ISOPRENOIDS OF *Euphorbia sororia*. I. TRITERPENOIDS

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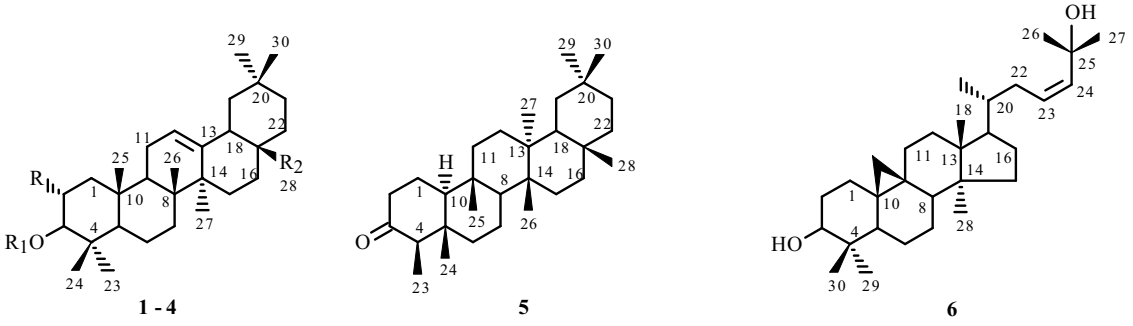
The traditional medicinal plant *Euphorbia sororia* A. Schrenk (Euphorbiaceae) is distributed mainly in northwestern China and certain regions of Central Asia. Seeds of *E. sororia* are used for treating chest pain, stomach stress, skin diseases, and stroke in addition to increasing mental capabilities and appetite [1]. Previous investigations of the aerial part of *E. sororia* isolated several sphingolipids, flavonoids, and coumarins [2–5].

Herein we report data on triterpenoids isolated from the slightly polar fraction of the MeOH extract of the whole plant. Chromatography and rechromatography of the fraction over a column of silica gel using various solvent systems isolated six triterpenoids **1**–**6**. PMR and ^{13}C NMR spectra in addition to DEPT experiments and two-dimensional HSQC and HMBC (Tables 1 and 2) and IR and electrospray positive- and negative-ion mass spectra (MS ES PI and NI) were interpreted to identify the isolated compounds as β-amyrin acetate (**1**) [6], β-amyrin (**2**) [7], oleanolic acid (**3**) [8], maslinic acid (**4**) [9], friedelin (**5**) [10], and cycloart-23*Z*-en-3β,25-diol (**6**) [11].



1 - 4 **5** **6**

1: R = H, R_1 = Ac, R_2 = CH_3; **2:** R = R_1 = H, R_2 = CH_3

3: R = R_1 = H, R_2 = COOH; **4:** R = OH, R_1 = H, R_2 = COOH

Chemical shifts of C-24, C-26, and C-29 in the ^{13}C NMR spectrum of maslinic acid (**4**) were revised relative to those published [9].

All triterpenoids described herein were isolated for the first time from *E. sororia*. Maslinic acid was found for the first time in the genus *Euphorbia*.

General Comments. PMR and ^{13}C NMR spectra in $CDCl_3$ or C_5D_5N were recorded on an INOVA-400 (Varian) spectrometer with TMS internal standard. ^{13}C NMR spectra were obtained with full C–H decoupling and under DEPT conditions; 2D spectra, using standard Varian programs.

IR spectra in KBr disks were recorded on a Shimadzu FTIR-8400s spectrophotometer; electrospray positive- and negative-ion mass spectra, in a VG autospec-3000 mass spectrometer.

Column chromatography used silica gel (200–300 mesh, Qingdao Haiang Chemical Co., Ltd.) and MCI-gel (CHP 20P, 75–150 μm).

