Oleanane Glycosides from *Eclipta alba*[1)]

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From the whole parts of *Eclipta alba* HASSK., six new oleanane triterpene glycosides, named eclalbasaponins I—VI (1—6), were isolated. The structures of 1—6 were characterized as echinocystic acid glycosides, and of those, 5 and 6 were revealed to be sulfated saponins on the basis of chemical and spectral data.

Keywords *Eclipta alba*; Compositae; triterpene glycoside; eclalbasaponin; echnocystic acid; sulfated saponin

Eclipta species are used in a traditional medicine in China and Asia. There are few reports on the constituents of this plant.[2)] In this paper we report the isolation of six new triterpenoid glycosides, eclalbasaponins I—VI (**1**—**6**), along with echinocystic acid (**7**) from the whole plants of *Eclipta alba* HASSK. (= *E. prostrata* L.) and Ecliptae Herba produced in China, as well as the structural elucidation of the new glycosides.

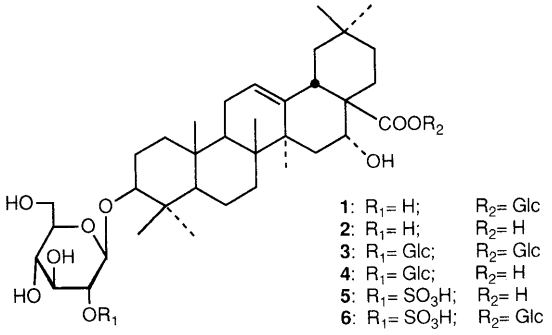
Eclalbasaponin I (**1**) showed a pseudo-molecular ion peak at *m/z* 795 $[M-H]^-$ in the negative ion fast atom bombardment mass spectrum (FAB-MS). The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum displayed signals caused by seven tertiary methyl groups [δ 0.89, 1.00, 1.01, 1.05, 1.13, 1.29 and 1.85], one olefinic proton [δ 5.62 (br s)] and two anomeric protons [δ 4.94 (d, J=7.7 Hz) and 6.32 (d, J=8.4 Hz)]. Acid hydrolysis of **1** liberated echinocystic acid (**7**) and D-glucose. Alkaline solvolysis of **1** produced a prosapogenin (**1a**), whose ^{13}C-NMR spectrum as listed in Table I showed signals due to one carbomethoxy group [δ 51.8 (q), 177.7 (s)], one β-glucopyranosyl residue and the O-glycosylated C-3 (δ 88.8) on echinocystic acid methyl ester residue. The structure of **1a** was elucidated as 3-*O*-β-D-glucopyranosyl echinocystic acid methyl ester. The ^{13}C-NMR spectrum of **1** exhibited 42 carbon signals ascribable to two β-glucopyranosyl residues consisting of one ether glucoside and one ester glucoside, which were attached to the 3-OH and 28-COOH of echinocystic acid signals[3)] (Table 1). Consequently, the structure of **1** was elucidated as 3,28-di-*O*-β-D-glucopyranosyl echinocystic acid.

Eclalbasaponin II (**2**) showed a pseudo-molecular ion peak at *m/z* 633 $[M-H]^-$ and a fragment ion peak at *m/z* 471 $[M-\text{hexose}]^-$ in the negative FAB-MS. Acid hydrolysis of **2** liberated D-glucose and echinocystic acid. The ^{13}C-NMR spectrum gave 36 carbon signals caused by one β-glucopyranosyl moiety and the O-glycosylated C-3 echinocystic acid moiety. Consequently, the structure of **2** was elucidated as 3-*O*-β-D-glucopyranosyl echinocystic acid.

Eclalbasaponin III (**3**) showed a pseudo-molecular ion peak at *m/z* 957 $[M-H]^-$, and fragment ion peaks at *m/z* 795 $[M-\text{hexose}]^-$, 633 [*m/z* 795 − hexose]$^-$, 471 [*m/z* 633 − hexose]$^-$ and 453 [*m/z* 633 − hexose − H_2O]$^-$ in the negative FAB-MS, indicating that **3** had one more hexosyl moiety than **1**. The ^{13}C-NMR spectrum of **3** exhibited the presence of the O-glycosylated C-2 of the β-glucopyranosyl unit. Alkaline hydrolysis of **3** gave a prosapogenin (**4**), whose ^{13}C-NMR spectrum exhibited 42 carbon signals due to a β-sophorosyl unit and the O-glycosylated C-3 of echinocystic acid. Based upon the above evidence, the structures of eclalbasaponins III (**3**) and IV (**4**) could be represented as shown in the formulae.

