Isomeric Eremophilane Lactones from *Senecio tsoongianus*

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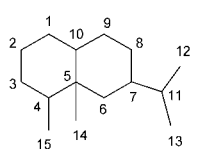
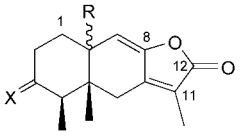
From the whole plant of *Senecio tsoongianus*, two pairs of epimers, tsoongianolides A (**1**) and B (**2**), and tsoongianolides C (**3**) and D (**4**), and two new sesquiterpene lactones, tsoongianolides E (**5**) and F (**6**), all of which possess the eremophilane skeleton, were isolated. Two known eremophilane dimers (**7**) and (**8**) were also obtained from this species. Their absolute structures were elucidated by 1D- and 2D-NMR techniques and by X-ray diffraction studies, along with chemical evidence.

Introduction. – The Senecioneae are a tribe of closely related genera of the family Asteraceae that are characterized by the nature of the pappus and the involucral phyllaries. They are also known for the abundance of antitumor pyrrolizidines and eremophilane (=decahydro-1,8a-dimethyl-7-(1-methylethyl)naphthalene) derivatives they contain [1][2]. During our systematic investigation of *Senecio* plants scattered in Yunnan Province, China we collected *S. tsoongianus* LING, which is traditionally used by local people for its anti-inflammatory properties. Phytochemical investigation of the petroleum ether extract afforded several known compounds, including β-sitosterol, stigmasterol, dotriacontene, and tritriacontane, as well as six new eremophilanolides, *i.e.*, tsoongianolides A–F (**1**–**6**), together with the known, but rare, eremophilane dimers **7** and **8**. Here, we present the structure elucidation and characterization of these compounds.

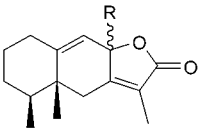
Results and Discussion. – *Compound Identification and Biogenetic Aspects.* The mass spectrum of tsoongianolide A (**1**) exhibited a molecular-ion peak at *m/z* 248, and a significant fragment due to loss of a H_2O molecule appeared at *m/z* 230. This suggested the presence of an OH group at a quaternary C-atom, which appeared at δ (C) 72.9 in the ^{13}C-NMR spectrum (see *Table 1* in the *Exper. Part*). Furthermore, a lactone C=O group at δ (C) 172.1, and resonances of a fully substituted C=C bond at δ (C) 149.3 and 122.9 were observed. The olefinic H-atom at δ (H) 5.48 (*s*) indicated a neighboring quaternary C-atom (see *Table 2* in the *Exper. Part*). Considering the three typical Me signals at δ (H) 0.83 (*s*), 0.85 (*d*, J = 6.6 Hz), and 1.85 (*d*, J = 1.4 Hz), **1** was deduced to



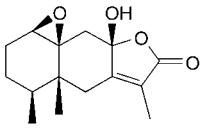
Eremophilane



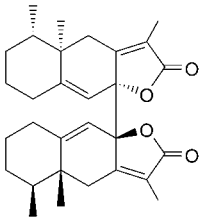
1 R = α-OH, X = H_2
2 R = β-OH, X = H_2
5 R = β-OH, X = O



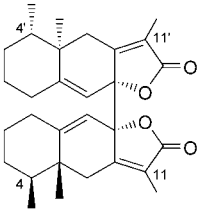
3 R = β-OH
4 R = α-OH



6



7 (8α/8'α)



8 (8α/8'β)

be an eremophilanolide, specifically a 7,11-en-8,12-olide [3–6]. Furthermore, the OH group had to be located at C(10), thus allowing the olefinic H–C(9) group to appear as a *singlet* in the ^{1}H-NMR spectrum.

The ^{13}C-NMR spectrum of **1** also showed four olefinic resonances and a quaternary C-atom at δ (C) 43.6, attributed to C(5) (*Table 1*). The typical *AB doublets* at δ (H) 2.59 (*d*, $J = 13.0$ Hz) and 2.64 (br. *d*, $J = 13.0$ Hz) could be assigned to CH_2(6). The broadened *doublet* at δ (H) 2.64 arises from homoallylic coupling with Me(13) [3].

The configuration of the 10-OH group of **1** was assigned by means of X-ray diffraction studies (see below). As shown in the *Figure* (*a*), the OH group adopts the α configuration. Thus, tsoongianolide A (**1**) was identified as (4*S*,5*R*,10*R*)-10-hydroxyeremophil-7(11),8-dien-8,12-olide[1]).

Tsoongianolide B (**2**) had the same molecular weight (*m/z* 248) and displayed similar fragments as **1** in its EI mass spectrum. The ^{13}C-NMR spectrum of **2** showed 15 resonances, three Me, four CH_2, and two CH groups, and six C_q, similar to that of **1** (*Table 1*). This suggested that **2** is an isomer of **1**. Scrutiny of the ^{1}H and ^{13}C-NMR spectra of these two lactones provide strong evidence in support of this hypothesis. Slight differences appeared at the CH_2(6) resonances. In the ^{1}H-NMR spectrum of **2**, the separated pair of *AB doublets* had disappeared in favor of a very close triplet-like signal centered at 2.58 ppm. Furthermore, the ^{1}H-NMR chemical shift of Me(14) in **2** was 1.02 ppm, compared to 0.83 ppm in the case of **1**. In contrast, H–C(4) of **2** was shifted from 2.23 to 1.76 ppm in the case of **1**. This indicated that the α-OH group of **1** was β-configured in **2**, thus deshielding Me(14). Moreover, because H–C(4) of **2** experienced no deshielding effect of the α-OH group present in **1**, its resonance appeared at a relatively low frequency.

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[1]) For systematic names, see *Exper. Part*.

