Androstane and Monoterpene Glucoside Sinapoyl Ester from *Cynanchum amplexicaule* Sieb. *et* Zucc.

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Received May 29, 2007; accepted June 22, 2007

A new androstane, 17β-hydroxy-androsta-4,6,15-trien-3-one (1) and a new monoterpene glucoside sinapoyl ester, (3*R*)-8-hydroxylinalool 3,8-di-*O*-β-D-(6′-*O*-*E*-sinapoyl)glucopyranoside (2) were isolated from the roots of *Cynanchum amplexicaule* Sieb. *et* Zucc. (Asclepiadaceae), along with two known monoterpenes, (3*R*)-8-hydroxylinalool (3) and (6*R*)-menthiafolic acid (4). Their structures were elucidated on the basis of analyses of physical, chemical, and spectral data.

Key words *Cynanchum amplexicaule*; androstane; monoterpene glucoside sinapoyl ester; monoterpene; Asclepiadaceae

Cynanchum amplexicaule Sieb. *et* Zucc. is widely distributed in China and used as a Chinese folk medicine for the treatment of rheumatoid arthritis, hectic fevers and abscesses. Two plant species, *Cynanchum atratum* and *Cynanchum versicolor* as a traditional Chinese medicine "Pai-Wei" are very similar in outward appearance with it. *Cynanchum amplexicaule* has been used as the substitute for the medicine "Pai-Wei" in some regions of China. Earlier phytochemical study of this plant had led to the isolation of two C-21 steroids.[1)] In this paper, we report the isolation and structure elucidations of a new androstane (**1**), a new monoterpene glucoside sinapoyl ester (**2**), as well as two known monoterpenes (**3**, **4**) from the roots of this plant.

Results and Discussion

Compound **1** was obtained as colorless needle, mp 187—190 °C. Its molecular formula was determined to be $C_{19}H_{24}O_2$ by the $[M+H]^+$ ion peak at *m/z* 285.1862 (Calcd 285.1855) in high-resolution (HR)-FAB-MS. The Lieberman–Burchard reaction is positive, which indicated **1** to be a steroid. The presence of a 4,6-dien-3-one system in **1** was suggested by the conjugated carbonyl carbon signal at δ 198.3 and the olefinic carbon signals at δ 163.3, 140.8, 127.8, 123.2 and confirmed by the olefinic proton signals at δ 5.62 (s), 6.21 (dd, J=10.2, 2.4 Hz) and 6.40 (br d, J=10.2 Hz).[2)] The cross peaks in the heteronuclear multiple bond connectivity (HMBC) spectrum between 17-OH/C-16 (δ 136.5), C-13 (δ 51.7); and H-15/C-13, C-14 (δ 54.4), C-16, C-17 (δ 83.5) indicated the presence of a 15-en-17-ol system. The β-oriented hydroxyl group at C-17 was assigned by the nuclear Overhouse effect spectroscopy (NOESY), in which the cross peaks between H-14/H-9, H-17; H-8/H-18, H-19 were observed. The structure of **1** was therefore elucidated as 17β-hydroxy-androsta-4,6,15-trien-3-one.

Compound **2** was obtained as white amorphous powder. Its molecular formula was determined to be $C_{44}H_{58}O_{20}$ by the $[M+H]^+$ ion peak at *m/z* 907.3612 (Calcd 907.3600) in HR-FAB-MS. Analysis of the ^{1}H- and ^{13}C-NMR spectra showed the presence of a monoterpene unit, two *trans*-sinapic acid units and two glucopyranosyl units. The aglycone was specified as 8-hydroxylinalool from the ^{1}H-NMR spectrum which exihibited an olefinic proton at δ 5.41 (t, J=7.2 Hz), three olefinic proton signals occurred as an ABX spin system at δ 6.00 (dd, J=18.0, 12.0 Hz), 5.15 (d, J=18.0 Hz) and 5.12 (d, J=12.0 Hz), two methyl signals at δ 1.62 (3H, s) and 1.27 (3H, s), a pair of nonequivalent methylene proton signals at δ 4.08 (d, J=11.4 Hz) and 3.98 (d, J=11.4 Hz), and two methylene signals at δ 2.09 (2H, m) and 1.60 (2H, m).[3)] The (*E*)-configuration of the trisubstituted double bond was deduced from the relatively low chemical shift of the C-9 (δ 12.8)[4)] and confirmed by the cross peak between H-5/H-9 and in NOESY. Furthermore, the ^{1}H-NMR spectrum indicated the

