. of the chiral 1-[(4*R*)-4-benzyl-2-thioxo-1,3-thiazolidin-3-yl]ethanone [8] (3.06 g, 12.19 mmol) in dry CH_2Cl_2 (25 ml) was added dropwise $TiCl_4$ (1.60 ml, 14.63 mmol), and the soln. was stirred for 5 min turning to a yellow color, followed by the addition of $EtN^{i}Pr_2$ (3.05 ml, 17.56 mmol) [9]. The suspension now turned to dark red (enolate) and was stirred for 20 min at 0° and then cooled to −78°. The above aldehyde (2.0 g, 12.19 mmol) in dry CH_2Cl_2 (15 ml) was added dropwise, and the mixture was stirred for 15 min at −78°. After completion, the reaction was quenched with sat. NH_4Cl (15 ml) soln., and the mixture was extracted with CH_2Cl_2 (3 × 40 ml). The combined org. extract was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 3:7): pure **5** (4.12 g, 82%). Yellow liquid. $[\alpha]^{25}_{D} = -281$ ($c = 1.65$, $CHCl_3$), determined by chiral HPLC (*DISCOVERY C8* 250 × 4.6 mm, 5 µm; MeCN/H_2O 60:40; flow rate, 1.0 ml/min, $\lambda =$ 210 nm): t_R 17.01 min (minor; 16%), 17.98 min (major; 84%). IR (neat): 3448, 3025, 2927, 2855, 1698, 1452, 1366, 1263, 1165, 1102, 1036, 916, 746, 701. ^{1}H-NMR: 7.36–7.08 (*m*, 10 H); 5.41–5.32 (*m*, 1 H); 4.14–4.04 (*m*, 1 H); 3.63 (*dd*, *J* = 2.2, 17.3, 1 H); 3.39 (*dd*, *J* = 7.5, 11.3, 1 H); 3.22 (*dd*, *J* = 3.0, 12.8, 1 H); 3.10–2.98 (*m*, 2 H); 2.89 (*d*, *J* = 12.0, 1 H); 2.63 (*t*, *J* = 7.5, 2 H); 1.73–1.23 (*m*, 6 H). ^{13}C-NMR: 201.3; 173.1; 142.4; 136.3; 129.3; 128.8; 128.3; 128.2; 127.2; 125.6; 68.2; 67.6; 45.8; 36.7; 36.1; 35.9; 31.8; 31.3; 25.1. ESI-MS: 436 ($[M + Na]^{+}$).

*(3*R*)-1-[(4*R*)-4-Benzyl-2-thioxo-1,3-thiazolidin-3-yl]-3-(methoxymethoxy)-7-phenylheptan-1-one* (**6**). To a cooled (0°) soln. of **5** (3.2 g, 7.74 mmol) in dry CH_2Cl_2 (10 ml), $EtN^{i}Pr_2$ (2.69 ml, 15.49 mmol) and then dropwise MOMCl (0.87 ml, 11.62 mmol) were added, and the mixture was stirred for 4 h. After completion of the reaction, the mixture was extracted with CH_2Cl_2, washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2:8): pure **6** (3.08 g,

87%). Colorless liquid. $[\alpha]_D^{25} = -232.72$ ($c = 0.55$, $CHCl_3$). IR (neat): 3425, 3025, 2927, 2854, 1688, 1601, 1493, 1451, 1342, 1261, 1163, 1042, 745, 700. ^{1}H-NMR: 7.37 – 7.24 (*m*, 8 H); 7.20 – 7.16 (*m*, 2 H); 5.36 – 5.27 (*m*, 1 H); 4.70 (*d*, $J = 6.7$, 1 H); 4.64 (*d*, $J = 6.7$, 1 H); 3.57 (*dd*, $J = 7.9$, 17.3, 1 H); 3.42 – 3.29 (*m*, 5 H); 3.23 (*dd*, $J = 3.7$, 12.4, 2 H); 3.09 – 2.98 (*m*, 1 H); 2.88 (*d*, $J = 11.5$, 1 H); 2.63 (*t*, $J = 7.5$, 2 H); 1.72 – 1.58 (*m*, 4 H); 1.49 – 1.37 (*m*, 2 H). ^{13}C-NMR: 201.2; 171.9; 142.5; 136.5; 129.4; 128.9; 128.4; 128.2; 127.2; 125.6; 96.6; 74.9; 68.6; 55.6; 44.2; 36.6; 35.8; 35.0; 32.1; 31.4; 24.8. ESI-MS: 480 ($[M + Na]^+$).

*(3*R*)-3-(Methoxymethoxy)-7-phenylheptanal* (**7**) [6e]. To a cooled ($-78°$) soln. of **6** (0.7 g, 1.53 mmol) in dry CH_2Cl_2 (10 ml) was added 1M DIBAL-H in toluene (1.83 ml, 1.83 mmol), and the mixture was stirred for 10 min at $-78°$. After completion, the reaction was quenched with sat. sodium potassium tartarate (10 ml), and the mixture was stirred for 0.5 h and then extracted with CH_2Cl_2 (3 × 15 ml). The combined org. extract was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): **7** (0.344 g, 90%). Colorless liquid. IR (neat): 3432, 3061, 3026, 2934, 2858, 2728, 1724, 1602, 1494, 1454, 1373, 1210, 1147, 1102, 1035, 917, 747. ^{1}H-NMR: 9.79 (*t*, $J = 2.2$, 1 H); 7.32 – 7.14 (*m*, 5 H); 4.65 (*s*, 2 H); 4.12 – 4.02 (*m*, 1 H); 3.33 (*s*, 3 H); 2.70 – 2.48 (*m*, 4 H); 1.74 – 1.52 (*m*, 4 H); 1.47 – 1.34 (*m*, 2 H). ^{13}C-NMR: 201.3; 142.2; 128.3; 128.2; 125.7; 95.8; 73.0; 55.5; 48.7; 35.7; 34.7; 31.2; 24.7. ESI-MS: 273 ($[M + Na]^+$).

*(6*R*)-6-(Methoxymethoxy)-10-phenyldec-1-en-4-ol* (**8**). To a cooled (0°) stirred soln. of **7** (0.3 g, 1.2 mmol), Zn dust (0.23 g, 3.6 mmol), and allyl bromide (C_3H_5Br) (0.16 ml, 2.4 mmol) in THF (15 ml), a sat. NH_4Cl soln. (0.4 ml) was added dropwise. The mixture was stirred for 4 h at r.t., until the aldehyde was totally consumed (TLC). The mixture was filtered, and the precipitate was thoroughly washed with AcOEt. The aq. layer was separated and treated with 5% HCl to dissolve the suspended turbid material. The clear soln. was extracted with AcOEt. The combined org. extract was washed with 10% $NaHCO_3$, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure **8** (0.32 g, 92%). Colorless liquid. IR (neat): 3454, 3026, 2935, 2858, 1640, 1602, 1494, 1448, 1211, 1148, 1035, 915, 746, 700. ^{1}H-NMR: 7.31 – 7.14 (*m*, 5 H); 5.92 – 5.76 (*m*, 1 H); 5.16 – 5.06 (*m*, 2 H); 4.72 – 4.60 (*m*, 2 H); 3.97 – 3.75 (*m*, 2 H); 3.38 (*s*, 3 H); 2.65 – 2.58 (*t*, $J = 7.5$, 2 H); 2.27 – 2.19 (*t*, $J = 7.5$, 2 H); 1.69 – 1.29 (*m*, 8 H). ESI-MS: 315 ($[M + Na]^+$).

*(6*R*)-6-(Methoxymethoxy)-10-phenyldec-1-en-4-one* (**9**). To a cooled (0°) soln. of **8** (0.2 g, 0.68 mmol) in dry CH_2Cl_2 (10 ml) was added *Dess–Martin* periodinane (DMP; 0.58 g, 1.36 mmol), and the mixture was stirred for 3 h at r.t. After completion, the reaction was quenched with sat. $Na_2S_2O_3$ soln., and the mixture was washed with sat. $NaHCO_3$, extracted with CH_2Cl_2 (2 × 5 ml), and washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure **9** (0.18 g, 90%). Colorless liquid. IR (neat): 3024, 2934, 2858, 1714, 1639, 1453, 1370, 1147, 1098, 1037, 918, 745, 700. ^{1}H-NMR: 7.31 – 7.31 (*m*, 5 H); 5.99 – 5.84 (*m*, 1 H); 5.21 – 5.09 (*m*, 2 H); 4.62 (*q*, $J = 6.7$, 2 H); 4.08 – 3.99 (*m*, 1 H); 3.31 (*s*, 3 H); 3.20 (*dt*, $J = 1.5$, 6.7, 2 H); 2.74 (*q*, $J = 6.7$, 1 H); 2.61 (*t*, $J = 7.5$, 2 H); 2.52 (*d*, $J = 4.5$, 1 H); 1.69 – 1.49 (*m*, 6 H). ^{13}C-NMR: 207.0; 142.3; 130.2; 128.3; 128.2; 125.6; 118.9; 95.9; 74.3; 55.5; 48.5; 47.4; 35.7; 34.5; 31.3; 24.7. ESI-MS: 313 ($[M + Na]^+$).

*(4*R*,6*R*)-6-(Methoxymethoxy)-10-phenyldec-1-en-4-ol* (**10**) [6f]. To a stirred soln. of **9** (0.15 g, 0.51 mmol) in THF (1 ml) at r.t. was added MeOH (0.6 ml, 15.3 mmol), followed by drtopwise addition of 0.1M SmI_2 in dry THF (17.83 ml, 1.78 mmol). The resulting mixture was stirred for 12 h before the septum was removed and stirring was continued until the color of the soln. changed. The reaction was then quenched with sat. $Na_2S_2O_3$ soln. (5 ml), the mixture was extracted with AcOEt (3 × 5 ml), and the combined org. extract was washed with H_2O (2 × 5 ml), dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure **10** (0.11 g, 76%).

*(6*R*)-5,6-Dihydro-6-[(1*E*,4*R*,6*R*)-4-hydroxy-6-(methoxymethoxy)-10-phenyldec-1-en-1-yl]-2*H*-pyran-2-one* (**17**). A soln. of **10** (0.03 g, 0.1 mmol) and **16** (0.038 g, 0.3 mmol) in dry CH_2Cl_2 (25 ml) in a 1 : 3 ratio was first bubbled with N_2 flow, then *Grubbs-II* catalyst (0.017 g, 0.02 mmol) was added at once, and the resulting mixture was heated under N_2 at 40° for 12 h. After completion of the reaction, the solvent was removed, and the residue was purified by CC (AcOEt/hexane 3 : 7): pure **17** (0.029 g, 74%). Colorless liquid. $[\alpha]_D^{25} = -4.37$ ($c = 0.8$, $CHCl_3$). IR (neat): 3455, 3024, 2926, 2855, 1718, 1456, 1382, 1248, 1147, 1033, 969, 816, 746. ^{1}H-NMR: 7.31 – 7.14 (*m*, 5 H); 6.92 – 6.84 (*m*, 1 H); 6.05 (*d*, $J = 9.8$, 1 H); 5.95 – 5.83 (*m*, 1 H); 5.68 (*dd*, $J = 6.7$, 15.8, 1 H); 4.90 (*q*, $J = 7.5$, 1 H); 4.64 (*s*, 2 H); 3.99 – 3.89 (*m*, 1 H); 3.85 – 3.74 (*m*, 1 H); 3.39 (*s*, 3 H); 2.62 (*t*, $J = 7.5$, 2 H); 2.47 – 2.39 (*m*, 2 H); 2.29 – 2.20 (*m*, 2 H); 1.71 – 1.48 (*m*,

6 H); 1.44 – 1.18 (*m*, 2 H). ^{13}C-NMR: 164.0; 144.6; 142.3; 131.3; 129.3; 128.3; 128.2; 125.6; 121.5; 96.3; 77.9; 75.9; 67.0; 55.8; 40.7; 40.1; 35.8; 34.6; 31.4; 29.6; 25.0. ESI-MS: 406 ($[M + NH_4]^+$).

*(6*R*)-6-[(1*E*,4*R*,6*R*)-4,6-Dihydroxy-10-phenyldec-1-en-1-yl]-5,6-dihydro-2*H*-pyran-2-one* (**1**) [5]. To a stirred soln. of **17** (0.02 g, 0.05 mmol) in a mixture of MeOH (3 ml) and MeCN (3 ml) was added $CeCl_3 \cdot 7\ H_2O$ (0.019 g, 0. 05 mmol) under N_2, then the mixture was stirred at reflux for 6 h. After completion, the reaction was quenched with solid $NaHCO_3$, the mixture was filtered, and the filtrate was concentrated, diluted with H_2O and extracted in to AcOEt (3 × 5 ml). The combined org. extract was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 4 : 6): **1** (0.015 g, 85%). White solid. M.p. 62 – 64°.

*(3*R*,5*R*)-1-[(4*R*)-4-Benzyl-2-thioxo-1,3-thiazolidin-3-yl]-3-hydroxy-5-(methoxymethoxy)-9-phenylnonan-1-one* (**18**). To a cooled (0°) soln. of the chiral 1-[(4*R*)-4-benzyl-2-thioxo-1,3-thiazolidin-3-yl]ethanone [8] (0.5 g, 2.0 mmol) in dry CH_2Cl_2 (15 ml) was added dropwise $TiCl_4$ (0.263 ml, 2.4 mmol), and the soln. was stirred for 5 min, the color turning to a yellow, followed by the addition of EtN^iPr_2 (0.41 ml, 2.4 mmol). The suspension now turned to dark red (enolate) and was stirred for 20 min at 0°. To this entirely dark red enolate, **7** (0.5 g, 2.0 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise, and the mixture was stirred for 15 min at – 78°. After completion, the reaction was quenched with sat. NH_4Cl (5 ml) soln., and the mixture was extracted with CH_2Cl_2 (2 × 20 ml). The combined org. extract was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 3 : 7): pure **18** (0.79 g, 79%). Yellow liquid. $[\alpha]_D^{25} = -128.1$ ($c = 2.2$, $CHCl_3$). IR (neat): 3445, 3032, 2931, 1690, 1496, 1455, 1343, 1282, 1271, 1169, 1032, 755, 740. ^{1}H-NMR: 7.39 – 7.24 (*m*, 8 H); 7.21 – 7.14 (*m*, 2 H); 5.43 – 5.34 (*m*, 1 H); 4.67 (*q*, *J* = 6.7, 2 H); 4.41 – 4.30 (*m*, 1 H); 3.87 – 3.77 (*m*, 1 H); 3.53 (*dd*, *J* = 3.0, 17.5, 1 H); 3.44 – 3.39 (*m*, 1 H); 3.38 (*s*, 3 H); 3.34 – 3.19 (*m*, 2 H); 3.05 (*dd*, *J* = 10.5, 13.4, 1 H); 2.89 (*d*, *J* = 11.5, 1 H); 2.63 (*t*, *J* = 3.3, 2 H); 1.88 – 1.75 (*m*, 1 H); 1.72 – 1.50 (*m*, 5 H); 1.46 – 1.34 (*m*, 2 H). ^{13}C-NMR: 201.2; 172.5; 142.4; 136.4; 129.4; 128.8; 128.3; 128.2; 127.2; 125.6; 95.2; 76.4; 68.4; 66.5; 55.7; 46.1; 40.6; 36.7; 35.8; 34.1; 32.0; 31.5; 24.5. ESI-MS: 524 ($[M + Na]^+$).

*(3*R*,5*R*)-1-[(4*R*)-4-Benzyl-2-thioxo-1,3-thiazolidin-3-yl]-3,5-bis(methoxymethoxy)-9-phenylnonan-1-one* (**19**). To a cooled (0°) soln. of **18** (0.35 g, 0.69 mmol) in dry CH_2Cl_2 (10 ml), EtN^iPr_2 (0.24 ml, 1.39 mmol) and then MOMCl (0.08 ml, 1.0 mmol) was added dropwise, and the mixture was stirred for 5 h. After completion of the reaction, the mixture was extracted with CH_2Cl_2 (2 × 10 ml), washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure **19** (0.31 g, 83%). Colorless liquid. $[\alpha]_D^{25} = -155.8$ ($c = 0.6$, $CHCl_3$). IR (neat): 3427, 3028, 2924, 1685, 1606, 1493, 1344, 1265, 1166, 1046, 754, 734. ^{1}H-NMR: 7.39 – 7.12 (*m*, 10 H); 5.37 – 5.27 (*m*, 1 H); 4.73 – 4.62 (*m*, 4 H); 4.36 – 4.25 (*m*, 1 H); 3.72 – 3.55 (*m*, 2 H); 3.44 – 3.40 (*m*, 1 H); 3.37 (*s*, 3 H); 3.35 (*s*, 3 H); 3.24 (*dd*, *J* = 3.0, 13.5, 1 H); 3.10 – 2.98 (*m*, 1 H); 2.89 (*d*, *J* = 11.3, 1 H); 2.63 (*t*, *J* = 7.5, 2 H); 1.78 – 1.55 (*m*, 5 H); 1.50 – 1.34 (*m*, 4 H). ^{13}C-NMR: 201.1; 171.7; 142.5; 136.5; 129.4; 128.8; 128.3; 128.2; 127.1; 125.6; 96.7; 95.4; 74.5; 72.5; 68.6; 66.5; 55.7; 44.5; 39.8; 36.5; 35.9; 34.3; 32.1; 31.5; 24.8. ESI-MS: 568 ($[M + Na]^+$).