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TABLE 1. Chemical Shifts of C and H Atoms of **1**–**4**. DEPT, HSQC, HMBC Data ($CDCl_3$, C_5D_5N, δ, ppm, J/Hz, 0 = TMS)

C atom	DEPT	Compound					
		1 ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** (C_5D_5N)		
		δ_C			δ_C	δ_H	HMBC
1	CH_2	38.23	38.24	37.14[a]	47.68	1.30, 2.23 dd (12.4, 4.4)	2, 3, 5, 10, 25
2	CH_2(CH)	23.57	26.89	27.17	68.50	4.07 td (11.2, 4.4)	3
3	CH	80.88	79.01	78.99	83.73	3.36 d (9.2)	1, 2, 4, 23, 24
4	C	37.11	38.75	38.76	39.78	–	
5	CH	55.21	55.23	55.25	55.83	1.02	
6	CH_2	18.23	18.35	18.35	18.78	1.41, 1.53	
7	CH_2	32.55	32.61	32.39	33.12[a]	1.32, 1.52	
8	C	39.77	39.75	39.26	39.74	–	
9	CH	47.52	47.59	47.68	48.08	1.82	8, 26
10	C	37.79	36.91	37.14[a]	38.46	–	
11	CH_2	23.52[a]	23.50	22.95	23.61	2.05, 2.05	
12	CH	121.51	121.69	122.66	122.39	5.45 t (3.2)	9, 14
13	C	145.20	145.18	143.56	144.78	–	
14	C	41.67	41.68	**	42.13	–	
15	CH_2	26.90	28.39	27.66	28.20	1.22, 1.22	
16	CH_2	26.10	26.12	23.36	23.86	1.98, 1.98	28
17	C	32.47	32.47	46.48	46.57	–	
18	CH	47.19	47.10	41.20	41.91	3.27 dd (13.6, 4.4)	12, 13, 14, 16, 28
19	CH_2	46.75	46.79	45.85	46.35	1.30, 1.80	13, 18, 20, 30
20	C	31.04	31.07	30.65	30.88	–	
21	CH_2	34.70	34.70	**	34.13	1.20, 1.45	
22	CH_2	37.09	37.11	32.74	33.12[a]	1.82, 2.04	28
23	CH_3	28.11	28.07	28.07	29.27	1.25 s	3, 4, 5, 24
24	CH_3	16.77	15.58	15.52	17.60	1.06 s	3, 4, 5, 23
25	CH_3	15.54	15.48	15.39	16.77	0.96 s	5, 10
26	CH_3	16.68	16.77	17.12	17.39	0.995 s	8, 14
27	CH_3	25.93	25.98	25.92	26.09	1.24 s	8, 14, 15
28	CH_3(C)	28.35	27.20	181.40	180.10	–	
29	CH_3	33.32	33.34	**	33.19	0.92 s	19, 20, 21, 30
30	CH_3	23.52[a]	23.67	23.55	23.68	0.98 s	19, 20, 21, 29
Ac	CH_3	21.32	–	–	–		
Ac	C	170.91	–	–	–		

[a]Resonances are superimposed; *assignment is ambiguous; **chemical shifts of resonances not found in spectrum.

E. sororia was grown in 2007 in Jimsaer (Xinjiang Uygur Autonomous Region, P. R. China) and was identified by Dr. G. M. Shen (Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences). A specimen with a voucher (XJIPC0236) was stored at Xinjiang Technical Institute of Physics and Chemistry, Academy of Sciences of the PRC.

Isolation and Separation of Isoprenoids from *E. sororia*. Air-dried ground whole plant (28 kg) was extracted exhaustively with MeOH (5 × 50 L) at room temperature. MeOH was evaporated from the total extracted substances, which were suspended in water and extracted with petroleum ehter. Solvent was evaporated to afford extract (2 kg) that was separated into two fractions by chromatography over a column of silica gel with elution by petroleum ether (fraction A) and MeOH (fraction B). Fraction B was rechromatographed over a column of MCI-gel CHP 20P with elution first by MeOH:H_2O (9:1) and then MeOH. The fraction eluting with MeOH:H_2O was evaporated (fraction C, 400 g) and rechromatographed over a column of silica gel with elution successively by petroleum ether and solvent systems petroleum ether:acetone (100:1→100:10), $CHCl_3$, and $CHCl_3$:acetone (100:1→0:100). Elution with petroleum ether:acetone (100:2) isolated β-amyrin acetate (**1**, 15 mg) and friedelin (**5**, 13 mg). Fractions eluted by petroleum ether:acetone (100:5) produced β-amyrin (**2**, 4 mg). Petroleum ether:acetone (100:7) eluted a cycloartane triterpenoid (**6**, 12 mg). Then, elution of the column by $CHCl_3$ isolated oleanolic acid (**3**, 21 mg). Continued elution of the column by $CHCl_3$:acetone (100:5) isolated maslinic acid (**4**, 42 mg).

