Two New Lignan Glycosides from *Saussurea laniceps*

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Two new lignan glycosides, lanicepsides A and B (**1** and **2**), along with the eight known compounds **3**–**6**, epipinoresinol, syringin, ethyl caffeate, and 5,6,7-trihydroxy-4′-methoxyflavone, including sesquiterpene lactones, lignans, and a flavone, were isolated from the ethanol extract of *Saussurea laniceps*. Their structures were determined by spectroscopic methods especially 1D- and 2D-NMR techniques.

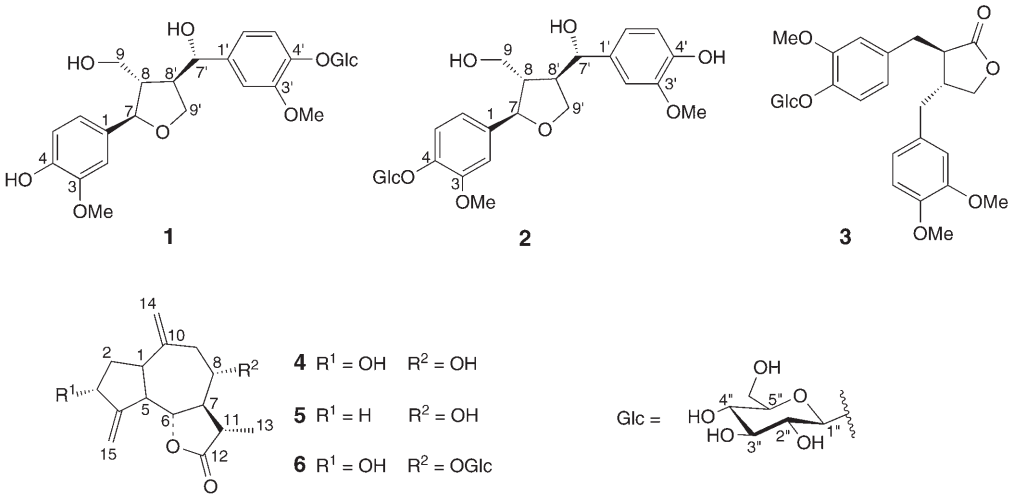
Introduction. – Plants of the genus *Saussurea* (Asteraceae) are used in both traditional Chinese medicine and Tibet folklore medicine for the treatment of rheumatic arthritis, dysmenorrhea, and gynopathy [1]. In previous studies on this genus, a variety of structurally diversified compounds including sesquiterpenoid derivatives [2], lignans [3], triterpenoids [4], and flavonoids [5] were reported. *Saussurea laniceps* HAND.-MAZZ. is mainly distributed in Tibet, Sichuan, and Yunnan Provinces of China. Previous investigations of this plant yielded sesquiterpenoids [6], two coumarins, and one anthraquinone [7]. In the current study, two new lignan glycosides, lanicepsides A and B (**1** and **2**), along with eight known compounds, have been isolated from the whole plant of *S. laniceps*. Herein, we report on the isolation and structural elucidation of the new compounds.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder. The molecular formula was deduced to be $C_{26}H_{34}O_{12}$ from a pseudomolecular-ion peak at *m*/*z* 561.1 ($[M+Na]^+$) in the ESI-MS and the ^{13}C-NMR data. It was further confirmed by HR-EI-MS which exhibited an ion corresponding to the loss of a hexose moiety at *m*/*z* 376.1538 ($[M-162]^+$, $C_{20}H_{24}O_7$; calc. 376.1522). The strong IR absorptions at 3367, 1603, and 1512 cm^{-1} showed the presence of OH groups and aromatic moieties.

The ^{1}H- and ^{13}C-NMR (*Table*), HMBC (*Fig.*), and ROESY (*Fig.*) data established the structure of **1** as (2*β*,3*α*,4*β*)-*α*^{4}-[4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]tetrahydro-2-(4-hydroxy-3-methoxyphenyl)furan-3,4-dimethanol, a new compound named lanicepside A[1]).

