A New Triterpene and a New Lignan from *Saussurea japonica*

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A new triterpene, 11α,12α-oxidotaraxerone (**1**) and a new lignan, saussurenoside (**2**), have been isolated from the aerial parts of *Saussurea japonica*. Their structures were determined using spectral methods.

Extensive chemical studies on the constituents of *Saussurea* species have been reported, and many compounds including coumarins, flavonoids, lignans, sesquiterpenes, steroids, and triterpenes have been isolated.[1−10] In the case of *S. japonica* (Thunb.) DC. (Compositae), a plant used in folk medicine as an antithrombic agent, three new flavones and one new sesquiterpene lactone, together with terpenes, β-sitosterol, and fatty acids, have been identified.[11,12] We have collected the aerial parts of *S. japonica* and investigated its chemical constituents. The $CHCl_3$-soluble fraction of the MeOH extract was chromatographed on Si gel (Merck 70−230 mesh) to give a number of compounds. Several known compounds were readily identified as oleic acid, a mixture of β-sitosterol and stigmasterol,[13] apigenin (5,7,4′-trihydroxyflavone),[14] 5-hydroxy-7,4′-dimethoxyflavone,[15] lupenone,[16] lupeol,[17] taraxasteryl acetate,[18] and *epi*-ψ-taraxastanonol[19] by analysis of their physical and spectral properties (mp, IR, MS, and ^{1}H-NMR). This paper deals with the structural elucidation of these two new compounds, 11α,12α-oxidotaraxerone (**1**) and saussurenoside (**2**), from this $CHCl_3$-soluble extract.

11α,12α-Oxidotaraxerone (**1**) was obtained as colorless needles, mp 204−206 °C. Elemental analysis indicated a molecular formula of $C_{30}H_{46}O_2$, with a molecular ion $[M]^+$ occurring in the EIMS at *m/z* 438. Analysis of the IR spectrum of **1** suggested that it contained a carbonyl group (1700 cm^{-1}) and a trisubstituted double bond (3040, 1640, and 830 cm^{-1}). The ^{1}H-NMR spectrum of **1** exhibited signals for eight singlet methyl groups (δ 0.79, 0.84, 0.95, 0.98, 1.07, 1.09, 1.10, and 1.21), a trisubstituted olefin proton [δ 5.55 (dd, J = 8.2, 3.4 Hz)] adjacent to a methylene group [δ 1.68 (1H, dd, J = 14.6, 8.2 Hz) and 1.96 (1H, dd, J = 14.6, 3.4 Hz)], a carbonyl group (δ 216.7) adjacent to a methylene group [δ 2.39 (1H, ddd, J = 16.2, 6.3, 2.6 Hz) and 2.64 (1H, ddd, J = 16.2, 12.0, 6.9 Hz)], and a methine proton [δ 1.02 (d, J = 4.6 Hz)] vicinal to an oxirane group [δ 51.7 (d) and 58.2 (d); δ 2.81 (1H, d, J = 4.6 Hz) and 3.14 (1H, t, J = 4.6 Hz)]. From the above evidence, compound **1** was assigned as a pentacyclic triterpene with carbonyl, oxirane, and trisubstituted olefin functional groups. The olefinic proton signal occurred as a doublet of doublets at low field (δ 5.55, J = 8.2, 3.4 Hz), suggesting that this compound is a taraxerene-type triterpene.[20] Comparison of the ^{1}H-NMR spectra (Table 1) of **1** with that of the known 11α,12α-oxidotaraxerol (**3**)[21] suggested that **1** possesses the same carbon skeleton as **3**, but has a ketone group instead of a hydroxy group. The ^{13}C-NMR data (Table 1) of **1** also agreed with the assigned structure. Reduction of **1** with $NaBH_4$ in MeOH yielded only one product, which was identical with **3** on comparison of spectral data.[21]

Table 1. ^{13}C- and ^{1}H-NMR (δ values) Data and HMBC Correlations of **1**[a]

