Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences; State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, People's Republic of China

Steroids from *Saussurea ussuriensis*

JIA-TAO FENG, YAN-PING SHI

Received June 23, 2004, accepted July 27, 2004

Prof. Yan-Ping Shi, PhD., Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China
shiyp@lzu.edu.cn/shiyp@ns.lzb.ac.cn

Pharmazie 60: 464–467 (2005)

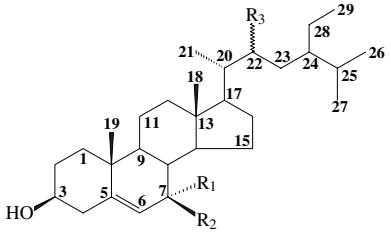
One novel and seven known steroids were isolated from the alcohol extract of the whole plant of *Saussurea ussuriensis*. The structure of the new compound was elucidated as stigmast-5-en-3β,7α,22-triol (**1**) by spectroscopic methods including intensive 2D NMR techniques (H^1-H^1 COSY, gHSQC, gHMBC) and HR-SI-MS and the known compounds were identified on the basis of comparing their NMR data with those of corresponding compounds in the literature.

1. Introduction

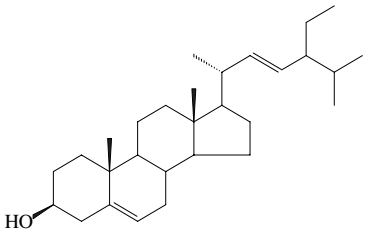
The genus *Saussurea* consists of about 400 species distributed throughout Asia and Europe. *Saussurea ussuriensis* is a perennial herb mainly grown in northwestern China and the rhizome has been used as a folk remedy for the treatment of cold, headache, and arthritis (Shih et al. 1999). Until now, its chemical constituents have not been investigated. In order to find its active principles, the chemical constituents of *Saussurea ussuriensis* were studied and a new steroid was isolated from the alcohol extract of the whole plant. In this paper we report the isolation and structural elucidation of the new and seven known steroids.

2. Investigations, results and discussion

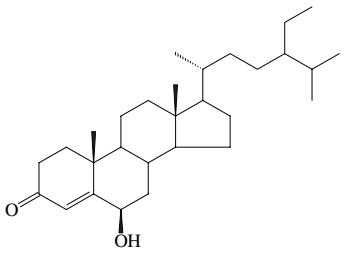
From the alcohol extract of the whole plant of *Saussurea ussuriensis*, a novel steroid, stigmast-5-en-3β,7α,22-triol (**1**), together with seven known steroids, stigmast-5-en-3β, 7α-diol (**2**) (Chaurasia et al. 1987; Greca et al. 1990; Mei et al. 1999), stigmast-5-en-3β, 7β-diol (**3**) (Chaurasia et al. 1987; Greca et al. 1990; Mei et al. 1999), stigmast-5-en-3β-ol-7-one (**4**) (Gao et al. 1997; Greca et al. 1990), β-sitosterol (**5**) (Chaurasia et al. 1987; Greca et al. 1990; Yoshiyasu et al. 1988); stigmasterol (**6**) (Rubinstein et al. 1976), stigmast-4-en-6β-ol-3-one (**7**) (Gao et al. 1997; Greca et al. 1990), ergostane-6, 22-dien-3β, 5α, 8α-triol (**8**) (Gao et al. 1997; Hou et al. 1999) were isolated. The structures of known compounds **2**–**4** were confirmed by comparing their corresponding properties (melting point,



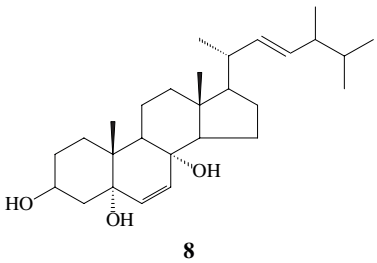
1 R_1=R_3=OH, R_2=H
2 R_1=OH, R_2=R_3=H
3 R_1=R_3=H, R_2=OH
4 R_1=R_2=O, R_3=H
5 R_1=R_2=R_3=H



6



7



8

MS, IR, ^{1}H NMR and ^{13}C NMR) with the reported values in the literature and compounds **5** and **6** were confirmed by comparison with authentic samples. To the best of our knowledge compound **1** is a new constituent isolated from this species.

