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TARAXASTANE GLYCOSIDES FROM *ECLIPTA ALBA**

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Key Word Index—*Eclipta alba*; Compositae; triterpene glycoside; saponin; eclalbasaponin; 3β,20β,16β-trihydroxytaraxastane; 3β,20β,28-trihydroxytaraxastane; sulphated saponin.

Abstract—From the dried whole plants of *Eclipta alba* (Ecliptae Herba, Chinese name Mo Han Lian) purchased in China, four new taraxastane triterpene glycosides, named eclalbasaponins VII–X were isolated, along with eclalbasaponins I–VI. The structures of eclalbasaponins VII–X were characterized as 3β,20β,16β- and 3β,20β,28-trihydroxytaraxastane glycosides, and their sulphated saponins on the basis of spectral data. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

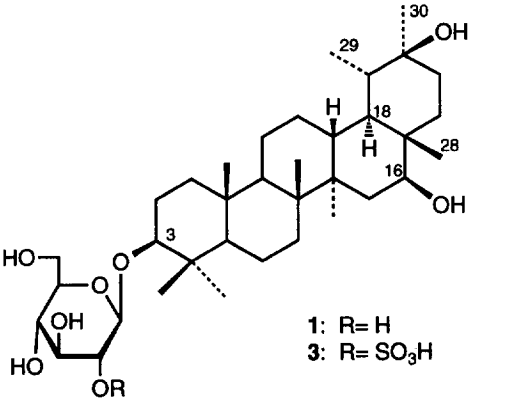
In the previous paper [1], we reported on the characterization of six new oleanene glycosides, named eclalbasaponins I–VI, obtained from whole plants of *Eclipta alba* HASSK. collected in Japan. As part of a continuing study on this crude drug, Ecliptae Herba, purchased in China, yielded another four new taraxastane glycosides, names eclalbasaponins VII (**1**), VIII (**2**), IX (**3**) and X (**4**). This paper deals with their structure elucidation.

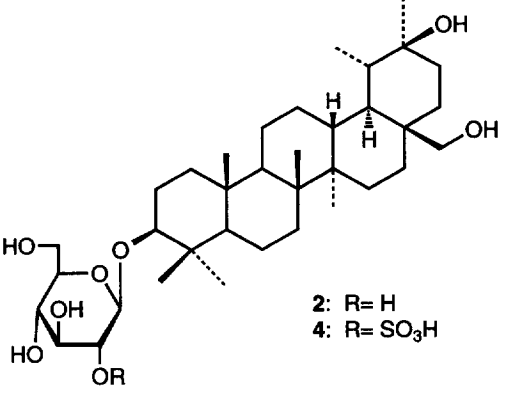
RESULTS AND DISCUSSION

The methanol extract prepared from the whole plants of *E. alba* purchased in China was separated and purified by CC on MCI gel CHP20P, Bondapak C_{18} and silica gel, to provide glycosides **1**–**4**, together with eclalbasaponins I–VI (**5**–**10**) obtained previously [1].

Eclalbasaponins I–VI (**5**–**10**) were identified by comparison of their physical and spectral data with those of authentic specimens.

Eclalbasaponin VII (**1**), $C_{36}H_{62}O_8$, gave a *quasi*-molecular ion peak at *m/z* 621 $[M-H]^-$ in its negative ion FAB-mass spectrum. The ^1H NMR spectrum (Table 1) displayed signals for seven tertiary methyl groups (δ 0.81, 1.02, 1.03, 1.16. 1.26, 1.33, and 1.38), one secondary methyl group (δ 1.45, *d*, *J* = 6.1 Hz), one methine proton (δ 3.79, *dd*, *J* = 4.5, 11.1 Hz) of an oxygen bearing group and one anomeric proton (δ 4.99, *d*, *J* = 7.6 Hz). The ^{13}C NMR spectrum (Table 1) of **1** exhibited 36 carbon signals attributable to one β-D-glucopyranosyl residue, and two oxygenated methines (δ 76.14, 88.93), one oxygenated quaternary carbon (δ 73.87), eight methyls, ten methylenes, five methines and five quaternary carbons in the aglycone moiety. A comparative study on the ^{13}C NMR spectra of **1** with those of eclalbasaponin I [1] and 16β-





*Part III in the series on the constituents of Compositae. For Part II see ref. [1].