Eclalbasaponin V (**5**) showed a $[M-H+2Na]^+$ peak at *m/z* 759.3361 ($C_{36}H_{57}Na_2O_{12}S$) in the high resolution positive FAB-MS. The negative FAB-MS of **5** gave a pseudo-molecular ion peak at *m/z* 713 $[M-H]^-$, and a fragment ion peak at *m/z* 633 interpreted as the loss of SO_3 from *m/z* 713; the molecular formula was estimated to be $C_{36}H_{58}O_{12}S$. Acid hydrolysis of **5** liberated D-glucose and echinocystic acid. Solvolysis using dioxane–pyridine[4)] afforded **2**. This evidence established that **5** was a sulfated derivative of **2**. The ^{1}H-NMR spectrum of **5** was similar to that of **2**, except for the H-2 signal (δ 5.06, br t, J=8.2 Hz) of glucose due to a sulfation shift.[4)] On comparative study of the ^{13}C-NMR data for **5** with that of **2**, the aglycone moiety was identical except for C-1 (−2.6 ppm), C-2 (+5.4 ppm) and C-3 (−0.5 ppm) of glucose. These experiments indicated that the sulfate group should be located at the C-2 of glucose in **2**. Therefore, the structure of **5** was characterized as shown in the formula.

Eclalbasaponin VI (**6**), showed a $[M-H+2Na]^+$ peak at *m/z* 921.3879 ($C_{42}H_{67}Na_2O_{17}S$) in the high resolution positive FAB-MS. The negative FAB-MS gave a pseudo-molecular ion peak at *m/z* 875 $[M-H]^-$, and a fragment ion peak at *m/z* 798 $[M-SO_3H]^-$. Solvolysis of **6** in the same manner as **5** gave **1**. The ^{13}C-NMR of **6** exhibited a pattern similar to that of **1** except for the C-1 (−2.3 ppm), C-2 (+5.6 ppm) and C-3 (−0.9 ppm) of the C-3-O-glucosyl moiety due to sulfation shifts. Therefore, the structure of **6** was elucidated as 3-*O*-[2-*O*-sulfonyl-β-D-glucopyranosyl] echinocystic acid 28-*O*-β-D-glucopy-



1: R_1= H; R_2= Glc
2: R_1= H; R_2= H
3: R_1= Glc; R_2= Glc
4: R_1= Glc; R_2= H
5: R_1= SO_3H; R_2= H
6: R_1= SO_3H; R_2= Glc

TABLE I. ^{13}C-NMR Data for **1**—**6**, **1a** and Aglycone (**7**) in Pyridine-d_5

Carbon	1	1a	2	3	4	5	6	7
1	38.6	38.8	38.8	38.8	38.7	38.6	38.7	39.0
2	26.3	26.6	26.5	26.5	26.5	26.3	26.4	27.2
3	88.6	88.8	88.7	89.0	88.9	89.5	89.6	70.8
4	39.2	39.5	39.4	39.4	39.4	39.5	39.5	39.4
5	55.7	55.9	55.8	55.9	55.8	55.8	55.8	55.9
6	18.3	18.5	18.4	18.5	18.4	18.3	18.4	18.9
7	33.2	33.3	33.3	33.4	33.4	33.4	33.4	33.4
8	39.8	39.8	39.9	40.0	39.8	39.8	40.1	39.9
9	46.9	47.0	47.2	47.1	47.0	47.0	47.1	47.3
10	36.8	37.0	36.9	36.8	36.8	36.9	37.0	37.4
11	23.6	23.7	23.7	23.7	23.7	23.7	23.8	23.9
12	122.4	123.8	122.0	122.6	122.2	122.2	122.7	122.4
13	144.2	144.5	145.1	144.4	145.0	145.0	144.5	145.1
14	41.8	41.9	41.7	42.0	42.0	42.0	42.0	42.1
15	35.8	35.9	36.0	35.9	36.0	36.0	35.9	36.2
16	74.0	74.3	74.6	74.3	74.6	74.6	74.4	74.6
17	48.8	49.0	49.0	49.0	48.7	48.9	49.1	48.9
18	41.0	41.2	42.1	41.2	41.3	41.4	41.2	41.5
19	46.9	47.0	47.2	47.1	47.2	47.2	47.1	47.3
20	30.6	30.9	30.8	30.1	30.9	30.9	30.8	30.0
21	35.7	35.9	36.0	36.0	36.1	36.0	36.1	36.2
22	32.0	32.5	33.3	32.1	32.7	32.6	32.2	32.8
23	28.0	28.2	28.2	28.1	28.6	28.2	28.3	28.8
24	16.8	17.1	16.9	16.8	16.7	17.0	17.0	16.6
25	15.5	15.6	15.7	15.6	15.5	15.4	15.6	15.7
26	17.3	17.2	17.6	17.5	17.3	17.4	17.5	17.5
27	27.0	27.1	27.9	27.2	27.1	27.2	27.2	28.1
28	175.7	177.7	181.1	175.9	179.9	180.5	175.9	180.0
29	33.0	33.2	33.3	33.1	33.2	33.3	33.2	33.6
30	24.4	24.6	28.1	24.6	24.6	24.8	24.6	24.7
$COOCH_3$		51.8						
3-*O*-Glc								
1	106.5	106.8	106.7	104.9	104.9	104.1	104.2	
2	75.4	75.7	75.6	83.1	83.2	81.0	81.0	
3	79.0	78.7	78.6	78.2	78.2	78.1	78.1	
4	71 4	71.8	71.7	71.5	71.5	71.6	71.7	
5	78.3	78.2	78.0	77.8	77.9	77.6	77.6	
6	62.6	63.0	62.8	62.6	62.6	62.6	62.6	
1′				105.8	105.8			
2′				76.9	76.9			
3′				78.1	78.1			
4′				71.4	71.4			
5′				77.7	77.8			
6′				62.6	62.6			
28-*O*-Glc								
1″	95.6			95.7			95.8	
2″	73.8			74.0			74.1	
3″	78.4			79.2			79.3	
4″	70.7			70.1			71.1	
5″	77.9			78.7			78.8	
6″	61.9			62.1			62.2	