TABLE 2. Chemical Shifts of C and H Atoms of **5**, **6**. DEPT, HSQC, HMBC Data ($CDCl_3$, C_5D_5N, δ, ppm, J/Hz, 0 = TMS)

C atom	Compound					
	5 ($CDCl_3$)		**6** ($CDCl_3$)			
	DEPT	δ_C	DEPT	δ_C	δ_H	HMBC
1	CH_2	**	CH_2	31.78	1.23, 1.54	
2	CH_2	41.53	CH_2	29.66	1.54, 1.69	
3	C	213.41	CH	78.31	3.25 dd (10.8, 4.8)	2, 29, 30
4	CH	58.24	C	40.20	–	
5	C	**	CH	46.94	1.26	
6	CH_2	41.30	CH_2	20.88	0.80, 1.59	
7	CH_2	18.26	CH_2	27.81	1.32, 1.90	
8	CH	53.01	CH	47.80	1.48	
9	C	**	C	19.71	–	
10	CH	59.49	C	25.85	–	
11	CH_2	35.50	CH_2	25.76	1.31, 1.31	
12	CH_2	**	CH_2	35.33	1.30, 1.30	
13	C	39.25*	C	45.05	–	
14	C	38.22*	C	47.86	–	
15	CH_2	32.43	CH_2	32.55	1.60, 1.60	
16	CH_2	**	CH_2	26.18	1.10, 1.97	
17	C	30.01	CH	51.81	1.55	
18	CH	42.80	CH_3	17.75	0.98 s	12, 13, 14, 17
19	CH_2	35.55	CH_2	29.63	0.34 d (4.4), 0.56 d (4.4)	1, 5, 8, 9, 11
20	C	**	CH	36.19	1.44	
21	CH_2	32.78	CH_3	17.88	0.87 d (6.4)	17, 22
22	CH_2	39.2	CH_2	38.86	1.80, 2.18	23, 24
23	CH_3	6.82	CH	138.87	5.58 m	22, 24, 25
24	CH_3	14.66	CH	125.26	5.58 m	22, 23, 25
25	CH_3	17.94	C	70.15	–	
26	CH_3	20.25	CH_3	29.25	1.30 s	
27	CH_3	**	CH_3	29.10	1.30 s	
28	CH_3	32.09	CH_3	18.93	0.90 s	14
29	CH_3	**	CH_3	25.03	0.96 s	4, 5, 30
30	CH_3	31.72	CH_3	13.66	0.81 s	4, 5, 29

[a]Resonances are superimposed; *assignment is ambiguous; **chemical shifts of resonances not found in spectrum.

***β*-Amyrin acetate (1)**, $C_{32}H_{52}O_2$, colorless needle-like crystals, mp 238–240°C (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 2960, 2846, 1735, 1456, 1366, 1241, 1032, 900. MS ES PI (*m*/*z*): 469 $[M + H]^+$. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 0.81 (s, CH_3-28), 0.85 (s, CH_3-23), 0.85 (s, CH_3-24), 0.94 (s, CH_3-29), 0.94 (s, CH_3-30), 0.98 (s, CH_3-25), 0.98 (s, CH_3-26), 1.13 (s, CH_3-27), 2.04 (s, CH_3COO), 4.45 (dd, $^3J_1 = 9$, $^3J_2 = 6$, H-3), 5.16 (t, $^3J_1 = {}^3J_2 = 3.5$, H-12) [6]. Table 1 lists the ^{13}C NMR spectrum.

***β*-Amyrin (2)**, $C_{30}H_{50}O$, colorless needle-like crystals, mp 196–198°C (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 2958, 2868, 1456, 1382. MS ES PI (*m*/*z*): 427 $[M + H]^+$. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 0.80, 0.86, 0.88, 0.89, 0.94, 0.98, 1.02, 1.11 (all s, $8 \times CH_3$), 3.15 (dd, $^3J_1 = 10.4$, $^3J_2 = 5.4$, H-3), 5.16 (t, $^3J_1 = {}^3J_2 = 3.5$, H-12) [7]. Table 1 lists the ^{13}C NMR spectrum.

