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Note

New steroids from *Adenophora stenanthina* subsp. *xifengensis*

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Two new steroids, 3β-vanilloyloxy-stigmast-5-ene-7β-ol (**1**) and 3β-vanilloyloxy-stigmast-5-ene-7β,15ξ-diol (**2**) have been isolated from the roots of *Adenophora stenanthina* subsp. *xifengensis* along with other eleven known compounds, ergost-6,22-dien-3β,5α,8α-triol (**3**), β-sitosterol (4), β-daucosterol (**5**), taraxerone (**6**), lupenone (**7**), glutinone (**8**), oleanolic acid (**9**), ursolic acid (**10**), dotriacontanoic acid (**11**), triacontanoic acid (**12**), and dodecylpalmitate (**13**). Their structures have been determined on the basis of spectroscopic methods (EIMS, IR, ^{1}H NMR, ^{13}C NMR, and DEPT) and by comparing with literature or authentic samples.

Adenophora stenanthina subsp. *xifengensis* (Campanulaceae), mainly distributed in Gansu province of China, has been used as a traditional chinese medicine since ancient time for the treatment of cough, phlegm, breathe heavily, especially, to cure lung disease[1]. Its chemical constituents have not been reported so far. We now report herein the isolation, characterization, and structural elucidation of two new steroid compounds, 3β-vanilloyloxy-stigmast-5-ene-7β-ol (**1**) and 3β-vanilloyloxy-stigmast-5-ene-7β,15ξ-diol (**2**) in addition to eleven known compounds, β-sitosterol[2], β-daucosterol[2], ergost-6,22-dien-3β,5α,8α-triol[3], taraxerone[4,5], lupenone[6], glutinone[7], oleanolic acid[8], ursolic acid[8], dotriacontanoic acid[2], triacontanoic acid[2], and dodecylpalmitate.

3β-Vanilloyloxy-stigmast-5-ene-7β-ol (**1**) $[\alpha]_D^{20}$-340° (c 1, $CHCl_3$), was obtained as white crystals crystallized from pet. ether-acetone and the molecular formula $C_{37}H_{56}O_5$ was deduced from elemental analyses and EIMS (M^+ at m/z 580). Compound **1** was recognized as a steroids linking a side chain of phenol from its positive Liebermann-Burchardt and $FeCl_3$ color reaction, as well as the appearance of IR absorption bands at 36-48, 3426 (hydroxyl), 1708 (carbonyl), 1579, 1514, 1462 (aromatic ring) cm^{-1}. The ^{1}H NMR spectrum of **1** showed signals at δ 7.64 (1H, dd, J=8.3, 1.6 Hz), 7.54 (1H, d, J=1.6 Hz), 6.93 (1H, d, J=8.3 Hz) due to 1,2,4-trisubstitutive benzene and at δ 3.95 due to a aromatic methoxy group. A comparison of ^{1}H- and ^{13}C NMR spectral data of **1** with those of a known compound methyl vanilloylate[9,10] revealed there was a vanilloyloxyl group as a side chain in the molecule of **1**, which was further supported by the identical ^{13}C NMR spectral data (Table I) of **1** with acetyl vanilloylate[11], and by the fragment peaks at m/z 412 (Basic peak, M^+-vaniloyloic acid), 168 (vanilloyloic acid), and 151 (vanilloyloxyl). In addition to the signals due to vanilloyloxyl moiety, the ^{1}H NMR spectrum displayed signals for six methyl groups at δ 0.71-1.11, a trisubstituted olefinic proton at δ 5.36 (1H, br s), and two oxymethine protons at δ 4.85 (1H, m), 3.89 (1H, br d, J=8.1 Hz). A combination of ^{13}C NMR and DEPT spectra (Table I) indicated the remaining moiety consisted of 29 carbons: Me (X6), CH_2 (X10), CH (X10, two CH−O) and three quaternary carbons. Furthermore, comparison of ^{1}H NMR and ^{13}C NMR (Table I) spectral data of **1** with those of β-sitosterol acetate[12], stigmast-5-ene-3β,7β-diol (**14**)[13] and stigmast-5-ene-3β,7α-diol[14] suggested that **1** was a stigmastane type compound, 3β-vanilloyloxy-stigmast-5-ene-7β-ol. The location of the ester function (vanilloylate at C-3) could establish by the acylation effects, such as comparison of the ^{1}H NMR spectra of **1** with **14** revealed that