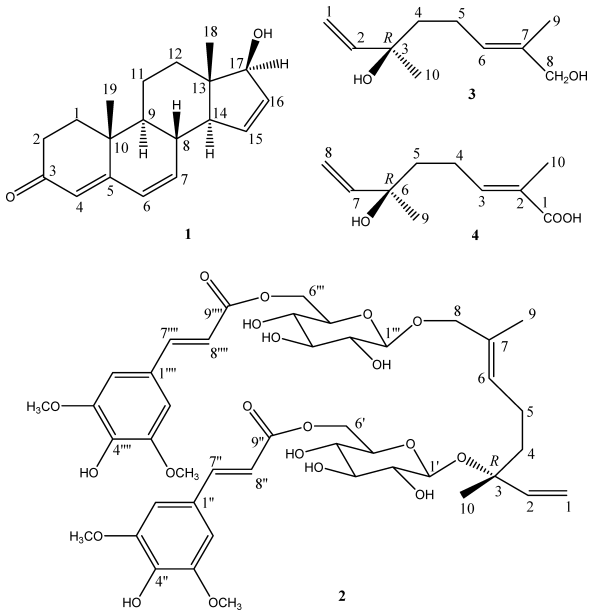

Fig. 1. Structures of Compounds **1**—**4**

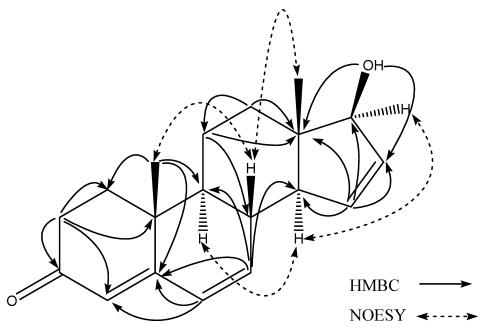

Fig. 2. Selected HMBC and NOESY Correlations of **1**

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existence of two *trans*-sinapic acid moieties by olefinic proton signals at δ 7.62 (1H, d, J=13.8 Hz), 7.60 (1H, d, J=13.8 Hz), 6.88 (2H, s), 6.86 (2H, s), 6.40 (2H, d, J=13.8 Hz) and four methoxyl signals at δ 3.84 (6H, s) and 3.85 (6H, s). The ^{13}C-NMR showed the characteristic signals of two 1,6-disubstituted glucoses (δ 101.1, 98.0, 76.7, 76.6, 73.9, 73.7×2, 73.6, 70.6, 70.5, 63.5, 63.3). The relatively large J values (7.8 Hz and 7.8 Hz) of the anomeric protons (δ 4.22 and 4.32) of glucoses indicated that the anomeric configurations were both β. Final structure proof came from the HMBC spectrum, in which the cross peaks between H-1′/C-3; H-1‴/C-8; H-6′/C-9″; and H-6‴/C-9⁗ were observed. Enzymatic hydrolysis of **2** gave a monoterpene identified as (3*R*)-8-hydroxylinalool (**3**). Therefore, the structure of **2** was elucidated as (3*R*)-8-hydroxylinalool 3,8-di-*O*-β-D-(6′-*O*-*E*-sinapoyl)glucopyranoside.

Compounds **3** and **4** were obtained as colorless oils. They were characterized as the known compounds (3*R*)-8-hydroxylinalool and (6*R*)-menthiafolic acid respectively, from their ^{1}H- and ^{13}C-NMR data and by comparison of their optical rotation values with those reported earlier for the compounds.[3,5,6]