Compound **1** was obtained as a white solid, $[\alpha]_D^{25}$ −74° (C 0.13, $CHCl_3$). Its molecular formula was assigned as $C_{29}H_{50}O_3$ on the basis of the HR-SI-MS spectral data [M-H_2O + H]$^+$ at m/z 429.3727, which could be supported by the ^{13}C NMR spectrum displayed 29 carbons including six methyls, nine methylenes, eleven methines and three quaternary carbons, assigned by DEPT experiment. The compound had five degrees of unsaturation. Its UV spectrum showed bands at 217 (log ε 3.15), 239 (log ε 3.28) and 282 nm (log ε 2.68), and the IR spectrum showed absorption bands for hydroxyl (3366 cm^{-1}) and double bond (1653 cm^{-1}) functions. In the downfield region of the ^{13}C NMR spectrum, there were two characteristic signals at $\delta_C = 146.36$ and $\delta_C = 123.80$ due to a double bond. The ^{1}H NMR spectrum indicated the presence of six methyl groups, of which two were singlets (δ 0.72, 1.00) positioned on tertiary carbons and three were doublets (δ 0.94, d, J = 6.8 Hz; δ 0.90, d, J = 6.8 Hz; δ 0.79, d, J = 6.8 Hz), an olefinic proton (δ 5.61, d, J = 5.6 Hz), as well as three hydroxymethine protons (δ 3.60, m; δ 3.72, br d, J = 10.8; δ 3.86, br s). The above spectral data suggested that compound **1** was a tetracyclic steroid with three hydroxyl groups and one trisubstituted double bond. Comparison of ^{13}C NMR spectroscopic data of **1** with those of known steroids (Chaurasia et al. 1987; Gao et al. 1997; Greca et al. 1990; Mei, et al. 1999; Luo et al. 2001) and compounds **2** and **3** showed that the chemical shifts of rings A, B, C and D are almost same as those of stigmast-5-en-3β,7α-diol (**2**) and the differences are from the side chain. So compound **1** was considered to be a stigmast-5-en-3β,7α-diol with an extra hydroxyl group at the side chain. The extra hydroxyl group could preliminarily be considered to be attached at C-22 because of the downfield shifts of C-20, C-22 and C-23, together with the upfield shift of C-21 from δ_C 18.99 in **2** to 12.30 in **1** caused by the γ-Gauch effect of 22-OH. To confirm the linking position of the only extra hydroxyl group, compound **1** was further subjected to 2D NMR experiments for gCOSY, gHSQC and gHMBC, respectively. The location of the hydroxyl group was further assigned using the gCOSY and gHSQC experiments: H-22 (δ_H 3.72, δ_C 71.25) showing correlation with H-21 (δ 0.94), H-20 (δ 1.26, m) and H-23 (δ 1.05, 1.24). This result is also supported by two pieces of evidence: the key correlation between H-21 and C-22 in the gHMBC experiment and the identical chemical shifts of the side chain in **1** and known taiwaniasterols owning 22-hydroxyl side chain (Lin et al. 1998). Hence, the structure of **1** was elucidated as stigmast-5-en-3β,7α,22-triol.