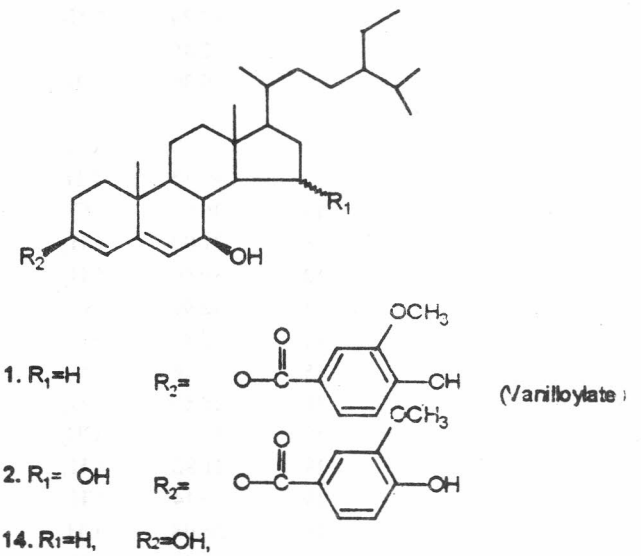

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Table I—^{13}C NMR data of compounds **1-3** recorded in $CHCl_3$ at 100 MHz (chemical shifts in δ, ppm downfield from TMS)

Carbon No.	1	DEPT	2	DEPT	3	DEPT
1	36.70	CH_2	6.72	CH_2	36.90	CH_2
2	27.84	CH_2	27.79	CH_2	30.07	CH_2
3	73.84	CH	73.84	CH	66.41	CH
4	37.74	CH_2	37.63	CH_2	34.88	CH_2
5	142.46	C	142.12	C	79.40	CH
6	126.30	CH	126.03	CH	130.72	CH
7	73.27	CH	73.71	CH	135.41	CH
8	40.79	CH	40.18	CH	82.14	C
9	48.17	CH	46.90	CH	51.08	CH
10	36.57	C	37.39	C	37.08	C
11	21.03	CH_2	20.44	CH_2	20.61	CH_2
12	39.50	CH_2	39.45	CH_2	39.33	CH_2
13	42.91	C	43.56	C	44.54	C
14	55.88	CH	61.60	CH	51.66	CH
15	26.36	CH_2	72.54	CH	28.62	CH_2
16	28.53	CH_2	38.85	CH_2	23.38	CH_2
17	55.33	CH	53.28	CH	56.18	CH
18	11.82	CH_3	13.16	CH_3	12.85	CH_3
19	19.14	CH_3	19.12	CH_3	18.15	CH_3
20	36.08	CH	35.89	CH	39.70	CH
21	18.82	CH_3	18.67	CH_3	19.62	CH_3
22	33.95	CH_2	33.83	CH_2	132.29	CH
23	26.07	CH_2	26.07	CH_2	135.19	CH
24	45.82	CH	45.73	CH	42.76	CH
25	29.12	CH	29.08	CH	33.04	CH
26	19.00	CH_3	18.96	CH_3	19.92	CH_3
27	19.79	CH_3	19.78	CH_3	20.86	CH_3
28	23.04	CH_2	23.03	CH_2	17.54	CH_3
29	11.96	CH_3	11.95	CH_3		
	Vanilloyloxyl groups					
1′	165.75	CO	165.77	CO		
2′	122.74	C	122.60	C		
3′	111.71	CH	111.76	CH		
4′	149.93	C	150.02	C		
5′	146.13	C	146.21	C		
6′	113.96	CH	114.04	CH		
7′	124.10	CH	124.07	CH		
OMe	56.09	CH_3	56.05	CH_3		

The ^{13}C NMR data of compounds **1** and **2** were assigned by comparison to those of **14** and methyl vanilloyloate; The ^{13}C NMR data of compound **3** was assigned by comparison to those of 5α,8α-peroxide ergosterol[17].

the shift of the signal at δ 3.57 (1H, m, H-3) in **14** shifted downfield to δ 4.85 (1H, m) in **1**, while the comparison of the ^{13}C NMR spectra of **14** and **1** indicated that the C-3 resonance of **14** shift downfield from δ 71.43 to δ 73.84, and the C-2, C-4 resonances shifted upfield from δ 31.58, 41.74 to δ 27.62, 37.74, respectively. Therefore **1** was 3β-vanilloyloxy-stigmast-5-ene-7β-ol.

3β-Vanilloyloxy-stigmast-5-ene-7β,15ξ-diol (**2**), white crystals from pet. ether-acetone, showed almost identical IR spectral data of those of **1**. The EIMS gave molecular ion peak at m/z 596 corresponding to molecular formula $C_{37}H_{56}O_6$. The ^1H, ^{13}C NMR (DEPT) spectra (Table I) were similar to those of **1** except different chemical shifts H-15, C-14, C-15, C-16 (Table I). Further comparison of ^1H HMR and ^{13}C NMR spectral data of **1** with those of **2**, the substituted effects[15] (α, β effects) of the extra hydroxyl group at C-15 of compound **2** made the H-15, C-14, C-15, C-16 in **1** shift down-

field from 1.3, 55.88, 26.36, 28.53 to 4.08, 61.60, 72.54, 38.85, respectively. Thus, the structure of **2** was also confirmed.