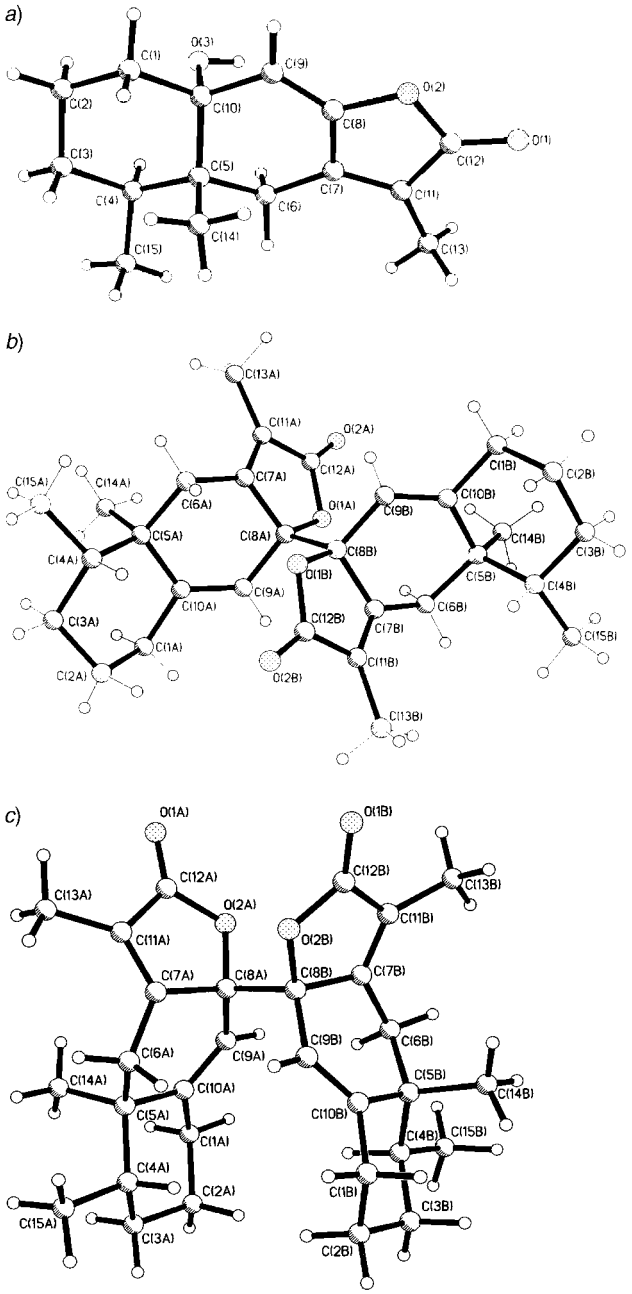
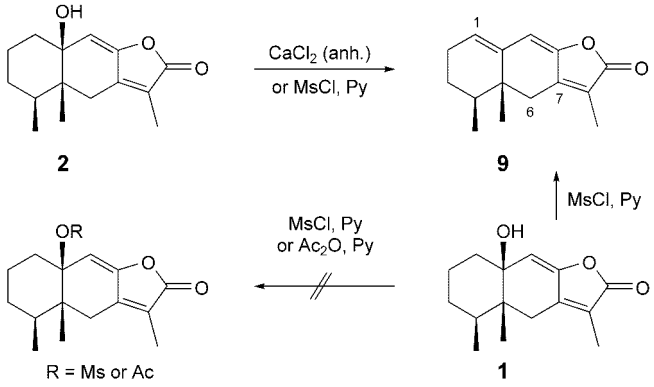


Figure. *X-Ray single-crystal structures of* a) *tsoongianolide A* (**1**), *and of the dimers* **7** (*b*) *and* **8** (*c*). For details, see text and *Exper. Part*.

Unexpectedly, **2** was not quite stable and was easily converted to its dehydrated form **9** during storage over $CaCl_2$ as a desiccant. Treatment of **2** with methanesulfonyl chloride (MsCl) and pyridine (*Scheme 1*) afforded a derivative whose melting point, TLC, optical rotation, and NMR properties were identical to those of an authentic sample of **9** [7]. To assign the absolute configurations at C(4) and C(5) of **2**, mesylation of tsoongianolide A (**1**) was performed. Again, the sole product was identical to **9**, which suggested that the orientations of Me(14) and Me(15) were identical in **1** and **2**. Therefore, tsoongianolide B (**2**) is the 10β-epimer of tsoongianolide A (**1**).

Scheme 1



Ac = acetyl, Ms = methanesulfonyl (mesyl), Py = pyridine

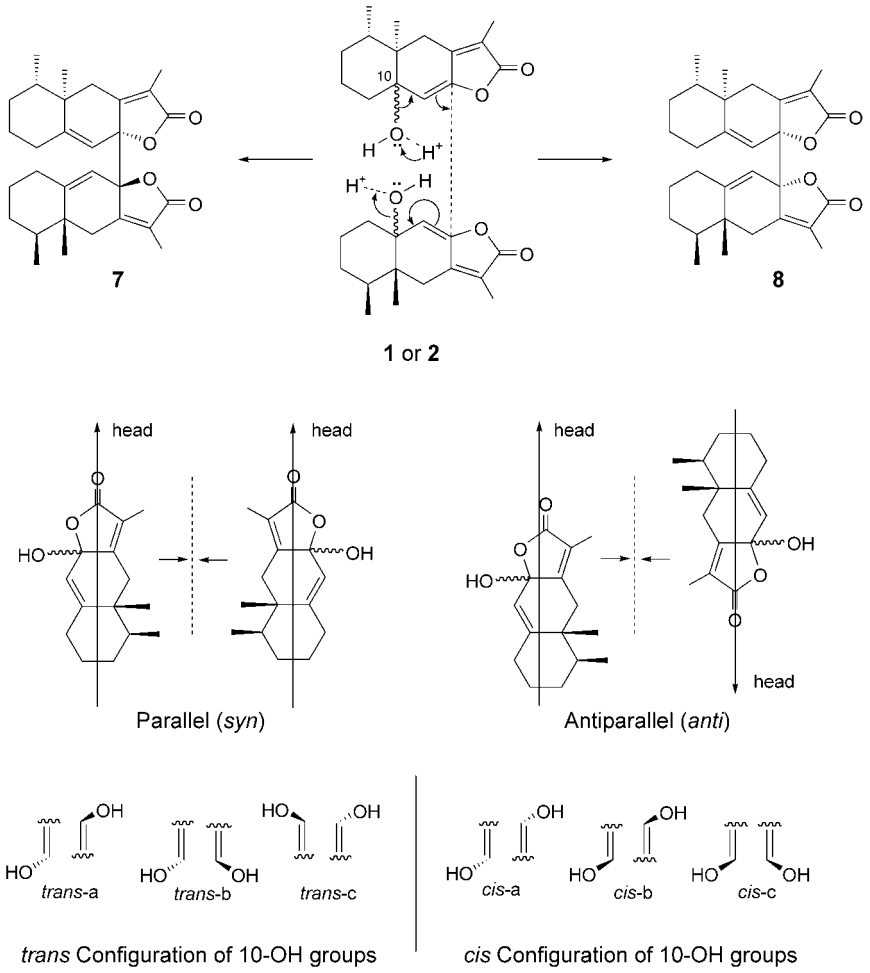
At first glance, compound **7** seemed to have the same skeleton as **1** and **2**. The ^{13}C-NMR spectral data also showed 15 resonances (*Table 1*), and so it was presumed to be another stereoisomer. Surprisingly, however, mesylation of **7** in pyridine also afforded **9**, as identified by its ^{1}H-NMR, $[\alpha]_D$, and MS data, as well as by mixed melting-point examination. In the mass spectrum of **7**, a very weak molecular-ion peak was found at *m/z* 462. This, along with the base peak at *m/z* 231, suggested the possibility of the presence of a dimer. X-Ray diffraction analysis finally confirmed this assumption (*Figure*, *b*). As reported by *Bohlmann* and *Van* [8], the two halves of this dimer are related by symmetry, so that its NMR data could easily be misinterpreted as arising from a monomer. 2D-NMR Experiments, including HMBC, HMQC, and NOESY spectra, were consistent with the X-ray diffraction results.