3. Experimental

3.1. Equipment

Melting points: X-4 Digital Display Micro-Melting point apparatus, uncorr. Optical rotation: Perkin Elmer 241 polarimeter, solvent $CHCl_3$. UV-spectra were measured on a Spect 50-UV/Vis spectrophotometer in MeOH solution (Analytic Jena AG). IR-spectra were recorded on a Nicolet NEXUS 670 FT-IR infrared spectrophotometer. ^{1}H NMR (400.13 MHz), ^{13}C NMR (100.62 MHz) and 2D NMR were recorded on a Varian INOVA-400 FT-NMR spectrometer (USA) in $CDCl_3$ with TMS as internal standard. HR-SI-MS (High resolution secondary ionization mass spectrometry) was determined on a Bruker APEX II. Separation and purification were performed by chromatographic column (CC) over silica gel. Silica gel (200–300 mesh) used for CC and silica gel GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qindao, P. R. China. Spots were detected on the TLC under UV light or by heating to over 110 °C after spraying with 98% H_2SO_4-EtOH (v : v = 5 : 95).

3.2. Plant material

The plant material (No. 2001–02) was collected from Zhang county, Gansu province of P. R. China and was identified by adjunct Prof. Ji MA, Faculty of Pharmacy, First Military Medical University of PLA, Gangzhou, P. R. of China. A specimen has been deposited at our laboratory.

3.3. Extraction and isolation

The air-dried whole plant of *Saussurea ussuriensis* (5.2 kg) was powdered and extracted with 95% EtOH at room temperature (20 L × 4, each extraction lasted 7 days). The combined extracts were evaporated to dryness under reduced pressure. The residue (240 g) was then suspended in H_2O (1.5 L), and extracted with petroleum ether (60–90 °C) (1.0 L × 4), EtOAc (1.0 L × 4) and *n*-BuOH (1.0 L × 4), respectively. The petroleum ether (60–90 °C) extract (94 g) was subjected to column chromatography on silica gel (1000 g) using petroleum ether (60–90 °C) with an increasing volume of acetone (v : v = 40 : 1, 20 : 1, 15 : 1, 10 : 1, 7 : 1, 4 : 1, 2 : 1, 1 : 1, each about 4.0 L) as eluent. The fractions A1-A10 were collected according to TLC analysis. Compound **6** (100 mg) was obtained from fraction A1 (0.9 g) after CC on silica gel (50 g) with petroleum ether-EtOAc (v : v, 15 : 1) and repeated recrystallization. Fraction A2 (3.5 g) was further frac-

Table 1: ^{1}H (400.13 MHz) and ^{13}C NMR (100.62 MHz) data, ^{1}H-^{1}H COSY and HMBC for compound 1

No.	δ_H	δ_C	COSY	HMBC
1	1.89 m	37.00	H-2	
2	1.89 m 1.52 m	31.35	H-1, H-3	
3	3.60 m	71.32	H-2, H-4	
4	2.32 m	41.98	H-3, H-6	
5		146.36		H-19
6	5.61 d (5.6)	123.80	H-4, H-7	H-19
7	3.86 br s	65.32	H-6, H-8	
8	1.48 m	37.59	H-7, H-9, H-14	H-6
9	1.27 m	42.28	H-8, H-11	H-19
10		37.51		H-19
11	1.55 m	20.70	H-9, H-12	
12	1.22 m 2.01 m	39.16	H-11	H-18
13		42.47		H-18
14	1.46 m	49.09	H-8, H-15	H-18
15	1.77 m	24.41	H-14, H-16	
16	1.79 m	28.71	H-15, H-17	
17	1.21 m	52.80	H-16, H-20	H-18, H-21
18	0.72 s	11.64		
19	1.00 s	18.24		
20	1.26 m	42.47	H-17, H-21, H-22	H-21
21	0.94 d (6.8)	12.30	H-20	
22	3.72 br d (10.8)	71.25	H-20, H-23	H-21
23	1.05 m 1.24 m	29.83	H-22, H-24	
24	1.74 m	41.37	H-23, H-25, H-28	H-27, H-29
25	1.79 m	28.69	H-26, H-27	H-26, H-27
26	0.90 d (6.8)	20.57	H-25	H-27
27	0.79 d (6.8)	17.50	H-25	H-26
28	1.24 m 1.36 m	23.60	H-24, H-29	H-29
29	0.87 t (7.2)	11.89	H-28	