	δ_C		δ_H	HMBC carbon correlations
1	39.6	H-1a	2.05 m	C-2, C-5, C-9, C-25
2	33.9	H-1b	2.12 m	C-2, C-5, C-9
3	216.7	H-2a	2.39 ddd (16.2, 6.3, 2.6)	C-7, C-8, C-9
4	47.3	H-2b	2.64 ddd (16.2, 12.0, 6.9)	C-7, C-10
5	54.7	H-5	1.28 m	C-1, C-6, C-7, C-23, C-20, C-25
6	38.5	H-6	1.70 m	C-8, C-10
7	36.5	H-7a	1.18 m	C-6, C-10
8	36.4	H-7b	1.45 m	C-10
9	52.8	H-9	1.02 d (4.6)	C-5, C-10
10	38.8	H-11	3.14 t (4.6)	C-8, C-9
11	51.7	H-12	2.81 d (4.6)	C-13, C-18
12	58.2	H-15	5.55 dd (8.2, 3.4)	C-13, C-16
13	37.5	H-16a	1.68 dd (14.6, 8.2)	C-18, C-22, C-28
14	156.6	H-16b	1.89 dd (14.6, 3.4)	C-17, C-22, C-28
15	119.2	H-18	1.17 m	C-12, C-28
16	38.2	H-19	1.56 m	C-29, C-30
17	35.4	H-21	1.40 m	C-29, C-30
18	48.1	H-22a	0.98 m	C-20, C-21
19	20.0	H-22b	1.07 m	C-20, C-21
20	28.7	H-23	1.09 s	C-3, C-4, C-5, C-24
21	33.1	H-24	1.07 s	C-3, C-4, C-5, C-23
22	35.2	H-25	1.21 s	C-5, C-9, C-10
23	21.5	H-26	1.10 s	C-9
24	26.1	H-27	0.79 s	C-12, C-13, C-18
25	16.2	H-28	0.84 s	C-16, C-18, C-22
26	26.7	H-29	0.98 s	C-20, C-21, C-30
27	19.5	H-30	0.95 s	C-20, C-29
28	30.2			
29	33.7			
30	29.9			

[a] Numbers in parentheses are coupling constants in Hz.

Saussurenoside tetraacetate (**4**) was obtained from the purification of saussurenoside (**2**) after acetylation. Compound **4**, an amorphous solid, gave elemental analysis and EIMS data consistent with the molecular formula $C_{35}H_{42}O_{16}$. The IR absorption bands of **4** showed the presence of hydroxy (3421 cm^{-1}), γ-lactone (1759 cm^{-1}), acetoxy (1735, 1259, and 1076 cm^{-1}), and aromatic groups (1587 and 1510 cm^{-1}). Saussurenoside (**2**) was eluted only with a polar solvent system (20% MeOH in EtOAc), thus suggesting it was a glycoside. The ^{1}H-NMR spectrum (Table 2) of **4** exhibited signals for four acetyl groups (δ 2.00, 2.01, 2.04, and 2.04) and seven sugar protons [δ 3.72 (m, H-5″), 4.13 (dd, J = 12.3, 2.4 Hz, H-6a″), 4.25 (dd, J = 12.3, 4.9 Hz, H-6b″), 4.93 (d, J = 7.3 Hz, H-1″), 5.15 (t, J = 9.3 Hz, H-4″), 5.20−

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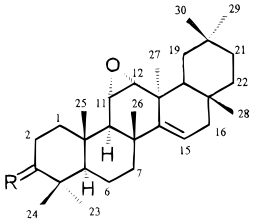
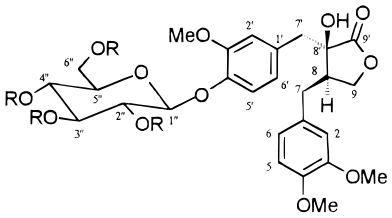
Table 2. ^{13}C- and ^{1}H-NMR (δ values) Data for 4[a]

