Interconvertible Eudesmanolides Containing a 6,12-Hemiketal Function from *Salvia castanea* DIELS f. *tomentosa* STIB.

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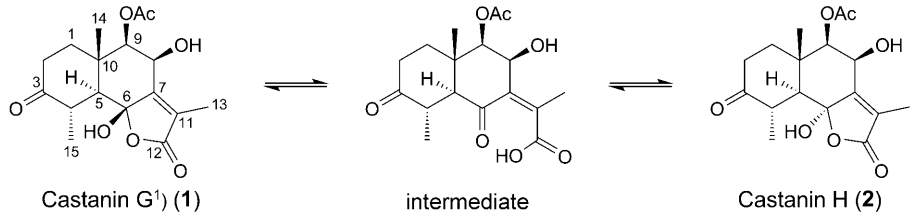
Two new eudesmanolide sesquiterpenoids containing a hemiacetal function, castanins G and H (**1** and **2**), were obtained as a pair of interconvertible isomers from the aerial parts of *Salvia castanea* DIELS f. *tomentosa* STIB., and separated as their uninterconvertible acetates **3** and **4**. Their structures were elucidated by unequivocal interpretation and comparative analysis of the NMR and MS data of the mixture **1**/**2** and of their acetates **3** and **4**, respectively. The inhibitory activity of **3** and **4** toward MCF-7, HeLa, and HepG2 cell lines was also evaluated.

Introduction. – The plants of the genus *Salvia* are a rich source of diterpenoids, especially abietane and clerodane diterpenoids [1]. To the best of our knowledge, there are more than 600 diterpenoids which have been reported from this genus, whereas only less than 30 sesquiterpenoids have been characterized from different *Salvia* plants [2–7]. *S. castanea* DIELS f. *tomentosa* is a herb with castaneous flowers that is distributed in the southwest of China [8]. Previous studies of its chemical constituents have resulted in the isolation of several sesquiterpenoids including two pairs of interconvertible germacranolides (=germacranolactones) containing a hemiacetal function, castanins C–F [2][9].

Hemiacetal metabolites, which usually exist as interconverting isomers, have been reported from different plants previously, such as enfumafungin [10], 30-hydroxy-3-oxofriedelan-28-al [11], 3-episkimmiarepin A [12], scutegalin B [13], and castanins C–F [9]. In this study, two new epimeric eudesmanolides (=eudesmanolactones) containing a hemiacetal function, castanins G[1]) (**1**) and H[1]) (**2**), were isolated from the aerial parts of *S. castanea* DIELS f. *tomentosa*. These two compounds were isolated as a mixture of interconvertible isomers and behaved like a single compound when examined by TLC (silica gel) and reversed-phase HPLC. Their ^{1}H- and ^{13}C-NMR spectra showed doubling of all signals, but the MS indicated a monomer. Extensive analysis of the NMR data of the mixture, in combination with the fact that some hemiketal and hemiacetal metabolites have been isolated from this species and several other plants as interconvertible forms [9–13], led to the recognition of the mixture as

[1]) Trivial atom numbering; for systematic names, see *Exper. Part*.

Scheme 1. *Equilibrium System of the Interconvertible Isomers* **1** *and* **2**



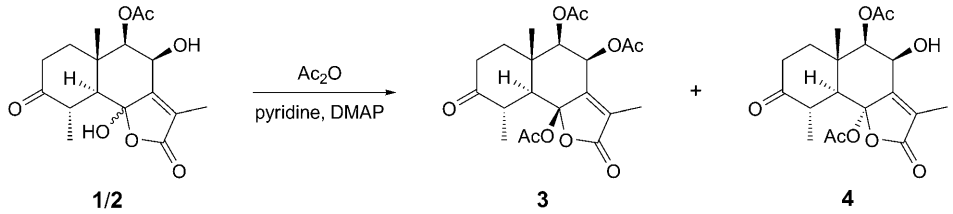
two interconvertible hemiketal sesquiterpenoids (*Scheme 1*). This pair of interconvertible isomers was separated as their uninterconvertible acetates **3** and **4**.