*Methyl (2*Z*,5*S*,7*R*)-5,7-Bis(methoxymethoxy)-11-phenylundec-2-enoate* (**20**). To a cooled (– 78°) soln. of **19** (0.25 g, 0.45 mmol) in dry CH_2Cl_2 (10 ml) was added 1M DIBAL-H in toluene (0.55 ml, 0.55 mmol), and the mixture was stirred at – 78° for 10 min. After completion, the reaction was quenched with sat. sodium potassium tartarate (3 ml) soln., and the mixture was srirred for 0.5 h and then extracted with CH_2Cl_2 (3 × 15 ml). The combined org. extract was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure aldehyde (0.12 g, 82%). Colorless liquid. The aldehyde was directly used for the next reaction. To a cooled (0°) suspension of NaH (0.017 g, 0.71 mmol) in dry THF (10 ml) under N_2 was added methyl [bis(2,2,2-trifluoroethoxy)phosphoryl]acetate (0.11 ml, 0.53 mmol), and the mixture was stirred for 30 min and then cooled to – 78°. The soln. of aldehyde (0.12 g, 0.35 mmol) in dry THF (5 ml) was added dropwise over a period of 5 min. The resulting mixture was stirred for 3 h at – 78°. After completion, the reaction was quenched with sat. NH_4Cl soln., and the mixture was extracted with Et_2O (3 × 5 ml). The combined org. extract washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 2 : 8): pure **20** (0.1 g, 74%). Colorless liquid. $[\alpha]_D^{25} = +12.7$ ($c = 0.9$, $CHCl_3$). IR (neat): 3433, 2931, 2856, 1722, 1645, 1442, 1406, 1175, 1099, 1036, 917. ^{1}H-NMR: 7.31 – 7.12 (*m*, 5 H); 6.34 (*dt*, *J* = 7.1, 11.5, 1 H); 5.92 – 5.85 (*m*, 1 H); 4.67 – 4.59 (*m*, 4 H); 3.88 – 3.78 (*m*, 1 H); 3.70 (*s*, 3 H); 3.69 – 3.60 (*m*,

1 H); 3.36 (*s*, 3 H); 3.34 (*s*, 3 H); 3.07 – 2.84 (*m*, 2 H); 2.61 (*t*, $J = 7.5$, 2 H); 1.90 – 1.77 (*m*, 1 H); 1.70 – 1.47 (*m*, 5 H); 1.45 – 1.30 (*m*, 2 H). ^{13}C-NMR: 166.6; 146.0; 142.5; 128.3; 128.2; 125.6; 121.0; 95.4; 95.2; 74.6; 73.8; 55.6; 55.5; 51.0; 39.4; 35.8; 34.3; 33.5; 31.5; 24.7. ESI-MS: 417 ([$M + Na$]$^+$).

(6S)-5,6-Dihydro-6-[(2R)-2-hydroxy-6-phenylhexyl]-2H-pyran-2-one (**2**) [6]. To a cooled (0°) soln. of **20** (0.04 g, 0.1 mmol) in THF (2 ml) was added 3M HCl (2 ml), and the mixture was stirred for 3 h. After completion of the reaction, the mixture was diluted with AcOEt, and the reaction was quenched with solid $NaHCO_3$, and the mixture was filtered. The filtrate was washed with brine, dried (anh. Na_2SO_4), and concentrated. The crude residue was purified by CC (AcOEt/hexane 1:1): compounds **2** and **2a** (70:30; 78% yield). Compound **2** (0.014 g): pale-yellow solid. M.p. 34 – 36°.

(1R,7R)-7-(4-Phenylbutyl)-2,6-dioxa-bicyclo[3.3.1]nonan-3-one (**2a**). White solid (0.006 g). M.p. 36 – 38°. $[\alpha]_D^{25} = -17.1$ ($c = 0.3$, $CHCl_3$). IR (KBr): 2924, 2855, 2102, 1736, 1494, 1078. ^{1}H-NMR: 7.31 – 7.16 (*m*, 5 H); 4.93 – 4.88 (*m*, 1 H); 4.38 – 4.33 (*m*, 1 H); 3.79 – 3.65 (*m*, 1 H); 2.87 – 2.78 (*m*, 2 H); 2.58 (*t*, $J = 7.5$, 2 H); 2.05 – 1.86 (*m*, 3 H); 1.77 – 1.27 (*m*, 7 H). ^{13}C-NMR: 169.7; 142.4; 128.2 (2); 125.6; 73.0; 65.6; 65.4; 36.8; 36.2; 35.7; 35.4; 31.2; 29.7; 24.6. ESI-MS: 297 ([$M + Na$]$^+$).

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Two Novel Abietane Diterpenoids from *Illicium wardii* A.C.SM.

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Two novel abietane diterpenoids, wardinols A and B (**1** and **2**, resp.), together with five known diterpenoids, **3**–**7**, were isolated from the fruits of *Illicium wardii* A.C.SM. Their structures were elucidated on the basis of intensive spectroscopic analysis, including 1D- and 2D-NMR spectroscopy. Wardinol A (**1**) is a rare 9,10-epoxy-9,10-secoabietane diterpenoid. All of the isolated compounds were evaluated for their cytotoxicities against three human tumor cell lines, A549, HCT116, and CCRF-CEM.

Introduction. – The genus *Illicium* (Illiciaceae family) is a rich source of prenylated C_6–C_3 compounds [1–3], neolignans [3], and secoprezizaane-type sesquiterpenes [4][5] with various activities, such as cancer chemopreventive activity [6], antibacterial activity [7], antioxidant activity [7], *etc. Illicium wardii* A.C.SM., mainly distributed in Nujiang Region of Yunnan Province, China, is a member of the genus *Illicium*. Although there are many reports about the chemical constituents and bioactivity of *Illicium* species, investigations on *I. wardii* were rarely reported, except for the isolation of seven compounds in 2007 [8]. During our search for bioactive compounds from medicinally important plants, two novel abietane diterpenoids, wardinols A and B (**1** and **2**, resp.), together with five known diterpenoids, **3**–**7** (*Fig. 1*), were obtained from the title plant. Herein, we report the isolation and structure elucidation of these compounds, as well their cytotoxicity evaluation against the three tumor cell lines A549, HCT116, and CCRF-CEM.

Results and Discussion. – The AcOEt-soluble fraction of the 95% EtOH extract of the fruits of *I. wardii* (3.5 kg) was submitted to repeated column chromatography on silica gel, octadecylsilyl (ODS), and *Sephadex LH-20*, in combination with preparative silica-gel TLC, to yield two new abietane-type diterpenoids, **1** and **2**, together with the five known diterpenoids **3**–**7** (*Fig. 1*). By comparing the physical and spectroscopic data with those reported in the literature, the structures of the known compounds were identified as angustanol (**3**) [9], 12-hyrdoxydehydroabietic acid (**4**) [10], 15-oxo-17-norabieta-8,11,13-trien-18-oic acid (**5**) [11], angustanoic acid E (**6**) [9], and dehydroabietic acid (**7**) [12], respectively.

Wardinol A (**1**) was obtained as a colorless oil. Its molecular formula was established as $C_{20}H_{30}O_2$ based on its positive-ion-mode HR-ESI-MS (*m/z* 303.2313 ($[M+H]^+$; calc. 303.2319)), implying six degrees of unsaturation. The ^{1}H-NMR spectrum (*Table*) of **1** displayed two Me *singlet*s (δ(H) 1.01 and 0.68), and signals of

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Fig. 1. *Chemical structures of compounds* **1**–**7**

one ^{i}Pr (δ(H) 2.81 (*sept.*, $J = 6.85$, 1 H) and 1.20 (*d*, $J = 6.85$, 6 H)) and one CH_2OH group (δ(H) 3.07 (*d*, $J = 11.28$), 3.46 (*d*, $J = 11.28$)), and three aromatic H-atoms (δ(H) 6.93 (*d*, $J = 2.25$), 6.71 (*d*, $J = 7.64$), and 6.92 (*dd*, $J = 2.25$, 7.64)). In the ^{13}C-NMR spectrum of **1** (*Table*), 20 C-atom resonances were observed, which were attributed to four Me (δ(C) 17.8, 21.4, 24.6, and 24.7), six CH_2 (δ(C) 21.0, 23.2, 35.5, 36.5, 43.1, and 71.1), and five CH groups (δ(C) 34.8, 52.4, 124.3, 125.5, and 128.2), and five quaternary C-atoms (δ(C) 40.6, 82.8, 137.2, 145.2, and 154.5). These data evidenced the presence of an aromatized *C*-ring abietane diterpenoid. Different from usual abietane-type diterpenoids, the ^{13}C- and DEPT NMR spectra of **1** showed signals of two O-bearing quaternary C-atoms at δ(C) 154.5 and 82.8, and one CH_2OH C-atom at δ(C) 71.1. In addition, the NMR spectra of **1** also displayed great similarity to those of the known compound karamatsuic acid [13] with the exception of the presence of a CH_2OH group instead of a COOH moiety, implying that compound **1** may be a 9,10-epoxy-9,10-secoabieta-8,11,13-triene.

In the HMBC spectrum (*Fig. 2*) of **1**, the Me(20), CH_2(2), and CH_2(6) signals displayed long-range correlations with a signal arising from an O-bearing C-atom at δ(C) 82.8, while H–C(12), H–C(14), and CH_2(7) signals correlated with that of another O-bearing C-atom at δ(C) 154.5. Additionally, two CH_2OH H-atom signals at δ(H) 3.46 (*d*, $J = 11.28$) and 3.07 (*d*, $J = 11.28$) exhibited HMBCs with those of Me(19), C(3), C(4), and C(5), in combination with their NOESY correlations with H–C(5) signal, and the NOESY correlation of Me(20) and Me signals at δ(H) 0.68, evidencing the CH_2(18)OH group. The relative configuration of **1** was determined to be identical with karamatsuic acid [13] by analysis of the NOESY spectrum of **1** (*Fig. 3*). Thus, the structure of **1** was determined to be 9,10α-epoxy-18-hydroxy-9,10-secoabieta-8,11,13-triene and named wardinol A.

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	**1**		**2**	
	δ(H)[a]	δ(C)[b]	δ(H)[a]	δ(C)[b]
1	1.86–1.89 (*m*, H_a), 1.64 – 1.71 (overlap, H_b)	43.1 (*t*)	1.86–1.89 (*m*, H_a), 1.18–1.26 (overlap, H_b)	37.3 (*t*)
2	1.64 – 1.71 (overlap, H_a), 1.57–1.62 (overlap, H_b)	21.0 (*t*)	1.80–1.83 (*m*, H_a), 1.68–1.76 (*m*, H_b)	19.0 (*t*)
3	1.58–1.66 (overlap, H_a), 1.20–1.25 (*m*, H_b)	36.5 (*t*)	1.40–1.47 (overlap, H_a), 1.18–1.26 (overlap, H_b)	42.5 (*t*)
4		40.6 (*s*)		34.4 (*s*)
5	2.01 (*dd*, J = 2.18, 11.85)	52.4 (*d*)	1.16 (*dd*, J = 1.93, 12.12)	51.7 (*d*)
6	1.80–1.84 (*m*, H_a), 1.33–1.40 (*m*, H_b)	23.2 (*t*)	1.53–1.58 (*m*, H_a), 1.40–1.47 (overlap, H_b)	19.8 (*t*)
7	2.74–2.77 (*m*, H_a), 2.68–2.72 (*m*, H_b)	35.5 (*t*)	2.35–2.40 (*m*, H_a), 2.02–2.08 (*m*, H_b)	25.3 (*t*)
8		137.2 (*s*)		131.5 (*s*)
9		154.5 (*s*)		163.1 (*s*)
10		82.8 (*s*)		40.2 (*s*)
11	6.71 (*d*, J = 7.64)	124.3 (*d*)	2.55–2.59 (*m*, H_a), 2.48–2.53 (*m*, H_b)	32.9 (*t*)
12	6.92 (*dd*, J = 2.25, 7.64)	125.5 (*d*)	4.20–4.23 (*m*)	69.7 (*d*)
13		145.2 (*s*)	2.19–2.21 (*m*)	59.6 (*d*)
14	6.93 (*d*, J = 2.25)	128.2 (*d*)		204.5 (*s*)
15	2.81 (*hept.*, J = 6.85)	34.8 (*d*)	2.14–2.18 (*m*)	25.4 (*d*)
16	1.20 (*d*, J = 6.85)	24.6 (*q*)	1.05 (*d*, J = 6.83)	23.1 (*q*)
17	1.20 (*d*, J = 6.85)	24.7 (*q*)	0.86 (*d*, J = 6.83)	22.3 (*q*)
18	3.46 (*d*, J = 11.28, H_a), 3.07 (*d*, J = 11.28, H_b)	71.1 (*t*)	0.92 (*s*)	33.6 (*q*)
19	0.68 (*s*)	17.8 (*q*)	0.90 (*s*)	22.1 (*q*)
20	1.01 (*s*)	21.4 (*q*)	1.10 (*s*)	20.1 (*q*)

[a]) Recorded at 600 MHz in CD_3OD. [b]) Recorded at 150 MHz in CD_3OD.

Fig. 2. $^1H,^1H$-*COSY* (—) and *key HMB* (H → C) *correlations of* **1** *and* **2**

Fig. 3. *Key NOESY correlations* (H ↔ H) *of* **1** *and* **2**

Wardinol B (**2**), a colorless oil, had the molecular formula $C_{20}H_{32}O_2$ as deduced from positive-ion-mode HR-ESI-MS (*m/z* 305.2475 ($[M+H]^+$; calc. 305.2487)), implying five degrees of unsaturation. Analysis of the NMR spectra (*Table*) revealed typical features of a usual abietane-type diterpenoid, exhibiting signals of five Me (δ(C) 23.1, 22.3, 33.6, 22.1, and 20.1), six CH_2 (δ(C) 37.3, 19.0, 42.5, 19.8, 25.3, and 32.9), and four CH groups (δ(C) 51.7, 69.7, 59.6, and 25.4), and five quaternary C-atoms (δ(C) 34.4, 131.5, 163.1, 40.2, and 204.5), *i.e.*, altogether 20 C-atom resonances in ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum (*Table*) displayed H-atom resonances for three Me groups (δ(H) 0.90 (*s*), 0.92 (*s*), and 1.10 (*s*)), an ^{i}Pr group (δ(H) 0.86 (*d*, $J = 6.83$, 3 H), 1.05 (*d*, $J = 6.83$, 3 H), and 2.14–2.18 (*m*, 1 H)), and an O-bearing CH H-atom (δ(H) 4.20–4.23 (*m*)). These data implied that there are one α,β-unsaturated C=O moiety and a secondary OH substituent in the structure of **2**. In ^{1}H,^{1}H-COSY spectrum, the O-bearing H-atom signal at δ(H) 4.20–4.23 showed cross-peaks with those of CH_2(11) (δ(H) 2.55–2.59, 2.48–2.53) and H–C(13) (δ(H) 2.19–2.21), respectively, indicating that the OH group was attached to C(12). This deduction was confirmed by key HMBCs from the signals of CH_2(11) and H–C(15) to that of the O-bearing C-atom at δ(C) 69.7. The α,β-unsaturated C=O moiety was comprised to C(8), C(9), and C(14), respectively, based on the HMBCs of the signals of CH_2(7) and CH_2(11) with those of the two olefinic C-atoms at δ(C) 131.5 and 163.1, and of the signals of H–C(15) and H–C(13) with that of the C=O C-atom (δ(C) 204.5). According to biogenetic considerations, Me(19), Me(20), and H–C(13) of **2** should be β-oriented, while Me(18), H–C(5), and ^{i}Pr were assumed to be in α-orientation, as depicted in *Fig. 1.* In the NOESY spectrum of **2** (*Fig. 3*), H_a–C(11) at δ(H) 2.55–2.59 correlated with those

of Me(20) and H–C(12) (δ(H) 4.20–4.23), respectively, assigning the relative configuration of H–C(12) as β. Additionally, H–C(12) signal showed key NOESY correlations with those of H–C(13) (δ(H) 2.19–2.21) and Me(16) (δ(H) 1.05), indicating that the ^{i}Pr group was α-oriented.

Cytotoxicity. – Compounds **1**–**7** were tested for their cytotoxicities against human lung cancer (A549), colon cancer (HCT116), and leukemia (CCRF-CEM) cell lines using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-terazolium bromide) colorimetric assay. None of these compounds showed significant cytotoxicity against the above cell lines ($IC_{50} > 100$ µg/ml).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Marine Chemical Factory*, Qingdao, P. R. China) and *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). TLC: SiO_2 plates (*Marine Chemical Factory*, Qingdao, P. R. China); visualization by spraying with 10% H_2SO_4 in EtOH. Optical rotations: *PerkinElmer 343* polarimeter (*PerkinElmer*, Norwalk, CT, USA). UV Spectra: *Shimadzu UV-2550* spectrophotometer; λ_{max} (log ε) in nm. NMR Spectra: *DRX-600* spectrometer (600 MHz); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Agilent-1100-LC/MSD-Trap* (ESI-MS) and *Agilent Micro-Q-Tof* (HR-ESI-MS) spectrometer; in *m/z*.

Plant Material. The fruits of *I. wardii* A.C.SM. were collected in Gongshan Mountain, Nujiang prefecture, Yunnan Province, P. R. China, in August 2011, and authenticated by Prof. *Yuan Chuan Zhou*, the Director of Nujiang Institute of Medical Plant. A voucher specimen is deposited with the School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The fruits of *I. wardii* A.C.SM. (3.5 kg) were powdered and extracted with 95% EtOH at r.t. (3 × 10 l; each 48 h). The pooled extract was concentrated *in vacuo* to afford a residue (360 g), which was suspended in H_2O (2 l) and extracted with AcOEt (4 × 2 l). The AcOEt-soluble fraction (150 g) was submitted to CC (SiO_2; petroleum ether/AcOEt 30:1, 20:1, 10:1, 5:1, 2:1, and 100% MeOH, resp.), to give six fractions, *Frs. 1–6*. *Fr. 1* (10 g) was purified by repeated CC (*ODS*; MeOH/H_2O 3:1 → 10:1; *Sephadex LH-20*; MeOH), and prep. TLC (SiO_2; $CHCl_3$/MeOH 30:1) to provide compounds **1** (6.0 mg) and **4** (7.8 mg). Similarly, compounds **2** (3.8 mg) and **3** (6.0 mg) were obtained from *Fr. 2* (8 g) by repeated CC (*ODS*; MeOH/H_2O 3:1 → 10:1; and *Sephadex LH-20*; $CHCl_3$/MeOH 1:1) and prep. TLC (SiO_2; $CHCl_3$/MeOH 30:1). Isolation and purification of *Fr. 4* (26 g) by a similar procedure gave compounds **5** (6.0 mg), **6** (23.0 mg), and **7** (14.0 mg).

Wardinol A (= *9,10α-Epoxy-18-hydroxy-9,10-secoabieta-8,11,13-triene* = *(1*R*,4a*S*,11a*S*)-1,2,3,4,4a,10,11,11a-Octahydro-1,4a-dimethyl-8-(1-methylethyl)dibenz[*b,f*]oxepin-1-methanol*; **1**). Colorless oil. $[\alpha]_D^{20} = -0.018$ ($c = 1.0$, MeOH). UV (MeOH): 270 (1.77). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table*. ESI-MS (pos.): 325 ($[M + Na]^+$), 627 ($[2M + Na]^+$). HR-ESI-MS (pos.): 303.2313 ($[M + H]^+$, $C_{20}H_{31}O_2^+$; calc. 303.2319).

Wardinol B (= *(12α)-12-Hydroxyabiet-8-en-14-one* = *(2*S*,3*S*,4b*S*,8a*S*)-3,4,4b,5,6,7,8,8a,9,10-Decahydro-3-hydroxy-4b,8,8-trimethyl-2-(1-methylethyl)-1(2*H*)-phenanthrenone*; **2**). Colorless oil. $[\alpha]_D^{20} = 0.049$

($c = 1.0$, MeOH). UV (MeOH): 247 (3.87). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table*. ESI-MS (pos.): 327 ($[M+Na]^+$), 631 ($[2M+Na]^+$). HR-ESI-MS (pos.): 305.2475 ($[M+H]^+$, $C_{20}H_{33}O_2^+$; calc. 305.2487).