	δ_C	δ_H		δ_C	δ_H
1	130.8		8′	76.2	
2	112.3	6.61 br s	9′	178.1	
3	149.1		1″	100.5	4.93 d (7.3)
4	147.8		2″	71.1	5.20−5.30
5	120.8	6.78 d (8.0)	3″	71.9	5.20−5.30
6	111.4	6.64 br d (8.0)	4″	68.3	5.15 t (9.3)
7	31.5	2.50 m	5″	72.5	3.72 m
		2.92 m	6″	61.9	4.13 dd
8	43.9	2.50 m			(12.3, 2.4)
9	69.9	4.02 d (5.8)			4.25 dd
1′	130.7				(12.3, 4.9)
2′	114.8	6.72 br s	OMe	55.9	3.75
3′	150.5			56.0	3.83
4′	145.3			56.0	3.83
5′	119.7	6.78 d (8.0)	OAc	169.3	2.04
6′	122.5	6.62 d (8.0)		169.4	2.04
7′	42.1	2.92 d (13.7)		170.2	2.00
		3.06 d (13.7)		170.5	2.01

[a] Numbers in parentheses are coupling constants in Hz.



1 R=O
3 R=β-OH, α-H



2 R=H
4 R=Ac

5.30 (2H, m, H-2″, H-3″)]. Using ^{1}H−^{1}H COSY and decoupling NMR experiments, the sequence of the seven sugar protons could be determined. The larger coupling constants of anomeric proton of sugar at lower field suggested that the sugar moiety was β-D-glucose linked to an aromatic ring.[22] In addition, two systems due to 1,3,4-trisubstituted phenyl protons [δ 6.61 (br s), 6.64 (br d, J = 8.0 Hz), and 6.78 (d, J = 8.0 Hz); 6.62 (br d, J = 8.0 Hz), 6.72 (br s), and 6.78 (d, J = 8.0 Hz)] and three phenyl methyl ethers [δ 3.75, 3.83, and 3.83] were evident in the ^{1}H-NMR spectrum of **4**. The ^{1}H-NMR spectrum of **4** also exhibited signals due to two benzylic protons at δ 2.92 and 3.06 (1H each, d, J = 13.7 Hz), two geminal protons on carbon atoms bearing the oxygen of a γ-lactone at δ 4.02 (2H, d, J = 5.8 Hz), and another three protons at δ 2.50 (2H, m) and 2.92 (1H, m, obscured by one of the benzylic protons). Using ^{1}H−^{1}H COSY and decoupling NMR experiments, the two protons at 2.92 and 2.50 were found to be linked to the same carbon (δ 31.5), and one proton at δ 2.50 (H-8) was placed between the proton at δ 4.02 (H-9) and the proton at δ 2.92 (H-7). The hydroxyl group was assigned as a tertiary alcohol because of its lack of reactivity on acetylation. The carbon bearing the hydroxy group (δ 76.2) was a quarternary carbon as determined by the

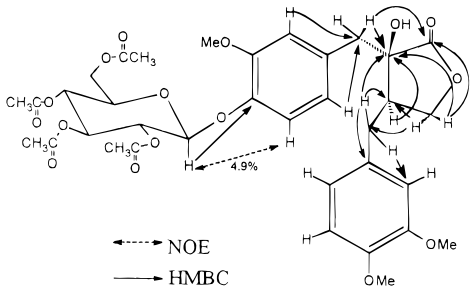


Figure 1.

DEPT technique. A HMQC experiment was used to determine the ^{1}H- and ^{13}C-NMR vicinal correlations, and the HMBC technique revealed further correlations in **4** (Figure 1). Saussurenoside tetraacetate (**4**) is a 8,8′-linked lignan with a γ-lactone and a glucose moiety linked to a phenyl group. The anomeric proton and H-5′ exhibited a NOE correlation (4.9% enhancement), and therefore the glucose unit was linked to the C-4′ position with an ether linkage. Because the signals of H-7a and H-8 (also H-7b and H-7a′) obscured each other, the NOE technique could not be utilized to recognize the stereochemistry between the two benzyl groups. Fortunately, the chemical shifts of the H-7 and H-7′ protons could be used to decide on the stereochemistry. The chemical shift difference between H-7a′ and H-7b′ (δ 3.06 and 2.92) was 0.14 ppm and between H-7a and H-7b (δ 2.52 and 2.92) was 0.4 ppm. The larger chemical shift difference between H-7a and H-7b suggested that one of the H-7 protons was deshielded by the hydroxy group.[23,24] Therefore, H-7 is *cis* to the hydroxy group, and the structure of saussurenoside could be unambiguously assigned as **2**.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were run on a Bruker AM-300 spectrometer. EIMS, FABMS, UV, and specific rotations were taken on a JEOL JMS-HX 300, a JEOL JMS-HX 110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on Si gel (Merck 3374, 70−230 mesh).

