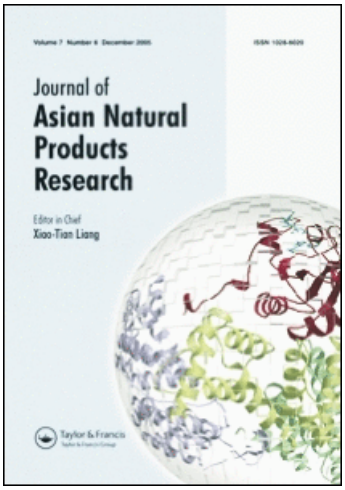

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Note: Sterols from the pericarp of Sphaerophysa salsula DC.

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Note

Sterols from the pericarp of *Sphaerophysa salsula* DC.

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A new stigmasterol, (24*S*)-stigmast-5-en-7β-ethoxy-3β-ol (**2**) together with three known sterols have been isolated from the ethanolic extract of the pericarp of *Sphaerophysa salsula* (Pall.) DC, and their structures elucidated mainly on the basis of the spectral data and comparison with the literature.

Keywords: *Sphaerophysa salsula* DC.; Pericarp; Stigmasterols; Ergosterols

1. Introduction

Sphaerophysa salsula DC. is a widely distributed plant in the northwest of China. Previous phytochemical studies on the whole plant have revealed the presence of isoflavans, coumarins, flavonoids, alkaloids, sterols, etc [1]. However, there are no reports on the chemical constituents of the pericarp. In the present investigation, we obtained sterols **1–4** from the pericarp, including a new stigmasterol, (24*S*)-stigmast-5-en-7β-ethoxy-3β-ol (**2**), and the three known sterols (24*S*)-stigmast-5-ene-3β,7α-diol (**1**), ergosterol peroxide (5α,8α-epidioxyergosta-6,22*E*-dien-3β-ol, **3**) and cerevisterol (ergosta-7,22*E*-diene-3β,5α,6β-triol, **4**). Compounds **1**, **3** and **4** were isolated from the *Sphaerophysa* genus for the first time.

2. Results and discussion

Compound **2** was obtained as colorless needles, mp 109–111°C. The $[M]^+$ peak at *m/z* 458 (8.1) in the EI-MS spectrum along with ^1H, ^{13}C NMR spectral data suggest a molecular formula of $C_{31}H_{54}O_2$ for **2**, which is supported by HR-EIMS $[M]^+$, at *m/z* 458.4065 (calcd for $C_{31}H_{54}O_2$, 458.4124). In addition, the EI-MS of **2** gave fragment ions at *m/z* 440

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($[M - H_2O]^+$, 9.9) and 412 ($[M - CH_3CH_2OH]^+$, 100), which indicate a hydroxyl and an ethoxyl substitution in the molecule. The signals at δ 15.8 and 63.1 in the ^{13}C NMR spectrum, together with the signals at δ1.17 (3H, t, $J = 6.9$ Hz), 3.64 (1H, dq, $J = 6.9, 8.1$ Hz) and 3.29 (1H, dq, $J = 6.9, 8.1$ Hz) in the ^1H NMR spectrum, also reveal the presence of the ethoxyl group, which was confirmed further by the HMQC experiment. The IR absorption bands reveal hydroxyl (3310 cm^{-1}) and olefinic carbon (1671 cm^{-1}) groups. The signals at δ 122.4 and 143.3 in ^{13}C NMR spectrum, together with the olefinic proton signal at δ 5.42 (1H, br s) in ^1H NMR spectrum, agree with the presence of a double bond. The signal at δ 71.3 in the ^{13}C NMR spectrum, together with the proton signal at δ 3.54 (1H, br s) in the ^1H NMR spectrum indicate the presence of a hydroxyl group. Except for the signals assigned to the ethoxyl (δ 15.8, 63.1 and δ 1.17), the ^{13}C NMR spectrum of **2** contains 29 carbon signals, and the ^1H NMR spectrum of **2** contains six methyl signals. The above facts suggest that **2** has a stigmastane skeleton. Upon comparing the remaining 29 signals in the ^{13}C NMR spectrum with those of stigmast-5-ene-3β,7α-diol and stigmast-5-ene-3β,7β-diol in the literature (table 1) [2,3], compound **2** showed a close resemblance with the latter, except for the signals at δ 122.4, 81.0 and 37.4. The ^{13}C NMR differences [a downfield shift of the signal at δ 81.0 (C-7) and upfield shifts of signals at δ 122.4 (C-6) and 37.4 (C-8)] compared to those of stigmast-5-ene-3β,7β-diol are explained by ether formation on C-7 in compound **2**. Thus, compound **2** might be a derivative of stigmast-5-ene-3β,7β-diol.

