47. Structure Determination of a New Spirobicyclic Triterpenoid from *Iris foetidissima*

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Dedicated to Professor *André Pirson*, co-founder of today's botany, on the occasion of his 80th birthday

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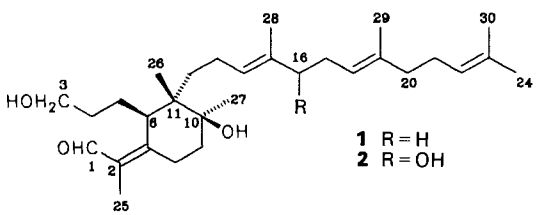
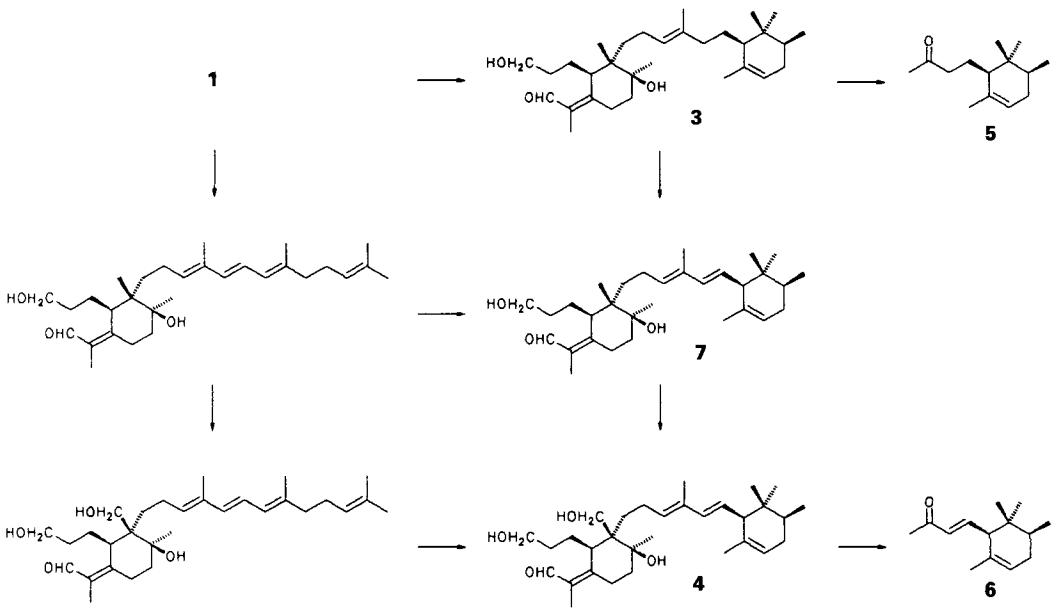
The novel iridal **10** has been isolated from rhizome extracts of *Iris foetidissima*. Its structure was established by spectroscopic methods and oxidative degradation. Final proof of the spirobicyclic nature of the compound – a new feature in the triterpenoid field – was afforded by the correlations observed in the 2D-HMBC- (^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence) spectrum. The possible biogenesis of this unusual compound is discussed.

Introduction. – The iridals constitute a family of triterpenoids which are the main lipid constituents of *Iris* rhizomes. Typically, they are monocyclic seco-ring-A compounds with various degrees of unsaturation and/or hydroxylation (*e.g.* **1** and **2**) or bycyclic C_{31}-triterpenoids (*e.g.* **3** and **4**) [1], the second ring system of which is formed by cyclization of the homofarnesyl side chain initiated by the transfer of a Me group from *S*-adenosylmethionine to the terminal double bond [2] *(Scheme 1)*. These cycloiridals serve as precursors of the dihydroirones and irones (*e.g.* **5** and **6**). The latter are responsible for the violet-like scent of the *Iris* oil [1]. Two possible pathways may lead from iridal **1** to the irone precursor **4** either *via* the cycloiridals with the dihydroirone moiety **3** and compound **7** (found as a minor component in *I. pallida* [3]) or *via* the postulated iridals with a conjugated triene grouping in the side chain *(Scheme 1)*. In our efforts to investigate the biosynthesis of the irone precursors, the search for iridals with this structural feature has been one of our main aims.