Plant Material. The aerial parts of *S. japonica* were collected in Nan-Tou, Taiwan, in 1990. The plant material was identified by Mr. Gun Muh-Tsuen, formerly a technician of the Department of Botany of the National Taiwan University, and a voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The aerial parts of *S. japonica* (1.5 kg) were extracted with MeOH (40 L) at room temperature (7 days × 3). To the MeOH extract were added 500 mL of H_2O, and this phase was then partitioned with 500 mL of $CHCl_3$ three times. The combined $CHCl_3$ extracts (99.3 g) were chromatographed on Si gel (Merck 3374, 70−230 mesh) repeatedly with hexane/EtOAc gradient solvent systems, with taraxasteryl acetate (10 mg), lupenone (15 mg), oleic acid (10 mg), lupeol (20 mg), a mixture of β-sitosterol and stigmasterol (100 mg), 11α,12α-oxidotaraxerone (**1**) (20 mg), *epi*-ψ-taraxastanonol (13 mg), 5-hydroxy-7,4′-

dimethoxyflavone (22 mg), and 5,7,4′-trihydroxyflavone (17 mg) being isolated sequentially. With 20% MeOH in EtOAc, a mixture containing saussurenoside was eluted, but it proved difficult to purify on subsequent chromatography. Therefore, after detecting the absence of any acetyl group by ^{1}H NMR, the crude saussurenoside was acetylated with Ac_2O in pyridine overnight at room temperature to afford pure saussurenoside tetraacetate (**4**) (15 mg) on subsequent column chromatography.

11α,12α-Oxidotaraxerone (1): mp 204−206 °C; $[\alpha]_D$ +12.5° (*c* 0.6, $CHCl_3$); UV λ max, no significant absorption above 210 nm; IR (KBr) ν max 3040, 1698, 1640, 1379, 870 cm^{-1}; ^{13}C- and ^{1}H-NMR data, see Table 1; EIMS *m/z* [M$^+$] 438 (28), 423 (M$^+$ − CH_3, 10), 405 (20), 259 (15), 231 (15), 204 (17), 135 (21), 123 (18), 108 (100). Anal. Calcd for $C_{30}H_{46}O_2$: C, 82.13, H, 10.57. Found: C, 83.25, H, 10.51.

Saussurenoside tetraacetate (4): amorphous solid; $[\alpha]_D$ −35.2° (*c* 0.9, $CHCl_3$); UV (MeOH) λ max (log ϵ) 226 (4.37), 279 (3.93) nm; IR (dry film) ν max 3421, 3043, 1759, 1735, 1587, 1510, 1259, 1232, 1076, 1001 cm^{-1}; ^{13}C- and ^{1}H-NMR data, see Table 2; FABMS *m/z* [M$^+$] 718 (11), 308 (10), 331 (62), 221 (8), 169 (100), 151 (29), 137 (37), 109 (58). Anal. Calcd for $C_{35}H_{42}O_{16}$: C, 58.49; H, 5.89. Found: C, 58.35; H, 5.94.

Reduction of 1 with $NaBH_4$. Excess $NaBH_4$ was added to a solution of **1** (10 mg) in 5 mL of MeOH, and the reaction mixture was allowed to stand for 4 h. Excess H_2O (40 mL) was added and the reaction mixture extracted with Et_2O (30 mL × 3). The product was purified and yielded 11α,12α-oxidotaraxerol (**3**) (8 mg), which exhibited physical and spectral data comparable to literature values.[22]

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