Cytotoxicity. A MTT colorimetric assay was performed in 96-well plates. Cell cultures were diluted with fresh medium consisting of *Dulbecco*'s modified eagle's medium (DMEM), 10% fetal bovine serum (FBS), and penicillin, as well as streptomycin to $4-6 \times 10^4$ cells/ml and placed in 96-well microplates at 100 μl/well. After 24 h incubation at 37° in a 5% CO_2 atmosphere, the tested compounds at different concentrations were added to the microplates in 10-μl amounts. The three tumor cell lines, A549, HCT116, and CCRF-CEM, were exposed to the drugs for another 72 h. Then, 20 μl of MTT soln. (5 mg/ml) were added to each well, and the plate was incubated for 4 h. Then, DMSO (100 μl) was added to each well. The OD of each well was measured on a *Wellscan* reader (*Varioskan Flash*, *Thermo Scientific*) at 570 nm. Doxicyclin was used as positive control. The assay was performed in triplicate. The data were represented as mean ± S.D. The cell lines were all preserved in Shanghai Institute for Pharmaceutical Industrial, P. R. China.

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Suberosanones A – C, New Metabolites Possessing Cyclopentenone System from the South China Sea Gorgonian Coral *Subergorgia suberosa*

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Two unusual novel bicyclic lactones, suberosanones A and B (**1** and **2**, resp.), characterized by the co-occurrence of cyclopentenone and butanolide rings within the same molecule, along with one tricyclic cyclopentenone derivative, suberosanone C (**3**), were isolated from the South China Sea gorgonian coral *Subergorgia suberosa*. Their structures were unambiguously established by detailed spectroscopic analyses (NMR, IR, and HR-MS). The absolute configurations of **1** and **2** were determined by quantum-chemical calculations using the time-dependent density-functional theory (TDDFT) method. All compounds showed neither antifouling activity against *Balanus amphitrite* larvae settlement nor antibacterial activity against a panel of bacterial strains at concentrations up to 25 μg/ml.

Introduction. – Natural products possessing cyclopentenone moieties have been established to exhibit a broad spectrum of biological features, including antitumor [1–3], antibacterial [4], anti-inflammatory [5], antifouling [6], tyrosinase inhibitory [7], and nitric oxide (NO) production-inhibitory [8][9] activities, and have attracted a great deal of attention from the synthetic chemistry community [10–14]. In our continuous efforts to discover structurally unique and biologically active metabolites from marine organisms, the gorgonian coral *Subergorgia suberosa*, which had been studied by our and other groups previously [15–22], prompted us to reinvestigate, due to its high ability in metabolizing structurally diverse compounds. This time, the title animal, collected from the Naozhou Island, South China Sea, in May 2011, was systematically studied, leading to the isolation of three unusual novel cyclopentenone derivatives (*Fig. 1*), bicyclic lactones suberosanones A and B (**1** and **2**, resp.), and tricyclic metabolite suberosanone C (**3**). Herein, we report the isolation, structure elucidation, and bioactivities of these secondary metabolites.

Results and Discussion. – The gorgonian coral *S. suberosa* (6.5 kg, wet weight) was cut into pieces and extracted with 95% EtOH at room temperature. The AcOEt-soluble portion of the extract was purified by column chromatography on SiO_2, octadecyl silane (ODS), repeatedly, and finally the compounds **1**–**3** were obtained by preparative RP-HPLC.

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Fig. 1. *Structures of compounds* **1**–**3**

Suberosanone A (**1**) was obtained as an optically active yellow oil. The molecular formula, $C_{12}H_{16}O_3$, was deduced from the HR-CI-MS pseudo-molecular-ion peak at *m*/*z* 209.1176 ($[M+H]^+$; calc. 209.1178), which corresponded to five degrees of unsaturation. The ^{1}H-NMR spectrum exhibited two Me *singlet*s (δ(H) 1.75 (*s*, Me(11)) and 2.07 (*s*, Me(12))), one Me *triplet* (δ(H) 0.99 (*t*, $J=7.5$, Me(10))), and signals of one CH H-atom (δ(H) 2.92–3.03 (*m*, H–C(5))) and three CH_2 H-atoms (*Table 1*). In addition, HSQC and ^{13}C-NMR spectra of **1** indicated the presence of three Me, three CH_2, one CH groups, and one O-bearing quaternary C-atom (δ(C) 92.2 (C(4))), as well as four sp^2 quaternary C-atoms (δ(C) 174.4 (C(7)), 204.6 (C(1)), 139.0 (C(2)), and 167.1 (C(3))) (*Table 2*). The distinct HMBCs of Me(12) signal (δ(H) 2.07 (*s*)) with those at δ(C) 204.6 (C(1)), 139.0 (C(2)), 167.1 (C(3)), 92.2 (C(4)); of Me(11) signal with those of C(1), C(2), C(3); and of H–C(5) signal with those of C(1), C(3), and C(4) (*Fig. 2*), were indicative for the presence of 2,3-dimethylcyclopentenone moiety in **1**, which was consistent with the strong IR absorption bands at 1710 and 1652 cm^{-1}. Furthermore, the ^{1}H,^{1}H-COSY correlations CH_2(6) (δ(H) 2.65 (*dd*, $J=$ 28.0, 13.0), 2.92–3.03 (*m*))/H–C(5); and CH_2(8) (δ(H) 1.77–1.81 (*m*), 1.98–2.03 (*m*))/CH_2(9) (δ(H) 1.14–1.22 (*m*), 1.31–1.38 (*m*)) and Me(10) revealed two independent ^{1}H,^{1}H spin systems for **1**, as depicted in *Fig. 2*. Direct connectivity of C(8) to C(4) was supported by the HMBCs of CH_2(8) with C(3), C(5) (δ(C) 46.6), and C(4); and of H–C(5) with C(8) (δ(C) 36.7), while diagnostic HMBC cross-peaks of CH_2(6) with C(7), C(1), C(5), C(4); and of H–C(5) with C(7) established the linkage of C(6) to C(7). The molecular formula and unsaturation degrees of **1**, combined with the strong IR absorption (1770 cm^{-1}) characteristic of ester C=O group, enabled us to establish the butanolide ring for **1** by connecting C(7) to C(4) *via* an O-atom. Therefore, the planar structure of suberosanone A was elucidated as shown in *Fig. 1*.

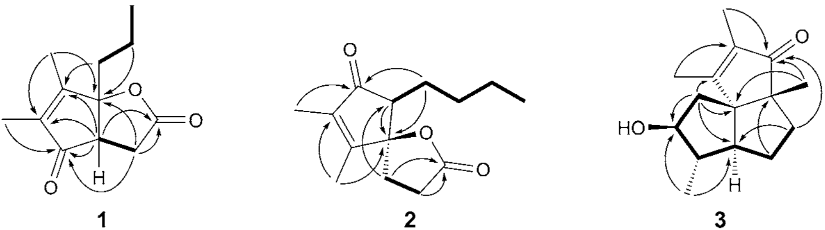

Fig. 2. *Key ^{1}H,^{1}H-COSY* (—) *and HMB* (H → C) *correlations of compounds* **1**–**3**

Table 1. *^{1}H-NMR Data of* **1**–**3**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	**1**[a])	**2**[a])	**2**[b])	**3**[a])
1				2.03–2.07 (*m*, 1 H)
2				1.21–1.24 (*m*, H_a), 1.36–1.40 (*m*, H_b)
3				1.42–1.47 (*m*, H_a), 2.03–2.07 (*m*, H_b)
5	2.92–3.03 (*m*, 1 H)	2.66 (br. *t*, J = 7.0, 3 H)	2.55 (*t*, J = 7.0, 3 H)	
6	2.65 (*dd*, J = 28.0, 13.0, H_a), 2.92–3.03 (*m*, H_b)	1.42–1.46 (*m*, H_a), 1.83–1.88 (*m*, H_b)	1.43–1.47 (*m*, H_a), 1.65–1.69 (*m*, H_b)	
7		1.34–1.38 (*m*, H_a), 1.58–1.61 (*m*, H_b)	1.28–1.30 (*m*, H_a), 1.43–1.47 (*m*, H_b)	
8	1.77–1.81 (*m*, H_a), 1.98–2.03 (*m*, H_b)	1.35–1.38 (*m*, 2 H)	1.30–1.34 (*m*, 2 H)	
9	1.14–1.22 (*m*, H_a), 1.31–1.38 (*m*, H_b)	0.93 (*t*, J = 7.0, 3 H)	0.88 (*t*, J = 7.0, 3 H)	1.64–1.67 (*m*, 1 H)
10	0.99 (*t*, J = 7.5, 3 H)	2.12–2.16 (*m*, H_a), 2.27–2.32 (*m*, H_b)	2.16–2.19 (*m*, H_a), 2.24–2.29 (*m*, H_b)	4.26 (br. *t*, J = 5.0, 1 H)
11	1.75 (*s*, 3 H)	2.74 (br. *t*, J = 7.0, 2 H)	2.72–2.78 (*m*, 2 H)	1.82–1.85 (*m*, H_a), 2.06–2.11 (*m*, H_b)
12	2.07 (*s*, 3 H)			2.08 (*s*, 3 H)
13		1.75 (*s*, 3 H)	1.65 (*s*, 3 H)	1.69 (*s*, 3 H)
14		2.00 (*s*, 3 H)	1.97 (*s*, 3 H)	1.01 (*s*, 3 H)
15				1.02 (*d*, J = 6.5, 3 H)

[a]) Recorded at 500 MHz in $CDCl_3$. [b]) Recorded at 300 MHz in (D_6)DMSO.

Table 2. *^{13}C-NMR Data of* **1**–**3**. δ in ppm.

Position	**1**[a])	**2**[a])	**2**[b])	**3**[a])
1	204.6	203.8	203.5	53.8
2	139.0	138.0	136.4	25.0
3	167.1	163.9	165.0	35.3
4	92.2	92.1	91.5	57.6
5	46.6	55.4	54.6	214.0
6	32.4	25.4	25.3	133.9
7	174.4	30.1	29.4	174.2
8	36.7	22.9	22.2	66.3
9	17.1	13.8	13.8	43.1
10	14.1	26.1	25.3	76.9
11	8.2	29.3	28.8	37.5
12	12.2	175.9	176.3	13.8
13		8.2	7.9	8.5
14		10.9	10.7	21.9
15				12.6

[a]) Recorded at 75 MHz in $CDCl_3$. [b]) Recorded at 75 MHz in (D_6)DMSO.

Structurally, **1** is very similar to sinularone C, isolated from soft coral *Sinularia* sp. [6], with the major difference between them being the length of the side chain. The relative configuration of **1** was determined on the basis of a NOESY experiment (*Fig. 3*). NOE Correlations of H–C(5) with both $CH_2(8)$ and $CH_2(9)$ indicated that these H-atoms were located on the same face of butanolide ring, thereby establishing the *cis*-fused junctions of the cyclopentenone and butanolide rings across the bond formed between C(5) and C(4).

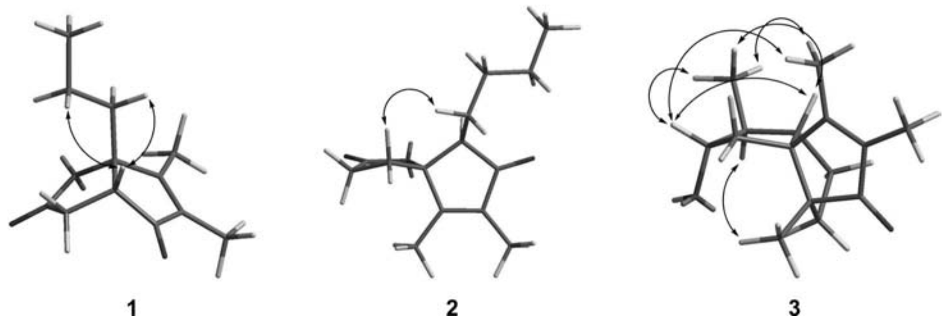

Fig. 3. *Key NOESY* (H ↔ H) *correlations of compounds* **1**–**3**

Suberosanone B (**2**) was obtained as an optically active yellow oil. Its molecular formula, $C_{14}H_{20}O_3$, was established based on HR-CI-MS with an M^+ ion peak at *m/z* 236.1413 (calc. 236.1412). The similarity of UV, IR, and NMR spectra of **1** and **2** (*Tables 1* and *2*) allowed us to assume that **2** might also contain both cyclopentenone and butanolide rings, as in **1**. This assumption was confirmed by careful analyses of the 2D-NMR spectra (^1H,^1H-COSY, HSQC, HMBC, and NOESY) of **2**. The HMBCs of

Me(13) (δ(H) 1.75 (*s*)) with C(1) (δ(C) 203.8), C(2) (138.0), and C(3) (163.9); of Me(14) (δ(H) 2.00 (*s*)) with C(4) (δ(C) 92.1), C(2), and C(3); and of H–C(5) (δ(H) 2.66 (br. *t*, $J = 7.0$)) with C(4), C(1), C(2), and C(3), evidenced the presence of the dimethylcyclopentenone moiety in **2**. ^{1}H,^{1}H-COSY Cross-peaks H–C(5)/CH_2(6) (δ(H) 1.42–1.46 (*m*), 1.83–1.88 (*m*))/CH_2(7) (δ(H) 1.34–1.38 (*m*), 1.58–1.61 (*m*))/CH_2(8) (δ(H) 1.35–1.38 (*m*))/Me(9) (δ(H) 0.93 (*t*, $J = 7.0$)), in combination with HMBCs of CH_2(6) with C(4), C(5), and C(1), disclosed that a butyl moiety was attached to the C(5). The spiro bicyclic lactone with the spiro-C-atom C(4) was deduced from the distinct HMBCs of CH_2(10) (δ(H) 2.12–2.16 (*m*), 2.27–2.32 (*m*)) with C(4), C(5), and C(3); of H–C(11) (δ(H) 2.74 (br. *t*, $J = 8.0$)) with C(12) (δ(C) 175.9), C(10) (δ(C) 26.1), and C(4), and was supported by the molecular formula and IR spectrum indicating the presence of an ester C=O group (1777 cm^{-1}). Thus, the planar structure of suberosanone B (**2**) was elucidated as shown in *Fig. 1*. The relative configuration of **2** was deduced from the NOESY spectrum (*Fig. 3*). NOESY Correlations of H_a–C(6) (δ(H) 1.42–1.46 (*m*)) with H_b–C(10) (δ(H) 2.27–2.30 (*m*)) indicated the *cis*-orientations of CH_2(6) and CH_2(10) relative to the cyclopentenone ring. Therefore, the relative configuration of **2** was determined.

The comparison of calculated and experimental electronic circular dichroism (ECD) curves has been proven to be powerful for the absolute-configuration determination of natural products [23][24]. To establish the absolute configurations of **1** and **2**, the time-dependent density-functional theory (TD-DFT) method was used to calculate their theoretical ECD spectra. The results showed that the calculated ECD spectra of compounds **1** and **2** were in good agreement with their experimental ones (*Fig. 4*). Thus, the absolute configurations of **1** and **2** were determined to be (4*S*,5*R*) and (4*R*,5*R*), respectively. Interestingly, despite great structural similarity between compound **1** and sinularone C, the configuration at both C(4) and C(5) of them is completely opposite to each other. It was also noteworthy that the compound **2** was an enantiomer of sinularone D [6], a metabolite recently isolated from soft coral *Sinularia* sp. The NMR data (in (D_6)DMSO) of **2** was identical to those of sinularone D, whereas the directions of both its optical rotation ($[\alpha]_D^{25} = +12.5$ ($c = 0.43$, MeOH)) and CD spectrum (λ_{max} nm ($\Delta\varepsilon$)[nm]: 225 (+2.46)) were opposite to those of sinularone D ($[\alpha]_D{}^{23} = -3.22$ ($c = 0.09$, MeOH), λ_{max} nm ($\Delta\varepsilon$)[nm]: 222 (−1.3), resp.) [6].

Suberosanone C (**3**) was obtained as an optically active yellow oil. The molecular formula, $C_{15}H_{22}O_2$, was deduced from the HR-CI-MS pseudo-molecular-ion peak at *m*/*z* 235.1699 ($[M + H]^+$; calc. 235.1698), indicating five degrees of unsaturation. The IR spectrum suggested the presence of an α,β-unsaturated ketone (1680 and 1635 cm^{-1}). The distinct ^{1}H-NMR signals for two Me groups at δ(H) 2.08 (*s*, Me(12)) and 1.69 (*s*, Me(13)), combined with the ^{13}C-NMR signals for three sp^2 quaternary C-atoms at δ(C) 214.0 (C(5)), 133.9 (C(6)), and 174.2 (C(7)), and two Me *singlet*s at δ(C) 13.8 (C(12)) and 8.5 (C(13)), allowed us to infer the presence of a dimethylcyclopentenone moiety (ring *A*) in **3**, as in **1**. This was supported by the HMBCs of Me(13) with C(5), C(6), and C(7); and of Me(12) with C(6), C(7), and C(8) (δ(C) 66.3). The direct connection of Me(14) (δ(H) 1.01 (*s*), δ(C) 21.9) to the sp^3 quaternary C-atom C(4) (δ(C) 56.7) was deduced from the HMBCs of Me(14) with C(4), C(5), and C(8). In addition, the bicyclic ring system consisting of ring *A* and *B* was established by the ^{1}H,^{1}H-COSY cross-peaks showing correlations from CH_2(2) (δ(H) 1.21–1.24 (*m*), 1.36–1.40 (*m*)) to

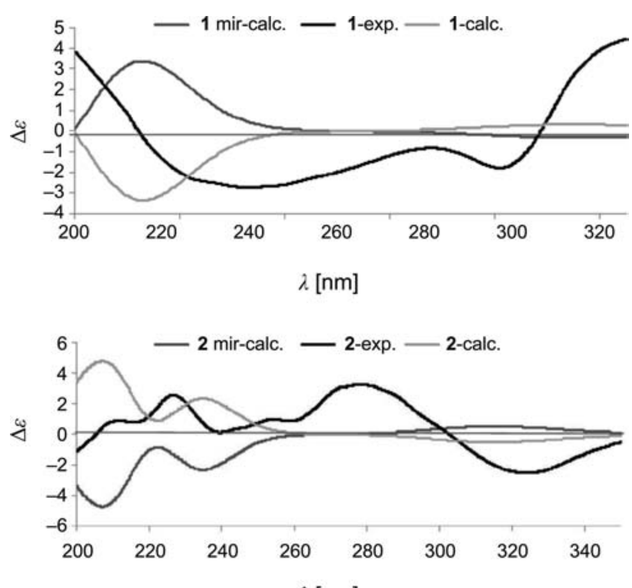

Fig. 4. *Experimental and calculated ECD spectra of compounds* **1** and **2**, *and their enantiomers*, $\mathbf{1}_{mir}$ *and* $\mathbf{2}_{mir}$, *respectively*

both H–C(1) (δ(H) 2.03–2.07 (*m*)) and CH_2(3) (δ(H) 1.42–1.47 (*m*), 2.03–2.07 (*m*)) (*Fig. 2*), and by HMBCs of H–C(1) with C(2) (δ(C) 25.0), C(3) (δ(C) 35.3), C(4), C(7), and C(8); of CH_2(3) with C(4), and C(5); and of Me(14) with C(3). Furthermore, the constructed bicyclic moiety was further extended from C(1) to C(11) by the ^{1}H,^{1}H-COSY cross-peaks H–C(1)/H–C(9) (δ(H) 1.64–1.67 (*m*))/H–C(10) (δ(H) 4.26 (br. *t*, *J* = 7.0))/CH_2(11) (δ(H) 1.82–1.85 (*m*), 2.06–2.11 (*m*)) (*Fig. 2*), where C(11) was connected to C(8) to form a unique tricyclic core for **3** as evidenced by the HMBCs of CH_2(11) with C(1), C(4), C(7), and C(8). A combination of ^{1}H,^{1}H-COSY cross-peak Me(15) (δ(H) 1.02 (*d*, *J* = 6.5))/H–C(9) and HMBCs of Me(15) with C(1), and C(9) (δ(C) 43.1) was indicative of the connection Me(15) to C(9), while the location of a OH group at the C(10) was deduced from the downfield chemical shift of CH(10) (δ(H) 4.26 (br. *t*, *J* = 7.0); δ(C) 76.9), and the HMBCs of H–C(10) with C(1), and C(8). As a result, the planar structure of suberosanone C (**3**) was established (*Fig. 1*). The relative configuration of **3** was determined by a NOESY experiment (*Fig. 3*). The NOE correlations of H–C(10) with Me(12), Me(15) and H–C(1), and Me(15) and with H–C(1) and Me(12) indicated that these H-atoms were located on the same face of ring *C*, whereas Me(14) and H–C(9) were oriented on the other side based on the NOE correlation of Me(14) with H–C(9) (*Fig. 3*). Literature survey indicated that suberosanone C (**3**) was similar to 5-oxosilphiperfol-6-ene from the stem of plant *Espeletiopsis guacharaca* [25], except for the additional presence of an OH group at C(10) of **3**.