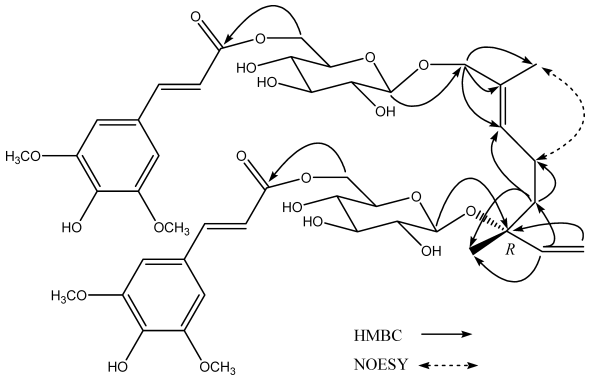

Fig. 3. Selected HMBC and NOESY Correlations of **2**

Experimental

General Experimental Procedures Optical rotations were obtained at 25 °C, using a P-E 241 MC. IR spectra were recorded on a Bruker IFS-55 infrared spectrophotometer. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) with TMS as internal standard. The HR-FAB-MS data were obtained on the Micross Mass Autospec-Ultima ETOF spectrometer. Preparative HPLC: Shimadzu LC-8A *vp*, Inertsil Prep-ODS 10×250 mm (6L Sciences Inc.), Shimadzu SPD-10A *vp* detector, detection 210 nm. *Anal.* HPLC: column, Diamonsil C_{18} 4.6×250 mm (Dikma).

Plant Material The roots of *Cynanchum amplexicaule* were collected in August 2005 at Xinxiang, Henan province, China. A voucher specimen was identified by Professor Qishi Sun and has been deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University (No. 6039).

Extraction and Isolation The roots (10 kg) of *C. amplexicaule* were extracted three times with hot 95% EtOH for 2 h, and the combined solution was concentrated *in vacuo* to give a syrup (1100 g), followed by suspension in water. The suspension was then extracted with petroleum ether, chloroform, and *n*-butanol successively.

The chloroform extract (150 g) was further fractionated by silica gel column chromatography (eluted with petroleum ester and acetone in increasing polarity) to obtain eight fractions. Fr. 5 was rechromatographed on silica gel column with petroleum ester–actone (7 : 1, v/v) to give five subfractions. Fr. 5-1 was separated by sephadex LH-20 eluted with $CHCl_3$–MeOH (1 : 1, v/v) to give compound **1** (21 mg). Fr. 5-3 was separated by preparative HPLC eluted with 57% aqueous CH_3CN to give compounds **3** (47 mg) and **4** (18 mg). The *n*-butanol fraction (252 g) was subjected to a silica gel column eluted with $CHCl_3$ and MeOH in increasing polarity to obtain nine fractions. Fr. 3 was further separated by preparative HPLC eluted with 42% aqueous MeOH to give compound **2** (39 mg).

Compound **1**: Colorless needle, mp 187—190 °C, $[\alpha]_D^{25}$ +40.6° (c=0.05, $CHCl_3$), HR-FAB-MS m/z: 285.1862 $[M+H]^+$ (Calcd for $C_{19}H_{25}O_2$: 285.1855). IR (KBr) cm^{-1}: 3415, 1707, 1644, 1617, 1269, 1199, 878. ^{1}H-

Table 1. The NMR Spectral Data of Compound **1** (in DMSO-d_6) and **2** (in CD_3OD) (600 MHz for ^{1}H, 150 MHz for ^{13}C)

1			**2**			**2**		
Position	δ_H (J Hz)	δ_C	Position	δ_H (J Hz)	δ_C	Position	δ_H (J Hz)	δ_C
1	1.65 (td, 13.2, 4.8)	33.3	1	5.15 (d, 18.0)	113.9	5″		148.0
	1.93 (dd, 13.2, 5.4)			5.12 (d, 12.0)		6″	6.88 (s)	105.0
2	2.25 (dd, 18.0, 3.6)	33.7	2	6.00 (dd, 18.0, 12.0)	143.0	7″	7.60 (d, 13.8)	145.9
	2.55 (m)					8″	6.40 (d, 13.8)	114.4
3		198.3	3		79.8	9″		167.6
4	5.62 (s)	123.2	4	1.60 (m)	39.1	–OMe×2	3.84 (s)	55.5
5		163.3	5	2.09 (m)	21.8	1‴	4.22 (d, 7.8)	101.1
6	6.21 (dd, 10.2, 2.4)	127.8	6	5.41 (t, 7.2)	129.4	2‴	3.20 (m)	73.7
7	6.40 (dd, 10.2, 2.4)	140.8	7		131.3	3‴	3.36 (m)	76.7
8	2.34 (br t, 12.0)	34.8	8	4.08 (d, 11.4)	74.7	4‴	3.33 (m)	70.6
				3.98 (d, 11.4)		5‴	3.41 (m)	73.7
9	1.19 (m)	50.9	9	1.62 (s)	12.8	6‴	4.42 (dd, 12.0, 1.8)	63.5
10		35.8	10	1.27 (s)	22.7		4.29 (dd, 12.0, 6.6)	
11	1.45 (m)	20.2	1′	4.32 (d, 7.8)	98.0	1⁗		125.2
	1.55 (m)					2⁗	6.86 (s)	105.5
12	1.45 (m)	34.2	2′	3.20 (m)	73.6	3⁗		148.0
	1.80 (d, 9.0)					4⁗		138.2
13		51.7	3′	3.36 (m)	76.6	5⁗		148.0
14	1.87 (ddd, 12.0, 1.8, 1.0)	54.4	4′	3.35 (m)	70.5	6⁗	6.86 (s)	105.0
15	6.11 (ddd, 6.0, 1.8, 1.0)	129.4	5′	3.47 (m)	73.9	7⁗	7.62 (d, 13.8)	145.9
16	5.68 (dt, 6.0, 1.0)	136.5	6′	4.52 (dd, 12.0, 1.8)	63.3	8⁗	6.40 (d, 13.8)	114.4
				4.33 (dd, 12.0, 6.6)		9⁗		167.5
17	4.15 (br s)	83.5	1″		125.2	–OMe×2	3.85 (s)	55.5
18	0.80 (s)	12.6	2″	6.88 (s)	105.5			
19	1.08 (s)	16.1	3″		148.0			
17-OH	4.89 (d, 5.4)		4″		138.2			