Assignments were aided by spin splitting patterns, DEPT, gCOSY, gHSQC, gHMBC experiments, and chemical shift values (δ) The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.00 ppm)

tionated on a silica gel column (80 g) using petroleum ether-EtOAc (v : v, 8 : 1) to give impure compound **5**, which was purified: firstly, on a silica gel column (60 g) using $CHCl_3$-Et_2O (v : v, 30 : 1); then on another silica gel column (30 g) with $CHCl_3$-EtOAc (v : v, 30 : 1) to yield pure **5** (6 mg). Fraction A4 (2.3 g) was further subjected to column chromatography on silica gel column (75 g) eluting with $CHCl_3$-acetone (v : v, 30 : 1) to give raw compound **7** (15 mg), which was further purified by CC (30 g) with petroleum ether-EtOAc (v : v, 5 : 1) as eluent and another CC (20 g) with $CHCl_3$-Et_2O (v : v, 4 : 1). Fraction A5 (2.9 g) was further fractionated on a silica gel column (90 g) eluting with $CHCl_3$-acetone (v : v, 30 : 1) to give three subfractions (fr.A5a, fr.A5b and fr.A5c). Fr. A5a (0.7 g) was further subjected to column chromatography on a silica gel column (50 g) eluting with petroleum ether-EtOAc (v : v, 4 : 1) to obtain impure **4** (35 mg), which was further purified by CC (25 g) with $CHCl_3$-Et_2O (v : v, 3 : 1). Fr.A5b (1.0 g) was purified using CC on silica gel (60 g) with petroleum ether-EtOAc (v : v, 4 : 1) and CC on silica gel (30 g) with $CHCl_3$-acetone (v : v, 20 : 1) to yield pure **8** (50 mg). Fraction A6 (1.4 g) was further subjected to column chromatography over silica gel (60 g) eluting with $CHCl_3$-acetone (v : v, 20 : 1) to give a mixture of compounds **2** and **3**, that was further isolated and purified: firstly, on a silica gel column (40 g) using petroleum ether-EtOAc (v : v, 2 : 1); then on another silica gel column (20 g) with petroleum ether-acetone (v : v, 3 : 1) to yield compound **2** (30 mg) and 3 (10 mg). Fraction A7 (3.1 g) was chromatographed on a silica gel column (120 g) and eluted with $CHCl_3$-acetone (v : v, 15 : 1) to afford impure **1** (5 mg), which was repeatedly purified by CC over silica gel (25 g) with petroleum ether-acetone (v : v, 3 : 1) as eluent.

Table 2: ^{1}H NMR (400.13 MHz) data for compounds 2, 3, 4, 7, 8

Proton	2	3	4	7	8
H-3	3.58 m	3.56 m	3.66 m		3.94 m
H-4				5.81 s	
H-6	5.61 d (4.0)	5.29 s	5.68 d (1.6)	4.35 s	6.22 d (8.4)
H-7	3.86 m	3.86 d (4.4)			6.48 d (8.4)
H-18	0.68 s	0.68 s	0.67 s	0.74 s	0.82 s
H-19	1.00 s	0.99 s	1.19 s	1.38 s	0.88 s
H-21	0.93 d (6.4)	0.93 d (6.4)	0.92 d (6.4)	0.92 d (6.4)	0.94 d (6.4)
H-22					5.12 dd (15.4, 8.1)
H-23					5.20 dd (15.4, 7.3)
H-26	0.84 d (6.4)	0.84 d (6.4)	0.84 d (6.4)	0.83 d (6.4)	0.84 d (6.4)
H-27	0.81 d (6.4)	0.82 d (6.4)	0.87 d (6.4)	0.81 d (6.4)	0.80 d (6.4)
H-29	0.86 t (6.8)	0.85 d (6.8)	0.82 t (6.8)	0.85 t (7.2)	0.99 t (7.2)

(δ, in $CDCl_3$, TMS as int. standard, coupling constants in parentheses)