The ^{1}H- and ^{13}C-NMR (DEPT) data of **1** (*Table*) revealed the presence of two 1,3,4-trisubstituted benzene rings (*δ*(H) 6.93 (*dd*, *J* = 8.2, 2.0 Hz, 1 H), 7.09 (*d*, *J* = 2.0 Hz, 1 H), and 7.14 (*d*, *J* = 8.2 Hz, 1 H);

[1]) See *Exper. Part* for systematic names.



and δ(H) 6.76 (*d*, *J* = 8.1 Hz, 1 H), 6.79 (*dd*, *J* = 8.1, 1.8 Hz, 1 H), and 6.94 (*d*, *J* = 1.8 Hz, 1 H)). The ^{1}H- and ^{13}C-NMR data also revealed the presence of two aromatic MeO groups at δ(H) 3.89 (*s*, 3 H) and 3.86 (*s*, 3 H), and one β-glucopyranosyl moiety. The remaining structure parts were identified by ^{1}H- and ^{13}C-NMR (DEPT) as four CH (two oxygenated) and two oxygenated CH_2 groups. The aforementioned groups accounted for nine out of the ten degrees of unsaturation in compound **1**, indicating the presence of an additional ring. The spectral data mentioned above together with HMBC correlations (*Fig.*), suggested **1** to possess a C(7)–O–C(9′) tetrahydrofuran lignan skeleton, similar to those of tinosposide B [8] and tanegool [9]. In the HMBC, two oxygenated CH groups at δ(C) 86.3 and 77.8 were firstly

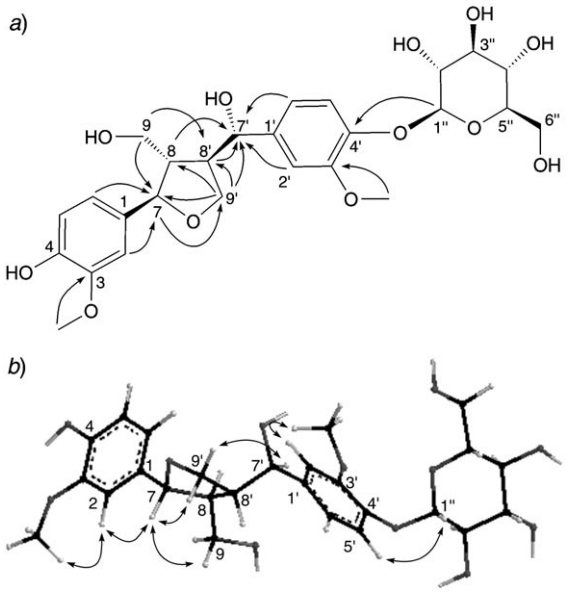


Figure. a) *Selected HMBC* (H → C) *correlations of* **1**. b) *Key ROESY* (↔) *correlations of* **1**.