Experimental Section

Melting points were recorded on a Kofler Melting point apparatus and uncorrected. All optical rotation were determined with a JASCO-20C automatic recording spectropolarimeter. Mass spectra were obtained on a VG ZAB-HS mass spectrometer using a 70 eV electron impact ionization, IR spectra were run on a Nicolet 170 SX FT-IR instrument and ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.16 MHz) spectra on a Bruker AM 400 FT-NMR spectrometer in $CDCl_3$ with TMS as internal standard. Silica gel (200-300, 300-400 mesh) was used for column chromatography and silica gel GF_{254}, G and H for TLC. Spots were detected on the TLC under UV light or by heating after spraying with 5% H_2SO_4.

Collection of plant material. The plant material was collected in August 1994 in Qingyang county, Gansu Province of China and identified by Y.S. Zhou of Lanzhou University. A voucher specimen has been preserved at the Herbarium of our institute.

Extraction and isolation of compounds. The air-dried roots of *A. stenanthina* subsp. *xifengensis* (6.9 kg) were powdered and extracted three times (each 3 day) with 95% and 70% EtOH at room temperature, respectively. The portion that dissolved in EtOAc (30.5 g) out of all extract (501 g) was subjected to column chromatography over silica gel (700 g, 200-300 mesh) with pet. ether (60-90°)-Me_2CO gradient, when seven crude fractions were obtained (fractions 1-7). Fraction 1 (pet. ether-Me_2CO; 40:1) was further separated by repeated column chromatography over silica gel using pet. ether-Et_2O (30:1) and pet. ether-EtOAc (50:1) as eluants, and purified by recrystallization with Me_2CO, $CHCl_3$, EtOAc giving 30 mg of **8**, 50 mg of **6** and 40 mg of **7** respectively. Fraction 2 (pet. ether-Me_2CO; 30:1) on recrystallization gave compound **11** (150 mg); **12** (200 mg) and **13** (80 mg). Compound **4** was obtained from fraction 3 (pet. ether-Me_2CO; 20:1) by recrystallization with MeOH. Fraction 4 (pet. ether-Me_2CO; 15:1) on repeated chromatographic purification over a silica gel column and eluting with pet. ether-$CHCl_3$-Me_2CO (5:5:1), and by crystallization several times with heating MeOH gave pure compound **9** (30 mg) and **10** (152 mg). Fraction 5 (pet. ether-Me_2CO; 8:1) was further separated by repeated column chromatography, over silica gel using pet. ether-Me_2CO (5:1) and C_6H_6-Et_2O (4:1) as eluants giving 20 mg of **1**, which was purified by TLC (development C_6H_6-Et_2O, 3:1), and 30 mg of **3**. Fraction 6 (pet. ether-Me_2CO; 5:1) was purified by a silica gel column (eluting C_6H_6-Et_2O; 2:1) and TLC (running: $CHCl_3$-EtOAc; 7:1) to give pure **2** (20 mg). Fraction 7 (pet. ether-Me_2CO; 2:1) was subjected to column chromatography over silica gel and eluted with $CHCl_3$-MeOH (10:1) to yield **5** (1.2 g).

The known compounds were identified either by comparing their corresponding properties (mps, Mass, IR, ^{1}H- and ^{13}C NMR) with literature values or comparing with authentic samples.

3β-Vanilloyloxy-stigmast-5-ene-7β-ol (1). White crystals, m.p. 187-90°C; $[\alpha]_D^{20}$-340°C (c 1, $CHCl_3$), (Found: C, 76.45; H, 9.70. Calcd for $C_{37}H_{56}O_5$: C, 76.51; H, 9.72%); IR (KBr): 3648, 3426, 2952, 2870, 1708, 1597, 1514, 1462, 1428, 1376, 1284, 1220, 1108, 1033, 949, 910, 878, 763 cm^{-1}; EIMS (70 eV): m/z 580 $[M]^+$ (4%), 562 (2), 412 (100), 394 (26), 269 (9), 253 (11), 168 (54), 151 (38); ^{1}H NMR ($CDCl_3$, TMS): 7.64 (1H, dd, J=8.3, 1.6 Hz), 7.54 (1H, d, J=1.6 Hz), 6.93 (1H, d, J=8.3 Hz), 5.36 (1H, br s), 4.85 (1H, m), 3.95 (3H, s), 3.89 (1H, br d, J=8.1 Hz).