Results and Discussion. – The mixture **1/2** was isolated as a white powder. Although the mixture presented a single spot on TLC (silica gel) plates developed in several solvent systems and only one peak in the HPLC, its ^{1}H- and ^{13}C-NMR spectra initially appeared to be unduly complex. Two sets of seventeen C-atom signals including two Ac groups, two α,β-unsaturated γ-lactone groups, and two C=O groups (*Table 1*) in the ratio of 2:1 were observed in the ^{1}H- and ^{13}C-NMR spectra. In the EI-MS of this mixture, a M^+ ion peak at m/z 338 for the molecular formula $C_{17}H_{22}O_7$ together with the $[M-H_2O]^+$ and $[M-CH_2CO]^+$ ion peaks at m/z 320 and 296 can all be found. These observations indicated the presence of a 2:1 mixture of two interconverting sesquiterpenoid isomers (*Scheme 1*). This deduction was rationalized by the separation and identification of **3** and **4**, the acetates of **1** and **2** (*Scheme 2*). The structures of **3** and **4** were identified by unequivocal interpretation and comparative analysis of the NMR and MS data.

Table 1. *^{13}C-NMR Data* (100 MHz, CD_3OD) *of Compounds* **1**–**4**[1]). δ in ppm.

	1	**2**	**3**	**4**
C(1)	39.5 (*t*)	38.5 (*t*)	37.8 (*t*)	37.6 (*t*)
C(2)	34.3 (*t*)	37.1 (*t*)	36.6 (*t*)	36.7 (*t*)
C(3)	211.6 (*s*)	213.0 (*s*)	209.8 (*s*)	210.8 (*s*)
C(4)	42.4 (*d*)	46.7 (*d*)	42.2 (*d*)	42.1 (*d*)
C(5)	55.5 (*d*)	51.4 (*d*)	55.6 (*d*)	55.3 (*d*)
C(6)	105.7 (*s*)	106.2 (*s*)	104.1 (*s*)	103.2 (*s*)
C(7)	153.0 (*s*)	153.1 (*s*)	148.0 (*s*)	147.1 (*s*)
C(8)	70.0 (*d*)	70.0 (*d*)	66.2 (*d*)	66.9 (*d*)
C(9)	77.6 (*d*)	78.8 (*d*)	77.3 (*d*)	77.7 (*d*)
C(10)	40.7 (*s*)	39.8 (*s*)	39.6 (*s*)	39.8 (*s*)
C(11)	130.1 (*s*)	128.2 (*s*)	132.6 (*s*)	131.8 (*s*)
C(12)	171.0 (*s*)	171.5 (*s*)	170.4 (*s*)	170.6 (*s*)
Me(13)	8.7 (*q*)	8.5 (*q*)	9.0 (*q*)	9.0 (*q*)
Me(14)	14.0 (*q*)	12.9 (*q*)	14.1 (*q*)	13.8 (*q*)
Me(15)	16.6 (*q*)	14.4 (*q*)	13.7 (*q*)	13.8 (*q*)
*Ac*O–C(6)	–	–	169.0 (*s*), 21.8 (*q*)	169.6 (*s*), 20.8 (*q*)
*Ac*O–C(8)	–	–	170.1 (*s*), 20.6 (*q*)	–
*Ac*O–C(9)	170.9 (*s*), 21.0 (*q*)	171.0 (*s*), 21.0 (*q*)	169.9 (*s*), 20.4 (*q*)	170.4 (*s*), 20.7 (*q*)

Scheme 2. *Acetylation of* **1** *and* **2**



Compound **3** was isolated as a white powder. Its molecular formula $C_{21}H_{26}O_9$ was deduced by the positive-mode HR-ESI-MS (*m/z* 363.1438 ($[M-AcO]^+$)) and the NMR data (*Tables 1* and *2*). The IR spectrum of **3** showed the presence of α,β-unsaturated γ-lactone and Ac groups at 1786 and 1751 cm^{-1}. The NMR data indicated that **3** contained three AcO, one C=O, an α,β-unsaturated γ-lactone, three Me, two CH_2, and four CH groups (including two oxygenated ones), as well as two quaternary C-atoms (including a dioxygenated one). Considering the characteristic ^{13}C-NMR signals at δ(C) 9.0 (*q*), 14.1 (*q*), and 13.7 (*q*) due to Me(13), Me(14), and Me(15), two CH groups at δ(C) 42.2 (*d*, C(4)) and 55.6 (*s*, C(5)), the sp^3 quaternary C-atom at δ(C) 39.6 (C(10)), together with the α,β-unsaturated γ-lactone group at δ(C) 148.0 (*s*, C(7)), 132.6 (*s*, C(11)), and 170.4 (*s*, C(12)), and the noticeable dioxygenated quaternary C-atom at δ(C) 104.1 (C(6)), compound **3** was deduced to be a eudesmanolide containing a hemiketal function [3][14][15].