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[a]). δ in ppm, J in Hz. Trivial atom numbering.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		134.5		136.6
H–C(2)	6.94 (*d*, *J* = 1.8)	111.8	7.00 (*d*, *J* = 1.9)	112.4
C(3)		149.6		151.4
C(4)		148.0		148.1
H–C(5)	6.76 (*d*, *J* = 8.1)	116.5	7.14 (*d*, *J* = 8.3)	118.3
H–C(6)	6.79 (*dd*, *J* = 8.1, 1.8)	121.1	6.89 (*dd*, *J* = 8.3, 1.9)	120.9
H–C(7)	4.52 (*d*, *J* = 8.6)	86.3	4.56 (*d*, *J* = 8.7)	86.0
H–C(8)	2.25–2.31 (*m*)	56.3	2.23–2.30 (*m*)	56.5
CH_2(9)	3.58–3.63 (*m*)	63.8	3.60–3.64 (*m*)	63.8
C(1′)		140.1		138.3
H–C(2′)	7.09 (*d*, *J* = 2.0)	112.7	6.98 (*d*, *J* = 2.0)	111.9
C(3′)		151.4		149.6
C(4′)		148.2		148.0
H–C(5′)	7.14 (*d*, *J* = 8.2)	118.2	6.76 (*d*, *J* = 8.1)	116.5
H–C(6′)	6.93 (*dd*, *J* = 8.2, 2.0)	121.3	6.81 (*dd*, *J* = 8.1, 2.0)	121.3
H–C(7′)	4.53 (*d*, *J* = 8.8)	77.8	4.46 (*d*, *J* = 8.7)	78.0
H–C(8′)	2.59–2.66 (*m*)	53.3	2.58–2.65 (*m*)	53.2
H_α–C(9′)	3.58–3.63 (*m*)	71.6	3.60–3.64 (*m*)	71.8
H_β–C(9′)	3.73 (*dd*, *J* = 8.4, 6.4)		3.74 (*dd*, *J* = 8.7, 6.0)	
MeO–C(3)	3.86 (*s*)	57.0	3.86 (*s*)[b])	57.3
MeO–C(3′)	3.89 (*s*)	57.3	3.86 (*s*)[b])	56.9
H–C(1″)	4.88 (*d*, *J* = 7.6)	103.3	4.88 (*d*, *J* = 7.8)	103.3
H–C(2″)	3.49 (*dd*, *J* = 9.2, 7.5)	75.4	3.43–3.51 (*m*)	75.4
H–C(3″)	3.46 (*t*, *J* = 9.2)	78.4	3.43–3.51 (*m*)	78.3
H–C(4″)	3.39–3.41 (*m*)	71.9	3.38–3.40 (*m*)	71.8
H–C(5″)	3.39–3.41 (*m*)	78.7	3.38–3.40 (*m*)	78.7
H–C(6″)	3.87[b]), 3.69 (*dd*, *J* = 12.2, 4.1)	63.0	3.87 (*dd*, *J* = 12.1, 2.1), 3.66–3.70 (*m*)	63.0

[a]) Recorded at 400 (^{1}H) and 100 (^{13}C) MHz in CD_3OD. [b]) Overlapped signals.

assigned to C(7) and C(7′), respectively, by the HMBC correlations of H–C(2) and H–C(6) to C(7), and H–C(2′) and H–C(6′) to C(7′). Then the 7,9′-epoxy bridge was established from the HMBC correlations between CH_2(9′) and C(7), and between H–C(7) and C(9′). It was further supported by the chemical shifts of C(7) and C(9′). HMBC Correlations of CH_2(9′) to C(7), C(8′), and C(8) then completely established the tetrahydrofuran ring skeleton. The HMBC correlations of CH_2(9) to C(7) and C(8′) located the hydroxymethyl group at C(8). The connection between C(7′) and C(8′) was suggested by the HMBC correlation between H–C(8′) and C(7′). The HMBC correlations between MeO–C(3) and C(3), and between MeO–C(3′) and C(3′) allocated two MeO groups to C(3) and C(3′), respectively (*Fig.*), which was further confirmed by the ROESY correlations MeO–C(3)/H–C(2) and MeO–C(3′)/H–C(2′). Finally, a HMBC correlation between H–C(1″) and C(4′) indicated that the glucopyranose moiety was located at C(4′) (*Fig.*).

The relative configuration of **1** was established on the basis of ^{1}H-NMR data and a ROESY experiment. The chemical shift of H–C(7) at δ(H) 4.52 indicated that H–C(7) and H–C(8) were *trans*-oriented (in a *cis*-arrangement, H–C(7) normally appears at δ(H) *ca.* 5.5) [10], and this was confirmed by the ROESY correlation between H–C(7) and CH_2(9). Arbitrarily assuming a β-configuration of H–C(8), the correlations between H–C(8′) and CH_2(9), and between H–C(7′) and H_β–C(9′) indicated that H–C(7) and H–C(8′) were both in α-orientation. The relative configuration at C(7′) was determined by comparison of the chemical shift and coupling constant of H–C(7′) with those of

tinosposide B [8]. The latter was also supported by the ROESY correlation between H–C(7′) and H_{β}–C(9′).

Lanicepside B (**2**), obtained as an amorphous powder, had the same molecular formula as compound **1**, on the basis of the ESI-MS and ^{13}C-NMR (DEPT) data. The NMR (*Table*), HSQC, and ROESY data established the structure of **2** as (2β,3α,4β)-2-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]tetrahydro-α^4-(4-hydroxy-3-methoxyphenyl)furan-3,4-dimethanol, named lanicepside B[1]).

