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Enantiomeric Sesquiterpene Lactones from *Senecio tsoongianus*

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Abstract: From *Senecio tsoongianus*, two pairs of enantiomeric isomers, tsoongianolides A (**1**) and B (**2**), tsoongianolides C (**3**) and D (**4**) were isolated. Their structures were elucidated by 1D and 2D-NMR techniques and X-ray diffractions. The cytotoxicity to KB cell of **1** and **2** is also reported.

Keywords: Sesquiterpenes, eremophilanes, X-ray, cytotoxicity.

Tribe Senecioneae is known as plentiful of pyrrolizidines and eremophilanes[1,2]. The crude extract of *Senecio tsoongianus* Ling is found to possess a cytotoxicity to KB cell with an inhibition ratio of 100% in 100 μmol/L[3]. Twelve components including four eremophilanolides, tsoongianolides A (**1**), B (**2**), C (**3**) and D (**4**) were isolated from this species.

The MS of tsoongianolide A (**1**) exhibited its molecular ion peak at *m/z* 248, and a significant fragment due to loss of a H_2O molecule appeared at *m/z* 230, thus suggesting the presence of a hydroxyl group in the molecule. The quaternary carbon at δ 72.9 in the ^{13}C-NMR spectrum revealed the presence of the quaternary hydroxyl group. Furthermore, the ^{13}C-NMR spectrum of **1** also showed one lactonic carbon at δ 172.1, a full-substituted double bond at δ 149.3 and δ 122.9. The olefinic proton appeared at δ 5.48 as a singlet disclosing the neighboring carbon of the olefinic methane is a quaternary one. Considering that three typical methyl signals appeared at 0.83 (s), 0.85 (d, J=6.6 Hz), and 1.85 ppm (d, J=1.4 Hz), the skeleton of compound **1** should be an eremophilanolide with a 7,11-en-8,12-olide[4-7]. Furthermore, the quaternary hydroxyl

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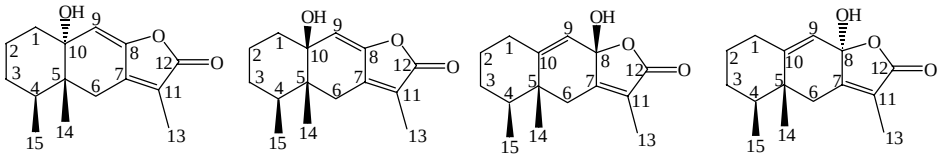
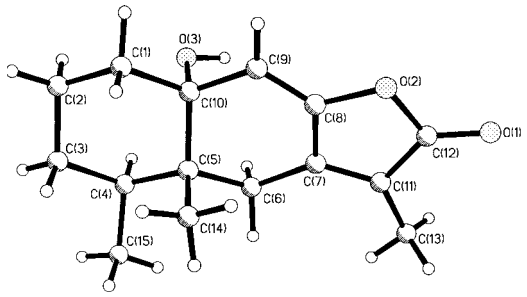


Tsoongianolide A (**1**) Tsoongianolide B (**2**) Tsoongianolide C (**3**) Tsoongianolide D (**4**)

group should be located at C-10, thus allowing the olefinic H-9 appeared as a singlet as mentioned above. The ^{13}C-NMR spectrum of **1** also showed four methene resonances[10], along with one methine carbon at δ 43.6, which was attributed to C-4 of **1**. In addition, the diagnostic AB doublets at δ 2.59 (d, J=13.0 Hz) and δ 2.64 (br d, J=13.0 Hz) could be assigned to C-6 methene signal. The broadened doublet at δ 2.64 was arose from the homoallylic coupling with Me-13[4]. X-ray diffraction analysis of the compound finally disclosed that the 10-hydroxyl group of **1** adopted an α-configuration, therefore the whole stereochemistry of tsoongianolide A is (10*R, 4*S, 5*S)-10α-hydroxy-7(11),8-dien- eremophila-8,12-olide.