Dimers are rare among the hundreds of eremophilane derivatives reported; to date, only compounds **7** and **8** have been reported [8]. Thereby, **8** had been isolated from the title plant. Indeed, we independently isolated dimer **8** and solved its structure spectroscopically and by X-ray diffraction (*Figure*, *c*).

From a biogenetic point of view, the dimers **7** and **8** may be generated inside the plant from **1** and **2** (*Scheme 2*). We hypothesize a mechanism in which the lactone ring is the head of a precursor molecule. When two monomers approach to form a transition state, from which elimination leads to a dimer, they can adopt two different relative orientations (*Scheme 2*). When the two olide heads are on the same side, we call this *parallel* access (*syn* arrangement), while, when the two heads are on opposite sides, the

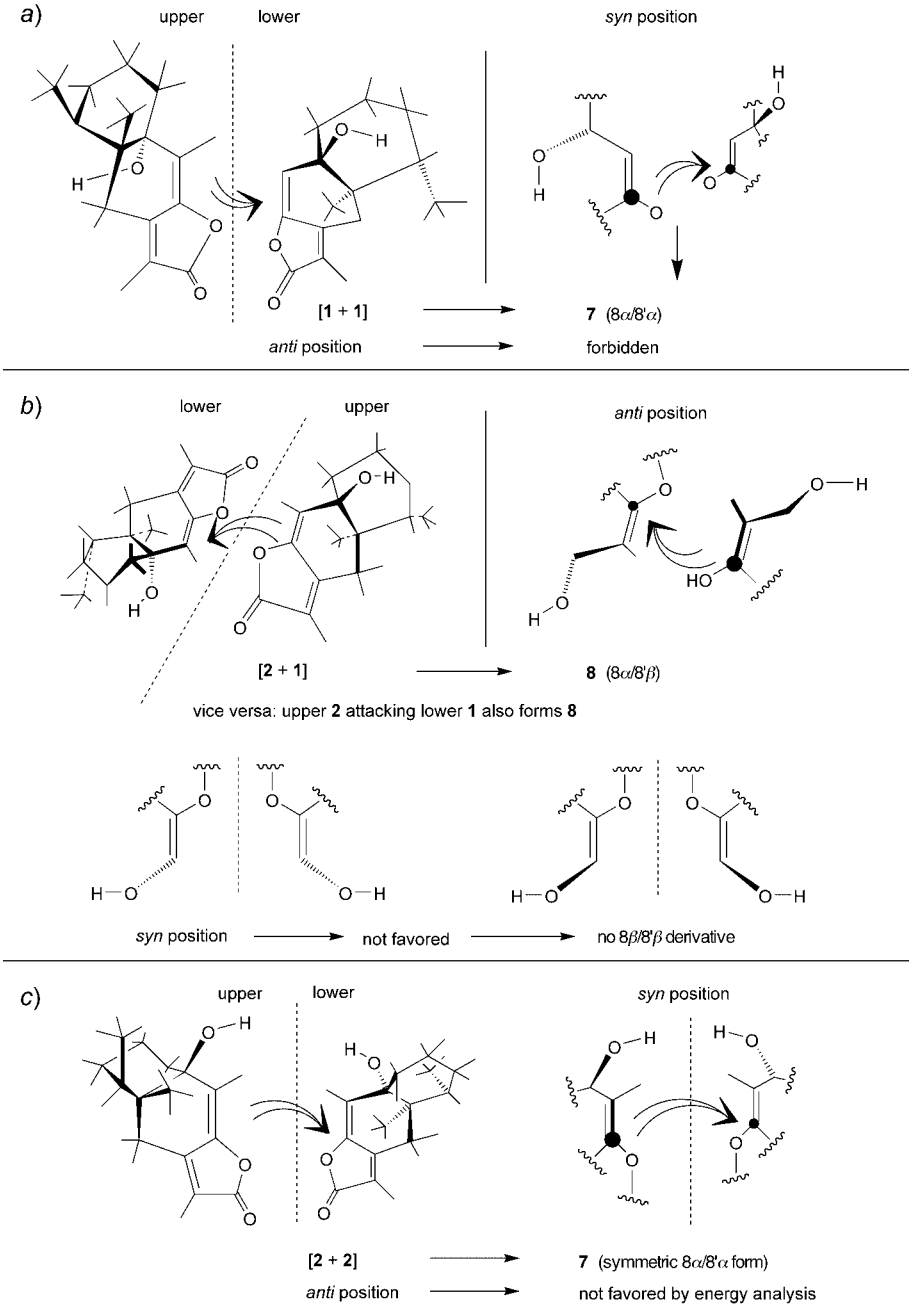
two molecules access each other in an *antiparallel* way (*anti* arrangement; *Scheme 2*). Elimination can occur only when the two OH leaving groups adopt a mutually *trans* configuration. Combining the requirements for both the *syn/anti* positions of the two monomers and the *cis/trans* configurations of the two 10-OH groups, the most-favorable situations for dimerization to **7** and **8** are shown in *Scheme 3*.

Scheme 2. *Proposed Mechanism for the Biogenesis of the Dimers* **7** *and* **8**, *and Possible* syn/anti *and* cis/trans *Arrangements of the Two Monomeric Units.* See text.



By making use of molecular models, it could be seen that the most-favorable transition state for reaction of two molecules of **1** is when they adopt a *syn* arrangement, so that the two OH groups are in a *trans* position, thus facilitating the elimination process to the 8α/8′α derivative **7** (*Scheme 3, a*). Similarly, *anti* arrangement of the two different monomers **1** and **2** will form the 8α/8′β type dimer **8** (*Scheme 3, b*), regardless of whether **1** or **2** is regarded as the 'upper' molecule. In these two situations,

Scheme 3. *Stereochemical Analysis of Dimer Formation*. See text.