Results. – On screening extracts of different *Iris* species by HPLC, *I. foetidissima* was found to be a very rich source of iridals showing the typical UV spectrum of conjugated trienes. These compounds are extremely labile, rapidly decomposing under the conditions of silica-gel or reversed-phase chromatography or both. One of the main trienes of this

Scheme 1

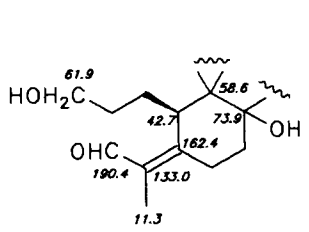


extract, however, allows separation on silica gel. On fractionation of the crude oil, it is eluted with $Et_2O/CHCl_3$ 7:3 (*v/v*) together with free fatty acids (mainly myristic acid). After esterification of the fatty acids with CH_2N_2 and re-chromatography of the mixture on silica gel, the iridal was obtained chromatographically pure. Mass spectrometry revealed a molecular ion at *m/z* 470 and fragment ions at *m/z* 452 and 434 arising from successive loss of two molecules of H_2O. A base peak at *m/z* 69 made the presence of a terminal isoprene-unit probable. The molecular composition was established as $C_{30}H_{46}O_4$ by mass and NMR spectroscopy.

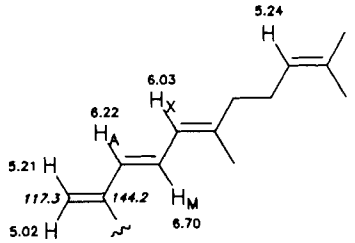
The conjugated triene moiety was readily deduced from the UV spectrum, which showed the typical triplet structure with λ_{max} 273 nm and side-maxima at 266 and 285 nm. 1 D- and 2 D-NMR spectra revealed essential structural features of the compound. The eight double-bond equivalents were found as one C=O group, five olefinic double bonds, and two ring systems. One of these was the seco-ring-A system typical of the iridals. Its NMR data are completely consistent with the values previously recorded for the other iridals (*e.g.* [3]).

In the ^{13}C-NMR spectra, the signals at 190.4 (*d*), 162.4 (*s*), 133 (*s*), and 11.3 (*q*) are due to the α,β-unsaturated aldehyde function and the attached Me group. The aldehyde H-atom shows a signal at 10.49 ppm (*s*) and the *H*–C(6)[1]) appears at 3.65 (br. *d*). In the H,H- and H,C-COSY spectra, the corresponding C-atom (42.7 ppm) can be connected *via* two CH_2 groups with diastereotopic protons to the terminal primary alcohol function, thus establishing the $CH_2CH_2CH_2OH$ side chain. By analogy with the other known iridals [1], a signal for one quaternary C-atom at 73.89 ppm was assigned to C(10),

[1]) As shown for compound **1**, the C-skeleton of the iridals is numbered in analogy to squalene.



A



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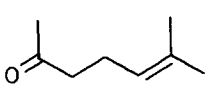

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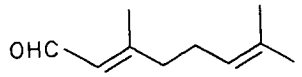

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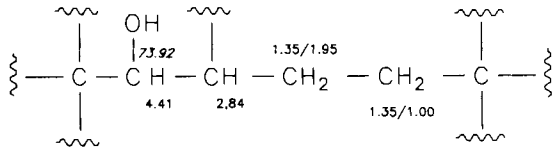
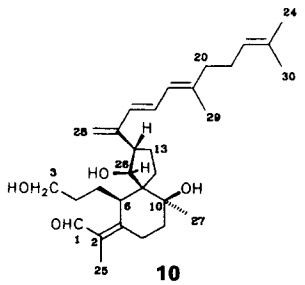
substituted with an OH group, and a second quaternary C-atom at 58.6 ppm to C(11). An unusual substitution pattern had to be expected for this C-atom from the extremely low-field shift of this signal. The remaining four double bonds are located in a branched terpenoid side chain. As suggested by the mass spectrum, one of these is found in an isolated isoprene unit, the olefinic H-atom of which showed a *triplet* at 5.24 ppm coupled with the protons of an allylic CH_2 group at 2.18 ppm. The ^1H signals of the Me groups attached to this double bond appear at 1.67 and 1.58 ppm as shown by long-range coupling to the olefinic proton. The other three double bonds form the conjugated triene. One of these had to be a methylidene group, as an olefinic methylene C-atom showed a ^{13}C-signal at 117.3, and the corresponding protons gave signals at 5.02 and 5.21 ppm. This double bond is connected to a diene moiety, which appears as an *AMX* system at 6.7, 6.22, and 6.03 ppm. The coupling constants ($J_{AM} = 15.4$, $J_{MX} = 10.8$ Hz) are indicative of the (*E*)-configuration. Since these protons show no direct coupling to any other H-atoms, the partial structure had to be as shown in formula **B**. A plausible isoprenoid chain results, when the fragments **A** and **B** are connected by an allylic CH_2 group, the protons of which give a signal at 2.16 ppm.