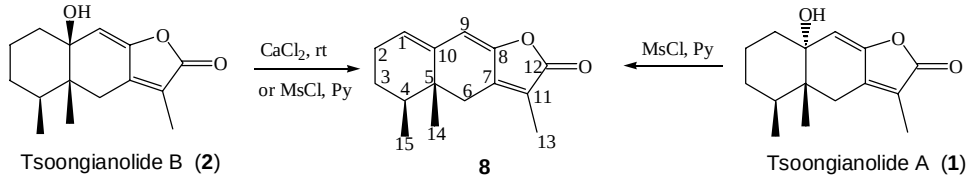


X-ray structure of tsoongianolide A (**1**)

Tsoongianolide B (**2**) showed the same molecular weight (*m/z* 248) and the similar fragments to those of **1**. The ^{13}C-NMR spectrum of **2** gave fifteen resonances which consisted of $CH_3\times3$, $CH_2\times4$, $CH\times2$, and $C\times6$, exactly consistent with that of **1**. This suggested that compound **2** is reasonably an enantiomer of **1**. Scrutiny of the ^{1}H and ^{13}C-NMR spectra of these two lactones evidenced this assumption[10]. Slight differences between these two enantiomers appeared at their C-6 methenes. The H-6 signal in the ^{1}H-NMR of **2** showed no longer a separated pair of AB doublets, but a very closed triplet-like signal centered at 2.58 ppm. Furthermore, the chemical shift of Me-14 in molecule **2** was down-field shifted to 1.02 ppm from that of 0.83 ppm in the case of **1**. However, the H-4 of **2** was high-field shifted to 1.76 ppm from that of 2.23 ppm in the case of **1**, thus indicated that the 10α-OH was changed to a 10β configuration in the case of **2**, and therefore deshielded the Me-14 signal of lactone **2**. Meanwhile, the H-4 of **2** lost the deshielding 10α-OH moiety, which existed in the case of **1**, and therefore appeared at a relative high field. Unexpectedly, the natural product **2** is not quite stable and easily to be changed to its dehydration metabolite **8** during storage under $CaCl_2$ as a drying agent. Mesylation of **2** by mesyl chloride and pyridine afforded a derivative, whose melting point, R_f value, NMR properties and $[\alpha]_D$ value were all

identical to the authentic sample **8**. Furthermore, to identify the absolute stereochemistry of 14-Me and 15-Me of **2**, a mesylation of tsoongianolide A (**1**) was performed and the sole product showed its identity with authentic sample of **8**. This suggested that the stereochemistries of 14-Me and 15-Me of **2** are the same with those of **1**. Therefore the absolute streochemistry of **2** should be (10*S, 4*S, 5*S)-10β-hydroxy-7(11),8-dien- eremophila-8,12-olide.

The IC_{50} of **1** and **2** on KB cell exhibited as 2.5×10^{-4} mol/L, 3.2×10^{-5} mol/L, respectively.



Tsoongianolide B (**2**) **8** Tsoongianolide A (**1**)

Tsoongianolide C (**3**) exhibited the same molecular ion peak with those of **1** and **2** at *m/z* 248, it also showed a quite similar ^{13}C-NMR spectrum with that of **2**. However, the quaternary carbon resonance of C-8 in compound **2** was invisible and was replaced in the case of **3** by a ketal quaternary carbon signal resonanced at δ 100.4 (C-8). The down-field shifted C-7 and C-9 of **3** also agreed with the presence of an 8-hydroxy group[10]. Therefore, the existence of an 8-OH-7(11),9-dien-8,12-olide moiety in the molecule of **3** is clear. As for the stereochemistry, the spliting H-6α and H-6β appeared at δ 2.38 (brd, J=12.6 Hz) and δ 2.69 (d, J=13.0 Hz) were exactly identical with a 10β-hydroxyl eremophilane lactone, which was a synthetic artifact by Aclinou *et al*[8]. This lactone is first isolated as a natural product herewith. The assignments of the carbons and hydrogens are based on the 2D-NMR spectroscopy including HMBC, HMQC and NOESY.