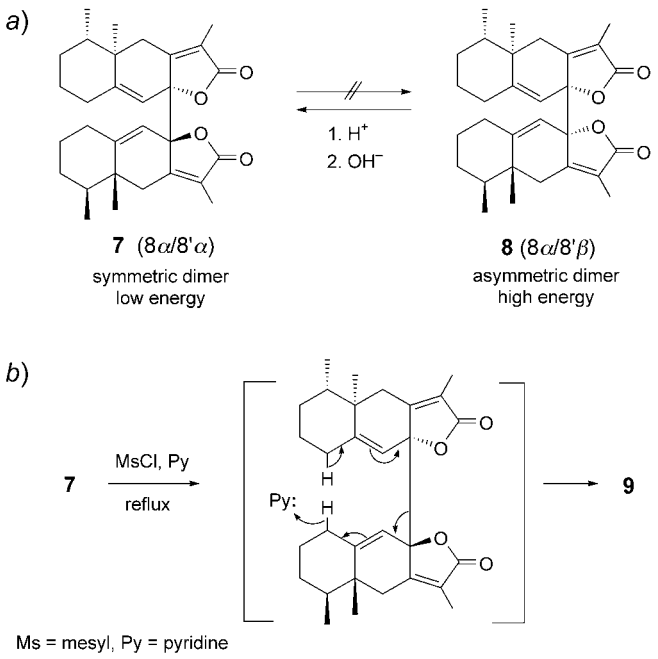
the steric hindrance is almost the same, and the two 10-OH groups can easily adopt *trans* positions for elimination. However, the *syn* position (both OH groups on the same side) will give rise to a much higher energy barrier. This might explain why the combination involving two different monomers produce only the 8α/8′β-dimer, but no 8β/8′β analog.

We anticipate that the dimerization involving two molecules of **2** will proceed *via* the *syn* arrangement, giving rise to the 8α/8′α product **7** (*Scheme 3*,*c*). Only in this way can the two molecules avoid the steric interactions between the four Me groups in 14- and 15-positions, and the *syn* arrangement allows the two α-OH groups to attain the *trans* position, facilitating elimination.

The asymmetric dimer **8** should be much more unstable than symmetric **7**. This is also evidenced in *Scheme 4*,*a*. Opening the lactone ring of **8** by acidic treatment followed by neutralization afforded the recycled lactone **7**. In contrast, the same procedure did not transform **7** to **8**.

The formation of **9** from **7** may be attributed to a base-catalyzed rearrangement (*Scheme 4*,*b*). The reaction is initiated by base attack on the allylic H–C(1), and the linkage of the dimer is then broken to form two monomeric molecules of **9**. Interestingly, treatment of **8** with MsCl/pyridine also afforded **9**. This reaction may also involve a similar, base-induced rearrangement, indicating that the different configurations do not influence the reaction.

Scheme 4



Tsoongianolide C (**3**) exhibited the same molecular-ion peak as **1** and **2** at *m/z* 248, and a rather similar ^{13}C-NMR spectrum to that of **2**. The quaternary C(8) resonance in **2** was replaced in the case of **3** by a ketal-type resonance at δ (C) 100.4. The downfield shifts of C(7) and C(9) of **3** also agreed with the presence of an 8-OH group (*Table 1* in the *Exper. Part*). The split H_α–C(6) and H_β–C(6) signals at δ (H) 2.38 (br. *d*, $J =$ 12.6 Hz) and 2.69 (*d*, $J = 13.0$ Hz) were consistent with a 10β-OH compound, reported earlier as a synthetic artifact [9]. The present work, thus, describes the first isolation of this lactone as a true natural product. The assignment of the ^{1}H- and ^{13}C-NMR spectra was based on HMBC and HMQC as well as NOESY 2D-NMR experiments. Finally, tsoongianolide C (**3**) was identified as (4*S*,5*R*,8*S*)-8-hydroxyeremophil-7(11),9-dien-8β,12-olide[1]).

Tsoongianolide D (**4**) exhibited its molecular ion peak at *m/z* 248, thus indicating another isomer of **1**, **2**, and **3**. The ^{1}H-NMR spectrum of **4** was very similar to that of **3**, the difference being in the split pattern of CH_2(6) (*Table 2*). In the case of **4**, the two *doublets* ($J = 14.6$ Hz) centered at 2.74 ppm disclosed the different configuration of the C(8) substituent, and ^{13}C-NMR data also provided evidence for a shift change at C(6). In addition, when compared with compound **3**, C(9) of **4** was downfield shifted by 8.6, and C(10) was upfield shifted by 15.5 ppm, respectively, but the ketal C-atom (C(8)) still resonated at δ (C) 103.0 (*Table 1*). All these data suggested that the configuration of the 8-OH group was α in the case of **4**, which was, thus, identified as the 8α isomer of **3**.

A molecular formula of $C_{15}H_{18}O_4$ for tsoongianolide E (**5**) was established by its EI-MS (*m/z* 262 (M^+)) and ^{13}C-NMR data (*Table 1*). Its IR spectrum exhibited absorptions for an α,β-unsaturated γ-lactone (1702 cm^{-1}) and a C=O group (1779 cm^{-1}). The ^{13}C-NMR spectrum of **5** showed signals for a Me-substituted, α,β-unsaturated lactone with an endocyclic C=C bond (δ (C)172.0, 147.5, 123.9, and 8.4), and a trisubstituted C=C bond (δ (C) 151.8 and 104.8), suggesting an eremophil-7(11),8-dien-8,12-olide structure. In addition, its ^{13}C-NMR spectrum revealed the presence of an OH group at quaternary C(10) (δ (C) 78.2). In the ^{1}H-NMR spectrum of **5**, one Me group at a tertiary (δ (H) 0.41) and two Me groups at secondary centers (δ (H) 1.67 and 0.72) were observed, while the only vinyl signal appeared at δ (H) 5.74. Based on the biogenetic consideration of eremophilane derivatives isolated from *Senecio* species, Me(14) and Me(15) were both assigned the β-orientation. The Me(14) resonance at relatively high field (δ (H) 0.41) indicated the presence of a *cis*-fused naphthalene ring [3][10][11]. The placement of the C=O group at C(3) was evident from the signal for H–C(4) (δ (H) 2.42 ($J = 6.8$ Hz)). Together, these data identified tsoongianolide E (**5**) as (4*R*,5*R*,10*S*)-10-hydroxy-3-oxo-eremophil-7(11),8-dien-8,12-olide[1]).

A molecular formula of $C_{15}H_{20}O_4$ for **6** was deduced from its EI-MS (*m/z* 266 (M^+)) and ^{13}C-NMR spectral data. On the basis of an IR absorption for an unsaturated lactone (1752 cm^{-1}), characteristic ^{1}H-NMR Me signals (δ (H) 1.80, 0.92, and 0.85), and a ^{13}C-NMR signal for a quaternary C-atom (δ (C) 98.3), an 8-hydroxy-eremophil-7(11)-en-8,12-olide skeleton was established, with an epoxy group at C(1)/C(10), as deduced from δ (C) 63.9 and 68.6 in the ^{13}C-NMR spectrum, and from two independent *AB* systems for H–C(6) (δ (H) 1.78, 2.44 (*d*, $J = 13.5$ Hz)) and H–C(9) (δ (H) 2.22, 2.42 (*d*, $J = 13.5$ Hz)) in the ^{1}H-NMR spectrum. The β-orientation of the epoxy ring was

deduced from H–C(1) (δ (H) 3.38 (d, $J = 8.0$ Hz)) [12][13] and by comparison with literature data [12]. The structure of tsoongianolide F (**6**) was, thus, determined as (1*R*,4*S*,5*R*,8*S*,10*S*)-1,10-epox-8-hydroxyeremophil-7(11)en-8α,12-olide[1]).

