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Two new steroids from *Adenophora stenanthina* subsp. *xifengensis*

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Two new steroids, 3β-vanilloyloxy-stigmast-5-ene-7-one, and stigmast-5-ene-3β,7β,15α-triol were isolated from the roots of *Adenophora stenanthina* subsp. *xifengensis* along with four known compounds, stigmast-5-ene-3β-ol-7-one,stigmast-5-ene-3β,7β-diol, syringin, sinapyl alcohol 1, 3′-di-O-β-D-glucopyranoside. Their structures were elucidated on the basis of spectral methods.

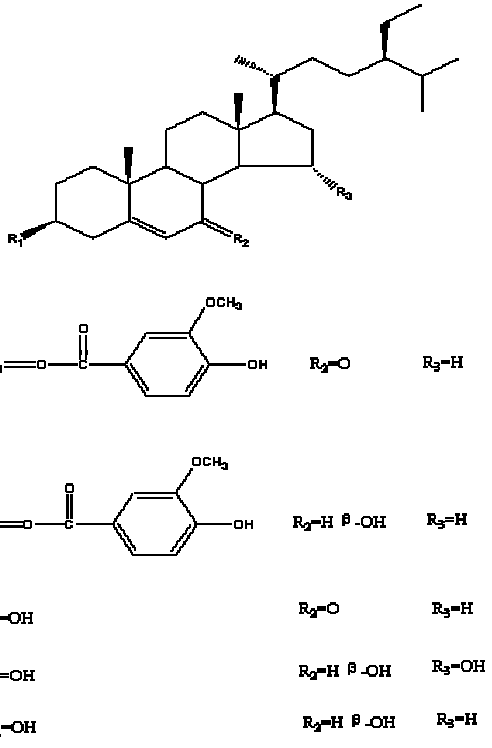
1. Introduction

Adenophora stenanthina subsp. *xifengensis,* mainly distributed in Gansu province of China, has been used as a traditional Chinese medicine since ancient time for the treatment of cough, phlegm, and especially to cure lung disease [1]. We earlier reported the isolation and structural elucidation of two new steroids, 3β-vanilloyloxy-stigmast-5-ene-7β-ol and 3β-vanilloyloxy-stigmast-5-ene-7β,15ξ-diol, along with eleven known compounds [2]. The present paper describes the further isolation and structural elucidation of two other new steroids and four known compounds.

2. Investigations, results and discussion

From the EtOAc and n-BuOH extracts of the roots of *Adenphora stenanthina* subsp. *xifengensis* two new steroids, 3β-vanilloyloxy-stigmast-5-ene-7-one (**1**) and stigmast-5-ene-3β-,7β,15α-triol (**4**), along with four known compounds, stigmast-5-ene-3β-ol-7-one (**3**), stigmast-5-ene-3β,7β-diol (**5**) [3], syringin (**6**), sinapyl acohol 1,3′-di-O-β-D-glucopyranoside (**7**) [4] were isolated by repeated column chromatography and preparative TLC. The known compounds were identified by comparison of their physical and spectral properties with those reported in the literature, whereas the structures of new compounds were deduced as follows.