Naturally occurring bicyclic or tricyclic compounds possessing 2,3-dimethylcyclopentone system are very rare, with only a few examples reported in the literature,

including sinularones C and D isolated from soft coral *Sinularia* sp. by *Lin* and co-workers recently [6], 5-oxosilphiperfol-6-ene from the stem of plant *Espeletiopsis guacharaca* [25], and α-tocospiros A and B from the aerial roots of *Ficus microcarpa* [26]. Not only will the isolation of suberosanones A – C (**1** – **3**, resp.) extend the big family of marine natural products with unusual structures, it would also attract further attention from synthetic organic chemists.

Compounds **1** – **3** were evaluated for their antifouling activities against *Balanus amphitrite* larvae settlement and antibacterial activities against *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Micrococcus luteus*. Unfortunately, all compounds tested showed no antifouling and antibacterial activities at concentrations up to 25 μg/ml.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 230 – 400 mesh, *Merck*) and *RP-18* gel (*Fuji Silysia Chemical Ltd.*). TLC: Precoated SiO_2 *G$_{60}$* aluminium sheet (*Merck*), visualization under UV light or by spraying with 5% H_2SO_4 in 95% EtOH followed by heating. Prep. HPLC: *Waters 2545* liquid chromatograph with an *XBridge* RP18 column (5 μm, 19 × 150 mm). Optical rotations: *JASCO P-2000* digital polarimeter. UV Spectra: *Shimadzu UV-1601* UV/VIS spectrophotometer; λ_{max} ($\Delta\varepsilon$), in nm. CD Spectra: *JASCO J-180* spectropolarimeter; λ in nm (deg cm^2 $dmol^{-1}$). IR Spectra: *PerkinElmer 16 PC* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian Inova*-500 NMR spectrometer and *Varian Mercury VX 300* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-CI-MS: *QSTAR XL* mass spectrometry system (*Applied Biosystem Co.*); in *m/z*.

Animal Material. The gorgonian coral *S. suberosa* was collected at Naozhou Island, South China Sea, P. R. China, in May 2011, and was identified by Dr. *Rob van Soest* (University of Amsterdam, The Netherlands). A voucher specimen (2011-05) has been deposited with the Laboratory of Natural Products, Department of Chemistry of Jinan University, Guangzhou, P. R. China.

Extraction and Isolation. The gorgonian coral *S. suberosa* (6.5 kg, wet weight) was extracted with EtOH three times (each time for 30 d) at r.t. The EtOH extract was concentrated under vacuum to give a brown gum (200 g). The residue obtained was partitioned between AcOEt and H_2O. The AcOEt fraction (140 g) was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt 10 – 100%; and MeOH/AcOEt 10 – 30%), to yield eleven fractions, *Frs. 1 – 11*. *Fr. 4* (7.0 g) was further separated by CC (SiO_2; PE/AcOEt 20 – 30%) into three subfractions. *Subfr. 2* (2.0 g) was then subjected to CC (*ODS*; MeOH/H_2O 50 – 100%) to furnish five fractions, *Subfr. 2a – 2e*. Finally, *Subfr. 2a* (0.3 g) was purified by prep. HPLC (35% MeCN/H_2O, 15.0 ml/min) to yield **1** (t_R 7.80 min; 6.5 mg), **2** (t_R 19.85 min; 5.5 mg), and **3** (t_R 11.63 min; 4.8 mg).

Suberosanone A (= *(3a*R*,6a*S*)-5,6-Dimethyl-6a-propyl-3a,6a-dihydro-2*H*-cyclopenta[*b*]furan-2,4(3*H*)-dione*; **1**). Yellow oil. $[\alpha]_D^{25} = +3.8$ ($c = 0.25$, MeOH). UV (MeCN): 234 (3.35). CD ($c = 0.25$, MeOH): 249 (−2.66). IR (film): 2962, 2935, 2874, 1777, 1710, 1652, 1420, 1324, 1265, 1218, 982. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-MS: 209.1176 ($[M + H]^+$, $C_{12}H_{17}O_3^+$; calc. 209.1178).

Suberosanone B (= *(5*R*,6*R*)-6-Butyl-8,9-dimethyl-1-oxaspiro[4.4]non-8-ene-2,7-dione*; **2**). Yellow oil. $[\alpha]_D^{25} = +12.5$ ($c = 0.43$, MeOH). UV (MeCN): 232 (3.28). CD ($c = 0.43$, MeOH): 225 (+2.46). IR (film): 2956, 2928, 2864, 1777, 1711, 1654, 1454, 1320, 1162, 1016, 906. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-MS: 236.1413 (M^+, $C_{14}H_{20}O_3^+$; calc. 236.1412).

Suberosanone C (= *(3a*R*,5a*S*,6*S*,7*S*,8a*R*)-7-Hydroxy-1,2,3a,6-tetramethyl-4,5,5a,6,7,8-hexahydrocyclopenta[*c*]pentalen-3(3a*H*)-one*; *10-Hydroxy-5-oxosilphiperfol-6-ene*; **3**). Yellow oil. $[\alpha]_D^{25} = -28.6$ ($c = 0.51$, MeOH). UV (MeCN): 250 (3.19). IR (film): 3442, 2931, 2868, 1680, 1635, 1511, 1461, 1387, 1336. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-MS: 235.1699 ($[M+H]^+$, $C_{15}H_{23}O_2^+$; calc. 235.1698).

Antifouling Assay. The antifouling activity was examined using the method described in by *Thiyagarajan et al.* [27]. Fresh cyprids were used in the testing. Larval settlement assays were examined using 24-well polystyrene plates (*Becton–Dickinson*). The tested samples were dissolved in small amount of DMSO and then diluted with 0.22 μm filtered seawater (FSW) to achieve final concentrations of 25.0, 12.5, 6.25, and 3.13 μg/ml. About 15–20 competent larvae were added to each well with 1 ml of test soln. in triplicate, and wells containing only FSW, DMSO, and larvae served as a control. The plates were incubated at 25° for 48 h. The effects of the test samples against biofouling were determined by examining the plates under a microscope to count settled, unsettled, and swimming larvae, and, where appropriate, potential toxic effects were recorded. The number of settled larvae was expressed as a percentage of the total number of larvae per well. The EC_{50} value was calculated as the concentration where 50% of the larval individuals were inhibited to settle as compared to the control.

Antibacterial Assay. The antibacterial activities were evaluated using a modified microdilution method described in [28]. In brief, the strains *B. subtillis*, methicillin-resistant *S. aureus*, *E. coli*, *C. albicans*, and *M. luteus* were inoculated in lysogeny broth (LB; (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, 1 l of double-distilled H_2O) and were incubated at 28° for 12 h, resp. Stock solns. of the samples were prepared at 25 mg/ml concentration in small amount of DMSO and then further diluted with LB broth to achieve various concentrations in 96-well plates. The bacteria were incubated at 28° overnight. Cell growth was checked by measuring the optical density at 595 nm, with penicillin G and streptomycin as positive controls.

ECD Calculation. The structures of all isomers of the two compounds **1** and **2** are fully scanned at HF/STO-3G level. All local minimum-energy conformations are calculated at B3LYP/6-31G(d)/B3LYP/3-21G(d) level to obtain global minima. Conformers within an energy range of 3 kcal/mol from the global minima were subjected to geometrical optimization (DFT/B3LYP/6-31G(d)) in the gas-phase combined with calculation of vibrational modes to confirm these minima. No imaginary frequencies were found. After conformational searches, seven conformations for **1** and 17 conformations for **2** with low energy were found. For these conformations, ECD spectra were calculated at the B3LYP/6-311+G(d,p) level in MeOH (SCRF/IEFPCM). Each calculated ECD spectrum was assigned a *Boltzmann* weight according to the energy of the minimized conformers at 298.15 K and overlaid prior to the comparisons with particular experimental results. All of the DFT calculations reported in this study were performed with the Gaussian 03 package [29].

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A Novel One-Pot Sulfonyloxylactonization of Alkenoic Acids Mediated by Hypervalent Iodine Species Generated *in situ* from Ammonium Iodide

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A novel and efficient one-pot procedure was designed for the sulfonyloxylactonization of alkenoic acids by the reaction of alkenoic acids with *m*-chloroperbenzoic acid and sulfonic acids in the presence of a catalytic amount of ammonium iodide in a mixture acetonitrile/2,2,2-trifluoroethanol (4:1) at room temperature for 24 h, which provided the corresponding sulfonyloxy lactones in moderate-to-good yields.

Introduction. – To extend the scope of catalytic use of hypervalent iodine reagents for organic synthesis, we have investigated the sulfonyloxylactonization of alkenoic acid in the presence of a catalytic hypervalent iodine reagent [1], and we also developed a novel one-pot method for preparation of α-tosyloxy ketones using a catalytic amount of NH_4I [2]. Herein, we report a novel sulfonyloxylactonization of alkenoic acids using a catalytic amount of inorganic NH_4I, which, to the best of our knowledge, has not been reported before.

Results and Discussion. – At the beginning of our work, we investigated the reaction of pent-4-enoic acid with *m*-chloroperbenzoic acid (MCPBA) and *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$) in the presence of catalytic amounts of iodides in organic solvents at room temperature for 24 h. Generally, the reaction proceeded well and afforded the desired product (*Table 1*).

In the light of the successful formation of 5-tosyloxypentano-4-lactone, the reaction conditions were optimized, and the results are compiled in *Table 1*. It was realized that solvents influenced the yield strongly, and when 1.0 equiv. of pent-4-enoic acid was mixed with 1.0 equiv. of $TsOH \cdot H_2O$, 1.2 equiv. of MCPBA and 0.2 equiv. of NH_4I in a mixture MeCN/2,2,2-trifluoroethanol (TFE) 4:1 at room temperature for 24 h, yields of up to 49% were obtained (*Entries 1–8*). When more MCPBA was added, the yield increased, and use of 2.0 equiv. of MCPBA resulted in a yield of 74% (*Entries 8–12*); in contrast, in the absence of MCPBA, no product was observed (*Entry 13*). Other oxidants such as potassium hydrogen persulfate (*Oxone*®) and $NaBO_3 \cdot 4\,H_2O$ were also studied; however, low yields were obtained (*Entries 14* and *15*). NaI and KI were efficient providing moderate yields (*Entries 16* and *17*). The effect of the amount of NH_4I was also investigated, and 0.3 equiv. turned out to be the best choice (*Entries 8*, *10*, *19–24*). When I_2 was used instead of NH_4I, the product was obtained in 66% yield,

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Table 1. *Optimization of the Sulfonyloxylactonization of Pent-4-enoic Acid*

TsOH (1 equiv.), oxidant, I^-; Solvent, r.t., 24 h

Entry	Solvent	Oxidant (equiv.)	I^- (equiv.)	Yield [%][a])
1	MeCN	MCPBA (1.2)	NH_4I (0.2)	42
2	TFE	MCPBA (1.2)	NH_4I (0.2)	39
3	MeOH	MCPBA (1.2)	NH_4I (0.2)	17
4	EtOH	MCPBA (1.2)	NH_4I (0.2)	21
5	THF	MCPBA (1.2)	NH_4I (0.2)	30
6	AcOEt	MCPBA (1.2)	NH_4I (0.2)	28
7	CH_2Cl_2	MCPBA (1.2)	NH_4I (0.2)	35
8	MeCN/TFE 4:1	MCPBA (1.2)	NH_4I (0.2)	49
9	MeCN/TFE 4:1	MCPBA (1.5)	NH_4I (0.2)	52
10	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0.2)	74
11	MeCN/TFE 4:1	MCPBA (2.5)	NH_4I (0.2)	52
12	MeCN/TFE 4:1	MCPBA (3.0)	NH_4I (0.2)	50
13	MeCN/TFE 4:1	–	NH_4I (0.2)	0
14	MeCN/TFE 4:1	Oxone® (2.0)	NH_4I (0.2)	48
15	MeCN/TFE 4:1	$NaBO_3 \cdot 4H_2O$ (2.0)	NH_4I (0.2)	39
16	MeCN/TFE 4:1	MCPBA (2.0)	NaI (0.2)	62
17	MeCN/TFE 4:1	MCPBA (2.0)	KI (0.2)	57
18	MeCN/TFE 4:1	MCPBA (2.0)	I_2 (0.2)	66
19	MeCN/TFE 4:1	MCPBA (1.2)	NH_4I (0.3)	67
20	MeCN/TFE 4:1	MCPBA (1.2)	NH_4I (0.4)	65
21	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0.3)	77
22	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0.4)	75
23	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0.1)	39
24	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0.05)	33
25	MeCN/TFE 4:1	MCPBA (2.0)	NH_4I (0)	0

[a]) Yield of the isolated lactones.

which was somewhat low compared with that obtained using NH_4I under the same conditions (Entry *18*).

With the optimal conditions in hand, and to assess their generality and applicability, a series of alkenoic acids were investigated (*Table 2*).

It is obvious from *Table 2* that the sulfonyloxylactonization was compatible with most of the alkenoic acids, and the corresponding sulfonyloxy lactones were obtained in moderate-to-good yields (*Entries 1–7*, *10*, and *11*). Similar treatment of but-3-enoic acid and (*E*)-hex-3-enoic acid, only the unsaturated lactones were obtained, not the desired sulfonylactones (*Entries 12* and *13*). The ^{1}H-NMR analysis revealed that the desired five-membered and four-membered lactones were first formed, and then transformed to the unsaturated lactones by elimination during workup procedure. Efforts for the preparation of the six-membered lactone using hex-5-enoic acid were only partially successful: the ^{1}H-NMR spectrum of the crude product indicated lactone formation, but purification was not achieved. Interestingly, when camphor-10-sulfonic

Table 2. *Sulfonyloxylactonization of Alkenoic Acids in the Presence of a Catalytic Amount of NH_4I*[a])

R–COOH (**1**) + R^1–SO_2OH (**2**) → (NH_4I (0.3 equiv.), MCPBA (2.0 equiv.), MeCN/TFE 4:1, r.t., 24 h) → lactone–$CH_2OSO_2R^1$ (**3**)

Entry	Alkenoic acid **1**	R	Sulfonic acid **2**	R^1	Sulfonyloxy lactone **3**	Yield [%][b])
1	**1a**	CH_2=$CHCH_2CH_2$	**2a**	4-Me–C_6H_4	**3a**	77
2	**1b**	CH_2=$CHCH_2CH(Me)$	**2a**	4-Me–C_6H_4	**3b**	63 (0.27:0.73)[c])
3	**1c**	CH_2=$CHCH(Me)CH_2$	**2a**	4-Me–C_6H_4	**3c**	61 (0.34:0.66)[c])
4	**1d**	CH_2=$CHCH_2C(Me_2)$	**2a**	4-Me–C_6H_4	**3d**	78
5	**1e**	Cyclopent-2-en-1-ylmethyl	**2a**	4-Me–C_6H_4	**3e**	50

Table 2 (cont.)

Entry	Alkenoic acid **1**	R	Sulfonic acid **2**	R^1	Sulfonyloxy lactone **3**	Yield [%][b])
6	**1a**	$CH_2{=}CHCH_2CH_2$	**2b**	4-Cl–C_6H_4	**3f**	62
7	**1b**	$CH_2{=}CHCH_2CH(Me)$	**2b**	4-Cl–C_6H_4	**3g**	57
8	**1c**	$CH_2{=}CHCH(Me)CH_2$	**2b**	4-Cl–C_6H_4	**3h**	46 (0.30 : 0.70)[c])
9	**1e**	(Cyclopent-2-en-1-yl)methyl	**2b**	4-Cl–C_6H_4	**3i**	43
10	**1a**	$CH_2{=}CHCH_2CH_2$	**2c**	Ph	**3j**	60
11	**1b**	$CH_2{=}CHCH_2CH(Me)$	**2c**	Ph	**3k**	66 (0.35 : 0.65)[c])
12	**1f**	$CH_2{=}CHCH_2$	**2a**	4-Me–C_6H_4	**3l**	63

Table 2 (cont.)