It is interesting that no alkaloids were isolated from *S. tsoongianus* although pyrrolizidine alkaloids are characteristic components of *Senecio* species [13–15]. The fact that no furo-eremophilanes were isolated also indicated the specificity of this plant from a chemotaxonomic point of view. Since the plant had been collected while in bloom, this might have led to high contents of highly oxidized metabolites such as eremophilanolides. Nevertheless, both eremophilane and eremophilanolides are characteristics of Senecioneae plants, especially those belonging to the genus *Senecio* [12][13][16][17]. Our investigation, therefore, provides chemotaxonomic evidence that *S. tsoongianus* may reasonably be regarded as belonging to the genus *Senecio*.

Determination of Absolute Structures. Although elucidation of chemical connectivities by single-crystal X-ray-diffraction analysis usually is not a problem, identification of a specific enantiomer may be ambiguous, particularly in the case of structures in which the heaviest atom is a second-period element. In a crystallographic structure determination, one absolute structure is refined competitively against the alternative. This procedure was devised by *Flack* [18], and the result is expressed as the *Flack* parameter ($x(u)$), which can be interpreted as a mole fraction of the alternative enantiomer in the sample used to collect diffraction data. The physical range of x lies between zero and unity, and, even if the bulk material is known to be enantiomerically pure, the standard uncertainty u (referred to as the estimated standard deviation in the older literature) should be in the region of 0.10 before any firm conclusions regarding its absolute structure can be drawn [19]. This has proved to be an extremely demanding criterion for light-atom structures.

In an attempt to improve the precision of the determinations of the X-ray crystal structures of compounds **1**, **7**, and **8**, diffraction data were collected at three different wavelengths (MoK_α, $\lambda = 0.71073$ Å; CuK_α, $\lambda = 1.54184$ Å; CrK_α, $\lambda = 2.2909$ Å) with the same crystal for each data collection. The data sets were combined and refined against a single model, using the facilities available for such refinements in SHELXL [20]. These procedures improved the standard uncertainty of x by 0.05 to 0.10, leading to values of the *Flack* parameter that unambiguously established the absolute structures of the compounds discussed here. These are, nevertheless, rather modest improvements given the experimental effort involved, and this is ascribable to the magnitude of the systematic errors present in the CrK_α data sets.

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Experimental Part

General. Melting points were measured on a *Kofler* hot-stage apparatus (uncorrected). Optical rotations were measured on a *JASCO P-1010* polarimeter. IR Spectra were recorded on a *Bruker Vector-22* instrument; in cm^{-1}. 1D- and 2D-NMR spectra were obtained on *Bruker AM-400* and *AMX-500* spectrometers, with Me_4Si as

internal standard; δ in ppm, *J* in Hz. EI and HR-EI Mass spectra (70 eV) were recorded on a *VG AutoSpec-3000* instrument; in *m/z* (rel. %)

Plant Material. Whole plants of *S. tsoongianus* were collected in Simao, Yunnan Province, P. R. China, in January 2000, and were identified by Prof. Dr. *Hua Peng*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 200001S6) has been deposited at the herbarium of the Kunming Institute of Botany.

Compound Purification. The air-dried, powdered whole plants (2.8 kg dry weight) of *Senecio tsoongianus* LING were extracted twice with 95% EtOH at r.t. The combined EtOH extracts were evaporated, and the dark green gummy residue (*ca.* 210 g) was taken up in H_2O and subsequently extracted with petroleum ether (b.p. 60–90°), AcOEt, and BuOH, affording 52, 63, and 44 g of crude extracts, resp.

The petroleum ether extract (40 g) was purified by column chromatography (CC) (600 g SiO_2, 200–300 mesh; gradient of petroleum ether/acetone 1:0–0:1): 25 main fractions (*STP1 – STP25*). *STP4* (1.3 g) was re-subjected to CC (60 g SiO_2; 200–300 mesh; petroleum ether/AcOEt 10:1 → 3:1). From this column, fractions *Fr. 15–18* and *26–29*, resp., afforded *tritriacontane* (68 mg) and *dotriacontene* (129 mg). *STP7* (0.8 g) was subjected to CC (40 g SiO_2, 200–300 mesh; petroleum ether/AcOEt 10:1 → 3:1). From this column, *Fr. 14–17*, and *Fr. 18* and *19*, afforded β-sitosterol (65 mg) and stigmasterol (42 mg), resp. *STP9* (1.2 g) was re-chromatographed (100 g SiO_2, 200–300 mesh; petroleum ether/AcOEt 10:1 → 2:1). From this column, *Fr. 9–11* were combined and subjected to prep. TLC (SiO_2; CH_2Cl_2/AcOEt 5:1) to afford **8** (20 mg). From the same column, *Fr. 13–16* were combined and, after recrystallization from AcOEt, afforded **7** (43 mg). *STP13* (1.6 g) was further chromatographed (120 g SiO_2, 200–300 mesh; petroleum ether/AcOEt 10:1 → 3:1). From this column, *Fr. 26–29* were combined and subjected to CC (100 g *Sephadex LH 20*; acetone), affording **2** (26 mg). From the same SiO_2 column, *Fr. 31–36* were re-chromatographed (30 g *Silica H*; CH_2Cl_2/AcOEt 20:1 → 10:1) to afford **1** (32 mg).

The original, crude AcOEt extract (30 g) was subjected to CC (400 g SiO_2, 200–300 mesh; CH_2Cl_2/AcOEt 100:1 → 1:1): ten crude fractions (*STE1– STE10*). *STE2* (1.8 g) was subjected to CC (150 g SiO_2; 200–300 mesh; CH_2Cl_2/acetone 10:1 → 3:1). The resulting *Fr. 10–16* were combined and subjected to reverse-phase CC (25 g *C18* SiO_2; MeOH/H_2O 85:15) to afford **3** (15 mg) and **4** (16 mg). *STE3* (0.8 g) was re-chromatographed (50 g SiO_2; 200–300 mesh; benzene/acetone 20:1 → 4:1). The resulting *Fr. 18–22* were combined, and a sample was purified to afford **6** (24 mg). *STE5* (2.1 g) was also chromatographed (80 g SiO_2; 200–300 mesh; benzene/MeOH 20:1 → 5:1). The resulting *Fr. 12–16* afforded, after recrystallization from acetone, compound **5** (19 mg).