and ^{13}C-NMR spectral data, see Table 1.

Compound **2**: White amorphous powder, $[\alpha]_D^{25}$ −17.1° (c=0.05, MeOH), HR-FAB-MS m/z: 907.3612 $[M+H]^+$ (Calcd for $C_{44}H_{59}O_{20}$: 907.3600). IR (KBr) cm^{-1}: 3350, 2920, 2860, 1710, 1610, 1518, 1110. ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

Enzymatic Hydrolysis of 2 A solution of the sample (30 mg) in a Na_2HPO_4–citric acid buffer (pH 4.0, 2 ml) was treated with β-glucosidase (142 u) and the whole mixture was kept stirred at 37 °C for 3 d, then extracted with $CHCl_3$ (5 ml×3). After dried over Na_2SO_4, the $CHCl_3$ layer was concentrated to give the aglycone (4 mg), which was identified as 8-hydroxylinalool, by TLC comparison with **3**. The stereochemistry at C-6 has been deduced to be *R* by comparison of its $[\alpha]_D^{25}$ (−5.70°, c=0.05, $CHCl_3$) with those of (*R*)- (−5.95°, $CHCl_3$) and (*S*)-8-hydroxylinalool (+7.13°, $CHCl_3$).[3] The aqueous layer was concentrated to dryness and purified by a silica gel column to give 6-*O*-[*E*]-sinapoyl-glucopyranose (**2a**), by direct comparison with authentic sample[7] on TLC.

(3*R*)-8-Hydroxylinalool (**3**): Colorless oil, $[\alpha]_D^{25}$ −5.70° (c=0.05, $CHCl_3$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.89 (1H, dd, J=17.4, 10.5 Hz, H-2), 5.39 (1H, t, J=7.2 Hz, H-6), 5.20 (1H, dd, J=17.4, 1.0 Hz, H-1a), 5.05 (1H, dd, J=10.5, 1.0 Hz, H-1b), 3.95 (2H, s, H_2-8), 2.03 (2H, m, H_2-5), 1.63 (3H, s, H_3-9), 1.59 (2H, m, H_2-4), 1.27 (3H, s, H_3-10). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 144.8 (C-2), 134.9 (C-7), 125.7 (C-6), 111.8 (C-1), 73.3 (C-3), 68.5 (C-8), 41.7 (C-4), 27.7 (C-10), 22.3 (C-5), 13.6 (C-9).

Alkaline Hydrolysis of 2a **2a** (18 mg) was dissolved in 0.5 M NH_4OH (5 ml) and the mixture was stirred at room temperature for 4 h. The solution was neutralized with 0.1 M HCl then patitioned between EtOAc and H_2O. The anqueous phase was passed through a silica gel column to afford D-glucose ($[\alpha]_D^{25}$ +47.4°, c=0.10, H_2O).

Acknowledgements This research work was financially supported by the National Nature Science Foundation of China, Project NO. 30672612. Special thanks are due to the Analytical Detective Center, Shenyang Pharmaceutical University, for recording NMR data.

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