Oleanane Glycosides from *Eclipta prostrata*

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ABSTRACT: Two oleanane-type glycosides eclalbasaponin II (**1**) and eclalbasaponin I (**2**) along with the ubiquitous steroid, stigmasterol were isolated from an *n*-hexane extract of the stem bark of *Eclipta prostrata*. The structures of the isolated compounds were confirmed by extensive spectroscopic studies, notably high field NMR and MS. The ^{13}C NMR data of the parent saponins **1** and **2** are reported here for the first time.

Key words: *Eclipta prostrata*, Compositae, Oleanane glycoside, Eclalbasaponin I, Eclalbasaponin II.

INTRODUCTION

Eclipta prostrata (Bengali name- Kalokeshi; Family- Compositae) is an annual weed of moist places that grow all over Bangladesh, Mayanmar, Malay Penisula, Central India.[1,2] The roots of *E. prostrata* are emetic and it is applied externally as an antiseptic to ulcers and wounds.[3] The whole plant is astringent, deobstruent, depurative, emetic, febrifuge, ophthalmic, purgative, styptic and tonic.[3] Previous phytochemical investigation of *E. prostrata* resulted in the isolation of thiophene derivatives, glycosides[4], alkaloids[5].

We, herein, report the further isolation of eclalbasaponin II (**1**) and eclalbasaponin I (**2**) and complete ^{1}H and ^{13}C NMR spectral data of the parent compounds for the first time.

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MATERIALS AND METHODS

The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) or Varian VXR-500S (500 MHz) instruments. The ^{13}C NMR spectra were acquired on the same instrument at 100 or 125 MHz while the 2D-NMR spectra were recorded with a Varian 500 VXR or Bruker 400 MHz instruments using the standard microprograms. For NMR studies deuterated chloroform and methanol were used and the δ values for ^{1}H and ^{13}C spectra were recorded in respect to the residual non-deuterated solvent signals. Fast atom bombardment mass spectra (FAB-MS) were obtained as a JEOL SX102 spectrometer.

Plant Material. The whole plant of *E. prostrata* was collected from Savar in the month of August 2004. A voucher specimen has been submitted in Bangladesh National Herbarium (ACC-31253), Dhaka, Bangladesh.

Extraction and Isolation. The powdered plant (500 g) of *E. prostrata* was soaked in 1.2 L of methanol for 7 days and then filtered off through whatman filter paper number 1. The extract was concentrated with a rotary evaporator. A portion

(5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[6] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions and evaporated to dryness to get *n*-hexane (0.55 g), carbon tetrachloride extract (1.50 g), chloroform (1.50 g) and aqueous soluble materials.

The *n*-hexane soluble fraction was then chromatographed by column chromatography (CC) over silica gel (60-120 mesh) using *n*-hexane, ethyl acetate and methanol mixtures of increasing polarities to give a total of 61 fractions, of 25 ml each. Repeated preparative thin layer chromatography (stationary phase- silica gel F_{254}, mobile phase- 8% methanol in chloroform, thickness of plates-0.5 mm) of fraction 53 afforded needle shaped crystal of **1**. Compound **2** was obtained as crystals from fraction 55 by preparative TLC over F_{254} silica gel using 10 % methanol in chloroform as the mobile phase, while stigmasterol was isolated as colorless needles from fraction 30.

Properties of Compounds. Eclalbasaponin II (**1**): Amorphous white powder; FAB-MS: *m/z* $[M-H]^-$ 633.64, $C_{36}H_{58}O_9$; For NMR data see Table 1 .

Eclalbasaponin I (**2**): White powder; FAB-MS: *m/z* $[M+Na]^+$ 819, $[M-H]^+$ 795, $C_{42}H_{68}O_{14}$; For NMR data see Table 1 .

Stigmasterol. Colorless needles; ^{1}H NMR (400 MHz, $CDCl_3$) spectral data was identical with published values.[7]

RESULTS AND DISCUSSION

The *n*-hexane soluble fraction of the methanol extract of *E. prostrata* was subjected to extensive chromatographic separation and purification to yield a total of three compounds. The structures of the isolated compounds were deduced by careful NMR and mass spectral analyses. The FAB-MS of compound **1** provided a pseudomolecular ion peak $[M-H]^-$ at *m/z* 633.64 in the negative ion mode, appropriate for a molecular formula, $C_{36}H_{58}O_9$. The ^{13}C NMR spectrum of compound **1** (Table 1) displayed 36 carbon resonances, while the HSQC and DEPT experiments indicated that 28 out of the 36 carbons had attached protons. The DEPT 135 and 90 spectral data showed resonances for 7 methyl, 10 methylene, 11 methine and 8 quaternary carbons.