Tsoongianolide A (=*(4a*R,*5*S,*8a*R*)-4a,5,6,7,8,8a-Hexahydro-8a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **1**). Colorless needles. M.p. 168° (dec.) ($CHCl_3$). R_f 0.76 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = +16$ ($c = 0.76$, $CHCl_3$). IR (KBr): 3534, 2936, 2857, 1767, 1664, 1464, 1440, 1378, 1322, 1002, 922, 876, 758. EI-MS: 248 (74, M^+), 231 (52), 230 (50), 220 (36), 215 (60), 202 (21), 192 (16), 187 (26), 177 (52), 175 (46), 163 (44), 149 (51), 139 (46), 121 (46), 109 (61), 91 (52), 82 (68), 69 (100). ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. HR-EI-MS: 248.1413 (M^+, $C_{15}H_{20}O_3^+$; calc. 248.1412).

X-Ray Crystal Data for **1**. See *Figure* (*a*). Crystal of $0.26 \times 0.24 \times 0.10$ mm size; $C_{15}H_{20}O_3$; $M_r = 248.31$; orthorhombic, space group $P\,2_12_12_1$; $a = 6.3660(5)$, $b = 12.4853(9)$, $c = 16.0971(11)$ Å; $V = 1279.42(16)$ Å^3; $Z = 4$; $D_{calc} = 1.289$ Mg/m^3; $F(000) = 536$; $\mu = 0.09$ (MoK_α), 0.71 (CuK_α), and 2.18 mm^{-1} (CrK_α); $R_1 = 0.0404$ (based on *F* and 5633 data points with $F > 4\sigma(F)$), $wR_2 = 0.1194$ (based on F^2 and all 5823 data points), $S = 1.704$ for 166 parameters; $\Delta F_{max} = 0.23$, $\Delta F_{min} = -0.18$ e/Å^3; *Flack* parameter refined to 0.07(11).

Tsoongianolide B (=*(4a*R,*5*S,*8a*S*)-4a,5,6,7,8,8a-Hexahydro-8a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **2**). Colorless needles. M.p. 107–108° ($CHCl_3$). R_f 0.2 (hexane/AcOEt 3:1). $[\alpha]_D^{25} = +110$ ($c = 0.87$, $CHCl_3$). IR (KBr): 3427, 2964, 2935, 2879, 1768, 1650, 1461, 1385, 1323, 1102, 982, 948, 757. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 248 (68, M^+), 230 (95), 220 (11), 219 (30), 215 (44), 205 (36), 192 (12), 187 (24), 178 (94), 177 (86), 167 (29), 163 (54), 160 (61), 150 (54), 125 (100), 109 (41), 107 (34), 95 (27), 91 (39), 77 (37), 69 (39), 55 (83). HR-EI-MS: 248.1412 (M^+, $C_{15}H_{20}O_3^+$; calc. 248.1412).

Tsoongianolide C (=*(4a*R,*5*S,*9a*S*)-4a,5,6,7,8,9a-Hexahydro-9a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **3**). Colorless gum. R_f 0.35 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = -26$ ($c = 0.69$, $CHCl_3$). IR (KBr): 3303, 2928, 2858, 1734, 1694, 1649, 1443, 1338, 1284, 1137, 1105, 939, 748, 577, 501. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 248 (4, M^+), 230 (8), 220 (68), 2 (14), 203 (100), 189 (10), 187 (9), 175 (16), 161 (14), 147 (18), 135 (15), 123 (26), 119 (26), 105 (25), 91 (39), 77 (28). HR-EI-MS: 248.1410 (M^+, $C_{15}H_{20}O_3^+$; calc. 248.1412).

Tsoongianolide D (=*(4a*R,*5*S,*9a*R*)-4a,5,6,7,8,9a-Hexahydro-9a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **4**). Colorless gum. R_f 0.31 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = -256$ ($c = 0.02$, $CHCl_3$). IR (KBr): 3319, 2921, 2850, 1747, 1698, 1462, 1377, 1320, 1215, 1134, 1000, 974, 750, 720. ^{1}H- and ^{13}C-NMR: see

Table 1. *^{13}C-NMR Spectral Data for Compounds* **1**–**9**. Recorded in $CDCl_3$ unless otherwise stated; δ(C) in ppm.

C-Atom	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a])	**8**[b])	**9**
C(1)	34.1	29.8	36.5	37.2	33.6	63.1	32.8	33.7	33.6	131.2
C(2)	30.1	29.4	30.7	27.2	30.6	29.0	26.9	29.5	27.6	26.1
C(3)	21.3	22.6	26.6	25.8	209.8	22.9	30.7	31.7	31.6	26.5
C(4)	34.7	34.0	43.7	40.8	33.6	39.4	43.3	40.1	44.1	38.8
C(5)	43.6	43.5	45.7	41.2	46.2	42.8	45.8	46.1	46.0	37.6
C(6)	31.9	36.4	32.3	45.2	30.9	29.9	38.1	33.4	38.2	34.8
C(7)	149.3	150.8	158.7	158.7	147.5	157.5	160.9	160.9	160.9	147.3
C(8)	151.3	147.4	100.4	103.0	151.8	98.3	86.7	86.5	86.5	139.6
C(9)	113.0	111.2	118.1	126.8	104.8	31.9	117.0	115.1	115.6	109.5
C(10)	72.9	74.0	152.1	136.6	78.2	68.6	152.1	156.6	154.9	139.1
C(11)	122.9	122.1	122.2	123.1	123.9	123.4	122.8	124.3	124.0	120.2
C(12)	172.1	171.6	172.0	172.2	172.0	172.0	172.7	174.1	174.1	171.2
C(13)	8.4	8.3	8.0	8.1	8.4	8.3	8.4	8.4	8.4	8.4
C(14)	15.5	14.3	15.4	15.8	13.5	14.9	18.1	20.5	18.7	15.6
C(15)	15.7	16.0	17.5	17.8	14.7	15.9	15.4	16.8	15.7	19.5

[a]) Resonances of the 8β half; recorded in (D_6)acetone. [b]) Resonances of the 8α half; recorded in (D_6)acetone. Assignments for the two halves are interchangeable.

Tables 2 and *1*, resp. EI-MS: 248 (12, M^+), 230 (13), 220 (10), 215 (17), 203 (16), 187 (8), 175 (18), 159 (10), 147 (8), 133 (10), 123 (100), 107 (39), 91 (40), 79 (28), 53 (37). HR-EI-MS: 248.1418 (M^+, $C_{15}H_{20}O_3^+$; calc. 248.1412).

Tsoongianolide E (=*(4a*R,*5*R,*8a*S*)-4a,7,8,8a-Tetrahydro-8a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2,6(4*H,*5*H*)-dione*; **5**). Colorless needles. M.p. 148–149° ($CHCl_3$). R_f 0.45 (hexane/AcOEt 2:1). $[\alpha]_D^{25} = -81$ ($c = 0.20$). IR (KBr): 3428, 2922, 1779, 1702, 1665, 1465, 1420, 1365, 1321, 1001, 958. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 262 (15, M^+), 245 (4), 234 (65), 191 (35), 177 (100), 163 (28), 150 (36), 135 (15), 127 (41), 111 (22), 91 (22), 77 (20), 55 (53). HR-EI-MS: 262.1203 (M^+, $C_{15}H_{18}O_4^+$; calc. 262.1205).