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Table 1. ^{13}C NMR and ^{1}H NMR assignments and ^{1}H–^{13}C long-range correlations of compound **1** by ^{1}H–^{1}H COSY, HMBC and HSQC in pyridine-d_5

C	δ_C	δ_H	Cross peaks (δ_C) in HMBC spectrum
1	38.92 CH_2	0.86 (*m*), 1.55 (*m*)	
2	26.80 CH_2	1.87 (*m*), 2.30 (*m*)	
3	88.93 CH	3.43 (*dd*, 4.5, 11.1)	16.87 (24), 28.16 (23), 39.62 (4), 107.01 (g-1)
4	39.62 C		
5	55.72 CH	0.74 (*m*)	16.35 (25), 16.87 (24), 18.53 (6), 36.82 (10), 39.62 (4)
6	18.53 CH_2	1.28 (*m*), 1.32 (*m*)	
7	34.83 CH_2	141 (*m*), 1.44 (*m*)	
8	41.60[a] C		
9	49.29 CH	1.26 (*m*)	
10	36.82 C		
11	21.55 CH_2	1.16 (*m*), 1.42 (*m*)	
12	28.90 CH_2	1.29 (*m*), 2.10 (*m*)	
13	38.88 CH	1.98 (*m*)	
14	43.09[a] C		
15	37.20 CH_2	1.64 (*dd*, 4.9, 12.8), 2.02 (*br t*, 12.8)	16.58 (27), 41.60 (8), 43.09[a] (14), 76.14 (16), 16.58 (27), 38.88 (13), 41.60 (8), 43.09[a] (14)
16	76.14 CH	3.79 (*dd*, 4.5, 11.1)	
17	41.97 C		
18	47.75 CH	1.34 (*dd*, 10.6, 10.6)	
19	42.03 CH	2.05 (*dd*, 6.4, 10.7)	18.12 (29), 22.06 (30), 47.75 (18), 73.87 (20)
20	73.87 C		
21	38.48 CH_2	1.92 (*ddd*, 3.3, 11.4, 11.4), 2.18 (*br dt*, 3.2, 14.0)	
22	36.38 CH_2	1.55 (*m*), 2.45 (*ddd*, 3.4, 3.4, 13.4)	13.32 (28), 41.97 (17), 47.75 (18), 73.87 (20)
23	28.16 CH_3	1.33 (*s*)	16.87 (24), 39.62 (4), 55.72 (5), 88.93 (3)
24	16.87 CH_3	1.02 (*s*)	28.16 (23), 39.62 (4), 55.72 (5), 88.93 (3)
25	16.35 CH_3	0.81 (*s*)	36.82 (10), 38.92 (1), 49.29 (9), 55.72 (5)
26	16.25 CH_3	1.03 (*s*)	34.83 (7), 41.60[a] (8), 43.09[a] (14), 49.29 (9)
27	16.58 CH_3	1.16 (*s*)	37.20 (15), 38.88 (13), 41.60[a] (8), 43.09[a] (14)
28	13.32 CH_3	1.26 (s)	36.38 (22), 41.97 (17), 47.75 (18), 76.14 (16)
29	18.12 CH_3	1.45 (*d*, 6.1)	42.03 (19), 73.87 (20)
30	22.06 CH_3	1.38 (*s*)	38.48 (21), 42.03 (19), 73.87 (20)
glc			
1	107.01 CH	4.99 (*d*, 7.6)	88.93 (3)
2	75.87 CH	4.07 (*br t*, 8.6)	
3	78.83 CH	4.28 (*t*, 8.9)	
4	71.92 CH	4.26 (*t*, 8.9)	
5	78.40 CH	4.05 (*m*)	
6	63.12 CH_2	4.44 (*dd*, 5.4, 11.7) 4.63 (*dd*, 2.4, 11.7)	