Entry	Alkenoic acid **1**	R	Sulfonic acid **2**	R[1]	Sulfonyloxy lactone **3**	Yield [%][b])
13	**1g**	(E)-EtCH=CHCH$_2$	**2a**	4-Me–C$_6$H$_4$	**3m**	75
14	**1a**	CH$_2$=CHCH$_2$CH$_2$	**2d**	(+)-Camphor-10-yl	**3n** [d]) OSO$_2$Camph	42
15	**1b**	CH$_2$=CHCH$_2$CH(Me)	**2d**	(+)-Camphor-10-yl	**3o** OSO$_2$Camph	38 (0.25 : 0.75)[c])
16	**1c**	CH$_2$=CHCH(Me)CH$_2$	**2d**	(+)-Camphor-10-yl	**3p** OSO$_2$Camph	32 (0.32 : 0.68)[c])

[a]) Reaction conditions: 1.0 equiv. of alkenoic acid, 0.3 equiv. of NH$_4$I, 2.0 equiv. of MCPBA, and 1.0 equiv. of TsOH · H$_2$O in MeCN/TFE 4 : 1 for 24 h. [b]) Yield of the isolated lactones. [c]) The ratio of diastereoisomers. [d]) Camph, (+)-camphor-10-yl.

acid, an aliphatic sulfonic acid, was used instead of $TsOH \cdot H_2O$ under similar condition, the corresponding sulfonyloxy lactones were also obtained; however, the yields were somewhat low (*Entries 13–16*). It was also found that alkenoic acids **1b** and **1c** usually resulted in the mixtures of diastereoisomers, and the ratios changed from 0.35 : 0.65 to 0.25 : 0.75 (*Entries 2*, *3*, *8*, *11*, *15*, and *16*).

According to the above results, a plausible reaction pathway for the present reaction is proposed in *Scheme 1*. Thus, NH_4I is first oxidized to I_2 and HOI with MCPBA, and both of them react with alkenoic acid to form the iodolactone, which is then transformed to the hypervalent iodine intermediate *in situ* by the continuing oxidation, and finally the sulfonyloxy lactone is formed with sulfonic acid as nucleophile in the following intermolecular nucleophilic substitution. To assess this proposal, pent-4-enoic acid was first reacted with I_2 and HOI [3], respectively, both providing nearly quantitatively the iodolactone. Then, the reaction of the iodolactone with sulfonic acids was examined (*Scheme 2*), with the result that, with only $TsOH \cdot H_2O$, the desired product of 5-tosyloxypentano-4-lactone was not detected after a long reaction time. However, when MCPBA was added to the reaction mixture, 5-tosyloxypentano-4-lactone was obtained in good yield. Therefore, the *in situ*-generated hypervalent iodine intermediate was responsible for the reaction. Recently, *Kang* and *Gade* reported another mechanism for an oxidative lactonization of alkenoic acids with AcOOH, AcOH, and Ac_2O in the absence of iodo species [4]. To identify that our protocol does not conform to the mechanism described in [4], we conducted our reaction in the absence of NH_4I, and no corresponding sulfonyloxy lactone was obtained (*Table 1*, *Entry 25*). We also examined the reaction of 4,5-epoxyhexanoic acid with $TsOH \cdot H_2O$ under our reaction conditions, but the desired sulfonyloxy lactone

Scheme 1. *A Plausible Reaction Pathway*

Scheme 2. *Identification of the Plausible Reaction Pathway*

was not observed after a long reaction time. Therefore, it is obvious that our reaction mechanism is different from that of *Afonso*'s reaction [5].

In summary, we have developed a novel and efficient method for the sulfonyloxylactonization of alkenoic acid using a catalytic amount of NH_4I with MCPBA in MeCN/TFE 4 : 1 at room temperature. The presented results highlight the advantageous role of inorganic iodide as catalyst and as a substitute for expensive aryl iodides. Moreover, it extends the scope of hypervalent iodine reagents in organic synthesis, as well as being more environmentally benign.

Experimental Part

General. Ammonium iodide (NH_4I), *m*-chloroperbenzoic acid (MCPBA), alkenoic acids, and sulfonic acids were commercially available. M.p.: *XT-4* melting-point apparatus; uncorrected. IR Spectra: *Thermo Nicolet 6700* instrument. ^{1}H- and ^{13}C-NMR spectra: in $CDCl_3$ on a *Bruker Avance III* (500 MHz) spectrometer. MS: *Thermo ITQ 1100* mass spectrometer.

Representative Procedure for the Catalytic Sulfonyloxylactonization of Alkenoic Acids Using a Catalytic Amount of NH_4I. To a mixture of MeCN (1.6 ml) and 2,2,2-trifluoroethanol (TFE; 0.4 ml), alkenoic acid **1** (0.3 mmol), MCPBA (0.6 mmol), NH_4I (0.09 mmol), and sulfonic acid **2** (0.3 mmol) were added. The resulting mixture was stirred at r.t. for 24 h. Then, H_2O (5 ml), sat. aq. $Na_2S_2O_3$ (2 ml), and sat. aq. Na_2CO_3 (2 ml), were poured into the mixture. The mixture was extracted with CH_2Cl_2 (3 × 5 ml), and the combined org. layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by prep. TLC (SiO_2; AcOEt/hexane 3 : 2) to give the pure product **3**.

(Tetrahydro-5-oxofuran-2-yl)methyl 4-Methylbenzenesulfonate (**3a**). Yield: 62 mg (77%). White solid. M.p. 78–80° ([1]: 79–81°). ^{1}H-NMR: 7.78 (*d*, *J* = 8.5, 2 H); 7.37 (*d*, *J* = 8.5, 2 H); 4.72–4.65 (*m*, 1 H); 4.19 (*dd*, *J* = 11.0, 3.0, 1 H); 4.13 (*dd*, *J* = 11.0, 4.0, 1 H); 2.61–2.48 (*m*, 2 H); 2.46 (*s*, 3 H); 2.39–2.31 (*m*, 1 H); 2.15–2.10 (*m*, 1 H). ^{13}C-NMR: 176.0; 145.4; 132.2; 130.0; 127.9; 76.4; 70.0; 27.8; 23.5; 21.6.

(Tetrahydro-4-methyl-5-oxofuran-2-yl)methyl 4-Methylbenzenesulfonate (**3b**). Yield: 54 mg (63%). White solid. M.p. 93–95° ([1]: 91–95°). ^{1}H-NMR: 7.80 (*dd*, *J* = 10.0, 5.0, 2 H); 7.37 (*d*, *J* = 10.0, 2 H); 4.68–4.62 (*m*, 0.27 H); 4.58–4.52 (*m*, 0.73 H); 4.22 (*dd*, *J* = 11.0, 3.5, 0.54 H); 4.15–4.11 (*m*, 1.46 H); 2.76–2.67 (*m*, 1 H); 2.51–2.35 (*m*, 1 H); 2.46 (*s*, 3 H); 2.06–2.03 (*m*, 0.27 H); 1.73–1.70 (*m*, 0.73 H); 1.27 (*d*, *J* = 7.0, 3 H). ^{13}C-NMR: 178.2; 145.4 (*d*, *J* = 12.5); 132.3; 130.0 (*d*, *J* = 6.3); 127.9 (*d*, *J* = 6.3); 74.5; 74.1; 70.2; 69.4; 35.0; 33.6; 32.1; 31.7; 21.6; 16.0; 15.0.

(Tetrahydro-3-methyl-5-oxofuran-2-yl)methyl 4-Methylbenzenesulfonate (**3c**) [1]. Yield: 52 mg (61%). Colorless oil. ^{1}H-NMR: 7.80 (*d*, *J* = 10.0, 2 H); 7.38 (*d*, *J* = 10.0, 2 H); 4.62–4.59 (*m*, 0.34 H); 4.17–4.13 (*m*, 0.66 H); 4.22–4.17 (*m*, 2 H); 2.81–2.71 (*m*, 1 H); 2.64–2.59 (*m*, 0.34 H); 2.52–2.50 (*m*, 0.66 H); 2.46 (*s*, 3 H); 2.29 (*dd*, *J* = 17.5, 8.0, 0.34 H); 2.19 (*dd*, *J* = 17.5, 8.0, 0.66 H); 1.18 (*d*, *J* = 10.0, 2 H); 1.12 (*d*, *J* = 5.0, 1 H). ^{13}C-NMR: 175.6; 175.2; 145.4 (*d*, *J* = 6.3); 132.1 (*d*, *J* = 23.8); 130.1 (*d*, *J* = 22.5); 127.9 (*d*, *J* = 2.5); 82.9; 78.8; 68.6; 67.7; 36.2 (*d*, *J* = 23.8); 31.8 (*d*, *J* = 25.0); 21.6; 17.0; 13.5.

(Tetrahydro-4,4-dimethyl-5-oxofuran-2-yl)methyl 4-Methylbenzenesulfonate (**3d**). Yield: 70 mg (78%). White solid. M.p. 99–101°. IR (film): 2977, 2934, 1787, 1459, 1355, 1210, 1188, 1136, 978. ^{1}H-NMR: 7.79 (*d*, *J* = 8.3, 2 H); 7.37 (*d*, *J* = 8.2, 2 H); 4.66–4.57 (*m*, 1 H); 4.21 (*dd*, *J* = 11.1, 3.5, 1 H); 4.09 (*dd*, *J* = 11.1, 5.2, 1 H); 2.46 (*s*, 3 H); 2.13 (*dd*, *J* = 12.9, 6.6, 1 H); 1.91 (*dd*, *J* = 12.9, 9.7, 1 H); 1.26 (*s*, 3 H); 1.25 (*s*, 3 H). ^{13}C-NMR: 180.7; 145.3; 132.4; 130.0; 127.9; 73.2; 69.6; 39.9; 38.5; 24.8; 24.7; 21.6. ESI-MS: 299.1 (100, $[M+1]^+$). HR-MS: 299.0944 ($[M+1]^+$, $C_{14}H_{19}O_5S^+$; calc. 299.0948).

*Hexahydro-2-oxo-2*H*-cyclopenta[*b*]furan-6-yl 4-Methylbenzenesulfonate* (**3e**). Yield: 44 mg (50%). Colorless oil. IR (film): 971, 2879, 1784, 1457, 1362, 1177, 1097, 984. ^{1}H-NMR: 7.79 (*d*, *J* = 8.3, 2 H); 7.37 (*d*, *J* = 8.1, 2 H); 4.84–4.83 (*m*, 1 H); 4.80 (*d*, *J* = 6.9, 1 H); 3.05–3.03 (*m*, 1 H); 2.79 (*dd*, *J* = 18.6, 10.3, 1 H); 2.45 (*s*, 3 H); 2.24 (*dd*, *J* = 18.7, 2.9, 1 H); 2.20–2.14 (*m*, 1 H); 1.91–1.87 (*m*, 2 H); 1.57–1.52 (*m*, 1 H). ^{13}C-NMR: 76.0; 145.3; 133.1; 130.0; 127.8; 87.1; 84.5; 36.1; 35.0; 30.6; 29.8; 21.6. ESI-ES: 297.1 (100, $[M+1]^+$). HR-MS: 297.0783 ($[M+1]^+$, $C_{14}H_{17}O_5S^+$; calc. 297.0791).

(Tetrahydro-5-oxofuran-2-yl)methyl 4-Chlorobenzenesulfonate (**3f**). Yield: 54 mg (62%). White solid. M.p. 99–100°. IR (film): 3092, 2958, 1774, 1477, 1398, 1359, 1281, 1190, 1169, 1088, 967. ^{1}H-NMR: 7.84 (*d*, *J* = 8.7, 2 H); 7.56 (*d*, *J* = 8.7, 2 H); 4.71–4.70 (*m*, 1 H); 4.24 (*dd*, *J* = 11.1, 3.2, 1 H); 4.17 (*dd*, *J* = 11.1, 4.5, 1 H); 2.62–2.49 (*m*, 2 H); 2.40–2.33 (*m*, 1 H); 2.15–2.08 (*m*, 1 H). ^{13}C-NMR: 175.8; 141.0; 133.8; 129.8; 129.4; 76.3; 70.4; 27.8; 23.4. ESI-MS: 291.0 (100, $[M+1]^+$). HR-MS: 291.0091 ($[M+1]^+$, $C_{11}H_{12}ClO_5S^+$; calc. 291.0088).

(Tetrahydro-4-methyl-5-oxofuran-2-yl)methyl 4-Chlorobenzenesulfonate (**3g**). Yield: 52 mg (57%). White solid. M.p. 118–120°. IR (film): 3095, 2996, 2938, 2879, 1768, 1457, 1370, 1283, 1181, 1144, 967. ^{1}H-NMR: 7.87 (*d*, *J* = 1.8, 1 H); 7.86 (*d*, *J* = 1.8, 1 H); 7.57 (*d*, *J* = 2.0, 1 H); 7.56 (*d*, *J* = 1.8, 1 H); 4.59–4.55 (*m*, 1 H); 4.28 (*dd*, *J* = 11.2, 3.3, 1 H); 4.15 (*dd*, *J* = 11.2, 5.2, 1 H); 2.72–2.69 (*m*, 1 H); 2.52–2.47 (*m*, 1 H); 1.74–1.68 (*m*, 1 H); 1.28 (*d*, *J* = 7.0, 3 H). ^{13}C-NMR: 178.0; 141.0; 133.9; 129.8; 129.4; 74.4; 69.8; 35.1; 32.2; 15.1. ESI-MS: 305.0 (100, $[M+1]^+$). HR-MS: 305.0241 ($[M+1]^+$, $C_{12}H_{14}ClO_5S^+$; calc. 305.0245).

(Tetrahydro-3-methyl-5-oxofuran-2-yl)methyl 4-Chlorobenzenesulfonate (**3h**). Yield: 42 mg (46%). Colorless oil. IR (film): 3093, 2969, 1788, 1477, 1368, 1280, 1188, 1088, 970. ^{1}H-NMR: 7.85 (*d*, *J* = 8.7, 2 H); 7.56 (*d*, *J* = 7.3, 2 H); 4.65–4.55 (*m*, 0.3 H); 4.27–4.17 (*m*, 0.7 H); 2.82–2.78 (*m*, 0.3 H); 2.73 (*dd*, *J* = 17.7, 8.7, 0.7 H); 2.63 (*dd*, *J* = 17.4, 8.6, 0.3 H); 2.50–2.46 (*m*, 0.7 H); 2.27 (*dd*, *J* = 17.4, 7.8, 0.3 H); 2.20 (*dd*, *J* = 17.7, 8.5, 0.7 H); 1.18 (*d*, *J* = 6.8, 0.9 H); 1.12 (*d*, *J* = 7.1, 2.1 H). ^{13}C-NMR: 175.0; 141.0; 133.8; 129.8 (*d*, *J* = 2.2); 129.4 (*d*, *J* = 2.4); 82.8; 78.7; 68.9; 68.2; 36.3; 36.1; 31.9; 31.8; 17.9; 13.6. ESI-MS: 322.0 (100, $[M+NH_4]^+$). HR-MS: 322.051 ($[M+NH_4]^+$, $C_{12}H_{17}NClO_5S^+$; calc. 322.0516).

*Hexahydro-2-oxo-2*H*-cyclopenta[*b*]furan-6-yl 4-Chlorobenzenesulfonate* (**3i**). Yield: 41 mg (43%). Colorless oil. IR (film): 3093, 2968, 1784, 1477, 1368, 1281, 1181, 1088, 983. ^{1}H-NMR: 7.87 (*d*, *J* = 8.6, 2 H); 7.56 (*d*, *J* = 8.5, 2 H); 4.89–4.87 (*m*, 1 H); 4.82 (*d*, *J* = 7.0, 1 H); 3.07–3.05 (*m*, 1 H); 2.81 (*dd*, *J* = 18.7, 10.3, 1 H); 2.27 (*dd*, *J* = 18.7, 2.9, 1 H); 2.21–2.17 (*m*, 1 H); 1.96–1.90 (*m*, 2 H); 1.59–1.54 (*m*, 1 H). ^{13}C-NMR: 175.9; 140.9; 134.7; 129.9; 129.3; 87.0; 85.2; 36.2; 35.0; 30.6; 30.0. ESI-MS: 339.0 (100, $[M+Na]^+$). HR-MS: 339.0061 ($[M+Na]^+$, $C_{13}H_{13}ClNaO_5S^+$; calc. 339.0070).

(Tetrahydro-5-oxofuran-2-yl)methyl Benzenesulfonate (**3j**). Yield: 46 mg (60%). Colorless oil. IR (film): 2957, 1781, 1449, 1362, 1181, 1096, 957. ^{1}H-NMR: 7.88–7.86 (*m*, 2 H); 7.68–7.65 (*m*, 1 H); 7.57–7.54 (*m*, 2 H); 4.69–4.65 (*m*, 1 H); 4.20 (*dd*, *J* = 11.1, 3.2, 1 H); 4.12 (*dd*, *J* = 11.2, 4.6, 1 H); 2.57–2.48 (*m*, 2 H); 2.35–2.28 (*m*, 1 H); 2.09–2.03 (*m*, 1 H). ^{13}C-NMR: 176.0; 135.1; 134.1; 129.3; 127.7; 76.3; 70.2; 27.7; 23.2. ESI-MS: 279.0 (100, $[M+Na]^+$). HR-MS: 279.0301 ($[M+Na]^+$, $C_{11}H_{12}NaO_5S^+$; calc. 279.0303).

(Tetrahydro-4-methyl-5-oxofuran-2-yl)methyl Benzenesulfonate (**3k**). Yield: 53 mg (66%). Colorless oil. IR (film): 3068, 2977, 2881, 1775, 1450, 1362, 1292, 1189, 1096, 972. ^{1}H-NMR: 7.87–7.83 (*m*, 2 H); 7.66–7.63 (*m*, 1 H); 7.55–7.52 (*m*, 2 H); 4.64–4.60 (*m*, 0.35 H); 4.55–4.50 (*m*, 0.65 H); 4.21 (*dd*, *J* = 11.3,

3.1, 0.65 H); 4.15 (*dd*, *J* = 11.0, 3.2, 0.35 H); 4.11 – 4.03 (*m*, 1 H); 2.73 – 2.59 (*m*, 1 H); 2.45 – 2.40 (*m*, 0.65 H); 2.32 – 2.27 (*m*, 0.35 H); 1.98 – 1.95 (*m*, 0.35 H); 1.66 – 1.59 (*m*, 0.65 H); 1.18 (*d*, *J* = 2.6, 3 H). ^{13}C-NMR: 178.9; 178.1; 135.2; 135.1; 134.1 (*d*, *J* = 8.4); 129.3 (*d*, *J* = 5.9); 127.6 (*d*, *J* = 4.7); 74.4; 74.0; 70.5; 69.7; 34.8; 33.4; 31.8; 31.4; 15.8; 14.8. ESI-MS: 288.1 (100, $[M + NH_4]^+$). HR-MS: 288.092 ($[M + NH_4]^+$, $C_{12}H_{18}NO_5S^+$; calc. 288.0906).