Compound **1** was obtained as white crystals from methanol, m.p. 198–200 °C. An $[M]^+$ at m/z 578 in EI-MS along with analysis of ^1H, ^{13}C NMR and DEPT spectra showed its molecular formula to be $C_{37}H_{54}O_5$. The ^1H NMR spectrum of **1** displayed aromatic ABX-type protons at δ 7.64 (1 H, dd, J = 8.3, 1.6 Hz), 7.54 (1 H, d, J = 1.6 Hz), 6.94 (1 H, d, J = 8.3 Hz), and the signal of a methoxy group at δ 3.96 (3 H, s). The ^{13}C NMR spectrum of **1** exhibited six aromatic carbons at δ 150.13, 146.20, 124.16, 122.37, 114.02, 111.74, a carbonyl carbon at δ 165.55, and the signal of a methoxy group at δ 56.11. The EI-MS gave the fragment peaks at m/z 410 (basic peak, M^+-vanillic acid), 168 (vanillic acid), and 151 (vanilloyl). Based on the above data, (**1**) was assumed to contain a vanilloyl group. In addition to the signals of the vanilloyl moiety, the ^1H NMR spectrum displayed an olefinic proton at δ 5.75 (br s), an oxymethine proton at δ 4.94 (tt, J = 11.4, 5.0 Hz), and the signals of six methyl groups at δ 1.26(s), 0.94 (d, J = 6.5 Hz), 0.85 (t, J = 7.8 Hz), 0.84 (d, J = 6.8 Hz), 0.82 (d, J = 6.8 Hz), 0.70 (s). The ^{13}C NMR and DEPT spectra indicated that the remaining moiety consisted of 29 carbons including the signals of an enone function at δ 202.02, 163.96, 126.75, an oxymethine carbon at δ 72.61, two quaternary carbons at δ 43.12, 38.38, and six methyl carbons at δ 19.78, 19.03, 18.92, 17.32, 11.96, 11.96. According to this information, and the presence of eleven degrees of unsaturation, **1** was supposed to possess a stigmastenone or poriferastenone skeleton with a vanilloyl moiety. Inspection of ^1H, ^{13}C NMR signals arising from the side chain of **1** and the comparison of chemical shifts of **1** with those of the relevant compounds, stigmast-5-ene-3β,6α-diol and poriferast-5-ene-3β,6α-diol [5], it was found that spectral data of **1** were in conformity with stigmast-5-ene-3β,6α-diol but not with poriferast-5-ene-3β,6α-diol. This revealed that **1** owned a stigmastenone skeleton. By further comparison of ^1H, ^{13}C NMR spectral data of **1** with those of known compounds, 3β-vanilloyloxy-stigmast-5-ene-7β-ol (**2**) [2] and stigmast-5-ene-7-one (**3**) [6], it was found that ^1H NMR spectral data of H-3, H-2′, H-5′, H-6′ of **1** were in accordance with those of **2**, and those of H-6 was in conformity with **3**, and ^{13}C NMR spectral data at C-1 to C-4, C-1′ to C-8′ of **1** were in areement with those of **2**, and those at C-5 to C-29 of **1** corresponded with **3**. The fact suggested that the location and orientation of vanilloyloxy were at C-3β, the location of enone group were at C-5 to C-7. Furthermore, compound **1** was subjected to methanolysis to yield stigmast-5-



ene-3β-ol-7-one and methyl vanilloylate which were identified by TLC with authentic compounds. On the basis of the above evidence, the structure of **1** was elucidated to be 3β-vanilloyloxy-stigmast-5-ene-7-one.