[a]Signals may be interchanged.

hydroxypseudotaraxasterol [2] suggested **1** to be a 3β,16β,20-trihydroxytaraxastane derivative. Full assignments of the ^{1}H and ^{13}C signals of **1** were secured by the ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Table 2). In the HMBC spectrum (8 Hz) of **1**, the H_3-28 (δ 1.26, *s*), H_3-29 (δ 1.45, *d*, $J = 6.1$ Hz) and H_3-30 (δ 1.38, *s*) signals showed $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ correlations with the ^{13}C signals at δ 36.38 (CH_2, C-22), 41.97 (C, C-17), 47.75 (CH, C-18) and 76.14 (CH-O, C-16); 42.03 (CH, C-19) and 73.87 (C, C-20); and 38.48 (CH_2, C-21), 42.03 (C-19) and 73.87 (C-20), respectively. Regarding the configuration at C-20, the differential NOE spectra of **1** showed interactions between H_3-28 (δ 1.26) and H-19 (δ 2.05), and between H_3-30 (δ 1.38) and H-18 (δ 1.34), H-16 (δ 3.79) thus the 20-OH had the β-configuration. Therefore, the structure of **1** was 3β,16β,20β-trihydroxytaraxastane 3-*O*-β-D-glucopyranoside.

Eclalbasaponin VIII (**2**) showed a *quasi*-molecular ion peak at *m/z* 621 $[M-H]^-$ in its negative ion FAB-mass spectrum. This molecular ion peak was identical with that of **1**, thus **2** was probably an isomer of **1**. The ^{13}C NMR spectrum was similar to that of **2**, except for the C-15–17 and C-28 signals of the aglycone. Singals at δ 27.00 (CH_2), 32.77 (CH_2), 39.92 (C) and 58.26 (CH_2) could be reasonably assigned to C-15–17 and 28, respectively. The ^{1}H and ^{13}C signals of **2** were also assigned unambiguously by the ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Table 2). In the HMBC spectrum (8 Hz) of **2**, the signals due to H_2-28 (δ 4.00, 4.25), H_3-29 (δ 1.37) and H_3-30 (δ 1.43) showed $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ correlations with the

Table 2. ^{13}C and ^{1}H NMR assignments ^{1}H–^{13}C long-range correlations of compound **2** by ^{1}H–^{1}H COSY, HMBC and HSQC in pyridine-d_5