Oleanolic acid (3), $C_{30}H_{48}O_3$, colorless needles, mp 303-305°C (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 3403, 2930, 1691, 1455, 1380, 1275, 1053, 812. MS ES NI (*m*/*z*): 455 $[M - H]^-$. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 0.78, 0.79, 0.90, 0.91, 0.92, 1.00, 1.15 (all s, $7 \times CH_3$), 2.81 (dd, $^3J_1 = 9$, $^3J_2 = 6.2$, H-18), 3.22 (dd, $^3J_1 = 9.1$, $^3J_2 = 4.7$, H-3), 5.30 (t, $^3J_1 = {}^3J_2 = 3.5$, H-12) [8]. Table 1 lists the ^{13}C NMR spectrum.

Maslinic acid (4), $C_{30}H_{48}O_4$, white powder. IR spectrum (KBr, ν_{max}, cm^{-1}): 3415, 3280, 2945, 1697, 1462, 1050. MS ES PI (*m*/*z*): 495 $[M + Na]^+$ [9]. Table 1 lists the PMR and ^{13}C NMR spectra.

Friedelin (5), $C_{30}H_{50}O$, colorless needles recrystallized from MeOH. IR spectrum (KBr, ν_{max}, cm^{-1}): 2925, 2865, 1715, 1460, 1385. MS ES PI (*m*/*z*): 427 $[M + H]^+$. PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 0.72 (s, CH_3-24), 0.87 (s, CH_3-25), 0.89 (d, $^3J = 6.8$, CH_3-23), 0.95 (s, CH_3-30), 1.00 (s, CH_3-29), 1.01 (s, CH_3-26), 1.05 (s, CH_3-27), 1.18 (s, CH_3-28), 2.24 (m, H-4), 2.30 (m, H-2), 2.38 (ddd, $^2J = 16$, $^3J_1 = 3.6$, $^3J_2 = 1.6$, H-2′) [10]. Table 2 lists the ^{13}C NMR spectrum.

Cycloart-23*Z*-en-3*β*,25-diol (6), $C_{30}H_{50}O_2$, colorless needles crystallized from $CHCl_3$:CH_3OH. IR spectrum (KBr, ν_{max}, cm^{-1}): 3655, 3324, 3038, 2970, 2931, 2866, 1467, 1377, 1358, 1224, 1163, 1104, 1051, 971, 890. MS ES PI (*m*/*z*): 443 $[M + H]^+$ [11]. Table 2 lists the PMR and ^{13}C NMR spectra.

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REFERENCES

1. Chinese Pharmacopoeia Commission, *Drug Standard of Ministry of Public Health of the People's Republic of China (Uygur Medicine Volumes)*, Xinjiang Health Science and Technology Publishing House, Urumqi, 1999, p. 26.
2. W. K. Zhang, G. E. Yang, Q. Li, X. Q. Zhang, and W. C. Ye, *China J. Chin. Mater. Med.*, **31**, 1694 (2006).
3. W. K. Zhang, J. K. Xu, X. Q. Zhang, X. S. Yao, and W. C. Ye, *Nat. Prod. Res.*, **22**, 353 (2008).
4. W. K. Zhang, X. Q. Zhang, and W. C. Ye, *J. China Pharm. Univ.*, **38**, 315 (2007).
5. W. K. Zhang, J. K. Xu, X. Q. Zhang, X. S. Yao, and W. C. Ye, *Chem. Phys. Lipids*, **148**, 77 (2007).
6. G. M. Fu, B. Y. Yu, and D. N. Zhu, *J. China Pharm. Univ.*, **34**, 377 (2003).
7. Y. S. Li, Z. D. Wang, and M. Zhang, *China Pharm. J.*, **37**, 12 (2002).
8. S. B. Mohuto and A. P. Kundu, *Phytochemistry*, **37**, 1517 (1994).
9. Q. S. Zhao, J. Tian, J. M. Yue, S. N. Chen, Z. W. Lin, and H. D. Sun, *Phytochemistry*, **48**, 1025 (1998).
10. J. G. Luo and L. Y. Kong, *Nat. Prod. Res. Dev.*, **17**, 166 (2005).
11. R. Tanka, T. Ida, S. Kita, W. Kamisako, and S. Matsunaga, *Phytochemistry*, **41**, 1163 (1996).