Tsoongianolide F (=*(1a*R,*4*S,*4a*R,*8a*S,*9a*S*)-1a,2,3,4,4a,5,8a,9-Octahydro-8a-hydroxy-4,4a,6-trimethyl-7*H*-oxireno[8,8a]naphtho[2,3-*b*]furan-7-one*; **6**). Colorless gum. R_f 0.29 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = +19$ ($c = 0.26$, $CHCl_3$). IR (KBr): 3458, 2928, 1752, 1655, 1325, 1101, 948. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 264 (38, M^+), 247 (4), 231 (5), 219 (15), 203 (4), 191 (8), 175 (16), 159 (15), 147 (11), 139 (32), 126 (42), 111 (25), 81 (100), 55 (62). HR-EI-MS: 264.1367 (M^+, $C_{15}H_{20}O_4^+$; calc. 264.1361).

*(4a*R,*4a′*R,*5*S,*5′*S,*9a*R,*9a′*R*)-4,4′,4a,4′a,5,5′,6,6′,7,7′,8,8′-Dodecahydro-3,3′,4a,4′a,5,5′-hexamethyl-2*H,*2′*H-*9a,9′a-binaphtho[2,3-*b*]furan-2,2′-dione* (**7**). Colorless needles. M.p. 167–168° ($CHCl_3$). R_f 0.41 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = +141$ ($c = 0.81$, $CHCl_3$). IR (KBr): 2925, 2856, 2360, 1751, 1685, 1462, 1379, 1094, 1003. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 462 (1, M^+), 447 (0.2), 418 (0.3), 391 (0.2), 374 (0.4), 328 (0.4), 303 (0.2), 281 (0.3), 261 (9), 247 (0.4), 231 (100), 215 (12), 203 (9), 202 (4), 189 (9), 175 (36), 161 (41), 149 (36), 91 (15). HR-EI-MS: 462.2772 (M^+, $C_{30}H_{38}O_4^+$; calc. 462.2770).

X-Ray Crystal Data for **7**. See *Figure* (*b*). Crystal of $0.50 \times 0.50 \times 0.44$ mm size; $C_{30}H_{38}O_4$; $M_r = 462.60$; orthorhombic, space group $P\,2_12_12_1$; $a = 12.9679(9)$, $b = 13.6192(9)$, $c = 15.1702(10)$ Å; $V = 2679.2(3)$ Å^3; $Z = 4$; $D_{calc} = 1.147$ Mg/m^3; $F(000) = 1000$; $\mu = 0.07$ (MoK_α), 0.59 (CuK_α), and 1.80 mm^{-1} (CrK_α); $R_1 = 0.0659$ (based on F and 5633 data points with $F > 4\sigma(F)$), $wR_2 = 0.1780$ (based on F^2 and all 13230 data points), $S = 1.827$ for 309 parameters; $\Delta F_{max} = 0.24$, $\Delta F_{min} = -0.32$ e/Å^3; *Flack* parameter refined to 0.04(12).

*(4a*R,*5*S,*9a*S*)-4a,5,6,7,8,9a-Hexahydro-3,4a,5-trimethyl-9a-[(4a*R,*5*S,*9a*R*)-4,4a,5,6,7,8-hexahydro-3,4a,5-trimethyl-2-oxonaphtho[2,3-*b*]furan-9a(2*H*)-yl]naphtho[2,3-*b*]furan-2(4*H*)-one* (**8**). Colorless needles. M.p. 185–186° ($CHCl_3$). R_f 0.51 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = +90$ ($c = 0.64$, $CHCl_3$). IR (KBr): 2926, 1764, 1749, 1685, 1647, 1442, 1338, 1183, 1099, 1008, 745. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 462 (3, M^+), 420 (2), 392 (4), 378 (1), 364 (6), 336 (2), 247 (1), 231 (100), 217 (5), 215 (5), 203 (6), 189 (8), 175 (37), 161 (42), 149 (37), 131 (13), 119 (18), 105 (19), 91 (26), 69 (34). HR-EI-MS: 462.2774 (M^+, $C_{30}H_{38}O_4^+$; calc. 462.2770).

X-Ray Crystal Data for **8**. See *Figure* (*c*). Crystal of $0.51 \times 0.30 \times 0.25$ mm size; $C_{30}H_{38}O_4$; $M_r = 462.56$; orthorhombic, space group $P\,2_12_12_1$; $a = 10.7301(12)$, $b = 11.2278(13)$, $c = 21.169(2)$ Å; $V = 2550.3(5)$ Å^3; $Z = 4$;

Table 2. *^{1}H-NMR Spectral Data for Compounds* **1**–**9**. Recorded in $CDCl_3$ unless stated otherwise; δ(H) in ppm, J in Hz. The assignments for the two halves of dimer **8** are interchangeable.