C	δ_C		δ_H	Cross peaks (δ_C) in HMBC spectrum
1	38.98	CH_2	0.86 (*m*), 1.57 (*m*)	
2	26.80	CH_2	1.88 (*m*), 2.31 (*m*)	
3	88.94	CH	3.44 (*dd*, 4.4, 11.7)	16.90 (24), 28.16 (23), 106.98 (g-1)
4	39.62	C		
5	55.70	CH	0.73 (*d*, 11.6)	16.90 (24), 18.50 (6), 34.81 (7), 36.86 (10), 39.62 (4)
6	18.50	CH_2	1.30 (*m*), 1.47 (*m*)	
7	34.81	CH_2	1.33 (2H, *m*)	
8	41.71	C		
9	49.95	CH	1.21 (*m*)	
10	36.86	C		
11	21.84	CH_2	1.21 (*m*), 1.46 (*m*)	
12	29.93	CH_2	1.28 (*m*), 1.86 (*m*)	
13	38.27	CH	1.89 (*m*)	
14	43.30	C		
15	27.00	CH_2	1.05 (*m*), 1.99 (*ddd*, 4.6, 4.6, 13.5)	
16	32.77	CH_2	1.33 (*m*), 2.11 (*ddd*, 2.2, 2.2, 13.5)	
17	39.92	C		
18	48.05	CH	1.83 (*dd*, 10.5, 10.5)	19.23 (29), 38.27 (13), 39.92 (I7), 43.30 (14), 58.26 (28)
19	39.60	CH	1.59 (*m*)	
20	72.74	C		
21	36.71	CH_2	1.89 (*m*), 2.22 (*m*)	
22	31.03	CH_2	1.90 (*m*), 2.24 (*m*)	
23	28.16	CH_3	1.34 (*s*)	16.90 (24), 39.62 (4), 55.70 (5), 88.94 (3)
24	16.90	CH_3	1.02 (*s*)	28.16 (23), 39.62 (4), 55.70 (5), 88.94 (3)
25	16.34	CH_3	0.823 (*s*)	36.86 (10), 38.98 (1), 49.95 (9), 55.70 (5)
26	16.44	CH_3	1.00 (*s*)	34.81 (7), 41.71 (8), 43.30 (14), 49.95 (9)
27	15.14	CH_3	1.00 (*s*)	27.00 (15), 38.27 (13), 41.71 (8), 43.30 (14)
28	58.26	CH_2	4.00 (*d*, 11.0), 4.25 (*d*, 11.0)	32.77 (15), 31.03 (22)
29	19.23	CH_3	1.37 (*d*, 6.1)	39.60 (19), 48.05 (18), 72.74 (20)
30	30.96	CH_3	1.43 (*s*)	36.71 (21), 39.60 (19), 72.74 (20)
glc				
1	106.98	CH	5.02 (*d*, 7.4)	88.94 (3)
2	75.87	CH	4.08 (*br t*, 8.3)	
3	78.83	CH	4.29 (*br t*, 8.9)	
4	71.94	CH	4.27 (*br t*, 8.9)	
5	78.38	CH	4.05 (*m*)	
6	63.12	CH_2	4.44 (*dd*, 5.5, 11.6) 4.62 (*dd*, 2.4, 11.6)	

^{13}C signals due to C-16 (δ 32.77) and C-22 (31.03); C-19 (39.60), C-18 (48.05) and C-20 (72.74); and C-21 (36.71), C-19 (39.60) and C-20 (72.74), respectively. Based on the above evidence the structure of **2** was suggested to be 3β,20,28-trihydroxytaraxastane 3-*O*-β-glucopyranoside. Regarding the configuration at C-20, the differential NOE spectra of **2** [H_2-28β (δ 4.00)/H-22 (δ 2.24) and H-19β (δ 1.59), H_3-30 (δ 1.43)/H-18 (δ 1.83)], led to the conclusion that C_{20}-OH was in the β-configuration. Therefore, the structure of **2** was 3β,20β,28-trihydroxytaraxastane 3-*O*-β-D-glucopyranoside.

Eclalbasaponin IX (**3**) showed a $[M-H+2Na]^+$ peak at *m/z* 747.3725 ($C_{36}H_{61}O_{11}Na_2S$) in its high resolution positive FAB-mass spectrum. The negative FAB-mass spectrum of **3** gave a *quasi*-molecular ion peak at *m/z* 701 $[M-H]^-$. Therefore, the molecular formula of **3** was estimated to be $C_{36}H_{62}O_{11}S$. The ^{1}H NMR spectrum of **3** was very similar to that of **1**, except for the H-2 signal (δ 5.08, *br t*, $J=9.2$ Hz) of the glucose moiety due to a sulphation shift [3], suggesting that the sulphate group should be located at C-2 of glucose in **1**. On comparing the ^{13}C NMR data for **3** with that of **1**, the aglycone moiety was identical except for C-1 (−2.8 ppm), C-2 (+5.3 ppm) and C-3 (−1.1 ppm) of the glucose moiety. Therefore, the structure of **3** was 3-*O*-(2-*O*-sulphonyl-β-D-glucopyranosyl) 3β,16β,20β-trihydroxytaraxastane.

