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Castanolide and *epi*-castanolide, two novel diterpenoids with a unique seco-norabietane skeleton from *Salvia castanea* Diels f. *pubescens* Stib.

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ABSTRACT

Castanolide (**1**) and *epi*-castanolide (**2**), two novel diterpenoids possessing a unique seco-norabietane skeleton, were isolated from *Salvia castanea* Diels f. *pubescens* Stib. Their structures and relative stereochemistry were elucidated by extensive NMR analysis and confirmed by single-crystal X-ray diffraction study. A possible biosynthetic pathway of these two compounds was also proposed.

Salvia, the largest genus in the family Labiatae, is distributed widely in the world.[1] Many species of this genus are used as folk medicine to treat various ailments throughout the world.[2] The genus of *Salvia* is a large pool of diterpenoids with structural diversity and biological properties.[3] Our previous studies of *Salvia* species have reported many new compounds including two novel diterpenoids.[4] *Salvia castanea* Diels *f. pubescens* Stib., a herb with castaneous flowers distributed in the southwest of China, has not been chemically studied before.[5] Aiming at searching for structurally interesting and bioactive diterpenoids from the *Salvia* species, we chemically investigated *S. castanea* Diels *f. pubescens* Stib. and isolated two novel diterpenoids, castanolide (**1**) and its epimer *epi*-castanolide (**2**). Compounds **1** and **2** have a unique seco-norabietane skeleton, which features a six-membered α,β-unsaturated lactone ring and a five-membered α-methyl-α,β-unsaturated γ-spirolactone moiety. To the best of our knowledge, this is the first report of norabietane diterpenoids with a six-membered α,β-unsaturated lactone ring. Described herein are the isolation, structural elucidation, and plausible biogenetic pathway of **1** and **2**.

The whole plant of *S. castanea* Diels f. *pubescens* Stib. was collected in Zhongdian county of Yunnan province, PRC, and identified by Professor X. W. Li of Kunming Institute of Botany, Chinese Academy of Sciences (voucher no. 200501). The air-dried and powdered sample (11.5 kg) was extracted with acetone (3 × 30 L × 24 h) at room temperature and evaporated in vacuum to give a crude extract, which was then partitioned between H_2O (3 L) and EtOAc (3 × 2 L). The EtOAc extract was subjected to column chromatogra-

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	1		2	
	δ_H (mult, *J*, Hz)	δ_C (mult)	δ_H (mult, *J*, Hz)	δ_C (mult)
1δ	1.67 m	32.7 t	1.29 m	32.6 t
1β	1.90 m		2.02 m	
2α	1.64 m	19.2 t	1.69 m	19.2 t
2β	1.73 m		1.76 m	
3α	1.41 m	40.5 t	1.32 m	40.7 t
3β	1.55 m		1.63 m	
4		32.9 s		33.2 s
5	2.33 dd (13.5, 1.5)	46.4 d	1.70 m	49.2 d
6α	1.82 m	16.9 t	1.82 m	17.6 t
6β	1.28 m		1.46 m	
7α	1.92 m	34.9 t	2.11 m	34.2 t
7β	2.16 m		2.35 m	
8		86.0 s		85.5 s
9		162.9 s		163.6 s
10		40.3 s		40.4 s
11	5.64 s	116.5 d	6.09 s	113.1 d
12		163.4 s		163.5 s
13	7.04 br d (1.0)	149.0 d	7.20 br d (1.0)	150.8 d
14		131.7 s		128.2 s
15		171.8 s		171.8 s
16	1.96 d (1.0)	10.7 q	1.90 d (1.0)	10.5 q
17	0.82 s	21.9 q	0.85 s	21.7 q
18	0.98 s	33.1 q	1.00 s	33.4 q
19a	4.85 d (11.0)	70.0 t	4.79 d (11.0)	69.6 t
19b	4.21 d (11.0)		4.22 d (11.0)	

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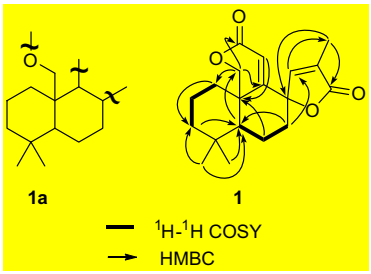


Figure 1. Fragment structure and key 2D correlations of **1**.