From the HMBC spectra (figure 1) of **2**, it was concluded that the ethoxyl group might be located at C-7. The chemical shift and coupling constant of Me-29 of **2** are consistent with those of stigmast-5-ene-3β, 7β-diol having a 24*S* configuration [4]. On the basis of the above evidence, the structure of **2** was established as (24*S*)-stigmast-5-en-7β-ethoxy-3β-ol.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yamaco micro-hot-stage apparatus and are uncorrected. NMR spectra were recorded on a Bruker-ARX-300 spectrometer. The IR (KBr) data were measured by Bruker-IFS-55. The MS spectra were determined with a Shimadzu QP5050A. HRMS was performed on a QSTAR LCQ mass spectrometer. The chromatographic silica gel (200–300 mesh) was produced by the Qingdao Ocean Chemical Factory.

3.2 Plant material

The pericarps of *Sphaerophysa salsula* (11.5 kg) were collected in the west part of the Autonomous Region of Inner Mongolia, China, in August, 2000, and identified by Professor Shuanglong Kang (Autonomous Region of Inner Mongolia Institute for Drug Controls). A voucher specimen (No.2000801) has been deposited in the Research Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang.

3.3 Extraction and isolation

The pericarps were air-dried and extracted with 95% EtOH to give a black crude material (457.0 g). The EtOH extract (400 g) was then suspended in H_2O, and partitioned with

Table 1. ^{13}C NMR data of compounds **1**–**4**.

No	**1** *(in $CDCl_3$)*	*Stigmast-5-ene-3β,7α-diol* [2,3]		*Stigmast-5-ene-3β,7β-diol* [3]	**2** *(in $CDCl_3$)*	**3** *(in Pyr-d_5)*	**4** *(in Pyr-d_5)*
1	37.0	37.00	36.95	36.93	37.1	37.5	33.8
2	31.4	31.37	31.31	31.56	31.7	31.3	32.6
3	71.3	71.35	71.27	71.42	71.6	65.8	67.6
4	42.0	42.01	41.94	41.71	42.0	35.5	42.0
5	146.2	143.88	146.16	143.48	143.3	82.3	76.1
6	123.8	123.86	123.78	125.42	122.4	136.2	74.3
7	65.3	65.36	65.78	73.34	81.0	130.9	120.5
8	37.5	37.51	37.45	40.91	37.4	79.3	141.6
9	42.3	42.23	42.07	48.26	48.5	52.0	43.8
10	37.4	37.39	37.34	36.42	36.5	38.3	38.1
11	20.7	20.71	20.64	21.06	21.2	21.1	22.4
12	39.2	39.17	39.11	39.54	39.7	40.0	39.9
13	42.1	42.13	42.20	42.91	43.0	44.7	43.8
14	49.4	49.42	49.36	55.37	55.5	52.2	55.2
15	24.3	25.90	24.23	26.36	25.8	23.7	23.5
16	28.3	28.31	29.22	28.51	28.6	29.0	28.5
17	55.7	55.70	55.66	55.96	56.5	56.4	56.1
18	11.6	11.63	11.56	11.79	11.9	13.0	12.5
19	18.2	18.25	18.96	19.12	19.8	18.4	18.8
20	36.1	36.10	36.04	36.07	36.1	39.6	40.8
21	18.8	18.80	18.17	18.81	18.9	21.1	21.4
22	33.9	33.91	33.85	33.97	34.0	136.0	136.2
23	25.9	25.97	28.88	26.11	26.1	132.3	132.1
24	45.8	45.82	45.77	45.84	45.8,	43.1	43.1
25	29.1	29.12	29.08	29.14	29.2	33.3	33.3
26	19.8	19.81	18.74	19.77	19.8	19.8	20.1
27	19.0	19.02	19.72	19.00	19.1	20.1	19.8
28	23.1	23.06	23.00	23.05	23.1	17.8	17.8
29	12.0	11.99	11.92	11.95	12.0		
1′					63.4		
2′					15.8		