3.4. Compounds isolated

3.4.1. Stigmast-5-en-3β,7α,22-triol (7α, 22-dihydroxysitosterol) (**1**)

White solid. M.p. 130–132 °C, $[\alpha]_D^{25}$ −74° (C 0.13, $CHCl_3$). HR-SI-MS: $[M\text{-}H_2O + H]^+$, found: 429.3727; Calcd.: 429.3724 for $C_{29}H_{49}O_2$, 411.3622 $[M\text{-}2H_2O + H]^+$, 393.3516 $[M\text{-}3H_2O + H]^+$. IR (KBr, cm^{-1}): 3366, 2926, 2855, 1742, 1712, 1653, 1462, 1376, 1262, 1166, 1099, 1056, 1098, 1024, 956, 887, 803, 720. UV (λ_{max}): 217 (log ε 3.15), 239 (log ε 3.28) and 282 nm (log ε 2.68). ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 1, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 1.

3.4.2. Stigmast-5-en-3β,7α-diol (7α-hydroxysitosterol) (**2**)

Colorless needle-shaped crystals from Me_2CO. M. p. 202–204 °C. IR (KBr, cm^{-1}): 3605, 3400, 2950, 2935, 2860, 1665, 1464, 1380, 1228, 1192, 1111, 1057, 1010, 952, 928, 892, 866. EI-MS m/z (%) (rel. int.): 430 ($[M]^+$, 3), 412 (M-18, 100). ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 2, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 3.

3.4.3. Stigmast-5-en-3β,7β-diol (7β-hydroxysitosterol) (**3**)

Colorless crystals from Me_2CO. M.p. 119–123 °C. IR (KBr, cm^{-1}): 3640, 2951, 2860, 1642, 1460, 1050, 1018. EI-MS m/z (%) (rel. int.): 430 ($[M]^+$, 3), 415, 412, 394, 289. ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 2, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 3.

3.4.4. Stigmast-5-en-3-ol-7-one (7-oxositosterol) (**4**)

Colourless needle crystals from Me_2CO. M.p. 108–110 °C. IR (KBr cm^{-1}): 3533, 3344, 2958, 2938, 2870, 1673, 1464, 1382, 1295, 1184, 1067, 1017, 948, 846. UV (MeOH, λ_{max}, nm): 202. EI-MS m/z (%) (rel. int.): 428 ($[M]^+$, 14). ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 2, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 3.

3.4.5. β-Sitosterol (**5**)

White needle-shaped crystals from Me_2CO. M.p. 139–140 °C. TLC and IR spectrum were identical with those of an author's sample.

3.4.6. Stigmasterol (**6**)

Colorless needles. M.p. 149–151 °C. TLC and IR spectrum were identical with those of an author's sample.

3.4.7. Stigmast-4-en-6β-ol-3-one (6β-hydroxyenone) (**7**)

Solids (Me_2CO). M.p. 192–194 °C. IR (KBr, cm^{-1}): 3500, 3403, 2985, 2869, 1681, 1466, 1384, 1232, 1194, 1039, 1018, 971, 879. UV (MeOH, λ_{max}, nm): 218, 233, 249 (logε 4.32, 4.28, 3.84 respectively). EI-MS m/z (%) (rel. int.): 428 ($[M]^+$, 75). ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 2, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 3.

3.4.8. Ergostane-6,22-diene-3β,5α,8α-triol (**8**)

White needle-shaped crystals from Me_2CO, M. p. 178–180 °C. $[\alpha]_D^{25}$ −16.8° (C, 0.5, $CHCl_3$). IR (Film, cm^{-1}): 3520, 3310, 2956, 2870, 1656, 1553, 1458, 1376, 1046, 968, 857, 778, EI-MS m/z (%) (rel. int.): 430(50), 412(100), 398(22), 285(30), 190(22), 178(60), 163(31), 181(16), 55(15). ^{1}H NMR ($CDCl_3$, TMS, 400.13 MHz): see Table 2, ^{13}C NMR ($CDCl_3$, TMS, 100.62 MHz): see Table 3.