H-Atom	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8** (8α half)[a])	**8** (8β half)[a])	**9**
H_a–C(1)	1.92 (*m*)	1.82 (*ddd*, *J* = 13.0, 13.0, 4.2)	2.12 (*m*)	2.52 (*dd*, *J* = 14.5, 2.6)	1.83 (*m*)	3.38 (*d*, *J* = 8.0)	2.17 (*m*)	2.19 (*ddd*, *J* = 13.6, 13.6, 4.2)	2.10 (*ddd*, *J* = 13.4, 13.4, 3.3)	5.90 (*s*)
H_b–C(1)	1.58 (*m*)	1.62 (*m*)	2.12 (*m*)	2.15 (*m*)	1.38 (*m*)	[b])	2.15 (*m*)	1.93 (*m*)	1.89 (*m*)	–
H_a–C(2)	1.36 (*m*)	1.28 (*m*)	1.35 (*m*)	1.45 (*m*)	1.95 (*m*)	[b])	1.82 (*m*)	1.77 (*m*)	1.76 (*m*)	2.20 (*m*)
H_b–C(2)	1.42 (*m*)	1.35 (*m*)	1.45 (*m*)	2.13 (*m*)	2.94 (*m*)	[b])	1.45 (*m*)	1.29 (*m*)	1.10 (*m*)	2.19 (*m*)
H_a–C(3)	1.49 (*m*)	1.58 (*m*)	1.38 (*m*)	1.34 (*m*)	–	[b])	1.34 (*m*)	1.31 (*m*)	1.26 (*m*)	1.56 (*m*)
H_b–C(3)	1.82 (*m*)	1.62 (*m*)	1.47 (*m*)	1.42 (*m*)	–	[b])	1.55 (*m*)	1.54 (*m*)	1.22 (*m*)	1.55 (*m*)
H_α–C(4)	2.23 (*m*)	1.76 (*m*)	1.55 (*m*)	1.67 (*m*)	2.47 (*q*, *J* = 6.8)	[b])	1.71 (*m*)	1.78 (*m*)	1.20 (*m*)	1.69 (*m*)
H_α–C(6)	2.64 (br. *d*, *J* = 13.0)	2.59 (br. *d*, *J* = 13.0)	2.38 (br. *d*, *J* = 12.6)	2.75 (*d*, *J* = 14.6)	2.54 (*d*, *J* = 13.5)	2.44 (*d*, *J* = 13.5)	2.35 (br. *d*, *J* = 13.0)	2.74 (br. *s*, *J* = 11.4)	2.65 (*d*, *J* = 12.9)	2.81 (*d*, *J* = 16.4)
H_β–C(6)	2.59 (*d*, *J* = 13.0)	2.57 (br. *d*, *J* = 13.0)	2.69 (*d*, *J* = 13.0)	2.73 (*d*, *J* = 14.6)	2.51 (*d*, *J* = 13.5)	1.78 (*d*, *J* = 13.5)	2.67 (*d*, *J* = 13.0)	2.91 (*d*, *J* = 13.9)	2.87 (br. *d*, *J* = 13.1)	2.20 (*m*)
H_a–C(9)	5.48 (br. *s*)	5.42 (br. *s*)	5.66 (br. *s*)	5.58 (*t*, *J* = 2.5)	5.74 (*s*)	2.42 (*d*, *J* = 13.5)	5.60 (br. *s*)	5.07 (br. *s*)	5.5 (*d*, *J* = 1.4)	5.77 (*t*, *J* = 4.0)
H_b–C(9)	–	–	–	–	–	2.22 (*d*, *J* = 13.5)	–	–	–	–
Me(13)	1.85 (*d*, *J* = 1.4)	1.84 (br. *s*)	1.76 (*d*, *J* = 1.4)	1.81 (*d*, *J* = 1.4)	1.67 (*s*)	1.80 (br. *s*)	1.79 (br. *s*)	1.83 (*d*, *J* = 1.3)	1.82 (*d*, *J* = 1.4)	1.88 (*d*, *J* = 1.5)
Me(14)	0.83 (*s*)	1.02 (*s*)	0.85 (*s*)	0.80 (*s*)	0.41 (*s*)	0.85 (*s*)	0.86 (*s*)	0.84 (*s*)	0.84 (*s*)	0.94 (*s*)
Me(15)	0.85 (*d*, *J* = 6.6)	0.76 (*d*, *J* = 6.8)	0.90 (*d*, *J* = 6.8)	0.97 (*d*, *J* = 6.8)	0.72 (*d*, *J* = 6.8)	0.92 (*d*, *J* = 6.0)	0.93 (*d*, *J* = 6.8)	0.92 (*d*, *J* = 7.4)	0.90 (*d*, *J* = 6.9)	0.97 (*d*, *J* = 6.8)
OH	3.76 (br.)	n.d.[c])	n.d.	3.38 (br.)	n.d.	n.d.	n.d.	n.d.	n.d.	–

[a]) Assignments for the two halves are interchangeable. [b]) The signals for H–C(1) to H–C(4) were not resolved (1.36–2.12 (*m*)). [c]) Not detected.

$D_{calc} = 1.189$ Mg/m^3; $F(000) = 976$; $\mu = 0.08$ (MoK_α), 0.72 (CuK_α), and 2.16 mm^{-1} (CrK_α); $R_1 = 0.0474$ (based on F and 21012 data points with $F > 4\sigma(F)$), $wR_2 = 0.2144$ (based on F^2 and all 22587 data points), $S = 1.031$ for 311 parameters; $\Delta F_{max} = 0.31$, $\Delta F_{min} = -0.21$ e/Å^3; *Flack* parameter refined to $-0.04(11)$

Mesylation Reactions. To a stirred soln. of **1** (8 mg) in anh. pyridine (1 ml), methanesulfonyl chloride (MsCl; 100 µl) was added at 0° under N_2 atmosphere. The mixture was stirred for 2 h at r.t., and then heated at 120° for 5 min. The pyridine was evaporated *in vacuo*, and the residue was partitioned between Et_2O and H_2O. The combined org. layers were subjected to prep. TLC (hexane/AcOEt 1:1) to afford **9** (4 mg). The product's physical and NMR spectral data were identical with those of an authentic sample of **9** [7]. The mesylation of **2** (8 mg) was carried out as for **1**, yielding 3 mg of **9**. A similar procedure was used for the mesylations of **7** (12 mg) or **8** (17 mg), but 2 ml of pyridine and 200 µl of MsCl were required, and heating at 120° was extended from 5 to 90 min. The yields of **9** were 6 and 8 mg from **7** and **8**, resp.

*(4a*R,5S*)-4a,5,6,7-Tetrahydro-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one* (**9**). Colorless gum. R_f 0.72 (hexane/AcOEt 3:1). $[\alpha]_D^{25} = -144$ ($c = 2.2$, $CHCl_3$). HR-EI-MS: 230.1308 (M^+, $C_{15}H_{18}O_2^+$; 230.1307).

Crystallography. X-Ray diffraction patterns were collected on a *Bruker SMART APEX CCD* diffractometer, with an MoK_α source ($\lambda = 0.71073$ Å), and on *Stoe Stadi-4* four-circle diffractometers, with CuK_α and CrK_α sources (see X-ray section in *Results and Discussion*). Each instrument was equipped with an *Oxford Cryosystems* low-temperature device, operating at 150 K. For a given determination, data sets were recorded immediately one after another, using the same sample and mount. The unit cells quoted in the supplementary data were taken from the MoK_α data collections.

Diffraction data collected by means of MoK_α radiation on the *SMART CCD* system were corrected with the multiscan method (SADABS) [20]; four-circle data-sets were corrected with the ψ-scan data (XPREP) [20]. The structures were solved by direct methods (SHELXS) [20], using the MoK_α data sets, and refined by full-matrix least-squares methods against F^2, using all data. H-atoms were included in calculated positions, and anisotropic displacement parameters were refined for all non-H-atoms (SHELXL) [20]. For the final, absolute structure refinement, the three data sets were combined into a single file conforming to the SHELX format *HKLF 2* (details are given in the program manual). Values of the dispersion correction terms f' and f'' were taken from *International Tables* [21].

The crystallographic data of **1**, **7**, and **8** have been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*), 12 Union Road, Cambridge CB2 1EZ, UK. The data can be obtained, free of charge, upon application to the CCDC *via* fax (+44-1223 336033), e-mail (deposit@ccdc.cam.ac.uk), or the internet (http://www.ccdc.cam.ac.uk/conts/retrieving.html), by referring to publications CCDC-248680, CCDC-248681, and CCDC-248682 for **1**, **7**, and **8**, resp.

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