ranosyl ester.

Saponins with a sulfate group have rarely been isolated[6] from the plant kingdom.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The optical rotations were measured with a JASCO DIP-360 digital polarimeter. The MS were measured with JEOL JMS-DX-303HF and HX-100 spectrometers and taken in a glycerol matrix containing NaI. The NMR spectra were recorded with a JEOL JNM-GX-400 spectrometer; chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Gas liquid chromatographic (GLC) analysis was performed on a Hewlett–Packard HP-5890A gas chromatograph with an H_2 flame ionization detector; the column was OV-1 (0.32 mm × 30 m); column temperature, 230 °C, carrier gas, He (2.2 kg/cm^2). Column chromatography was carried out with Kieselgel 60 (230—400 mesh, Merck) and Sephadex LH-20 (25—100 μ Pharmacia Co., Ltd.). TLC was performed on pre-coated Kieselgel 60 F_{254} plates (0.2 mm, Merck) using a $CHCl_3$–MeOH–H_2O system as the developing solvent, and detection was achieved by spraying with a 20% H_2SO_4 reagent followed by heating.

Isolation The dried whole plants (930 g) of Eclipta alba collected at Kumamoto, Japan in September 1990, were extracted with MeOH, and the extract was evaporated under reduced pressure to afford a residue (85 g) which was partitioned between *n*-hexane, 1-BuOH and water. The 1-BuOH phase was removed to furnish the residue (23 g), which was subjected to column chromatography on Sephadex LH-20 eluted with MeOH and silica gel eluted with $CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2—6 : 4 : 1, to provide echalbasaponins I—VI (**1**—**6**), 818, 76, 27, 21, 20 and 14 mg, respectively. Similarly to the method above, the crude drug of Echiptae Herba (1.6 kg), purchased in Nanjing, China in 1992, was extracted with MeOH and separated to afford eclalbasaponins I—VI (**1**—**6**), 174, 297, 110, 65, 32, and 32 mg respectively, along with echinocystic acid (15 mg).

Eclalbasaponin I (1) Colorless needles (from MeOH), mp 252—254 °C, $[\alpha]_D^{28}$ +1.0° (c = 1.27, MeOH). Positive FAB-MS m/z: 819.4501 ($[M+Na]^+$, $C_{42}H_{68}NaO_{14}$, require: 819.4507). Negative FAB-MS m/z: 795 $[M-H]^-$, 633 $[M-Glc]^-$, 471 [m/z 633 − Glc]$^-$, 453 [m/z 471 − H_2O]$^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.89, 1.00, 1.01, 1.05, 1.13, 1.29, 1.85 (each 3H, s, H_3-23, 24, 25, 26, 27, 29, 30), 3.41 (1H, dd, J = 4.3, 11.4 Hz, H-3), 4.05 (1H, m, Glc H-2), 4.94 (1H, d, J = 7.7 Hz, Glc H-1), 5.31 (1H, br s, H-16), 5.62 (1H, br s, H-12), 6.32 (1H, d, J = 8.4 Hz, 28-*O*-Glc H-1).