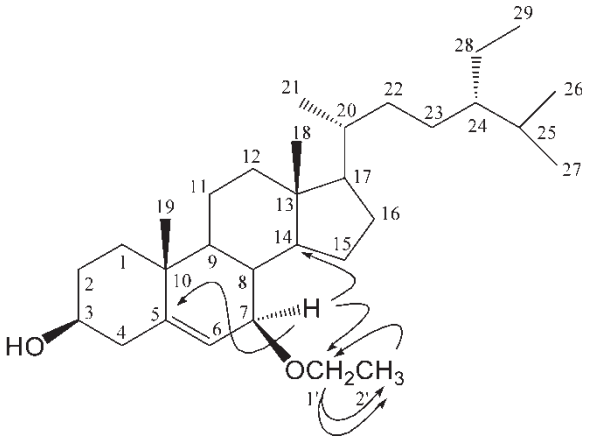

Figure 1. Important HMBC correlations of compound **2**.

chloroform and n-BuOH successively. The chloroform extract (101 g) was subjected to column chromatography over silica gel, eluting with light petroleum (60–90°C)–acetone (100 : 0 → 100:100) gradiently. The fractions eluted with light petroleum–acetone (100:4.5), (100:8), (100:17) and (100:30) were further separated by repeated column chromatography over silica gel with a gradient mixture of light petroleum–acetone–EtOAc as eluent. Compounds **2** (4 mg), **1** (11 mg), **3** (26 mg), and **4** (33 mg) were obtained from the fractions 1 (100:4.5), 2 (100:8), 3 (100:17) and 4 (100:30), respectively.

(24*S*)-*Stigmast-5-ene-3β,7α-diol* (**1**) [2–4]: colorless flakes (light petroleum-acetone), mp 202–204°C, Liebermann–Burchard reaction positive. ^{13}C and ^{1}H NMR see tables 1 and 2.

(24*S*)-*Stigmast-5-en-7β-ethoxy-3β-ol* (**2**): colorless needles (acetone), mp 109–111°C, Liebermann-Burchard reaction positive. IR (KBr) (cm^{-1}): 3310, 2959, 2865, 1671, 1462, 1381, 1314. HR-EIMS: $[M]^+$ *m/z* 458.4065 (calcd for $C_{31}H_{54}O_2$, 458.4124). EI-MS 70 eV, *m/z* (rel int %): 458 (M^+, 8.4), 440 ([M − H_2O^+] 9.9), 412 ([M − $CH_3CH_2OH]^+$, 100), 398 (8.0). ^{13}C, ^{1}H NMR see tables 1 and 2.

Ergosterol peroxide (5α,8α-epidioxyergosta-6,22E-dien-3β-ol) [5–7] (**3**): colorless needles (light petroleum–EtOAc), mp 181–183°C, Liebermann–Burchard reaction positive. EI-MS 70 eV, *m/z* (rel int %): 428 (M^+), 410 ([M − $H_2O]^+$), 396 ([M − $O_2]^+$, 53.2),

Table 2. ^{1}H NMR data of compounds **1** and **2**.

Proton	**1** *(in $CDCl_3$)*	*3β,7α-Stigmast -5-ene-3,7-diol* [2]	*3β,7β-Stigmas t-5-ene-3,7-diol* [2]	**2** *(in $CDCl_3$)*
H-3	3.59m	3.59m	3.56m	3.54m
H-6	5.61d (4.2)	5.62d (4.8)	5.30s	5.42brs
H-7	3.86m	3.86m	3.86d (4.4)	3.43brd (4.4)
H-18	0.69s	0.69s	0.70s	0.69s
H-19	1.00s	0.99s	1.05s	1.04s
H-21	0.94d (6.3)	0.93d (6.6)	0.93d (6.6)	0.95 (7.8)
H-26	0.82d (6.6)	0.83d (6.7)	0.85d (6.6)	0.82d (6.6)
H-27	0.84d (6.9)	0.82d (6.7)	0.81d (6.7)	0.83d (6.9)
H-29	0.86t (7.2)	0.85t (7.2)	0.85t (7.2)	0.86t (7.2)
H-1′				3.29dq, 3.64dq (6.9, 8.1), (6.9, 8.1)
H-2′				1.17t (6.9)