The NMR data (*Table*) and HSQC spectrum of **2** indicated that the three aromatic protons at δ(H) 6.89 (*dd*, J = 8.3, 1.9 Hz, 1 H), 7.00 (*d*, J = 1.9 Hz, 1 H), and 7.14 (*d*, J = 8.3 Hz, 1 H) belonged to a 1,3,4-trisubstituted benzene ring, with the corresponding C-signals observed at δ(C) 136.6, 112.4, 151.4, 148.1, 118.3, and 120.9. Similarly, the presence of another 1,3,4-trisubstituted benzene ring (δ(H) 6.76 (*d*, J = 8.1 Hz, 1 H), 6.81 (*dd*, J = 8.1, J = 2.0 Hz, 1 H), and 6.98 (*d*, J = 2.0 Hz, 1 H); δ(C) 138.3, 111.9, 149.6, 148.0, 116.5, and 121.3) was also established. In addition, two aromatic MeO groups (δ(H) 3.86 (br. *s*, 6 H); δ(C) 57.3 and 56.9) and one β-glucopyranosyl moiety (δ(H) 4.88 (*d*, J = 7.8 Hz, H–C(1″)); δ(C) 103.3, 75.4, 78.3, 71.8, 78.7, and 63.0) could also be discerned. Both the ^{1}H- and ^{13}C-NMR data of **2** showed high similarity to those of compound **1**, indicating that the structures of the two compounds were closely related (*Table*). Further comparison of the ^{1}H- and ^{13}C-NMR data of **1** and **2** revealed that the only difference was the location of the glucopyranosyl moiety. A ROESY correlation observed between H–C(1″) and H–C(5) revealed that in **2** the glucopyranosyloxy moiety was attached to C(4). The relative configuration of the tetrahydrofuran ring of compound **2** was determined to be the same as that of **1** by comparison with **1** and analysis of its ROESY plot.

The eight known compounds, arctiin (**3**) [11], 3α,8α-dihydroxy-11βH-11,13-dihydrodehydrocostuslactone (**4**) [12], 8α-hydroxy-11βH-11,13-dihydrodehydrocostuslactone (**5**) [12], 3α,8α-dihydroxy-11βH-11,13-dihydrodehydrocostuslactone 8-*O*-β-D-glucopyranoside (**6**) [12], epipinoresinol [11], syringin [13], ethyl caffeate [14], and 5,6,7-trihydroxy-4′-methoxyflavone [15] were identified by comparison of their ^{1}H- and ^{13}C-NMR as well as ESI-MS data with those reported in the literature. All of these known compounds were isolated from the title plant for the first time, and compounds **3**–**6**, epipinoresinol, and syringin have been reported from plants of the same genus previously [3b,c][12].

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Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai, P. R. China). Column chromatography (CC): silica gel (200–300 mesh), silica gel *H60*, C_{18} reversed-phase silica gel (*RP-18*, 250 mesh; *Merck*), *Sephadex LH-20* (*Amersham Biosciences*). TLC: pre-coated silica gel *GF254* plates (*Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China). Semi-prep. HPLC: *Waters-515* HPLC pump and *Waters-2487* dual λ absorption detector, *YMC-Pack-ODS-A* column (250 × 10 mm, 5 μm). Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-210A* spectrometer. IR Spectra: *Nicolet Magna-750* spectrometer; KBr disc. NMR Spectra (^{1}H, ^{13}C, HSQC, HMBC, and ROESY): *Bruker AM-400* spectrometer; chemical shifts δ in ppm relative to $SiMe_4$. EI-MS and HR-EI-MS (70 eV): *Finnigan MAT-95* mass spectrometer. ESI-MS: *Finnigan LCQ^{DECA}* instrument.