Table 2. *^{1}H-NMR Data* (400 MHz, CD_3OD) *of Compounds* **1**–**4**[1]). δ in ppm, *J* in Hz.

	1	**2**	**3**	**4**
H_α–C(1)	1.45–1.57 (*m*)	1.45–1.57 (*m*)	1.50–1.56 (*m*)	1.48–1.55 (*m*)
H_β–C(1)	1.91–2.00 (*m*)	1.91–2.00 (*m*)	1.93–1.98 (*m*)	1.89–1.96 (*m*)
H_α–C(2)	2.15–2.31 (*m*)	2.15–2.31 (*m*)	2.25–2.31 (*m*)	2.24–2.30 (*m*)
H_β–C(2)	2.50–2.61 (*m*)	2.50–2.61 (*m*)	2.55–2.63 (*m*)	2.55–2.61 (*m*)
H–C(4)	3.35–3.49 (*m*)	3.35–3.49 (*m*)	3.10–3.17 (*m*)	3.01–3.07 (*m*)
H–C(5)	1.90–1.99 (*m*)	1.90–1.99 (*m*)	1.91–1.96 (*m*)	1.91–1.95 (*m*)
H–C(8)	5.78 (*d*, *J* = 4.0)	5.74 (*d*, *J* = 4.0)	6.18 (*d*, *J* = 4.0)	6.00 (*d*, *J* = 4.0)
H–C(9)	4.48 (*d*, *J* = 4.0)	4.50 (*d*, *J* = 4.0)	4.72 (*d*, *J* = 4.0)	4.70 (*d*, *J* = 4.0)
Me(13)	1.90 (*s*)	1.91 (*s*)	1.92 (*s*)	1.88 (*s*)
Me(14)	1.65 (*s*)	1.54 (*s*)	1.64 (*s*)	1.65 (*s*)
Me(15)	1.18 (*d*, *J* = 6.8)	1.16 (*d*, *J* = 6.8)	1.22 (*d*, *J* = 6.8)	1.22 (*d*, *J* = 6.8)
AcO–C(6)	–	–	2.14 (*s*)	2.10 (*s*)
AcO–C(8)	–	–	2.09 (*s*)	–
AcO–C(9)	2.02 (*s*)	2.05 (*s*)	2.08 (*s*)	2.10 (*s*)

The HMBC spectrum of **3** displayed the following correlations (*Fig.*): H–C(1)/C(3), C(5), and C(14), H–C(2)/C(3), C(4), and C(10), H–C(5)/C(6), C(14), and C(15), H–C(8)/C(6), C(7), C(10), and C(11), H–C(9)/C(1), C(10), and C(14), Me(13)/C(7), C(11), and C(12), Me(14)/C(1), C(5), C(9), and C(10), and Me(15)/C(3), C(4), and C(5). On the basis of the above HMBCs, together with three H-atom spin systems, H–C(1)/H–C(2), H–C(8)/H–C(9), and Me(15)/H–C4)/H–C(5),

obtained by the analysis of the ^{1}H,^{1}H COSY plot, compound **3** was determined to be a 3-oxoeudesmano-12,6-lactone with three Ac substitutions at C(6), C(8), and C(9).

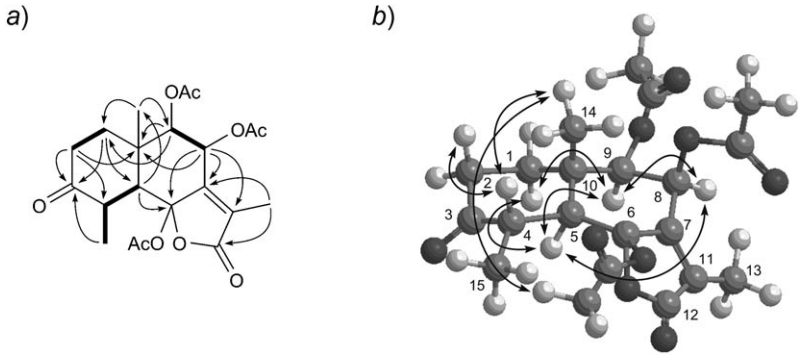


Figure. *Selected* a) *HMBC* (H → C) *and COSY* (—), *and* b) *NOE* (H ↔ H) *correlations of* **3**[1])