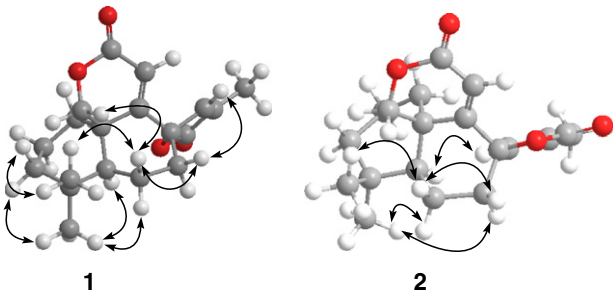


Figure 2. Key ROESY correlations of **1** and **2**.

phy over MCI gel and eluted with MeOH–H_2O (9:1 and 1:0). The MeOH–H_2O (9:1) fraction (420 g) was subjected to column chromatography over silica gel, eluting with a gradient of EtOAc in petroleum ether, to yield seven fractions (I–VII). Fraction IV was repeatedly chromatographed on silica gel, RP-18, and finally purified by semi-preparative HPLC (Agilent 1100 HPLC system, Zorbax SB-C18, 250 × 9.4 mm; UV detector; MeOH–H_2O 65:35) to afford compound **1** (14 mg) and **2** (17 mg).

Castanolide (**1**) was isolated as colorless crystals with a molecular formula of $C_{19}H_{24}O_4$ as established by HRESIMS (found $[M+Na]^+$ 339.1579; calcd 339.1572),[6] indicating eight degrees of unsaturation. The IR spectrum of **1** showed the absorptions for conjugated lactone (1752 and 1736 cm^{-1}) and olefinic (1659 and 1639 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) showed 19 carbon resonances due to two lactone groups, five quaternary carbons (two olefinic and one oxygenated), three methines (including two olefinic ones), six methylenes (one oxygenated), and three methyls.

According to the characteristic signals for normal abietane diterpenoids at δ_C 32.9 (s, C-4), 46.4 (d, C-5), 40.3 (s, C-10), 21.9 (q, C-17), and 33.1 (q, C-18), compound **1** should derive from an abietane diterpenoid.[7] The HMBC spectrum obviously displayed the following correlations: H-3 (δ_H 1.41 and 1.55, each 1H, m) with C-1, C-2, C-4, C-5, C-17, and C-18; H_3-17 (δ_H 0.82, s), and H_3-18 (δ_H 0.98, s) with C-3, C-4, and C-5; H_3-17 with C-18; H-5 (δ_H 2.33, dd, J = 13.5, 1.5 Hz) with C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-17, and C-18; H-6 (δ_H 1.28, m) with C-4, C-5, C-8, and C-10; H-7 (δ_H 2.16, m) with C-5, C-6, and C-13; H-19a (δ_H 4.85, d, J = 11.0 Hz) with C-1, C-9, C-10, and C-12. Moreover, two proton spin systems were observed from the ^{1}H–^{1}H COSY spectrum: H_2-1/H_2-2/H_2-3 and H-5/H_2-6/H_2-7. The above evidence revealed the existence of fragment **1a** (Fig. 1).

Further analysis of the HMBC experiment revealed the correlations from H-11 (δ_H 5.64, s) to C-8, C-9, C-10, and C-12, and from H-19 to C-8 and C-12, which suggested that C-19 exhibited two possible linkages: –C(19)–O–C(8)– and –C(19)–O–C(12)–. Moreover, the following HMBC correlations also appeared for **1**: δ_H 2.16 (1H, m, H-7) with C-13; δ_H 7.04 (1H, br d, H-13) with C-8, C-14, C-15, and C-16; δ_H 1.96 (3H, d, J = 1.0 Hz, H-16) with C-13, C-14, and C-15. Meanwhile, the correlation between H_3-16 (δ_H 1.96, d, J = 1.0 Hz), and H-13 (δ_H 7.04, br d, J = 1.0 Hz) was also observed in ^{1}H–^{1}H COSY spectrum. Since the NMR spectra could not provide sufficient information to establish the structure of **1**, a single-crystal X-ray diffraction study was conducted to clarify the uncertain structural details.[8] The result (Fig. 3) unambiguously confirmed the presence of the linkage of –C(19)–O–C(12)– and established the five-membered α-methyl-α,β-unsaturated γ-spirolactone moiety. Thus, the planar structure of compound **1** was elucidated as shown in Figure 1.