Eclalbasaponin X (**4**), showed a $[M-H+2Na]^+$ peak at *m/z* 747.3729 ($C_{36}H_{61}O_{11}Na_2S$) in its high resolution positive FAB-mass spectrum. The negative FAB-mass spectrum gave a *quasi*-molecular ion peak at

m/z 701 $[M-H]^-$, and a fragment ion peak at m/z 621 $[M-SO_3H]^-$. Therefore, the molecular formula of **4** was deduced to be $C_{36}H_{62}O_{11}S$. The ^{13}C NMR spectrum of **4** exhibited a similar pattern to that of **2**. But the chemical shifts of the C-1 (−2.6 ppm), C-2 (+5.4 ppm) and C-3 (−0.9 ppm) of the 3-*O*-glucosyl moiety were different from those of **2** due to sulphation shifts. For the same reason described in the structure elucidation of **3**, the structure of **4** was elucidated as 3-*O*-(2-*O*-sulphonyl-β-D-glucopyranosyl) 3β,20β,28-trihydroxy-taraxastane.

These saponins and their aglycones are new natural compounds.

EXPERIMENTAL

MS: JEOL JMS-DX-303HF and HX-100; NMR: JEOL JNM-GX-400 and α-500; CC: MCI gel CHP-20P (Mitsubishi Kasei), Kieselgel 60 (230–400 mesh, Merck) and Bondapak C_{18} (Waters); TLC: pre-coated Kieselgel 60 F_{254} plates (0.2 mm, Merck) using a $CHCl_3$–MeOH–H_2O (7:3:0.5) system as the developing solvent, and detection by spraying with a 20% H_2SO_4 reagent followed by heating.

Extraction and separation. The crude drug of Ecliptae Herba (1.6 kg), purchased in Nanjing, China in 1992, was extracted with MeOH, and the extract was evapd under reduced pressure to afford a residue (123 g) which was partitioned between benzene and water. The aq. phase was removed to furnish a residue, which was subjected to CC on MCI gel CHP-20P eluted with H_2O, 40% MeOH, 60% MeOH, 80% MeOH and 100% MeOH to provide five frs. Fr. 3 (609 mg, 60% MeOH eluate) was subjected to CC on silica gel ($CHCl_3$–MeOH–H_2O, 7:3:0.5) to give **10** (32 mg). Fr. 4 (1.03 g, 80% MeOH eluate) was also subjected to CC on Bondapak C_{18} (30–70% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2–7:3:0.5) to give **5** (174 mg), **7** (110 mg), **9** (32 mg), **3** (10 mg) and **4** (6 mg). Fr. 5 (972 mg, 100% MeOH eluate) was further sepd by CC on Bondapak C_{18} (50–60% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2–7:3:0.5, $CHCl_3$–MeOH, 8:1 and hexane–acetone, 4:1) to give echinocystic acid (15 mg), **6** (297 mg), **8** (65 mg), **1** (4 mg) and **2** (5 mg).

Eclalbasaponin VII (**1**). Amorphous powder, $[\alpha]_D^{26}$ −15 ($c = 0.31$, MeOH). Positive FAB-MS m/z: 645.4342 ($[M+Na]^+$, $C_{36}H_{62}O_8Na$, requires 645.4343). Negative FAB-MS m/z: 621 $[M-H]^-$. ^1H and ^{13}C NMR: Table 1.

Eclalbasaponin VIII (**2**). Amorphous powder, $[\alpha]_D^{26}$ −18 ($c = 0.33$, MeOH). Positive FAB-MS m/z: 645.4342 ($[M+Na]^+$, $C_{36}H_{62}O_8Na$, requires 645.4343). Negative FAB-MS m/z: 621 $[M-H]^-$. ^1H and ^{13}C NMR: Table 2.