Table 3: ^{13}C NMR (100 MHz) data for compounds 2, 3, 4, 7, 8

Carbon	2	3	4	7	8	Carbon	2	3	4	7	8
C-1	37.00	36.93	36.34	37.08	36.84	C-16	28.26	28.53	28.51	28.17	23.32
C-2	31.33	31.54	31.14	34.52	29.98	C-17	55.70	55.94	54.69	56.00	56.13
C-3	71.27	71.39	70.43	200.4	66.27	C-18	11.61	11.80	11.94	11.96	12.80
C-4	41.99	41.71	41.80	126.3	34.64	C-19	18.22	19.13	17.27	19.48	18.01
C-5	143.4	146.2	165.3	168.6	79.36	C-20	36.08	36.08	36.04	36.11	39.64
C-6	123.8	125.4	126.0	73.24	130.6	C-21	18.99	18.99	18.89	18.72	19.57
C-7	63.35	73.33	202.4	38.56	135.4	C-22	33.89	33.96	33.92	33.89	132.2
C-8	37.38	40.87	45.39	29.72	82.12	C-23	26.09	26.37	26.09	26.10	135.1
C-9	42.23	48.25	49.95	53.63	51.04	C-24	45.81	45.81	45.80	45.83	42.70
C-10	37.49	36.41	38.27	37.98	36.82	C-25	29.12	29.12	29.13	29.16	32.99
C-11	20.68	21.05	21.19	20.96	20.56	C-26	19.78	19.78	19.76	19.80	19.88
C-12	39.15	39.45	38.68	36.59	39.26	C-27	19.00	19.00	19.02	19.02	20.82
C-13	42.12	42.91	41.80	42.50	44.49	C-28	23.05	23.04	23.04	23.07	17.50
C-14	49.39	55.36	49.92	55.89	51.61	C-29	11.97	11.97	11.94	12.00	
C-15	25.91	25.91	26.29	24.14	28.56						

(δ, in $CDCl_3$, TMS as int. standard, types of carbons assigned by DEPT experiments)

Acknowledgements: This work was supported by the "Bairen Jihua" Foundation of the Chinese Academy of Sciences (CAS) in 2000 and the National Natural Science Foundation of China (NSFC 20021001 and 20372029). The authors are greatly indebted to adjunct Professor Ji Ma (Faculty of Pharmacy, First Military Medical University of PLA, Gangzhou, P. R. of China) for her help in identification of the plant material.

References

Chaurasia N, Wichtl M (1987) Sterols and steryl glycosides from *Urtica dioica*. J Nat Prod 50: 881–885.

Gao K, Jia ZJ (1997) Sterol compounds from *Ligularia dentate*. J Lanzhou Univ (Natural Sciences) 33: 77–80.

Greca MD, Monaco P, Previtera L (1990) Stigmasterols from *Typha latifolia*. J Nat Prod 53: 1430–1435.

Hou ZF, Shi YP, Li Y (1997) New stroids from *Adenophora stenanthina* subsp. *xifengensis*. Indian J Chem 36B: 293–296.

Lin WH, Fang JM, Cheng YS (1998) Diterpenoids and steroids from *Taiwania cryptomerioides*. Phytochemistry 48: 1391–1397.

Luo XD, Wu SH, MA YB, Wu DG (2001) The chemical constituents of *Amoora yunnanensis*. Acta Botanica Sinica 43: 426–430.

Mei SX, Pan XH, Hou ZF, Li Y (1999) Chemical constituents of *Pugionium cornutum*. Chin Pharm J 34: 366–367.

Rubinstein I, Goad LJ, Clague ADH, Mulhein LJ (1976) The 220 MHz NMR spectra of phytosterols. Phytochemistry 15: 195–200.

Shih C, Jin SY (1999) Flora Reipublicae Popolaris Sinicae, Science Press: Tomus 78: 173–175.

Yoshiyasu F, Yoshinori N, Geng PW, Wang R, Junko S, Bao XJ, Kazuyuki N (1988) *In vitro* fibrinolytic phytosterols isolated from the roots of *Spatholobus suberetus*. Planta Med 54: 34–36.