Compound **4** was obtained as white crystals from methanol, m.p. 168–170 °C. An $[M]^+$ at m/z 446 in EI-MS along with analysis of ^1H, ^{13}C NMR and DEPT spectra showed its molecular formula to be $C_{29}H_{50}O_3$. The ^1H NMR spectrum displayed an olefinic proton at δ 5.33 (br s), three oxymethine protons at δ 4.08 (td, J = 10.0, 3.0 Hz), 3.95 (br d, J = 8.1 Hz), 3.54 (tt, J = 11.4, 5.0 Hz), and the signals of six methyl groups at δ 1.06 (s), 0.92 (d, J = 6.5 Hz), 0.85 (t, J = 7.8 Hz), 0.84 (d, J = 6.8 Hz), 0.82 (d, J = 6.8 Hz), 0.78 (s). The ^{13}C NMR and DEPT spectra exhibited 29 carbons including two olefinic carbons at δ 143.14, 125.15, three oxymethine carbons at δ 73.78, 72.55, 71.42, two quaternary carbons at δ 43.56, 37.24, and six methyl carbons at δ 19.76, 19.10, 18.96, 18.66, 13.13, 11.94. Taking into account the above data for five degrees of unsaturation, **4** was assumed to own a stigmastene or periferastene skeleton with three hydroxyl. Comparison of ^1H, ^{13}C NMR spectral data of the side chain of **4** with those of the relevant compounds, stigmast-5-ene-3β,6α-diol and poriferast-5-ene-3β,6α-diol [5], it was found that ^1H, ^{13}C NMR spectral data of **4** were in conformity with stigmast-5-ene-3β,6α-diol but not with poriferast-5-ene-3β,6α-diol. This demonstrated that **4** possessed a stigmastene skeleton. The ^1H, ^{13}C NMR spectra were similar to those of the known compound stigmast-5-ene-3β,7β-diol (**5**) [3] except different H-15, C-14, C-15, C-16. Further comparison of spectral data of **4** with those of **5**, the substituted effects of extra hydroxyl [7] at C-15 of **4** made the H-15, C-14, C-15, C-16 in **4** shift downfield from 1.3, 55.37, 26.36, 28.51 to 4.08, 61.67, 72.55, 38.83, respectively. The fact suggested that the location and orientation of three hydroxyl groups were at C-3β, C-7β and C-15. The orientation of the 15-OH was presumed to be α based on comparison of the chemical shifts and splitting pattern of **4** with those of the relevant compounds, 15α- and 15β-hydroxysteroids [8]. The splitting pattern at H-15 of **4** was the same and the chemical shifts at H-15, CH_3-18 in the ^1H NMR spectrum and C-15, C-16, C-17, C-18 in ^{13}C NMR spectrum of **4** were parallel to 15α-hydroxysteroids, (22 E, 24 S, 25 S)-23-methyl-5α-ergost-22-en-3β,5,6β,15α,25,26-hexaol 26-sulfate, but not 15β-hydroxysteroids, (22 E, 25 S)-5α-cholest-22-en-3β,5,6β,15β,25,26-hexaol. Using a NOE difference spectra experiment, irradiation at δ 4.08 (15-H) enhanced the intensity of the signal at δ 0.78 (18-Me), and irradiation at δ 0.78 enhanced the intensity of the signal at δ 4.08. Thus, the orientation of 15-H was decided to be β, and in conclusion, 15-OH had α-orientation. Based on the above arguments, the structure of **4** was elucidated as stigmast-5-ene-3β,7β,15α-triol.

3. Experimental

3.1. Equipment

Melting points were recorded on a Kofler apparatus and were uncorrected. IR spectra were run on a Nicolet 170 SX FT-IR instrument; EI-MS and FAB-MS were determined on a VG ZAB-HS mass spectrometer using 70 eV electron impact ionization; ^1H NMR (400.13 MHz) and ^{13}C NMR (100.16 MHz) spectra were recorded with a Bruker AM 400 FT-NMR spectrometer in $CDCl_3$ and C_5D_5N using TMS as internal standard.

3.2. Plant material

Adenophora stenanthina subsp. *xifengensis* was collected in August 1997 in Qingyang county, Gansu Province, P.R. China and identified by Professor Y. S. Zhou of Lanzhou University.