Tsoongianolide D (**4**) exhibited its molecular ion peak at *m/z* 248, thus indicated that it is isomeric with lactones **1**, **2** and **3**. The ^{1}H-NMR spectrum of **4** is very similar to that of **3**, the difference between **3** and **4** demonstrated by their split patterns of H-6 methenes. In the situation of **4**, two doublets centered at 2.74 (J=14.6 Hz) suggested a different configuration of the substituent on C-8 when comparing with that of **3**. ^{13}C-NMR spectral data of **4** also evidenced the change of C-6 moiety[10]. Additionally, when comparing with those of compound **3**, the C-9 of **4** was down-field shifted for 8.6 ppm and C-10 of **4** was high-field shifted for 15.5 ppm, respectively. However, the ketal carbon of **4** still existed at δ 103.0. All information above suggested that the configuration of the 8β-hydroxy in the case of **3** was converted to an α moiety in lactone **4**. Therefore **4** was assigned as 8α-OH-7(11),9-dien-eremophila-8β,12-olide. The different optical rotation value of **4** with that of **3** also agreed with the deduction.

Acknowledgments

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10. ^{13}C-NMR spectral data of compounds **1**-**4**. (**1**): C-1 – C-15: 34.1, 30.1, 21.3, 34.7, 43.6, 31.9, 149.3, 151.3, 113.0, 72.9, 122.9, 172.1, 8.4, 15.5, 15.7; (**2**): C-1 – C-15: 29.8, 29.4, 22.6, 34.0, 43.5, 36.4, 150.8, 147.4, 111.2, 74.0, 122.1, 171.6, 8.3, 14.3, 16.0, (**3**): C-1 – C-15: 36.5, 30.7, 26.6, 43.7, 45.7, 32.3, 158.7, 100.4, 118.1, 152.1, 122.2, 172.0, 8.0, 15.4, 17.5, (**4**): C-1 – C-15: 37.2, 27.2, 25.8, 40.8, 41.2, 45.2, 158.7, 103.0, 126.8, 136.6, 123.1, 172.2, 8.1, 15.8, 17.8,
    ^{1}H-NMR spectral data of compounds **1**-**4**. (**1**): 1.92 m, H-1; 1.58 m, H-1′; 1.36 m, H-2; 1.42 m, H-2′; 1.49 m, H-3; 1.82 m, H-3′; 2.23 m, H-4 ; 2.64 br d (13.0), H-6 ; 2.59 d (13.0), H-6 ; 5.48 br s, H-9; 1.85 d (1.4), H-13; 0.83 s, H-14; 0.85 d (6.6), H-15; 3.76 br, OH. (**2**):1.82 ddd (13.0, 13.0, 4.2), H-1; 1.62 m, H-1′; 1.28 m, H-2; 1.35 m, H-2′; 1.58 m, H-3; 1.62 m, H-3′; 1.76 m, H-4 ; 2.59 br d (13.0), H-6 ; 2.57 br d (13.0), H-6 ; 5.42 br s, H-9; 1.84 br s, H-13; 1.02 s, H-14; 0.76 d (6.8), H-15; (**3**): 2.12 m, H-1 & H-1'; 1.35 m, H-2; 1.45 m, H-2′; 1.38 m, H-3; 1.47 m, H-3′; 1.55 m, H-4 ; 2.38 br d (12.6), H-6 ; 2.69 d (13.0), H-6 ; 5.66 br s, H-9; 1.76 d (1.4), H-13; 0.85 s, H-14; 0.90 d (6.8), H-15; (**4**): 2.52 dd (14.5, 2.6), H-1; 2.15 m, H-1′; 1.45 m, H-2; 2.13 m, H-2′; 1.34 m, H-3; 1.42 m, H-3′; 1.67 m, H-4 ; 2.75 d (14.6), H-6 ; 2.73 d (14.6), H-6 ; 5.58 t (2.5), H-9; 1.81 d (1.4), H-13; 0.80 s, H-14; 0.97 d (6.8), H-15; 3.38 br, OH.

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