*Furan-2(5*H*)-one* (**3l**) [6]. Yield: 16 mg (63%). Colorless oil. ^{1}H-NMR: 7.61 – 7.59 (*m*, 1 H); 6.19 – 6.17 (*m*, 1 H); 4.93 – 4.92 (*m*, 2 H). ^{13}C-NMR: 173.6; 152.7; 121.6; 72.1.

*5-Ethylfuran-2(5*H*)-one* (**3m**) [6]. Yield: 26 mg (75%). Colorless oil. ^{1}H-NMR: 7.47 – 7.45 (*m*, 1 H); 6.14 – 6.12 (*m*, 1 H); 5.03 – 5.00 (*m*, 1 H); 1.88 – 1.82 (*m*, 1 H); 1.77 – 1.70 (*m*, 1 H); 1.02 (*t*, *J* = 1.9, 3 H). ^{13}C-NMR: 173.1; 155.9; 121.8; 84.3; 26.3; 9.0.

*(Tetrahydro-5-oxofuran-2-yl)methyl [(1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]hept-1-yl]methansulfonate* (**3n**) [7]. Yield: 42 mg (42%). Colorless oil. ^{1}H-NMR: 4.82 – 4.79 (*m*, 1 H); 4.51 (*ddd*, *J* = 22.0, 11.5, 3.0, 1 H); 4.37 (*ddd*, *J* = 21.5, 11.0, 4.5, 1 H); 3.62 (*dd*, *J* = 15.5, 6.0, 1 H); 3.08 (*dd*, *J* = 15.0, 4.0, 1 H); 2.69 – 2.52 (*m*, 2 H); 2.43 – 2.37 (*m*, 3 H); 2.20 – 2.02 (*m*, 3 H); 1.97 (*d*, *J* = 17.5, 1 H); 1.75 – 1.65 (*m*, 1 H); 1.51 – 1.45 (*m*, 1 H); 1.10 (*s*, 3 H); 0.88 (*s*, 3 H). ^{13}C-NMR: 214.2; 176.1; 76.7 (*d*, *J* = 7.5); 70.1; 69.9; 57.8 (*d*, *J* = 2.5); 48.1; 47.2; 42.6 (*d*, *J* = 2.5); 42.4 (*d*, *J* = 1.3); 27.9; 26.8; 24.8 (*d*, *J* = 7.5); 23.3 (*d*, *J* = 8.8); 19.5 (*t*, *J* = 2.5).

*(Tetrahydro-4-methyl-5-oxofuran-2-yl)methyl [(1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]hept-1-yl]-methanesulfonate* (**3o**) [7]. Yield: 39 mg (38%). Colorless oil. ^{1}H-NMR: 4.79 – 4.72 (*m*, 0.25 H); 4.68 – 4.62 (*m*, 0.75 H); 4.51 (*ddd*, *J* = 19.0, 11.5, 3.0, 1 H); 4.37 – 4.31 (*m*, 1 H); 3.66 – 3.59 (*m*, 1 H); 3.09 – 3.03 (*m*, 1 H); 2.83 – 2.78 (*m*, 0.25 H); 2.77 – 2.70 (*m*, 0.75 H); 2.56 – 2.49 (*m*, 1 H); 2.46 – 2.37 (*m*, 2 H); 2.16 – 2.13 (*m*, 1 H); 2.10 – 2.03 (*m*, 1 H); 1.97 (*d*, *J* = 18.0, 1 H); 1.79 – 1.68 (*m*, 2 H); 1.50 – 1.44 (*m*, 1 H); 1.31 (*d*, *J* = 5.5, 2.25 H); 1.30 (*d*, *J* = 6.0, 0.75 H); 1.10 (*s*, 2.25 H); 1.09 (*s*, 0.75 H); 0.89 (*s*, 2.25 H); 0.88 (*s*, 0.75 H). ^{13}C-NMR: 214.4; 214.3; 179.2; 178.3; 74.9; 74.5 (*d*, *J* = 7.5); 70.5; 70.4; 69.7; 69.4; 57.9 (*d*, *J* = 3.8), 48.2 (*d*, *J* = 3.8); 47.4; 47.3 (*d*, *J* = 6.3); 42.7; 42.5 (*d*, *J* = 2.5); 35.2; 33.7; 32.2 (*d*, *J* = 8.8); 31.6 (*d*, *J* = 6.3); 26.9; 24.9 (*t*, *J* = 3.8); 19.6 (*d*, *J* = 5.0); 16.1; 15.1 (*d*, *J* = 3.8).

*(Tetrahydro-3-methyl-5-oxofuran-2-yl)methyl [(1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]hept-1-yl]-methanesulfonate* (**3p**) [7]. Yield: 33 mg (32%). Colorless oil. ^{1}H-NMR: 4.75 – 4.30 (*m*, 3 H); 3.63 (*dd*, *J* = 15.0, 4.5, 1 H); 3.07 (*dd*, *J* = 15.5, 4.0, 1 H); 2.85 – 2.78 (*m*, 1 H); 2.72 – 2.66 (*m*, 0.68 H); 2.56 – 2.49 (*m*, 0.32 H); 2.45 – 2.22 (*m*, 3 H); 2.15 – 2.13 (*m*, 1 H); 2.11 – 2.02 (*m*, 1 H); 1.97 (*d*, *J* = 18.5, 1 H); 1.75 – 1.65 (*m*, 1 H); 1.51 – 1.44 (*m*, 1 H); 1.23 (*dd*, *J* = 6.5, 3.0, 0.96 H); 1.17 (*d*, *J* = 7.5, 2.04 H); 1.10 (*s*, 3 H); 0.88 (*s*, 3 H). ^{13}C-NMR: 214.3; 175.7 (*d*, *J* = 2.5); 175.4; 83.3 (*d*, *J* = 2.5); 79.2 (*d*, *J* = 5.0); 68.8; 68.6; 68.2; 68.0; 57.9 (*d*, *J* = 3.8); 48.2; 47.4 (*d*, *J* = 3.8); 47.2 (*d*, *J* = 2.5); 42.7 (*d*, *J* = 3.8); 42.5 (*d*, *J* = 2.5); 36.4 (*d*, *J* = 3.8); 36.2; 31.9; 31.8; 31.7; 26.9; 24.9 (*d*, *J* = 2.5); 24.8; 19.6 (*d*, *J* = 3.8); 18.0; 13.5.

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Three New Chromanones from *Calea clausseniana*

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Three new chromanones, uniflorol A acetate (**1**), uniflorol B acetate (**2**), and 2,2-dimethyl-6-{1-[(4′-acetoxy)angeloyloxy]ethyl}chroman-4-one (**3**), together with the known chromanone **4**, and two known *p*-hydroxyacetophenone derivatives, **5** and **6**, were isolated from the CH_2Cl_2 crude extract of the underground parts of *Calea clausseniana* BAKER. The structures of the new compounds were elucidated by spectroscopic analyses, including 2D-NMR techniques.

Introduction. – The Asteraceae family comprises *ca.* 23,000 species included in 1,535 genera [1]. This family is still known by its older common name Compositae. It is mainly herbaceous with a few shrubs and trees. The genus *Calea* L. occurs in Mexico, Central and South America and contains *ca.* 110 species [2]. *Calea clausseniana* BAKER is a perennial herb with yellow flowers. The plant is not used in folk medicine, although other species of the same genus are used for treatment of stomach diseases [3–5]. Recently, we described the isolation of a new 5-deoxyflavone glycoside and one known quercetin glycoside from the EtOH extract of the aerial parts of the plant [6]. In addition to these compounds, herein we report the isolation and the structure elucidation of three novel chromanones, **1**, **2**, and **3**, one known chromanone, **4**, and two known *p*-hydroxyacetophenone derivatives, **5** and **6**, from underground parts of this plant.

Results and Discussion. – Phytochemical investigation of the CH_2Cl_2 extract of *C. clausseniana* underground parts by chromatographic techniques led to the isolation and structure elucidation of three new chromanones, **1**–**3**, along with four known compounds, *i.e.*, 2,2-dimethyl-6-(1-hydroxyethyl)chroman-4-one (**4**) [7], 2-senecioyl-4-[1-(angeloyloxy)ethyl]phenol (**5**) [7], and 2-senecioyl-4-[1-(acetylsarracinoyloxy)-ethyl]phenol (**6**) [8] (*Fig.*). All isolates were characterized by spectroscopic methods and by comparison of their data with those in the literature.

Compounds **1** and **2** (*Fig.*) were obtained as a mixture in an amorphous yellow gum. The molecular formula $C_{20}H_{24}O_6$ of the compounds was deduced from the *pseudo*-molecular-ion peak at *m/z* 383.1497 ($[M+Na]^+$; calc. 383.3927) in the positive-ion-mode HR-ESI-MS. The IR spectrum displayed the absorption bands of ketone (1692 cm^{-1}) and ester (1720 and 1735 cm^{-1}) functional groups. Two sets of data appeared in the ^1H- and ^{13}C-NMR spectra, indicating that this compound was an

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Figure. *Structures of compounds* **1**–**6**

inseparable mixture of two isomers ((*E*)/(*Z*)) in a ratio of 1:1. The ^{1}H-NMR spectrum (*Table 1*) showed signals for Me groups, with identical chemical shifts at δ(H) 1.45 (*s*, 4 Me), correlated to those of C(12) (δ(C) 26.6) and C(13) (δ(C) 26.6) in the HMQC spectrum. The signals at δ(H) 1.58–1.55 (*m*, were assigned to each of Me(8) of **1** and **2**), the signal at 2.08 (*d*, *J* = 7.2) to Me(4′) of **1**, and that at δ(H) 1.94 (*d*, *J* = 7.2) to Me(4′) of **2**. The presence of two AcO groups was evident from the ^{1}H-NMR resonances at δ(H) 2.03 (*s*, Me(7′) of **1**) and 2.04 (*s*, Me(7′) of **2**). Three signals at δ(H) 7.86–7.85 (*m*, 2 H), 7.51–7.47 (*m*, 2 H), and 6.93–6.89 (*m*, 2 H) were attributed to aromatic H-atoms at C(3), C(5), and C(6) (δ(C) 124.0, 134.2, 134.4 of **1** and δ(C) 123.9; 134.0; 134.3 of **2**). The olefinic H-atoms displayed signals at δ(H) 6.43 (*q*, *J* = 7.2, H–C(3′)) for **1** and 7.17 (*q*, *J* = 7.2, H–C(3′)) for **2**. Also, the spectrum exhibited signals for four CH_2 groups at δ(H) 2.71 (*s*, CH_2(10) of **1** and **2**), 4.75 (*s*, CH_2(5′) of **1**) and 4.87 (*s*, CH_2(5′) of **2**), and for two CH groups at δ(H) 5.98–5.90 (*m*, H–C(7) of **1** and **2**). A ketone CO function was indicated by the ^{13}C signal at δ(C) 192.3 (C(9)). The ^{1}H- and ^{13}C-NMR data of the mixture indicated chromanone-type structures and were partly reminiscent of those of uniflorol-A and uniflorol-B [9]. In addition to ^{1}H-NMR signals observed in uniflorol A and uniflorol B, the mixture of **1** and **2** showed in its spectrum two 3-H *singlet*s (δ(H) 2.03, Me(7′) of **1** and 2.04, Me(7′) of **2**) of two AcO groups. These data indicated that the OH groups of uniflorol A and uniflorol B were acetylated, and the following changes in chemical shifts were observed: deshielding of CH_2(5′) compared to uniflorol A (δ(H) 4.24) and uniflorol B (δ(H) 4.35) to δ(H) 4.75 and 4.87 for **1** and **2**. Based on the ^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC spectra, the structures of compounds **1** and **2** were established as uniflorol A acetate and uniflorol B acetate, respectively.

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2** (at 400 and 100 MHz, resp.; in $CDCl_3$; J in Hz). Atom numbering as indicated in the *Figure*.

Position	**1**			**2**		
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	HMBC
1	–	159.6 (*s*)	3, 5, 6	–	159.6 (*s*)	3, 5, 6
2	–	119.8 (*s*)	6	–	119.8 (*s*)	6
3	7.86 – 7.85 (*m*)	124.0 (*d*)		7.86 – 7.85 (*m*)	123.9 (*d*)	
4	–	134.2 (*s*)	3, 6, 7, 8	–	134.0 (*s*)	3, 6, 7, 8
5	7.51 – 7.47 (*m*)	134.4 (*d*)		7.51 – 7.47 (*m*)	134.3 (*d*)	
6	6.93 – 6.89 (*m*)	118.6 (*d*)		6.93 – 6.90 (*m*)	118.5 (*d*)	
7	5.98 – 5.90 (*m*)	71.9 (*d*)		5.98 – 5.90 (*m*)	71.8 (*d*)	
8	1.58 – 1.55 (*m*)	22.1 (*q*)		1.58 – 1.55 (*m*)	22.1 (*q*)	
9	–	192.3 (*s*)	3, 10	–	192.3 (*s*)	3, 10
10	2.71 (*s*)	48.8 (*t*)		2.71 (*s*)	48.8 (*t*)	
11	–	79.4 (*s*)	10, 12, 13	–	79.4 (*s*)	10, 12, 13
12	1.45 (*s*)	26.6 (*q*)		1.45 (*s*)	26.6 (*q*)	
13	1.45 (*s*)	26.6 (*q*)		1.45 (*s*)	26.6 (*q*)	
1′	–	165.1 (*s*)	5′	–	165.5 (*s*)	5′
2′	–	127.7 (*s*)	4′, 5′	–	128.2 (*s*)	4′, 5′
3′	6.43 (*q*, J = 7.2)	144.3 (*d*)		7.17 (*q*, J = 7.2)	144.7 (*d*)	
4′	2.08 (*d*, J = 7.2)	15.8 (*q*)		1.94 (*d*, J = 7.2)	14.6 (*q*)	
5′	4.75 (*s*)	65.6 (*t*)		4.87 (*s*)	57.7 (*t*)	
6′	–	170.9 (*s*)	7′	–	170.8 (*s*)	7′
7′	2.03 (*s*)	20.9 (*q*)		2.04 (*s*)	20.8 (*q*)	

Compound **3** (*Fig.*) was obtained as a yellow gum. It displayed a *pseudo*-molecular-ion peak at m/z 383.1350 ($[M + Na]^+$, calc. 383.3927) in the positive-ion-mode HR-ESI-MS. The IR spectrum indicated the presence of ketone and ester C=O functions (1694, 1712, and 1742 cm^{-1}), and an aromatic ring (1579, and 1321 cm^{-1}). The ^{1}H-NMR spectrum (*Table 2*) exhibited signals for four Me groups (δ(H) 1.46 (*s*, Me(12), Me(13)), 1.57 (*d*, J = 6.7, Me(8)), and 1.95 (*d*, J = 1.8, Me(5′))). The presence of an AcO group was evident from ^{1}H-NMR resonance at δ(H) 2.07 (*s*, Me(7′)). The ^{1}H-NMR spectrum also showed signals of aromatic H-atoms of a 1,3,4-trisubstituted benzene at δ(H) 7.85 (*d*, J = 2.3, H–C(3)), 7.49 (*dd*, J = 8.6, 2.3, H–C(5)), and 6.92 (*d*, J = 8.6, H–C(6)), of an olefinic H-atom at δ(H) 6.02 – 5.99 (*m*, H–C(3′)), of two CH_2 groups at δ(H) 2.72 (*s*, CH_2(10)) and 5.01 – 4.99 (*m*, CH_2(4′)), and of one CH group at δ(H) 5.90 (*q*, J = 6.70, H–C(7)). The ^{13}C-NMR spectrum along with HMBC features (*Table 2*), revealed 20 C-atom signals, including those of three O-bearing C-atoms at δ(C) 72.5 (C(7)), 79.8 (C(11)), and 63.5 (C(4′)). These spectral characteristics showed similarities to those of **1** and **2**. The location of the AcO group at C(4′) was established by HMBC of Me(5′) signal with that of C(1′). Thus, the structure of compound **3** was established as 2,2-dimethyl-6-[1-(4-acetoxyangeloyloxy)ethyl]chroman-4-one.

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Table 2. *¹H- and ¹³C-NMR Data of* **3** (at 400 and 100 MHz, resp.; in $CDCl_3$; *J* in Hz). Atom numbering as indicated in the *Figure.*

Position	δ(H)	δ(C)	HMBC
1	–	160.0 (*s*)	3, 5, 6
2	–	120.3 (*s*)	6, 10
3	7.85 (*d*, *J* = 2.3)	124.4 (*d*)	
4	–	134.3 (*s*)	3, 6, 7, 8
5	7.49 (*dd*, *J* = 8.6, 2.3)	134.8 (*d*)	
6	6.92 (*d*, *J* = 8.6)	119.1 (*d*)	
7	5.90 (*q*, *J* = 6.7)	72.5 (*d*)	
8	1.57 (*d*, *J* = 6.7)	22.5 (*q*)	
9	–	192.7 (*s*)	3, 10, 11, 12
10	2.72 (*s*)	49.2 (*t*)	
11	–	79.8 (*s*)	10, 12, 13
12	1.46 (*s*)	27.0 (*q*)	
13	1.46 (*s*)	27.0 (*q*)	
1′	–	166.4 (*s*)	5′, 7
2′	–	129.4 (*s*)	4′, 5′
3′	6.02 – 5.99 (*m*)	138.9 (*d*)	
4′	5.01 – 4.99 (*m*)	63.5 (*t*)	
5′	1.95 (*d*, *J* = 1.8)	20.3 (*q*)	
6′	–	171.2 (*s*)	4′, 7′
7′	2.07 (*s*)	21.3 (*q*)	

Experimental Part

General. Optical rotations: *Jasco DIP-370* polarimeter ($l = 1$ cm). IR Spectra: *Nicolet Protégé 460* spectrophotometer; KBr pellets; in cm^{-1}. Column chromatography (CC): silica gel *60 H* (SiO_2; 70 – 230 mesh; *Merck* No. 1.07736). TLC: SiO_2 GF_{254} (*Merck* No. 1.07730). ¹H- and ¹³C-NMR spectra: *Bruker DRX 400* spectrometer; $CDCl_3$ soln.; TMS as internal standard; chemical shifts (δ) in ppm, and coupling constants, *J*, in Hz; 2D-NMR experiments (¹³C,¹H-HMQC and ¹³C,¹H-HMBC) performed using a *Bruker DRX 500* spectrometer. HR-ESI-MS: *UltrOTOF Bruker–Daltonics* instrument (Billarica, USA) equipped with an ESI ion source and operating in positive-ion mode.