Additional proof for these partial structures and their connection mode was obtained by reaction of the compound with pyridine chlorochromate [4]. By this procedure, the side chain was cleaved at the conjugated double bonds to give 6-methylhept-5-en-2-one (**8**) and citral (**9**). The second ring system, therefore, had to be located between the iridal ring and the side chain. Of the five unassigned C-atoms, one is contained in a CHOH group ($\delta(C) = 73.92$), the proton of which appeared as a *doublet* at 4.41 ppm, coupling only with the H-atom of a CH group appearing at 2.84. The fourth substituent of the secondary alcohol, therefore, had to be one of the two quaternary C-atoms of the iridal ring. The CH group on the other side of this fragment is connected to two consecutive CH_2 groups ($\delta(C) = 26.1$, $\delta(H) = 1.35/1.95$; $\delta(C) = 34.5$, $\delta(H) = 1.35/1.00$), the last of which is again bound to a quaternary C-atom of the iridal ring. The third position at one of these two quaternary C-atoms is occupied by a Me group ($\delta(C) = 27.8$, $\delta(H) = 1.02$), the last C-atom to be assigned. From these data, the two alternative structures **10** and **11** could be formulated. However, a distinction between **10** and **11** could not be achieved by the usual spectroscopic methods. Also, all attempts to obtain more information by chemical degradation studies failed.





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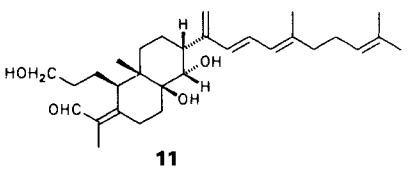

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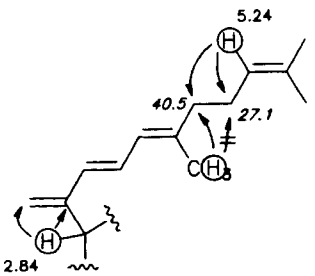
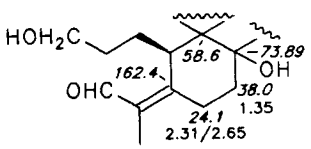
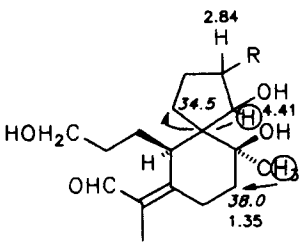
The problem was eventually resolved by recording inverse 2D-^{1}H,^{13}C-NMR spectra at high magnetic field (14 Tesla, 600 MHz, ^{1}H). The enormous gain in sensitivity of this method compared to conventional 2D-^{13}C-detected correlations allows experiments on very small amounts of sample and better definition of the observed signals. Thus, by the special pulse sequence introduced by *Bax et al.* [5] in 1986 a ^{1}H-detected multiple-bond ^{13}C-multiple-quantum-coherence spectrum was measured. The cross peaks in this spectrum are derived exclusively from H,C coupling *via* two or three bonds. ^{4}J(C,H) is normally too small to be observed. Detailed analysis of this spectrum allowed the elucidation of the constitution of the new iridal and assignment of all the ^{1}H- and ^{13}C-NMR signals.

The structure of the side chain was confirmed by coupling of the Me protons (δ(H) = 1.83) with the methylene C-atom at 40.5 but not at 27.1 ppm, and coupling of the olefinic proton (δ(H) = 5.24) with both of these allylic C-atoms. The methine H-atom (2.84) is coupled with the two C-atoms of the methylidene group, thus proving that this end of the triene moiety is connected to the second ring system. To resolve the nature of







this ring and its connection to the iridal ring, it was necessary to assign all C-atoms of the latter. The protons of the CH_2 groups corresponding to δ(C) = 24.1 (δ(H) = 2.31/2.65) and δ(C) = 38.0 (δ(H) = 1.35) both show cross peaks with C(7) (δ(C) = 162.4), whereas only the protons at 1.35 are coupled to the quaternary C-atom at 58.6 ppm. Thus, the ^{13}C assignments for C(8) to C(11) previously made from biogenetic arguments and by analogy with other iridals are unambiguous.