Plant Material. The whole plant of *Saussurea laniceps* was collected in September 2000 in the Tibet Autonomous Region of China, and was identified by Prof. *H. Li*. A voucher specimen (access No. Sl-

2004-4Y) was deposited at the Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The dried powder of *Saussurea laniceps* (2.5 kg) was extracted with 95% EtOH at r.t. for three times (3×2 l, each time for 7 days), and the combined EtOH soln. was concentrated to give a crude extract (250 g). The crude extract was suspended in H_2O (1.0 l) and then extracted successively with AcOEt (5×2 l) and BuOH (5×500 ml). The AcOEt-soluble fraction (132 g) was separated by CC (SiO_2, petroleum ether/Me_2CO $40:1 \rightarrow 0:1$): *Fractions 1–5*. *Fr. 4* (18 g) was purified by repeated CC (SiO_2, petroleum ether/Me_2CO $6:1 \rightarrow 2:1$; *Sephadex LH-20*, EtOH; *RP-18*, MeOH/H_2O $1:4 \rightarrow 1:1$): 3α,8α-dihydroxy-11βH-11,13-dihydrodehydrocostuslactone (**4**; 600 mg), ethyl caffeate (40 mg), epipinoresinol (60 mg), 8α-hydroxy-11βH-11,13-dihydrodehydrocostuslactone (**5**; 300 mg), and 5,6,7-trihydroxy-4′-methoxyflavone (50 mg). The BuOH-soluble fraction was separated by CC (*RP-18*, MeOH/H_2O $1:9 \rightarrow 2:3$): *Fractions 5a–5d*. *Fr. 5a* was purified by CC (*Sephadex LH-20*, EtOH): syringin (130 mg). *Fr. 5b* was subjected CC (*Sephadex LH-20*, EtOH) firstly, and then the major component was purified by CC (silica gel, $CHCl_3$/MeOH $10:1 \rightarrow 5:1$): 3α,8α-dihydroxy-11βH-11,13-dihydrodehydrocostuslactone 8-*O*-β-D-glucopyranoside (**6**; 300 mg). By the same separation and purification procedures, *Fr. 5c* gave arctiin (**3**; 120 mg). *Fr. 5d* was subjected to semi-prep. HPLC (MeOH/H_2O 3:7, 3 ml min^{-1}) to afford **1** (14 mg) and **2** (12 mg).

Lanicepside A (= rel-*4-{(*R*)-Hydroxy[(3*S*,4*R*,5*S*)-tetrahydro-5-(4-hydroxy-3-methoxyphenyl-4-(hydroxymethyl)furan-3-yl]methyl}-2-methoxyphenyl* β-D-*Glucopyranoside*; **1**): White powder. $[\alpha]_D^{25} = -63$ ($c = 0.16$, MeOH). UV (MeOH): 229 (4.2), 278 (3.8). IR (KBr): 3367, 2920, 1603, 1512, 1462, 1263, 1223, 1159, 1068, 1030, 814. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 561.1 ($[M + Na]^+$). EI-MS: 376 (6), 328 (17), 297 (7), 265 (5), 259 (100), 224 (5), 206 (58), 188 (26), 175 (73), 151 (46), 137 (56), 131 (25), 115 (13), 91 (8), 73 (19). HR-EI-MS: 376.1538 ($[M - Glc]^+$, $C_{20}H_{24}O_7^+$; calc. 376.1522).

Lanicepside B (= rel-*2-Methoxy-4-{(2*R*,3*S*,4*R*)-tetrahydro-4-[(*S*)-hydroxy(4-hydroxy-3-methoxyphenyl)methyl]-3-(hydroxymethyl)furan-2-yl}phenyl* β-D-*Glucopyranoside*; **2**): White powder. $[\alpha]_D^{25} = -61$ ($c = 0.15$, MeOH). UV (MeOH): 229 (4.2), 279 (3.7). IR (KBr): 3367, 2920, 1651, 1601, 1514, 1464, 1427, 1265, 1223, 1159, 1126, 1070, 1032, 814. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 561.1 ($[M + Na]^+$). EI-MS: 358 (57), 328 (8), 297 (6), 206 (98), 188 (55), 175 (100), 151 (54), 137 (58), 131 (19), 124 (18), 115 (14), 91 (10), 73 (11), 60 (20). HR-EI-MS: 358.1429 ($[M - Glc - H_2O]^+$, $C_{20}H_{22}O_6^+$; calc. 358.1416).

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