The relative configuration of **1** was deduced by the ROESY experiment (Fig. 2). The ROESY correlations of H-5 with H-7α and H-7β with H-13 indicated that H-13 was in β-orientation, which was further confirmed by X-ray analysis (Fig. 3).

epi-Castanolide (**2**), colorless crystals,[9] had the molecular formula $C_{19}H_{24}O_4$ as determined by HRESIMS (found $[M+Na]^+$ 339.1566; calcd 339.1572). The 1D (Table 1) and 2D NMR spectra data of **2** were similar to those of **1**, except for the presence of ROESY correlation of H-5α with H-13 instead of H-7β with H-13, indicating that compound **2** is the C-8 epimer of **1**. The X-ray diffraction analysis of **2**,[10] as shown in Figure 3, finally confirmed its structure and relative stereochemistry.

Considering ginkgolide B is a well-known potent platelet activating factor (PAF) antagonist and possesses the similar lactone moieties with **1** and **2**, the PAF antagonistic activity of **1** and **2**

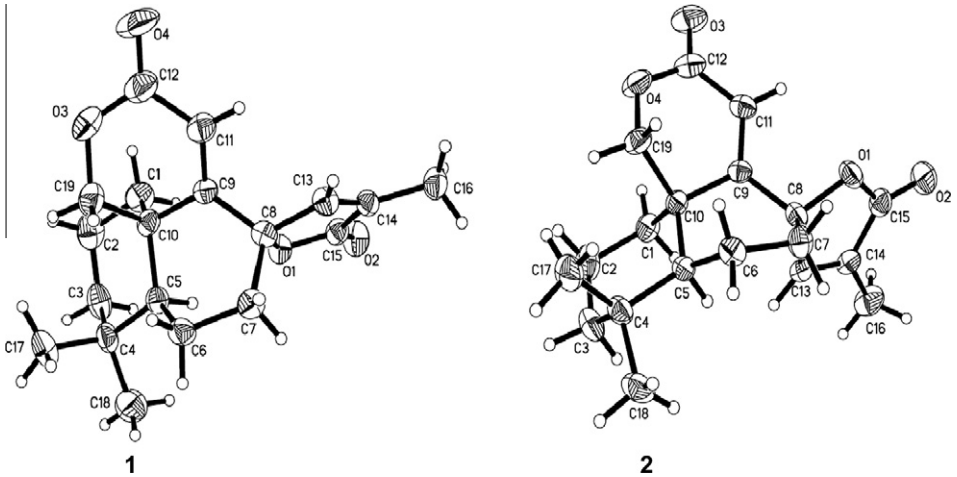
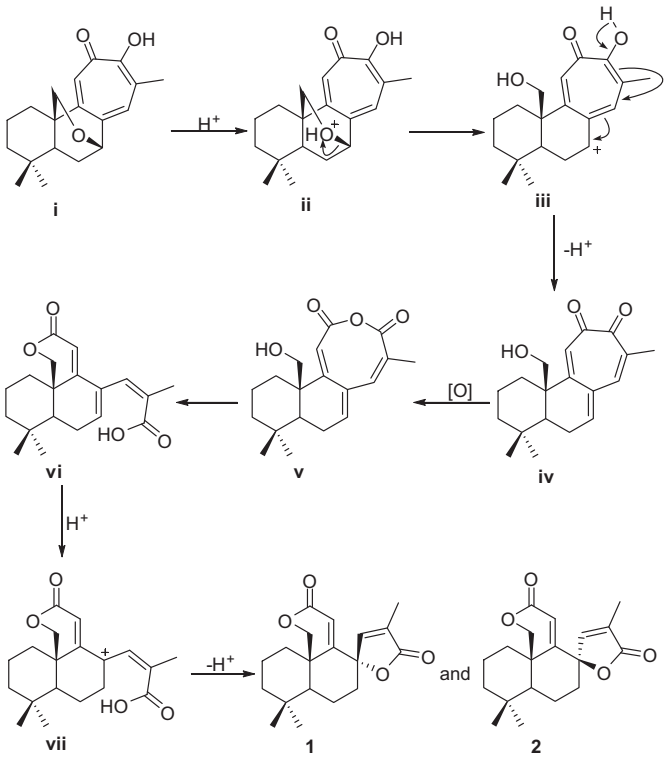