Several correlations definitely show that the compound isolated has the spiro-bicyclic structure **10**. The two most important arguments are: the proton of the C*H*OH group in the second ring is coupled to the methylene C-atom at 34.5 ppm. Only in the spiro system are these two atoms three bonds apart; in the anellated ring system, this would be a coupling over four bonds. The Me group (δ(H) = 1.02) has to be connected to the quaternary C-atom (δ(C) = 73.89) bearing the tertiary OH group, since the Me protons are coupled to C(9) at 38.0 (δ(H) = 1.35).

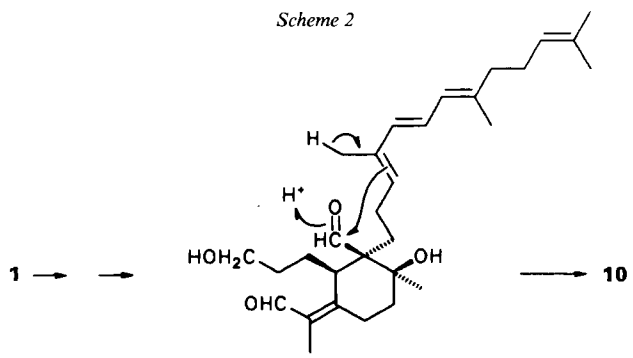
A positive NOE can be observed between the methylidene proton at 5.21 ppm and H_A (δ(H) = 6.22) of the *AMX* system as well as between the *Me*–C(19) and H_M (δ(H) = 6.7), indicating a s-*trans*-arrangement of the triene system.

It has been already shown that the configuration of the iridal ring system is identical throughout all the *Iris* species studied [6]. We, therefore, assume, that this holds true for the compounds from *I. foetidissima* as well. Hence, the absolute configuration of the spiro systems and the conformation of the conjugated triene could be ascertained from NOE experiments.

The observation of positive NOE's at the *Me*–C(10) upon irradiation of the *H*–C(26) (4.41 ppm) and H–C(14) (2.84 ppm) indicates that both are located on the same side of the cyclopentane ring and facing this Me group. The compound is, therefore, (*6*R,*10*S,*11*S,*14*S,*26*R)*-26-hydroxy-15-methylidenespiroirid-16-enal* (**10**).

Discussion. – To our knowledge, the structure of this iridal from *I. foetidissima* is the first example of a spirobicyclic triterpenoid found in nature. A proposal for its formation is depicted in *Scheme 2*. Starting with iridal **1**, an additional unsaturation is introduced into the side chain to give a conjugated triene, and the Me group at C(11) is oxidized to the corresponding aldehyde. In a subsequent (enzymatic) step, acid-catalyzed ring closure between C(26) and C(14) takes place, and, by loss of a proton from C(28), the methylidene group is formed. We suppose that the triene moiety arises by dehydrogenation of the side chain of iridal **1** (similar to that in carotene biosynthesis) rather than by dehydration

Scheme 2



of 16-hydroxiridal **2**, since, on ^3H labelling of the latter, no incorporation into any triene was observed [7]. Extracts of different *Iris* species show that iridals with the triene moiety are always present, and we have recently succeeded in isolating several of these compounds from *I. pseudacorus* extracts, with structures related to **10** [8]. Hence, the formation of the irone precursors *via* the conjugated trienes may still be possible, and we are continuing our search for the appropriate intermediates.

Financial support of the *Deutsche Forschungsgemeinschaft*, Bad Godesberg, and the *Fonds der chemischen Industrie*, Frankfurt, is gratefully acknowledged. We wish to thank Dr. *H. Schmickler*, Köln, and Dr. *H. Röttele*, Karlsruhe, for carrying out numerous NMR experiments.

Experimental Part

General. Plant Material. Rhizomes of *I. foetidissima* were obtained from *Bornträger & Schlemmer*, D–6521 Offstein, in October 1988. GLC: *Shimadzu GC 8A*, cap. column *OV 1* (15 m, 0.25 mm i.d.). HPLC: *Kontron* model *200*, column: *Lichrocart RP 18* (125 mm, *Merck*); *Hewlett-Packard-1040A* diode-array detector. $[\alpha]_{578}$: *Zeiss 0.05* precision polarimeter, in CH_2Cl_2 (*c* in g/100 ml). UV spectrum: *Zeiss PMQ-2*. NMR spectra: *Bruker WH-300* (Cologne; ^1H: 300 MHz, ^{13}C: 75.4 MHz), *Bruker WM-400* (Karlsruhe; ^1H: 400 MHz, ^{13}C: 100.6 MHz), *Bruker AM-600* (Braunschweig; ^1H: 600 MHz, ^{13}C: 150.9 MHz), in (D_6)benzene, chemical shifts in ppm δ relative to TMS (= 0 ppm), coupling constants *J* in Hz. MS: *Finnigan-MAT 4510* GC/MS (EI: 70 eV, CI: NH_3), *m/z* (rel. intensity in %).