Eclalbasaponin IX (**3**). Amorphous powder, $[\alpha]_D^{25}$ −12 ($c = 0.46$, MeOH). Positive FAB-MS m/z: 747.3725. ($[M-H+2Na]^+$, $C_{36}H_{61}O_{11}Na_2S$, requires 747.3731). Negative FAB-MS m/z: 701 $[M-H]^-$, 683 $[M-H-H_2O]^-$. ^1H NMR (pyridine-d_5): δ 0.74, 0.98, 1.13, 1.18, 1.23, 1.36, 1.47 (each 3H, *s*, H_3-23–28, H_3-30), 1.42 (3H, *d*, $J = 6.2$ Hz), 3.36 (1H, *dd*, $J = 4.0$, 11.7 Hz, H-3), 3.76 (1H, *dd*, $J = 4.7$, 11.4 Hz), 3.96 (1H, *m*, glc H-5), 4.18 (1H, *t*, $J = 9.2$ Hz), 4.32 (1, *dd*, $J = 5.5$, 11.7 Hz, glc H-6*a*), 4.47 (1H, *t*, $J = 9.2$ Hz, glc H-3), 4.53 (1H, *br d*, $J = 11.7$ Hz, glc H-6*b*), 5.06 (1H, *d*, $J = 7.7$ Hz, glc H-1), 5.08 (1H, *br t*, $J = 9.2$ Hz, glc H-2). ^{13}C NMR: Table 3.

Table 3. ^{13}C NMR data for compounds **3** and **4** in pyridine-d_5

C	3	4
1	38.86	38.86
2	26.56	26.56
3	89.68	89.64
4	39.64	39.86
5	55.68	55.68
6	18.45	18.46
7	34.82	34.79
8	41.56[a]	41.68
9	49.24	49.91
10	36.79	36.86
11	21.52	21.80
12	28.87	29.60
13	38.86	38.25
14	43.05[a]	43.26
15	37.13	27.00
16	76.14	32.73
17	41.96	39.59
18	47.72	48.00
19	41.96	39.69
20	73.86	72.74
21	38.86	36.67
22	36.34	31.00
23	28.23	28.26
24	16.88	16.91
25	16.27	16.30
26	16.21	16.36
27	16.55	15.12
28	13.29	58.26
29	18.07	19.16
30	22.01	30.91
glc 1	104.28	104.25
2	81.09	81.21
3	77.77	77.84
4	71.83	71.86
5	78.29	78.48
6	62.78	62.84

[a]Signals may be interchanged.

Eclalbasaponin X (**4**). Amorphous powder, $[\alpha]_D^{25}$ −5 ($c = 0.29$, MeOH). Positive FAB-MS m/z: 747.3729. ($[M-H+2Na]^+$, $C_{36}H_{61}O_{11}Na_2S$, require: 747.3731). Negative FAB-MS m/z: 701 $[M-H]^-$, 683 $[M-H-H_2O]^-$. ^1H NMR (pyridine-d_5): δ 0.76, 0.97 × 2, 1.19, 1.42, 1.50 (each 3H or 6H, *s*, H_3-23–27, H_3-30), 1.35 (3H, *d*, $J = 5.5$ Hz, H_3-29), 3.38 (1H, *br d*, $J = 11.0$ Hz, H-3), 3.97 (1H, *m*, glc H-5), 4.22 (3H, *m*, H_2-28, glc H-4), 4.32 (1H, *dd*, $J = 5.5$, 11.5 Hz, glc H-6*a*), 4.51 (1H, *t*, $J = 9.0$ Hz, glc H-3), 4.54 (1H, *br d*, $J = 11.5$ Hz, glc H-6*b*), 5.05 (1H, *d*, $J = 7.5$ Hz, glc H-1), 5.10 (1H, *br t*, $J = 9.0$ Hz, glc H-2). ^{13}C NMR: Table 3.

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