Figure 3. X-ray crystal structures of **1** and **2**.



Scheme 1. Plausible biogenetic pathway of **1** and **2**.

was tested; However, neither of the compounds showed activity. Moreover, compounds **1** and **2** were also evaluated for their cytotoxicity against HL-60, A-549, SMMC-7721, PANC-1, and SK-BR-3 cell lines and their effect on the differentiation of neurons. Unfortunately, no positive results were founded.

Biogenetically, miltipolone (**i**) has been considered as a precursor of several norabietanoid-type diterpenoids.[11] The biogenetic pathway of **1** and **2** from miltipolone was thus proposed. As shown in Scheme 1, miltipolone **i** underwent a cleavage of the oxygen bridge in acidic condition, thus giving **iii**, which then converted to the intermediate **iv**. This intermediate **iv** further underwent oxidation reaction, esterification reaction, and subsequently, an intermolecular nucleophilic attack to produce compounds **1** and **2**.

Acknowledgments

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6. Castanolide (**1**): colorless crystals (MeOH); mp 240–242 °C; $[\alpha]_D^{25.8}$ +46.67 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (3.31) nm; IR (KBr) ν_{max}: 1752, 1736, 1659, 1639 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 339 $[M+Na]^+$; HRESIMS *m*/*z* 339.1579 $[M+Na]^+$ (calcd for $C_{19}H_{24}O_4Na$, 339.1572).
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8. Crystal data for castanolide (**1**): $C_{19}H_{24}O_4$, M = 316.38; orthorhombic, space group $P2_12_12_1$; a = 6.5492 (12) Å, b = 11.936 (2) Å, c = 21.835 (4) Å, α = 90.00, β = 90.00, γ = 90.00, V = 1706.9 (5) $Å^3$, Z = 4, d = 1.231 g/cm^3, crystal dimensions 0.21 × 0.14 × 0.08 mm were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 11131, of which 4058, were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0584, wR_2 = 0.0777. The crystal structure of **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Center (deposition number: CCDC 746691). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/ retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; or desposit@ccdc.cam.ac.uk).
9. *epi*-Castanolide (**2**): colorless crystals (MeOH); mp 207–209 °C; $[\alpha]_D^{25.7}$ +129.75 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (3.24) nm; IR (KBr) ν_{max}: 1770, 1716, 1658, 1630 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 339 $[M+Na]^+$; HRESIMS *m*/*z* 339.1566 $[M+Na]^+$ (calcd for $C_{19}H_{24}O_4Na$, 339.1572).
10. (1) Crystal data for *epi*-castanolide (**2**): $C_{19}H_{24}O_4$, M = 316.38; monoclinic, space group $P21$; a = 8.4020 (14) Å, b = 11.1434 (18) Å, c = 9.4696 (15) Å, α = 90.00, β = 110.341 (2), γ = 90.00, V = 831.3 (2) $Å^3$, Z = 2, d = 1.264 g/cm^3, crystal dimensions 0.23 × 0.18 × 0.12 mm were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 5381, of which 3394, were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0448, wR_2 = 0.0940. The crystal structure of **2** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Center (deposition number: CCDC 746692). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; or desposit@ccdc.cam.ac.uk).
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