3.3. Extraction and isolation

The air-dried roots of *Adenophora stenanthina* subsp. *xifengensis* (5.8 kg) were powdered and extracted two times (each three days) with 95% and 70% EtOH at room temperature, respectively. The extract was concentrated under reduced pressure. The residue suspended in H_2O and then extracted with EtOAc and n-BuOH. The EtOAc extract (38 g) was subjected to CC over silica gel with pet. ether–Me_2CO (40 : 1–1 : 1) gradient, when seven crude fractions were obtained (fractions 1–7). Fraction 4 (pet. ether–Me_2CO; 15 : 1) was further separated by repeated CC over silica gel using pet. ether–Et_2O (4 : 1) and pet. ether–EtOAc (6 : 1) as eluants giving **3** and **5** (35 mg). Fraction 5 (pet. ether–Me_2CO; 8 : 1) was further separated by repeated CC over silica gel using C_6H_6–Et_2O (5 : 1) and C_6H_6–Me_2CO (8 : 1) as eluants giving **1** (30 mg) and **4** (25 mg). The n-BuOH extract (87 g) was chromatographed on a column of Amberlite XAD-4 nonionic polystyrene resin eluting initially with H_2O followed by 95% EtOH. The latter (27 g) was chromatographed on a silica gel with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.05–2 : 1 : 0.5) gradient, when five crude fractions were obtained (fractions 8–12). Fraction 8 ($CHCl_3$–MeOH–H_2O; 9 : 1 : 0.05) was subjected to CC over silica gel and eluted with EtOAc–MeOH–H_2O (20 : 1 : 0.1) to yield **6** (20 mg). Fraction 10 ($CHCl_3$–MeOH–H_2O; 4 : 1 : 0.2) was subjected to CC over silica gel and eluted initially with EtOAc–MeOH–H_2O (8 : 1 : 0.2) followed by EtOAc–EtOH–H_2O (8 : 1 : 0.2) to obtain **7** (50 mg).

Table 1: ^{13}C NMR spectral data of compounds 1, 2, 3, 4 and 5 (100 MHz, $CHCl_3$)

No.	1	2	3	4	5	No.	1	2	3	4	5
1	37.93	36.70	36.4	36.95	36.93	20	36.07	36.08	36.1	35.88	36.07
2	27.52	27.84	31.2	31.63	31.56	21	18.92	18.81	18.9	18.66	18.81
3	72.61	73.84	70.5	71.42	71.42	22	33.93	33.95	33.9	33.85	33.97
4	38.67	37.74	41.8	41.60	41.71	23	26.10	26.07	26.1	26.11	26.11
5	163.96	142.46	165.2	143.44	143.48	24	45.82	45.82	45.8	45.75	45.84
6	126.75	126.30	126.1	125.45	125.42	25	29.13	29.12	29.1	29.10	29.14
7	202.02	73.27	202.4	73.78	73.34	26	19.03	19.00	19.0	18.96	19.00
8	45.44	40.79	45.4	40.30	40.91	27	19.78	19.79	19.8	19.76	19.77
9	49.96	48.17	49.9	46.99	48.26	24^1	23.04	23.04	23.0	23.04	23.05
10	38.38	36.57	38.3	37.24	36.42	24^2	11.96	11.96	12.0	11.94	11.95
11	21.19	21.03	21.2	20.47	21.06						
12	38.55	39.50	38.7	39.44	39.54	1′	122.37	122.74			
13	43.12	42.91	43.1	43.56	42.91	2′	111.74	111.71			
14	49.83	55.88	49.9	61.67	55.37	3′	150.13	149.93			
15	26.31	26.36	26.3	72.55	26.36	4′	146.20	146.13			
16	28.54	28.53	28.5	38.83	28.51	5′	114.02	113.96			
17	54.69	55.33	54.7	53.32	55.95	6′	124.16	124.10			
18	11.96	11.82	12.0	13.13	11.79	7′	165.55	165.75			
19	17.32	19.14	17.3	19.10	19.12	8′	56.11	56.09			

Table 2: ^{1}H NMR spectral data of compounds 1, 2, 3, 4 and 5 (400 MHz, $CHCl_3$)

No.	1	2	3	4	5
3	4.94 (tt,11.4,5.0)	4.85 (m)	3.68 (tt, 11.0, 5.5)	3.54 (tt, 11.4, 5.0)	3.56 (m)
6	5.75 (br s)	5.36 (br s)	5.69 (d, 1.8)	5.33 (br s)	5.30 (br s)
7		3.89 (br d, 8.1)		3.95 (br d, 8.1)	3.86 (br d, 8.2)
15				4.08 (td, 10.0, 3.0)	1.3
2′	7.54 (d, 1.6)	7.54 (d, 1.6)			
5′	6.94 (d, 8.3)	6.93 (d, 8.3)			
6′	7.64 (dd, 8.3, 1.6)	7.64 (dd, 8.3, 1.6)			
$-OCH_3$	3.96 (s)	3.95 (s)			