3β-Vanilloyloxy-stigmast-5-ene-7β,15ξ-diol (2). White crystals, m.p. 194-96°C; $[\alpha]_D^{20}$-332°C (c 0.98, $CHCl_3$); IR (KBr): 3645, 3421, 2950, 2872, 1708, 1599, 1515, 1462, 1428, 1377, 1283, 1220, 1108, 1031, 950, 911, 878, 765 cm^{-1}; EIMS (70 eV): m/z 596 $[M]^+$ (4%), 578 (2), 428 (70), 410 (100), 392 (23), 269 (38), 267 (37), 251 (19), 168 (81), 151 (63); ^{1}H NMR ($CDCl_3$, TMS): 7.64 (1H, dd, J=8.3, 1.6 Hz), 7.54 (1H, d, J=1.6 Hz), 6.93 (1H, d, J=8.3 Hz), 5.39 (1H, br s), 4.85 (1H, m), 4.08 (1H, m), 3.98 (1H, br d, J=8.1 Hz), 3.93 (3H, s).

Ergost-6,22-dien-3β,5α,8α-triol (3). White crystals from pet. ether-acetone, m.p. 178-180°C; $[\alpha]_D^{20}$-2.1° (*c* 1, $CHCl_3$); (Found: C, 77.98; H, 10.58%, Calcd: C, 78.09; H, 10.76%); IR (KBr): 3501, 3292, 2957, 2926, 2857, 1460, 1376, 1301, 1224, 1159, 1104, 1075, 1043, 970, 860, 777, 721, 695 cm^{-1}; EIMS (70 eV): m/z 430 $[M^+]$ (17%), 412 (100), 398 (61); ^{1}H NMR ($CDCl_3$, TMS): 6.51 (1H, d, J=8.5 Hz, H-7), 6.25 (1H, d, J=8.5 Hz, H-6), 5.21 (1H, dd, J=15.3, 7.4 Hz, H-22), 5.16 (1H, dd, J=15.3, 8.2 Hz, H-23), 3.97 (1H, m, H-3). The ^{1}H NMR and ^{13}C NMR data have been reported herein for the first time.

Taraxerone (6). White plates from $CHCl_3$, m.p. 247-48°C; IR, EIMS and ^{1}H NMR data identical with those reported in the literature[4,5]; ^{13}C NMR ($CDCl_3$, TMS):38.34 (C-1, CH_2), 34.14 (C-2, CH_2), 217.61 (C-3, CO), 47.58 (C-4, C), 55.77 (C-

5, CH), 17.44 (C-6, CH_2), 36.57 (C-7, CH_2), 38.67 (C-8, C), 48.69 (C-9, CH), 35.77 (C-10, C), 19.95 (C-11, CH_2), 33.35 (C-12, CH_2), 37.53 (C-13, C), 157.61 (C-14, C), 117.20 (C-15, CH), 33.06 (C-16, CH_2), 48.77 (C-17, C), 48.69 (C-18, CH), 40.62 (C-19, CH_2), 28.77 (C-20, C), 35.09 (C-21, CH_2), 37.68 (C-22, CH_2), 26.09 (C-23, CH_3), 21.03 (C-24, CH_3), 14.80 (C-25, CH_3), 29.84 (C-26, CH_3), 25.56 (C-27, CH_3), 29.84 (C-28, CH_3), 21.48 (C-29, CH_3), 33.34 (C-30, CH_3). The ^{13}C NMR spectral data have not been reported in the literature so far.

Lupenone (7). White crystals from EtOAc, m.p. 170-71°C; EIMS and ^1H NMR data were identical with those reported in the literature[6].

Glutinone (8). White tetrahedrons from acetone, m.p. 223-25°C; ^1H NMR ($CDCl_3$, TMS): 0.83 (3H, s), 0.97 (3H, s), 1.00 (3H, s), 1.04 (3H, s), 1.10 (3H, s), 1.18 (3H, s), 1.25 (6H, s), 2.45 (2H, m), 5.70 (1H, m); m.p. and EIMS was identical with those reported in the literature[7,16].

Dodecylpalmitate (13). White wax, m.p. 46-47°C; EIMS (70 eV); 424 [M^+] (1%), 409 (0.3), 395 (0.3), 381 (0.3), 367 (0.3), 353 (0.3), 339 (0.3), 325 (0.3), 311 (0.3), 297 (0.3), 283 (0.3), 269 (0.3), 256 (13), 213 (10), 185 (7), 171 (6), 157 (6), 129 (22), 115 (9), 111 (5), 97 (17), 85 (17), 83 (19) 73 (81), 71 (28), 69 (31), 60 (82), 57 (64), 55 (58), 43 (100).

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