Isolation. The chopped rhizomes (1160 g) were extracted with $CHCl_3$/MeOH 2:1 (*v/v*). After evaporation of the solvent, the residue was partitioned between CH_2Cl_2 and H_2O. The org. phase was washed with sat. NaCl soln., dried ($MgSO_4$), and evaporated to give 28 g of crude oil; 24.35 g of this oil were separated on a silica-gel column (100 cm; 2.5 cm i.d.; silica gel: *Merck* 0.063·0.2 mm) using a petroleum ether/Et_2O/$CHCl_3$/acetone/MeOH gradient. The fraction eluting with Et_2O/$CHCl_3$ 7:3 (*v/v*; 760 mg) contained **10** together with free fatty acids. The latter were esterified with CH_2N_2 and the fraction re-chromatographed on silica gel (12 g) to give 120 mg of chromatographically (HPLC) pure **10** as glasslike solid.

*(+)-(6*R*,10*S*,11*S*,14*S*,26*R*)-26-Hydroxy-15-methylidenespiroirid-16-enal (= (1'*R*,2'*S*,5'*S*,6'*R*,10'*S*)-2-[1,10-Dihydroxy-6-(3-hydroxypropyl)-2-(5,9-dimethyl-1-methylidenedeca-2,4,8-trienyl)spiro[4.5]dec-2-ylidene]propanal;* **10**). $[\alpha]_{578}^{20}$ = +137 (*c* = 1.09). UV (EtOH): 265 (15350), 273 (15500), 284 (sh, 11000). ^1H-NMR: 10.49 (*s*, H–C(1)); 6.7 (*dd*, *J*(17,16) = 15.4, *J*(17,18) = 10.8, H–C(17)); 6.22 (*d*, *J*(17,16) = 15.4, H–C(16)); 6.03 (*d*, *J*(18,17) = 10.8, H–C(18)); 5.24 (*t*, *J*(22,21) = 6.9, H–C(22)); 5.21, 5.02 (2*s*, 2 H–C(28)); 4.41 (*d*, *J*(26,14) = 3.5, H–C(26)); 3.65 (br. *d*, *J*(6,5) = 9.7, H–C(6)); 3.62–3.52 (*m*, 2 H–C(3)); 2.84 (*m*, H–C(14)); 2.65–2.3 (*m*, 2 H–C(8)); 2.37–1.95 (*m*, 2 H–C(5)); 2.18 (*m*, 2 H–C(21)); 2.16 (*m*, 2 H–C(20)); 1.95–1.35 (*m*, 2 H–C(13)); 1.92 (*s*, 3 H–C(25)); 1.83 (*s*, 3 H–C(29)); 1.67 (*s*, 3 H–C(24)); 1.58 (*s*, 3 H–C(30)); 1.46–1.38 (*m*, 2 H–C(4)); 1.35 (*m*, 2 H–C(9)); 1.35–1.00 (*m*, 2 H–C(12)); 1.02 (*s*, 3 H–C(27)). ^{13}C-NMR: 190.4 (*d*, C(1)); 162.4 (*s*, C(7)); 144.2 (*s*, C(15)); 140.4 (*s*, C(19)); 133.0 (*s*, C(2)); 132.9 (*d*, C(16)); 131.8 (*s*, C(23)); 125.8 (*d*, C(18)); 125.7 (*d*, C(17)); 124.3 (*d*, C(22)); 117.3 (*t*, C(28)); 73.92 (*d*, C(26)); 73.89 (*s*, C(10)); 61.9 (*t*, C(3)); 58.6 (*s*, C(11)); 49.1 (*d*, C(14)); 42.7 (*d*, C(6)); 40.5 (*t*, C(20)); 38.0 (*t*, C(9)); 34.5 (*t*, C(12)); 31.7 (*t*, C(4)); 29.8 (*t*, C(5)); 27.8 (*q*, C(27)); 27.1 (*t*, C(21)); 26.1 (*t*, C(13)); 25.8 (*q*, C(24)); 24.1 (*t*, C(8)); 17.8 (*q*, C(30)); 17.1 (*q*, C(29)); 11.3 (*q*, C(25)). EI-MS: 470 (0.5, M^+), 452 (1), 434 (0.5), 383 (2), 365 (1), 281 (1), 109 (50), 69 (100). NCI: 470 (M^-).

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