3.4. 3β-Vanilloyloxy-stigmast-5-ene-7-one (1)

White crystals, m.p. 198–200 °C (MeOH). EI-MS (m/z, %): 578 (M^+, 6), 410 (100), 285 (8), 267 (12), 251 (19), 168 (57), 151 (41). ^{1}H NMR ($CDCl_3$): 7.64 (1 H, dd, J = 8.3, 1.6 Hz, H-6′), 7.54 (1 H, d, J = 1.6 Hz, H-2′), 6.94 (1 H, d, J = 8.3 Hz, H-5′), 5.75 (1 H, br s, H-6), 4.94 (1 H, m, H-3), 3.96 (3 H, s, $-OCH_3$), 1.26 (3 H, s, Me-19), 0.94 (3 H, d, J = 6.5 Hz, Me-21), 0.85 (3 H, t, J = 7.8 Hz, Me-24^2), 0.84 (3 H, d, J = 6.8 Hz, Me-26), 0.82 (3 H, d, J = 6.8 Hz, Me-27), 0.70 (3 H, s, Me-18). ^{13}C NMR: see Table 1.

3.5. Methanolysis of 1

Compound **1** (5 mg) was stirred in 10% NaOMe–MeOH (1 ml) for 2 h. H_2O was added and the product extracted with CH_2Cl_2. Removal of the solvent and subsequent CC on silica gel yielded stigmast-5-ene-3β-ol-7-one and methyl vanilloylate which were identified by TLC with authentic compounds.

3.6. Stigmast-5-ene-3β,7β,15α-triol (4)

White crystals, m.p. 168–170 °C (MeOH). EI-MS (m/z, %): 446 (M^+, 2), 428 (68), 410 (100), 392 (44), 305 (23), 287 (47), 269 (35), 229 (8), 213 (17), 175 (20), 135 (12), 69 (27), 43 (32). ^{1}H NMR ($CDCl_3$): 5.33 (1 H, br s, H-6), 4.08 (1 H, td, J = 10.0, 3.0 Hz, H-15), 3.95 (1 H br d, J = 8.1 Hz, H-7), 3.54 (1 H, tt, J = 11.4, 5.0 Hz, H-3) 1.06 (3 H, s, Me-19), 0.92 (3 H, d, J = 6.5 Hz, Me-21), 0.85 (3 H, t, J = 7.8 Hz, Me-24^2), 0.84 (3 H, d, J = 6.8 Hz, Me-26), 0.82 (3 H, d, J = 6.8 Hz, Me-27), 0.78 (3 H, s, Me-18). ^{13}C NMR: see Table 1.

3.7. Sinapyl alcohol 1,3′-di-O-β-D-glucopyranoside (7)

White powder. FAB-MS (m/z): 557 $[M + Na]^+$. ^{1}H NMR (C_5D_5N): 6.84 (2 H, s, H-2, H-6), 6.82 (1 H, d, J = 15.8 Hz, H-7), 6.58 (1 H, dt, J = 15.8, 5.1 Hz, H-8), 5.66 (1 H, d, J = 7.1 Hz, H-1′), 4.88 (1 H, d, J = 7.8 Hz, H-1″), 4.61 (1 H, dd, J = 12.0, 5.1 Hz, H-9a), 4.42 (1 H, dd, 12.0, 5.1 Hz, H-9b), 3.79 (6 H, s, $-OCH_3$). ^{13}C NMR (C_5D_5N): 153.93 (C-3, C-5), 135.50 (C-4), 134.03 (C-1), 130.97 (C-7), 129.56 (C-8), 105.14 (C-2, C-6), 104.76 (C-1′), 104.55 (C-1″), 78.05 (C-5′, C-5″), 77.94 (C-3′), 77.62 (C-3″), 75.61 (C-2′), 75.03 (C-2″), 71.44 (C-4′), 71.17 (C-4″), 69.29 (C-9), 62.72 (C- 6′), 62.51 (C-6″).

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