Acid Hydrolysis of 1—5 A solution **1** (50 mg) in 1 N HCl–50% MeOH (5 ml) was heated at 80 °C for 4 h in a hot bath, then poured into water and extracted with $CHCl_3$. Removal of the respective solvent furnished the water and $CHCl_3$ extracts. The $CHCl_3$ extract was purified by silica gel (*n*-hexane : acetone = 4 : 1) column chromatography to give echinocystic acid (**7**, 22 mg), colorless needles, mp 279—281 °C, $[\alpha]_D^{28}$ +20.4° (c = 1.20, EtOH). ^{1}H-NMR (pyridine-d_5) δ: 0.95, 1.04, 1.07, 1.08, 1.19, 1.24, 1.85 (each 3H, s, H_3-23, 24, 25, 26, 27, 29, 30), 3.47 (1H, dd, J = 5.9, 10.2 Hz, H-3), 5.26 (1H, br s, H-16), 5.68 (1H, br s, H-12). On the other hand, the water extract furnished D-glucose (Rf = 0.36), which was detected on TLC (impregnated 0.5 M NaH_2PO_4; solvent, 2-propanol : acetone : 0.1 M lactic acid = 4 : 4 : 2). Acid hydrolysis of **2**—**5** (1—3 mg) was performed by the same method as used for **1**, and the aglycone and sugar were detected by TLC.

Alkaline Solvolysis of 1 After a solution of **1** (50 mg) in 0.5 N NaOH–MeOH (3 ml) was heated at 37 °C for 3 h, the reaction mixture was diluted with water, and it was passed through an MCI gel CHP-20P column, washed with water until it became neutral, and subsequently eluted with MeOH. The methanolic fraction was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 9 : 1) to provide the prosapogenin methyl ester (**1a**, 15 mg), a white powder, $[\alpha]_D^{30}$ +1.1° (c = 1.54, $CHCl_3$). ^{1}H-NMR (pyridine-d_5) δ: 0.88, 0.90, 1.02, 1.03, 1.10, 1.32, 1.80 (each 3H, s, H_3-23, 24, 25, 26, 27, 29, 30), 3.42 (1H, dd, J = 4.0, 11.7 Hz, H-3), 3.70 (3H, s, OMe), 4.05 (1H, br t, J = 8.2 Hz, Glc H-2), 4.95 (1H, d, J = 7.7 Hz, Glc H-1), 5.09 (1H, br s, H-16), 5.55 (1H, br s, H-12).

Eclalbasaponin II (2) An amorphous powder, $[\alpha]_D^{28}$ +6.8° (c = 1.07, MeOH). Negative FAB-MS m/z: 633 $[M-H]^-$, 471 $[M-Glc]^-$, 453 [m/z 471 − H_2O]$^-$. Positive FAB-MS m/z: 657.3981 ($[M+Na]^+$, $C_{36}H_{58}NaO_9$, require: 657.3979). ^{1}H-NMR (pyridine-d_5) δ: 0.89, 1.00, 1.01, 1.04, 1.17, 1.30, 1.77 (H_3-23, 24, 25, 26, 27, 29, 30), 3.41 (1H, br d, J = 11.0 Hz, H-3), 4.03 (1H, m, Glc H-2), 4.92 (1H, d, J = 7.7 Hz, Glc H-1), 5.10 (1H, br s, H-16), 5.63 (1H, br s, H-12).

Eclalbasaponin III (3) An amorphous powder, $[\alpha]_D^{29}$ −3.1° (c = 1.33, MeOH). Negative FAB-MS m/z: 957 $[M-H]^-$, 795 $[M-Glc]^-$, 633 [m/z 795 − Glc]$^-$, 471 [m/z 633 − Glc]$^-$, 453 [m/z 471 − H_2O]$^-$. Positive FAB-MS m/z: 981.5032 ($[M+Na]^+$, $C_{48}H_{78}NaO_{19}$, require: 981.5035). ^{1}H-NMR (pyridine-d_5) δ: 0.88, 1.00, 1.04, 1.09, 1.12, 1.25, 1.82 (H_3-23, 24, 25, 26, 27, 29, 30), 3.29 (1H, dd, J = 4.0, 11.7 Hz, H-3), 4.89 (1H, d, J = 7.7 Hz, Glc H-1), 5.30 (1H, br s, H-16), 5.36 (1H, d, J = 7.4 Hz, Glc′ H-1), 5.61 (1H, br s, H-12), 6.30 (1H, d, J = 8.0 Hz, 28-*O*-Glc H-1).