Plant Material. The plant was collected in November 1997, in Minas Gerais, BR-050, km 131, Brazil, and was identified by Prof. *Edward E. Schilling* and Prof. *Jimi N. Nakajima*, Department of Botany, University of Tennessee and Department of Biology, Universidade Federal de Uberlândia-MG, resp. A voucher specimen (SPFR 04702) is deposited with the Herbarium of Department of Biology, FFCLRP/USP, Ribeirão Preto, Brazil.

Extraction and Isolation. Dried and powdered underground parts (46 g) were exhaustively extracted with CH_2Cl_2 at r.t. The solvent was evaporated under vacuum to afford 0.23 g of crude extract. The crude extract was chromatographed on SiO_2 *60* column (35 g) and eluted with hexane/AcOEt (gradient), AcOEt/MeOH (gradient) and MeOH to give twelve fractions. *Frs. 1* (33 mg) and *2* (25 mg) were then subjected to prep. TLC (hexane/AcOEt 9:1) to afford **5** (2 mg) and **6** (8 mg). *Fr. 3* (10 mg) was also purified by prep. TLC (hexane/AcOEt 8:2) to give the mixture **1**/**2** (4 mg). *Fr. 5* was purified by prep. TLC (hexane/AcOEt 7:3) to yield compounds **3** (8 mg) and **4** (2 mg).

Uniflorol A Acetate (=*1-(3,4-Dihydro-2,2-dimethyl-4-oxo-2*H*-chromen-6-yl)ethyl (2*Z*)-2-[(Acetyloxy)methyl]but-2-enoate*; **1**). Amorphous yellow gum. IR (KBr): 1735, 1720, 1692, 1577, 1315, 836, 755. ¹H- and ¹³C-NMR, and HMBC: *Table 1.* HR-ESI-MS: 383.1497 ($[M + Na]^+$, $C_{20}H_{24}NaO_6^+$; calc. 383.3927).

Uniflorol B Acetate (=*1-(3,4-Dihydro-2,2-dimethyl-4-oxo-2*H*-chromen-6-yl)ethyl (2*E*)-2-[(Acetyloxy)methyl]but-2-enoate*; **2**). Amorphous yellow gum. IR (KBr): 1735, 1720, 1692, 1577, 1315, 836, 755.

^{1}H- and ^{13}C-NMR and HMBC: *Table 1*. HR-ESI-MS: 383.1497 ([M + Na]$^+$, $C_{20}H_{24}NaO_6^+$; calc. 383.3927).

2,2-Dimethyl-6-[1-(4-acetoxyangeloyloxy)ethyl]chroman-4-one (= *1-(3,4-Dihydro-2,2-dimethyl-4-oxo-2*H*-chromen-6-yl)ethyl (2*Z*)-4-(Acetyloxy)-2-methylbut-2-enoate*; **3**). Amorphous yellow gum. $[\alpha]_D^{26} = -14$ (c = 0.0033, $CHCl_3$). IR (KBr): 1742, 1712, 1694, 1579, 1321, 895, 791. ^{1}H- and ^{13}C-NMR and HMBC: *Table 2*. HR-ESI-MS: 383.1350 ([M + Na]$^+$, $C_{20}H_{24}NaO_6^+$; calc. 383.3927).

2-Senecioyl-4-[1-(acetylsarracinoyloxy)ethyl]phenol (= *1-[4-Hydroxy-3-(3-methylbut-2-enoyl)phenyl]ethyl (2*Z*)-2-[(Acetyloxy)methyl]but-2-enoate*; **6**). ^{13}C-NMR (100 MHz, $CDCl_3$): 196.4 (C(9)); 1171.0 (C(19)); 65.5 (C(14)); 163.4 (C(1)); 159.2 (C(11)); 144.6 (C(16)); 134.1 (C(5)); 132.0 (C(4)); 128.5 (C(3)); 128.1 (C(15)); 120.5 (C(2)); 120.2 (C(10)); 119.2 (C(6)); 72.4 (C(7)); 65.9 (C(18)); 28.7 (C(12)); 22.6 (C(8)); 21.9 (C(13)); 21.3 (C(20)); 16.2 (C(17)). The ^{13}C-NMR data of **6** have not been published previously.

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New Orsellinic Acid Esters from Fungus *Chaetomium globosporum*

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Seven new orsellinic acid esters, **1**–**7**, and four known compounds were isolated from the solid, fermented rice culture of *Chaetomium globosporum* (cib-132). Their structures were elucidated by 1D- and 2D-NMR spectra, and the relative configuration of compound **1** was determined by X-ray crystallographic analysis.

Introduction. – Secondary metabolites from fungi are an important source for basic chemical intermediate or drugs such as penicillin, cyclosporine A, griseofulvin, and lovastatin [1–3]. In recent years, various bioactive metabolites from fungi were reported [4–7], which inspired us to search for new bioactive compounds from fungi. Previously, compounds of diverse structured types such as chaetoglobosins, orsellides, azaphilones, depsidones, diketopiperazines, anthraquinones, and terpenoids have been isolated and identified from the genus *Chaetomium* (Chaetomiaceae). Many compounds from this genus were shown to possess significant biological features such as cytotoxic, antibiotic, phytotoxic, and other activities [8–14]. In our continuing study on *Chaetomium globosporum* obtained from wheat roots, seven new orsellinic acid esters, **1**–**7** (*Fig. 1*), together with four known compounds, orsellinic acid, divaric acid, cochliodinol, and 1-*O*-hexadecanolenin, were isolated and identified from the solid fermented rice culture of this fungus. Herein, the isolation and structure elucidation of compounds **1**–**7** are described.

Results and Discussion. – Compound **1** was obtained as triclinic crystals (actone/H_2O, 100:1), and its molecular formula, $C_{13}H_{18}O_7$, was established from the *quasi*-molecular ion at *m/z* 309.0943 ($[M+Na]^+$) in HR-ESI-MS, indicating of five degrees of unsaturation. The IR spectrum of **1** revealed the presence of OH (3411 cm^{-1}) and phenyl groups (1622 cm^{-1}).

In the ^{1}H-NMR spectrum of **1**, signals of two *meta*-coupled aromatic H-atoms (δ(H) 6.20 (d, $J=2.4$) and 6.25 (d, $J=2.4$)) and two Me groups (δ(H) 2.55 (s) and 1.31 (d, $J=6.2$)) (*Table 1*) were detected. Its ^{13}C-NMR spectrum displayed signals of an aromatic ring ((δ(C) 102.2, 106.4, 112.9, 145.3, 164.3, and 166.5), four O-bearing C-atoms ((δ(C) 68.2, 68.9, 69.7, and 76.7), an ester CO group (δ(C) 173.3), and two Me groups ((δ(C) 24.9 and 20.8) (*Table 2*). These spectroscopic features revealed that compound **1** should be an orsellinic acid ester [9]. In the HMBC spectrum, correlations H–C(4)/C(2) ((δ(C) 106.4), C(3) ((δ(C) 166.5), and C(5) ((δ(C) 164.3), H–C(6)/C(4)

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Fig. 1. *Compounds* **1**–**7** *isolated from* C. globosporum

((δ(C) 102.2) and C(2); and H–C(8)/C(7) ((δ(C) 145.3), C(6), and C(2) (*Fig. 2*), supported the presence of an orsellinic acid unit. Due to the five degrees of unsaturation, there should be a linear side chain in compound **1**. The following HMBCs suggested a pentane-1,2,3,4-tetraol moiety and the connection of this moiety with orsellinic acid: H–C(1′)/C(1) (δ(C) 173.3), C(2′) (δ(C) 69.7), and C(3′) (δ(C) 76.7); H–C(2′)/C(1′) (δ(C) 68.2); H–C(3′)/C(4′) (δ(C) 68.9) and C(5′) (δ(C) 20.8), and H–C(5′)/C(4′). Therefore, the constitution of compound **1** was elucidated as 2,3,4-trihydroxypentyl orsellinate. The relative configuration, *rel*-(2′*R*,3′*R*,4′*R*), of **1** was determined by X-ray crystallographic analysis (*Fig. 3*).

Compounds **2** and **3** were isolated as amorphous white powders and possessed the same formula $C_{13}H_{18}O_7$ as **1** on the basis of their HR-ESI-MS. Compound **2** was a stereoisomer of **1** evidenced by 1D-NMR (*Tables 1* and *2*), HSQC, and HMBCs

Table 1. *^{1}H-NMR Data* (600 MHz, CD_3OD) *of Compounds* **1**–**3**. δ in ppm, *J* in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	**1**	**2**	**3**
4	6.20 (*d*, *J* = 2.4, 1 H)	6.22 (*d*, *J* = 2.4, 1 H)	6.18 (*d*, *J* = 1.8, 1 H)
6	6.25 (*d*, *J* = 2.4, 1 H)	6.27 (*d*, *J* = 2.4, 1 H)	6.22 (br. *s*, 1 H)
8	2.55 (*s*, 3 H)	2.59 (*s*, 3 H)	2.53 (*s*, 3 H)
1′	4.43–4.46 (*m*, 2 H)	4.64 (*dd*, *J* = 11.6, 2.6, 1 H), 4.47 (*dd*, *J* = 11.6, 6.7, 1 H)	1.41 (*d*, *J* = 6.5, 3 H)
2′	4.26–4.29 (*m*, 1 H)	3.96–3.99 (*m*, overlapped, 1 H)	5.48 (*qd*, *J* = 6.4, 3.3, 1 H)
3′	3.30 (overlapped, 1 H)	3.57 (*dd*, *J* = 7.3, 5.7, 1 H)	3.87 (*d*, *J* = 8.3, 3.3, 1 H)
4′	3.85–3.87 (*m*, 1 H)	3.96–3.99 (*m*, overlapped, 1 H)	3.56–3.59 (*m*, 1 H)
5′	1.31 (*d*, *J* = 6.2, 3 H)	1.29 (*d*, *J* = 6.4, 3 H)	3.82 (*dd*, *J* = 11.3, 3.1, 1 H), 3.66 (*dd*, *J* = 11.3, 6.1, 1 H)

Table 2. ^{13}*C-NMR Data* (150 MHz, CD_3OD) *of Compounds* **1**–**3**. δ in ppm. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	**1**	**2**	**3**
1	173.3	173.1	172.3
2	106.4	106.3	106.5
3	166.5	166.1	166.1
4	102.2	101.9	101.2
5	164.3	163.9	163.8
6	112.9	112.6	112.6
7	145.3	145.1	144.9
8	24.9	24.6	24.7
1′	68.2	68.1	14.0
2′	69.7	72.4	74.1
3′	76.7	76.5	73.7
4′	68.9	69.9	73.8
5′	20.8	18.4	65.1

(*Fig. 2*). The HMBCs H–C(2′)/C(1) (δ(C) 172.3) and C(1′) (δ(C) 14.0); H–C(3′)/C(2′) (δ(C) 74.1) and C(1′), H–C(5′)/C(4′) (δ(C) 73.8) and C(3′) (δ(C) 73.7) suggested that the pentane-1,2,3,4-tetraol moiety was also present in compound **3**. However, the

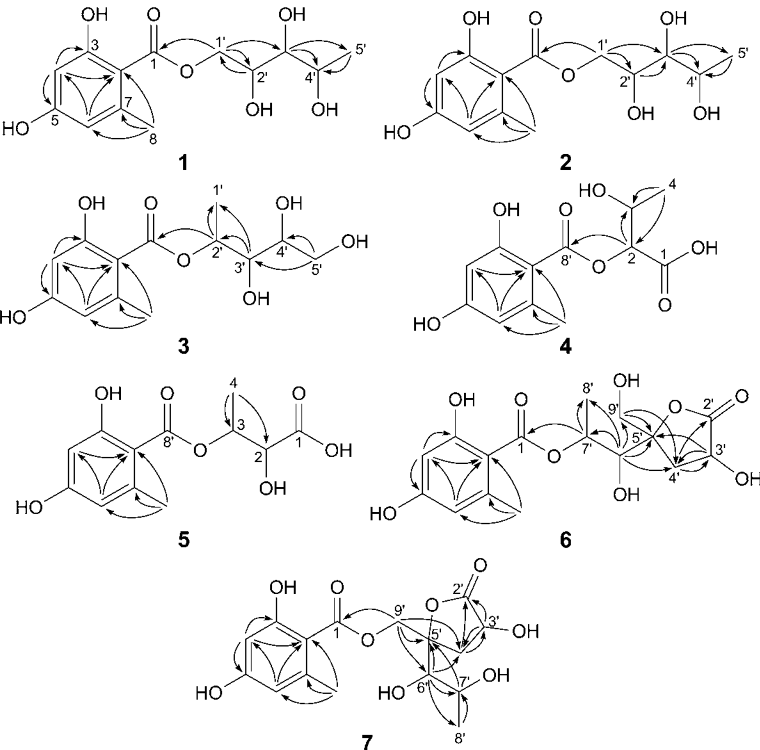

Fig. 2. *Key HMBCs of compounds* **1**–**7**

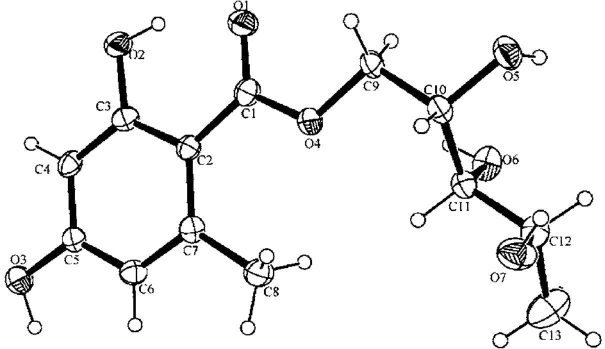

Fig. 3. *ORTEP Diagram of compound* **1**

pentane-1,2,3,4-tetraol unit was linked to orsellinic acid at C(2′) (*Fig. 2*). Thus, compound **3** was determined as 3,4,5-trihydroxypentan-2-yl orsellinate. The relative configurations of compounds **2** and **3** were not confirmed, but compound **3** in CD_3OD could be converted to **2** at room temperature within five days (*Fig. 4*), which was confirmed by HPLC analysis. Accordingly, the corresponding configurations at C(2′/4′), C(3′/3′) and C(4′/2′) in **2** and **3** could be deduced.

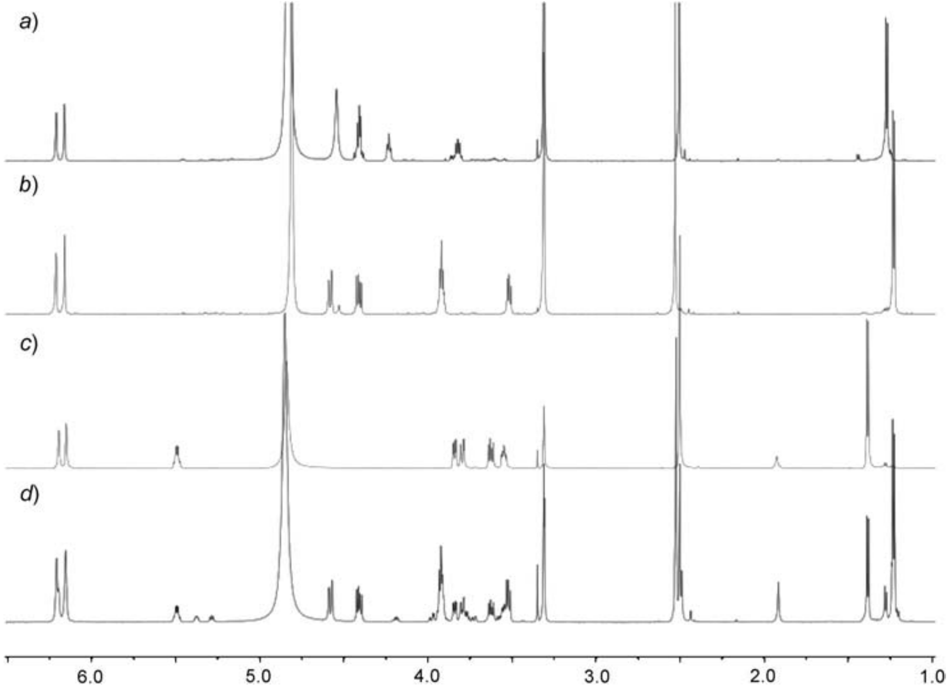

Fig. 4. *^{1}H-NMR Spectra recorded on a* Bruker Avance *600-MHz spectrometer in CD_3OD. a*) Spectrum of **1**; *b*) spectrum of **2**; *c*) spectrum of **3**; *d*) spectrum of **3** after standing at room temperature for 5 d.

Table 3. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp; CD_3OD) *of Compounds* **4** *and* **5**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	δ(H)		δ(C)	
	4	**5**	**4**	**5**
1′			106.2	106.1
2′			165.9	166.4
3′	6.18 (*d*, J = 2.2, 1 H)	6.21 (*d*, J = 1.7, 1 H)	101.9	101.9
4′			164.1	164.0
5′	6.24 (*d*, J = 2.2, 1 H)	6.25 (*d*, J = 1.7, 1 H)	112.6	112.7
6′			145.1	145.2
7′	2.57 (*s*, 3 H)	2.53 (*s*, 3 H)	24.5	24.7
8′			172.2	172.3
2	5.34 (*d*, J = 3.4, 1 H)	4.47 (*s*, 1 H)	78.8	73.8
3	4.31 – 4.35 (*m*, 1 H)	5.54 (br. *s*, 1 H)	68.0	74.4
4	1.33 (*d*, J = 6.5, 3 H)	1.44 (*d*, J = 6.1, 3 H)	18.8	15.2

Compound **4** was obtained as an amorphous white powder with a molecular formula of $C_{12}H_{14}O_7$ (six degrees of unsaturation) deduced from the HR-ESI-MS (*m/z* 293.0635 ($[M + Na]^+$; calc. 293.0632)). Comparing the ^{13}C-NMR data with those of compounds **1** – **3**, the orsellinic acid unit (δ(C) 24.5, 101.9, 106.2, 112.6, 145.1, 164.1, 165.9, 172.2) in compound **4** was identified (*Table 3*). A 2,3-dihydroxybutanoic acid moiety was elucidated from the ^{13}C-NMR signals, HR-ESI-MS analysis, and the HMBCs H–C(4)/C(3) (δ(C) 68.0) and C(2) (δ(C) 78.8), H–C(2)/C(3) (*Fig. 2*). The HMBC H–C(2)/C(8′)(δ(C) 172.2 suggested the orsellinic acid and 2,3-dihydroxybutanoic acid moieties were connected *via* an O-atom between C(8′) and C(2). Compound **4** was finally identified as 2-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-3-hydroxybutanoic acid. The configuration of compound **4** was determined by comparing the optical rotation of its hydrolysis product ($[\alpha]_D^{25} = -19.0$, $c = 0.1$, H_2O) with that of *threo*-L-2,3-dihydroxybutyric acid ($[\alpha]_D^{25} = -17.75$, H_2O; *erythro*-L-2,3-dihydroxybutyric acid: $[\alpha]_D^{25} = -9.5$, H_2O) [15]. Thus, compound **4** was finally determined as *threo*-L-2-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-3-hydroxybutanoic acid.