The relative configuration of **3** was established on the basis of a ROESY experiment (*Fig.*). The ROESY correlations H_α–C(1)/H–C(5), Me(14)/H–C(4), and H–C(4)/H_β–C(2) indicated the α-orientation of H–C(5) and Me(15), as well as the β-orientation of H–C(4) and Me(14). Furthermore, the NOEs H–C(5)/H–C(8), H–C(5)/H–C(9), H_α–C(1)/H–C(9), and H–C(8)/H–C(9) suggested that H–C(8) and H–C(9) were both α-oriented. The AcO at C(6) was suggested to be β-oriented by the NOE found between Me(14) and AcO–C(6). Accordingly, the structure of **3** was elucidated as 6,8,9-tris(acetyloxy)-3-oxoeudesmano-12,6-lactone.

The molecular formula of acetate **4**, $C_{19}H_{24}O_8$, was deduced from the positive-mode HR-ESI-MS (*m*/*z* 381.1551 (M^+)). Comparison of the 1D-NMR data of **4** (*Tables 1* and *2*) with those of **3** indicated that they were closely related, except for the lack of the Ac group at C(8) or C(6) in **4**, which can be confirmed by the HMBC from H–C(9) to the C=O group of AcO–C(9) at δ(C) 170.4. Considering the fact that **4** can be isolated as an uninterconvertible pure compound, the two AcO groups of **4** can be ascribed to be substituents at C(6) and C(9), respectively. So, the structure of **4** was determined to be the 8-deacetyl derivative of **3**.

The NOEs of **4** observed in the ROESY plot were also strikingly similar to those of **3**, except for the replacement of the NOE Me(14)/AcO–C(6) in **3** by the NOE H_α–C(1)/AcO–C(6) in **4**, which indicated that the AcO at C(6) was α-oriented. Moreover, the NOEs H_α–C(1)/H–C(5), Me(14)/H–C(4), H–C(4)/H_β–C(2), H–C(5)/H–C(8), H–C(5)/H–C(9), H_α–C(1)/H–C(9), and H–C(8)/H–C(9) were all present just like in **3**, which established the α-orientation of H–C(5), H–C(8), H–C(9), and Me(15), as well as the β-orientation of H–C(4) and Me(14).

The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** displayed two sets of 15 C-atom signals in the ratio of 2:1, which indicated that the mixture was composed of two interconverting sesquiterpenoid isomers in the ratio of 2:1. The prominent features distinguishing **1** from **3** in the NMR spectra (*Tables 1* and *2*) was the absence of two Ac groups at C(6) and C(8) of **1**, and the difference between **2** and **4** was the appearance of one more Ac group at C(6) in **4**. So, compounds **3** and **4** can be determined as the 6,8-diacetyl and 6-acetyl derivatives of **1** and **2**, respectively. In addition, considering that compounds **1**

and **2** were two interconvertible isomers, along with the fact that **3** and **4** were the 6,8-diacetyl and 6-acetyl derivatives of **1** and **2**, respectively, the AcO group of **1** and **2** could also be ascribed to be at C(9). The assignment of the 1D-NMR data of **1** and **2** (*Tables 1* and *2*) was achieved by the careful comparison with those of **3** and **4** in combination with the ratio (2 : 1) of **1** and **2** in the mixture. Therefore, the structures and relative configurations of **1** and **2** were established as (6α,8β,9β)-9-(acetyloxy)-6,8-dihydroxy-3-oxoeudesmano-12,6-lactone (= castanin G) and (6α,8β,9β)-9-(acetyloxy)-6,8-dihydroxy-3-oxoeudesmano-12,6-lactone (= castanin H), respectively.