Alkaline Hydrolysis of 3 After a solution of **3** (50 mg) in 1 N NaOH/H_2O (1.5 ml) was heated at 60 °C for 2 h, the reaction mixture was neutralized with 1N HCl and evaporated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) to give **4** (15.7 mg).

Eclalbasaponin IV (4) An amorphous powder, $[\alpha]_D^{29}$ +6.3° (c = 1.45,

MeOH). Negative FAB-MS *m/z*: 795 $[M-H]^-$, 633 $[M-Glc]^-$, 471 $[m/z\ 633-Glc]^-$, 453 $[m/z\ 471-H_2O]^-$. Positive FAB-MS *m/z*: 819.4514 ($[M+Na]^+$, $C_{42}H_{68}NaO_{14}$, require: 819.4507). ^{1}H-NMR (pyridine-d_5) δ: 0.86, 1.03, 1.07, 1.10, 1.19 1.27, 1.85 (H_3-23, 24, 25, 26, 27, 29, 30), 3.31 (1H, dd, J=4.4, 11.7 Hz, H-3), 4.92 (1H, d, J=7.3 Hz, Glc H-1), 5.25 (1H, br s, H-16), 5.37 (1H, d, J=7.7 Hz, Glc′ H-1), 5.65 (1H, br s, H-12).

Eclalbasaponin V (5) An amorphous powder, $[\alpha]_D^{22}$ −9.3° (c=1.70, pyridine). Negative FAB-MS *m/z*: 713 $[M-H]^-$, 633 $[M-SO_3H]^-$, 471 $[m/z\ 633-Glc]^-$, 453 $[m/z\ 471-H_2O]^-$. Positive FAB-MS *m/z*: 759.3361 ($[M-H+2Na]^+$, $C_{36}H_{57}Na_2O_{12}S$, require: 759.3366). ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.99, 1.06, 1.17, 1.18, 1.43, 1.84 (H_3-23, 24, 25, 26, 27, 29, 30), 3.34 (1H, dd, J=4.0, 11.3 Hz, H-3), 4.99 (1H, d, J=7.7 Hz, Glc H-1), 5.24 (1H, br s, H-16), 5.63 (1H, br s, H-12).

Eclalbasaponin VI (6) An amorphous powder, $[\alpha]_D^{22}$ +0.5° (c=1.43, pyridine). Negative FAB-MS *m/z*: 875 $[M-H]^-$, 795 $[M-SO_3H]^-$, 713 $[M-Glc]^-$. Positive FAB-MS *m/z*: 921.3879 ($[M-H+2Na]^+$, $C_{42}H_{67}Na_2O_{17}S$, require: 921.3895). ^{1}H-NMR (pyridine-d_5) δ: 0.83, 1.01, 1.04, 1.10, 1.17, 1.43, 1.83 (H_3-23, 24, 25, 26, 27, 29, 30), 3.35 (1H, br d, J=11.0 Hz, H-3), 4.99 (1H, d, J=7.7 Hz, Glc H-1), 5.31 (1H, br s, H-16), 5.60 (1H, br s, H-12), 6.32 (1H, d, J=8.1 Hz, 28-*O*-Glc H-1).

Solvolysis of 5 and 6 A solution of **5** (1 mg) in dioxane–pyridine (1 : 1, 0.5 ml) was kept at 140 °C in a sealed tube for 3 h. The mixture was evaporated under a N_2 brow. The product obtained was identical with compound **2** on TLC (*Rf* 0.74, $CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). A solution of **6** was treated in the same manner to give **1**, identified by TLC (*Rf* 0.47, $CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5).

Identification of Component Monosaccharides, 1—6 A solution of each compound (3—5 mg each) in 1 N aqueous HCl–dioxane (1 : 1, 0.5 ml) was heated at 90 °C for 2 h. The precipitate was removed by filtration and the supernatant was treated with Amberlite IRA-400. The neutralized solution was concentrated *in vacuo* to give a sugar fraction. The pyridine solution of the sugar fraction was derived into the trimethylsilyl ether of methyl 2-(polyhydroxyalkyl)thiazolidine-4(*R*)-carboxylate using Mihashi's method,[5] and analyzed by GLC to detect a peak at t_R (min): 16.2 (D-glucose). The standard monosaccharides were subjected to the same reaction, and GLC analysis was performed under the same condition. *C.f.* standard specimens, t_R (min): 17.4 (L-glucose), 16.2 (D-glucose).

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References and Notes

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