Compound **5** was obtained as an amorphous white powder with the same formula, $C_{12}H_{14}O_7$, as **4** from the *quasi*-molecular ion (*m/z* 293.0641 ($[M + Na]^+$, calc. 293.0632)) in its HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra are similar to those of **4** (*Table 3*). However, an obvious upfield shift of H–C(2) and C(2), and downfield shift of H–C(3) and C(3) in compound **5** were observed in the NMR spectra. These findings suggested that the 2,3-dihydroxybutanoic acid and orsellinic acid moieties were linked through an O-bridge between C(8′) and C(3). Compound **5** was thus identified as 3-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-2-hydroxybutanoic acid. The configuration of compound **5** was also deduced by the method as described for **4**. Compound **5** was, therefore, determined as *threo*-L-3-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-2-hydroxybutanoic acid.

Compound **6** was obtained as an amorphous white powder. The molecular formula, $C_{16}H_{20}O_9$, was deduced from the *quasi*-molecular ion at *m/z* 379.1002 ($[M + Na]^+$) in HR-ESI-MS, indicating seven degrees of unsaturation. On the basis of NMR data (*Table 4*), the orsellinic acid unit in **6** was easily recognized. Based on ^{13}C-NMR and

Table 4. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp; CD_3OD) *of* **6** *and* **7**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	δ(H)		δ(C)	
	6	**7**	**6**	**7**
1			172.1	172.8
2			106.3	105.7
3			164.0	164.2
4	6.20 (*d*, J = 2.1, 1 H)	6.20 (*d*, J = 2.0, 1 H)	101.9	101.9
5			166.2	166.5
6	6.24 (*d*, J = 2.1, 1 H)	6.24 (*d*, J = 2.0, 1 H)	112.7	112.7
7			144.9	145.0
8	2.53 (*s*, 3 H)	2.53 (*s*, 3 H)	24.6	25.3
2′			179.2	179.5
3′	4.68 (*dd*, J = 9.1, 1 H)	4.75 (*d*, J = 9.0, 1 H)	69.8	69.8
4′	2.74 (*dd*, J = 13.3, 9.4, 1 H), 2.06 (*dd*, J = 13.3, 8.2, 1 H)	2.82 (*dd*, J = 13.3, 9.5, 1 H), 2.09 (*dd*, J = 13.3, 8.7, 1 H)	34.3	34.6
6′	4.07 (*d*, J = 4.4, 1 H)	3.63 (*d*, J = 8.1, 1 H)	75.2	78.0
7′	5.37–5.42 (*m*, 1 H)	3.75–3.79 (*m*, 1 H)	72.6	68.8
8′	1.45 (*d*, J = 6.4, 1 H)	1.33 (*d*, J = 6.1, 1 H)	16.3	21.6
9′	3.78 (*d*, J = 12.2, 1 H), 3.66 (*d*, J = 12.2, 1 H)	4.71 (*d*, J = 12.2, 1 H), 4.61 (*d*, J = 12.2, 1 H)	65.4	68.5

HSQC experiments, one Me group (δ(C) 16.3), two CH_2 (δ(C) 34.3 and 65.4) and three O-bearing CH groups (δ(C) 72.6, 75.2, 69.8), an O-bearing quaternary C-atom (δ(C) 89.8), and one ester CO group (δ(C) 179.2) were identified. The remaining atoms should form a ring deduced from degrees of unsaturation. In the HMBC spectrum, the substructure from C(2′) to C(9′) could be postulated on the basis of following correlations: H–C(7′)/C(8′), H–C(6′)/C(9′), C(8′), C(7′), C(5′), and C(4′), H–C(4′)/ C(3′) and C(2′), H–C(3′)/C(4′) and C(5′), and H–C(9′)/C(4′) and C(5′) (*Fig. 2*). Meanwhile, a γ-lactone formed between C(2′) and C(5′) was deduced from the characteristic IR absorption at 1765 cm^{-1}, and the quaternary C-atom resonance at δ(C) 89.8 (C(5′)). The lactone moiety was linked with the orsellinic acid by an O-atom between C(1) and C(7′), which was supported by the correlation H–C(7′)/C(1). Hence, compound **6** was finally identified as 1-hydroxy-1-[4-hydroxy-2-(hydroxymethyl)-5-oxotetrahydrofuran-2-yl]propan-2-yl orsellinate.

Compound **7** was assigned the molecular formula $C_{16}H_{20}O_9$ based on the *quasi*-molecular-ion peak at *m/z* 379.0997 ($[M + Na]^+$), the same as that of **6**. That the NMR data of **7** (*Table 4*) were similar to thoes of **6**, suggesting that compound **7** was an isomer of **6**. The γ-lactone moiety was the same as in compound **6** on the basis of HMBCs. However, the HMBC H–C(9′)/C(1) indicated that C(9′) was connected with C(1) in **7** (*Fig. 2*). Therefore, compound **7** was determined as [2-(1,2-dihydroxypropyl)-4-hydroxy-5-oxotetrahydrofuran-2-yl]methyl orsellinate. Attempts to obtain single crystals of compounds **6** and **7** for X-ray crystallographic analyses have failed. The relative configurations of compounds **6** and **7** could not be determined.

Conclusions. – Orsellinic acid esters possessed diverse biological including cytotoxic, antibacterial, antiviral, and other activities [9][16–21]. In this work, the antibacterial activities of compounds **1**–**5** were evaluated. Compounds **6** and **7** could not be evaluated due to insufficient amounts. Compounds **1**–**5** showed no activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia colis*, or *Saccharomycetes* ($c > 50$ μg/ml).

This work was financially supported by the *National Natural Science Foundation of China* (No. 21272228), the *Knowledge Innovation Program of the Chinese Academy of Sciences* (KSCX2-EW-G-13-04), and the *National New Drug Innovation Major Project of China* (2011ZX09307-002-02).

Experimental Part

General. M.p.: *X-6* precision micro melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *PerkinElmer S2 Lambda 35* UV/VIS spectrometer; in MeOH; λ in nm. IR Spectra: *PerkinElmer Spectrum One* FT-IR spectrometer; as KBr tablets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance 600* MHz spectrometer; δ in ppm, J in Hz; residual solvent peak as reference. HR-ESI-MS: *BioTOF-Q* mass spectrometer.

Fungus Material. Chaetomium globosporum (cib-132) was isolated from wheat root collected in Ya'an of Sichuan Province, P. R. China, and identified by *T. Y.*, Chengdu Institute of Biology, Chinese Academy of Sciences (CAS), P. R. China.

Extraction and Isolation. The fungal seed culture and fermentation process were identical to those reported [14]. The fermented solid rice medium (7 kg) was soaked with AcOEt (13 l × 2, 1 d for each time) at r.t. The soln. was concentrated under reduced pressure to afford a residue (41.0 g). This residue was divided into four fractions, *Frs. A*, *B*, *C*, and *D*), by column chromatography (CC) (SiO_2 (400 g, 300–400 mesh, ϕ 45 cm × 8.5 cm); petroleum ether (PE)/acetone 6:1, 3:1, 1.5:1, 0:1, successively). *Fr. C* was further separated by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford *Frs. C1* and *C2*. Compounds **1**–**3** (5.5, 27.0, and 14.3 mg, resp.) and **6** (3.5 mg) were obtained from *Fr. C1* (2.1 g) by HPLC (MeOH/H_2O 37:63). Compounds **4** (23.0 mg) and **5** (18.2 mg) were obtained from *Fr. C2* (1.7 g) separated by HPLC (MeOH/H_2O 40:60). *Fr. C2* was further separated by HPLC (MeCN/H_2O 27:73) to give compound **7** (3.0 mg).

Esters Hydrolysis. Compounds **4** (5.1 mg) and **5** (3.4 mg) were hydrolyzed with 5% NaOH at 70° for 2 h, neutralized with 1N HCl, and extracted with BuOH (3 ×), to furnish the hydrolysis products of **4** and **5**, resp.

The crystallographic data of compound **1** were collected with a *Xcalibur, Eos*, diffractometer with graphite-monochromated MoK_α radiation; μ(MoK_α) = 0.070. *Crystal data:* $C_{13}H_{20}O_8$, M_r 304.29, triclinic system, crystal size/mm^3, 0.41 × 0.35 × 0.10. Space group: $P1$, $a = 7.1516$ (3) Å, $b = 7.9646$ (4) Å, $c = 13.0796$ (6) Å, $\alpha = 93.461$, $\beta = 95.811(4)$, $\gamma = 100.435(4)$, $V = 726.56$ (6) Å^3, $T = 293.15$ K, $Z = 2$, 6110 reflections measured, 4598 independent reflections [R(int) = 0.0182]. The final R_1 values were 0.0463 [$I > 2\sigma(I)$], and the final wR_2 values were 0.0765 [$I >= 2\sigma(I)$]. The final R_1 values were 0.0782 (all data), and the final wR_2 values were 0.0908 (all data). The crystal structure has been deposited with the *Cambridge Crystallographic Data Centre* under the deposition No. CCDC-948632. Copies of the data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK; e-mail:deposit@ccdc.cam.ac.uk; fax: +44(0)1223336033).

rel-*(2*′R,*3*′R,*4*′R*)-2,3,4-Trihydroxypentyl Orsellinate* (= rel-(*2*R,*3*R,*4*R*)-2,3,4-Trihydroxypentyl 2,4-Dihydroxy-6-methylbenzoate*; **1**): Triclinic crystals. M.p. 78–81°. $[\alpha]_D^{20} = -6.0$ ($c = 0.1$, MeOH). UV (MeOH): 225 (4.32), 264 (4.10), 300 (3.67). IR (KBr): 3411, 2975, 2930, 1644, 1457, 1316, 1260, 1204, 1169, 997, 845. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 309.0943 ($[M + Na]^+$, $C_{13}H_{18}NaO_7^+$; calc. 309.0950).

2,3,4-Trihydroxypentyl Orsellinate (= *2,3,4-Trihydroxypentyl 2,4-Dihydroxy-6-methylbenzoate*; **2**). White amorphous powder. $[\alpha]_D^{20} = -30.0$ ($c = 0.1$, MeOH). UV (MeOH): 225 (4.37), 264 (4.20), 300

(3.75). IR (KBr): 3433, 2980, 2936, 1642, 1457, 1316, 1264, 1168, 995, 845. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 309.0950 ([M + Na]$^+$, $C_{13}H_{18}NaO_7^+$; calc. 309.0950).

3,4,5-Trihydroxypentan-2-yl Orsellinate (= *3,4,5-Trihydroxypentan-2-yl 2,4-Dihydroxy-6-methylbenzoate*; **3**). White amorphous powder. $[\alpha]_D^{20} = -10.0$ (c = 0.1, MeOH). UV (MeOH): 225 (4.40), 264 (4.12), 300 (3.68). IR (KBr): 3401, 2978, 2935, 1642, 1452, 1314, 1265, 1204, 1169, 1061, 843. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 309.0948 ([M + Na]$^+$, $C_{13}H_{18}NaO_7^+$; calc. 309.0945).

threo-L-*2-[(2,4-Dihydroxy-6-methylbenzoyl)oxy]-3-hydroxybutanoic Acid* (**4**). White amorphous powder. $[\alpha]_D^{20} = -15.0$ (c = 0.1, MeOH). UV (MeOH): 225 (4.30), 264 (4.13), 300 (3.70). IR (KBr): 3430, 2982, 2935, 1732, 1650, 1623, 1449, 1258, 1102, 997. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-MS: 293.0635 ([M + Na]$^+$, $C_{12}H_{14}NaO_7^+$; calc. 293.0632).

threo-L-*3-[(2,4-Dihydroxy-6-methylbenzoyl)oxy]-2-hydroxybutanoic Acid* (**5**). White amorphous powder. $[\alpha]_D^{20} = -15.0$ (c = 0.1, MeOH). UV (MeOH): 225 (4.30), 264 (4.12), 300 (3.67). IR (KBr): 3435, 2983, 2930, 1717 1650, 1643, 1456, 1262, 1163, 1064, 995. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-MS: 293.0641 ([M + Na]$^+$, $C_{12}H_{14}NaO_7^+$; calc. 293.0632).

1-Hydroxy-1-[tetrahydro-4-hydroxy-2-(hydroxymethyl)-5-oxofuran-2-yl]propan-2-yl Orsellinate (= *1-Hydroxy-1-[tetrahydro-4-hydroxy-2-(hydroxymethyl)-5-oxofuran-2-yl]propan-2-yl 2,4-Dihydroxy-6-methylbenzoate*; **6**). White amorphous powder. $[\alpha]_D^{20} = +18.0$ (c = 0.1, MeOH). UV (MeOH): 225 (4.13), 264 (3.74), 300 (3.67). IR (KBr): 3430, 2982, 2935, 1732, 1650, 1623, 1449, 1258, 1102. ^{1}H- and ^{13}C-NMR: see *Table 4*. HR-ESI-MS: 379.1002 ([M + Na]$^+$, $C_{16}H_{20}NaO_9^+$; calc. 379.1000).

[2-(1,2-Dihydroxypropyl)tetrahydro-4-hydroxy-5-oxofuran-2-yl]methyl Orsellinate (= *[2-(1,2-Dihydroxypropyl)tetrahydro-4-hydroxy-5-oxofuran-2-yl]methyl 2,4-Dihydroxy-6-methylbenzoate*; **7**). White amorphous powder. $[\alpha]_D^{20} = -7.0$ (c = 0.1, MeOH). UV (MeOH): 225 (4.10), 264 (3.68), 300 (3.65). IR (KBr): 3446, 2927, 2855, 1771, 1622, 1451, 1316, 1260, 1112. ^{1}H- and ^{13}C-NMR: see *Table 4*. HR-ESI-MS: 379.0997 ([M + Na]$^+$, $C_{16}H_{20}NaO_9^+$; calc. 379.1000).

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Received August 29, 2013

2014
Vol. 97
No. 1
1 – 159

Contents

REVIEW

Confusing Quantitative Descriptions of *Brønsted–Lowry* Acid–Base Equilibria in Chemistry Textbooks – A Critical Review and Clarifications for Chemical Educators

E. C. Meister, *M. Willeke*, *W. Angst*, *A. Togni*, *P. Walde** 1

$$H_2O(l) + H_2O(l) \rightleftharpoons HO^-(aq) + H_3O^+(aq)$$

$$K = \frac{a_{HO^-(aq),c} \cdot a_{H_3O^+(aq),c}}{a^2_{H_2O(l),x}}$$

Irigermanone, a Noriridal with Unprecedented Methylketone Function, from *Iris germanica*

*O. Potterat**, *C. Herzog*, *M. Raith*, *S. N. Ebrahimi*, *M. Hamburger* 32

OH
OH
O
O

Insights into the Synthesis of Steroidal *A*-Ring Olefins

C. L. Varela, F. M. F. Roleira, S. C. P. Costa, A. S. C. T. Pinto, A. I. O. S. Martins, R. A. Carvalho, N. A. Teixeira, G. Correia-da-Silva, E. Tavares-da-Silva** 39

Perturbations of the *Dushman* Reaction with Piroxicam: Experimental and Model Calculations

N. D. Pejić, S. M. Blagojević, N. B. Sarap, J. P. Maksimović, S. R. Anić, Ž. D. Čupić, L. Z. Kolar-Anić* 47

Two New Cytotoxic Spirostane-Steroidal Saponins from the Roots of *Bletilla striata*

*J. E. Park, K. W. Woo, S. U. Choi, J. H. Lee, K. R. Lee** 56

Three New 24-Nortriterpenoids from the Roots of *Ilex asprella*

K. Jiang, J.-Q. Bai, J. Chang, J.-J. Tan, S.-J. Qu, H.-F. Luo, C.-H. Tan*, D.-Y. Zhu* 64

R^1	R^2	R^3
Me	Me	OH
Me	$=CH_2$	
H	Me	Me

Palladium- and Solvent-Free Synthesis of Ynones by Copper(I)-Catalyzed Acylation of Terminal Alkynes with Acyl Chlorides under Aerobic Conditions

E. Mohammadi, B. Movassagh, M. Navidi* 70

CuI/C22 Cat. (2.6 mol-%)

Et_3N, 60°

Unexpected Ring Enlargement of 2-Hydrazono-2,3-dihydro-1,3-thiazoles to 1,3,4-Thiadiazines

W.-D. Pfeiffer, K.-D. Ahlers, A. S. Saghyan, A. Villinger, P. Langer** 76

2M HCl

conc. HCl

Eudesmanolides and Guaianolides from *Carpesium triste*

H.-B. Wu, W.-S. Wang, T.-T. Liu, Y.-G. Jiao* 88

R = α/β-Me

R = α/β-Me

Rare C_{25} Steroids Produced by *Penicillium chrysogenum* P1X, a Fungal Endophyte of *Huperzia serrata*

*Y.-M. Ying, Z.-Z. Zheng, L.-W. Zhang, W.-G. Shan, J.-W. Wang, Z.-J. Zhan** 95

New Dammarane-Type Saponins from the Roots of *Panax notoginseng*

*L. Qiu, Y. Jiao, G.-K. Huang, J.-Z. Xie, J.-H. Miao, X.-S. Yao** 102

R^1 = H, S_1, Xyl(1→2)Xyl

R^2 = Xyl(1→6)Glc(1→6)Glc, H, H

A Stereoselective Aldol Approach for the Total Syntheses of Two 6-Alkylated 2*H*-Pyran-2-ones

S. Rajaram, *D. Ramesh*, *U. Ramulu*, *P. Prabhakar*, *Y. Venkateswarlu** 112

Two Novel Abietane Diterpenoids from *Illicium wardii* A.C.Sm.

Y.-P. Gao, *Y.-H. Shen**, *W.-D. Zhang** 122

Suberosanones A – C, New Metabolites Possessing Cyclopentenone System from the South China Sea Gorgonian Coral *Subergorgia suberosa*

J. Zhang, *Y. Liang*, *L.-C. Li**, *S.-H. Xu** 128

A Novel One-Pot Sulfonyloxylactonization of Alkenoic Acids Mediated by Hypervalent Iodine Species Generated *in situ* from Ammonium Iodide

J. Hu, *M. Zhu*, *Y. Xu*, *J. Yan** 137

Three New Chromanones from *Calea clausseniana*

*A. M. do Nascimento**, *D. C. R. de Oliveira* 146

New Orsellinic Acid Esters from Fungus *Chaetomium globosporum*

G.-B. Xu, *N.-N. Wang*, *J.-K. Bao*, *T. Yang*, *G.-Y. Li** 151

* Author to whom correspondence should be addressed
Korrespondenzautor