The cytotoxicity of compounds **3** and **4** were tested against MCF-7, HeLa, and HepG-2 cell lines *in vitro* using the method described previously [16]. All these compounds showed weak inhibitory activities with $IC_{50} > 150$ μg/ml.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Inc.*) and porous resin (*DM-130*; *Shandonglukang Inc.*). TLC: SiO_2 plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Optical rotations: *Horiba-SEPA-300* polarimeter. UV/VIS Spectra: *UV-2401-PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer, KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AM-400* spectrometer (at 400 and 100 MHz, resp.); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. 2D-NMR Spectra: *Bruker-DRX-500* instrument. EI-MS: *VG-Auto-Spec-3000* spectrometer; in *m*/*z* (rel. %). HR-ESI-MS: *API-Qstar-Pulsar* instrument.

Plant Material. Plants of *Salvia castanea* DIELS f. *tomentosa* STIB. were collected in Lijiang, Yunnan Province, in July 2000, and were identified by Prof. *Xi-Wen Li*, Kunming Institute of Botany. A voucher specimen (No. 200098) was deposited with the Kunming Institute of Botany, the Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried and powdered aerial parts (4.1 kg) of *S. castanea* DIELS f. *tomentosa* STIB. were extracted with acetone for 24 h at r.t. (3 × 10 l). The solvent was removed under vacuum, and the resulting gummy material was subjected to CC (*DM-130* porous resin, MeOH/H_2O 1 : 1 and 9 : 1). The residue obtained with MeOH/H_2O 9 : 1 was partitioned between H_2O and AcOEt (2 l). The org. extract (56 g dry weight) was subjected to CC (SiO_2, petroleum ether/acetone 1 : 0 → 0 : 1): *Fractions 1–4* (by TLC analysis). *Fr. 2* was resubjected to CC (SiO_2, petroleum ether/$CHCl_3$/acetone/H_2O 80 : 15 : 5 : 0.5): **1/2** (80 mg).

rel-*(4*R*,5*S*,5a*R*,9*R*,9a*R*,9b*S*)*/rel-*(4*R*,5*S*,5a*R*,9*R*,9a*R*,9b*R*)-5-(Acetyloxy)-5,5a,6,7,9a,9b-hexahydro-4,9b-dihydroxy-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,8(4*H*,9*H*)-dione* (= *Castanin G*/*Castanin H*; **1/2**): White powder. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 338 (6, M^+), 320 (15, $[M - H_2O]^+$), 296 (11), 278 (17), 260 (27), 124 (40), 69 (48), 55 (100).

Acetylation of the Interconverting Isomers **1/2**. To **1/2** (80 mg) dissolved in pyridine (3 ml), Ac_2O (0.5 ml) was added, and then the mixture stirred for 48 h. The solvent was evaporated, and the remaining powder was subjected to prep. HPLC (MeOH/H_2O 45 : 55): **3** (36 mg) and **4** (9 mg), resp.

rel-*(4*R*,5*S*,5a*R*,9*R*,9a*R*,9b*S*)-4,5,9b-Tris(acetyloxy)-5,5a,6,7,9a,9b-hexahydro-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,8(4*H*,9*H*)-dione* (= *Castanin G 6,8-Diacetate*; **3**): White powder. $[\alpha]_D^{25.0} = -24.96$ ($c = +0.18$, MeOH). UV (MeOH): 225.4 (1.37). IR (KBr): 2984, 2946, 2881, 1786, 1752, 1713, 1635, 1431, 1373, 1241, 1222, 1126, 1104, 1048, 1030, 972, 737, 622. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 867 ($[2M + Na]^+$), 445 ($[M + Na]^+$), 363 ($[M - AcO]^+$). HR-ESI-MS (pos.): 363.1438 ($[M - AcO]^+$, $C_{19}H_{23}O_7^+$; calc. 363.1443).

rel-*(4*R*,5*S*,5a*R*,9*R*,9a*R*,9b*R*)-5,9b-Bis(acetyloxy)-5,5a,6,7,9a,9b-hexahydro-4-hydroxy-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,8(4*H*,9*H*)-dione* (= *Castanin H 6-Acetate*; **4**): White powder. $[\alpha]_D^{25.0} = +10.95$ ($c = 0.38$, MeOH). UV (MeOH): 217.8 (0.61), 202.2 (0.64). IR (KBr): 3440, 2927, 1749, 1711, 1632, 1374, 1238, 1136, 1023, 944, 748, 593. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 403 ($[M+Na]^+$), 381 ($[M+H]^+$), 321 ($[M-AcO]^+$). HR-ESI-MS (pos.): 381.1551 ($[M+H]^+$, $C_{19}H_{25}O_8^+$; calc. 381.1549).

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