377 ($[M - 2H_2O - CH_3]^+$, 13.5), 363 ($[M - 2H_2O - 2CH_3]^+$, 22.7), 251 ($C_{19}H_{23}$, 23.9), 107 (53.98), 69 (100). ^{1}H NMR (300 MHz, C_5D_5N), δ (ppm): 0.78 (3H, s, Me-18), 0.86 (3H, d, $J = 6.7$ Hz, Me-26), 0.87 (3H, d, $J = 6.7$ Hz, Me-27), 0.90 (3H, s, Me-19), 0.96 (3H, d, $J = 6.6$ Hz, Me-28), 1.03 (3H, d, $J = 6.6$ Hz, Me-21); 6.32 (1H, d, $J = 8.4$ Hz, Hz, H-6), 6.32 (1H, d, $J = 8.4$ Hz, H-7); 5.20 (1H, dd, $J = 15.3$, 8.4 Hz, H-22), 5.26 (1H, d, $J = 15.3, 7.0$ Hz, H-23), 4.36 (1H, m, H-3). ^{13}C NMR (75 MHz, C_5D_5N) see table 1.

Cerevisterol (ergosta-7,22E-diene-3β,5α,6β-triol) [7] (**4**): colorless needles (EtOAc–MeOH), mp 234–236°C, Liebermann–Burchard reaction positive. EI-MS 70 eV, *m/z* (rel int %): 412 ($[M - H_2O]^+$, 32.88), 394 ($[M - 2H_2O]^+$, 53.17), 379 ($[M - 2H_2O - CH_3]^+$, 35.06), 251 ($C_{19}H_{23}$, 46.89), 107 (46.39), 95 (59.16), 69 (100). ^{1}H NMR (300 MHz, C_5D_5N) δ: 0.65 (3H, s, Me-18), 0.84 (3H, d, $J = 6.8$ Hz, Me-27), 0.85 (3H, d, $J = 6.7$ Hz, Me-26), 0.94 (3H, d, $J = 6.9$ Hz, Me-28), 1.05 (3H, d, $J = 6.6$ Hz, Me-21), 1.58 (3H, s, Me-19); δ 5.74 (1H, m, H-7); 5.20 (1H, dd, $J = 15.9, 8.3$ Hz, H-23), 5.26 (1H, d, $J = 15.9, 7.0$ Hz, H-22); 4.31 (1H, m, H-6), 4.84 (1H, m, H-3). ^{13}C NMR (75 MHz, C_5D_5N) see table 1.

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References

[1] Z.J. Ma, X. Li. *J. Shenyang Pharm. Univ.*, **17**, 452–455 (2000).
[2] D.G. Marina, M. Pietro, P. Lucio. *J. Nat. Prod.*, **53**, 1430–1435 (1990).
[3] F. Yosgutasu, N. Yoshinori, P.W. Geng, R. Wang, S. Junko, J. Bao, N. Kazuyuki. *Planta Med.*, **54**, 34–36 (1988).
[4] G. Notaro, V. Piccialli, D. Sica. *J. Nat. Prod.*, **55**, 1588–1594 (1992).
[5] T. Yoshihisa, A. Ryouko, M. Yoshito, O. Takashi, N. Kimiko, T. Toshiaki. *Phytochemistry*, **31**, 243–246 (1992).
[6] H. Wan. *Chin. Trad. Herb Drugs*, **31**, 328–330 (2000).
[7] T. Yoshihisa, U. Minoru, O. Takashi, N. Kimiko, M. Koutarou, T. Toshiaki. *Phytochemistry*, **30**, 4117–4120 (1991).