The ^{1}H NMR spectrum of compound **1** (Table 1) displayed a one proton broad singlet at δ 5.30, which indicated the presence of an olefinic proton. Seven singlets, each of three proton intensity, at δ 0.79, 0.86, 0.96, 0.96, 0.97, 1.06 and 1.37 in the ^{1}H NMR spectrum suggested the presence of seven tertiary methyl groups in compound **1**. On the other hand, the ^{13}C NMR resonance at δ 181.1 was characteristic for a carboxylic acid group. In the ^{1}H NMR spectrum signals were observed at δ 4.33, 3.19 (2H), 3.29, 3.28, 3.67 & 3.84 and form the HSQC spectrum, the chemical shifts of directly bonded carbons of these protons were determined at δ 106.6, 75.6, 78.2, 71.6, 77.6 and 62.8 with attached proton at δ 3.67 & 3.84, respectively. The spectral features revealed the presence of a sugar residue in compound **1.**

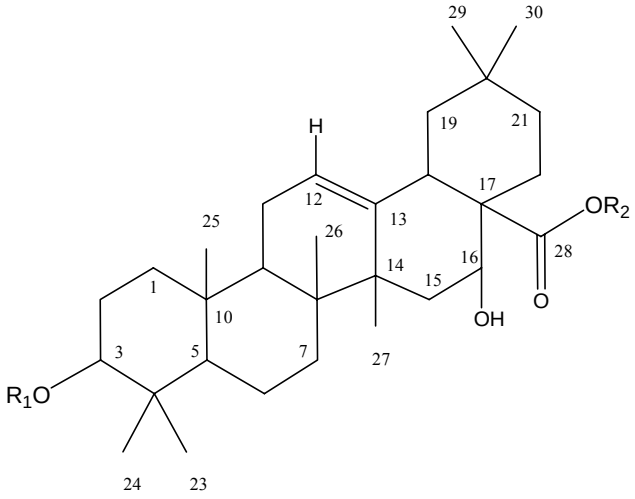
The ^{1}H - ^{1}H COSY data allowed to assign the complete spin system for the sugar moiety in compound **1**. The anomeric proton (H-1') at δ 4.33 (d, *J*=7.9 Hz) was found to couple with a proton at δ 3.19 (t, *J*=7.9 Hz, H-2'), which, in turn, revealed strong interaction with another proton resonating at δ 3.29 (m, H-3'). This proton (H-3') also demonstrated coupling with H-4' appeared as a multiplets at δ 3.19 which, in turn, exhibited connectivity with H-5' at δ 3.28 and 3.67. The hydroxymethyl protons ($-CH_2OH$) at δ 3.67 and δ 3.84 revealed geminal coupling (*J*=11.8 Hz), in addition to the vicinal couplings with 3.28 assignable to H-5'. This allowed to identify the sugar as D-glucose. The large coupling (*J*= 7.8 Hz) observed between H-1' and H-2' confirmed a beta linkage between the sugar and the aglycone moiety.

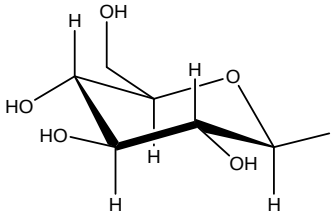
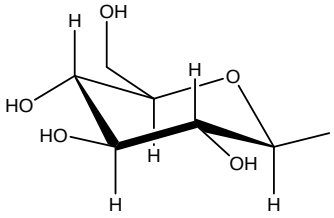
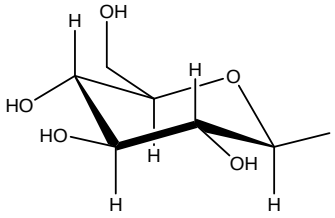
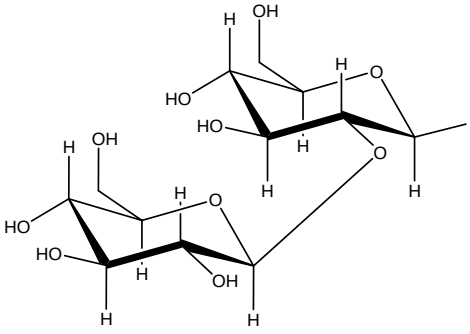
The identification of compound **1** and its ^{13}C assignments were established unambiguously by 2D NMR studies. The ^2J and ^3J connectivities as observed in the HMBC experiment are shown in Table 1. From the HSQC experiment, the connectivity of the specific proton(s) to the attached carbon was identified. The HMBC experiment

Table 1. NMR spectral data for compounds 1 and 2 in CD_3OD.*

Position	1				2	
	δ_C	δ_H	HMBC		δ_C	δ_H
			^2J	^3J		
1	38.0				38.6	
2	27.0				26.3	
3	90.8	3.20 m		19.3 (C-6)	90.9	3.20 m
4	40.1				39.2	
5	57.1				55.7	
6	19.3				18.3	
7	32.6				33.2	
8	40.6				39.8	
9	48.1				46.9	
10	37.8				36.8	
11	24.4	1.89	123.4 (C-12)		23.6	
12	123.4	5.30 br. s	24.4 (C-11)	48.1 (C-9), 42.6 (C-14), 42.6 (C-18)	122.4	5.32 m
13	145.0				144.2	
14	42.6				41.8	
15	39.8	1.35			35.8	
16	75.2	4.46 br. s	48.4 (C-17)	42.6 (C-14), 42.6 (C-18)	74.0	4.50 br.s
17	48.4				48.8	
18	42.6	3.01 dd (J = 7.7)	145.0 (C-13), δ 47.6 (C-19), 48.4 (C-17)	123.4 (C-12), 42.6 (C-14), 75.2 (C-16), 181.1 (-COOH)	41.0	
19	47.6	1.01		48.4 (C-17)	46.9	
20	31.3		31.3 (C-29)		30.6	
21	36.5				35.7	
22	36.2				32.0	
23	27.2	0.79		90.8 (C-3)	28.0	0.88 s
24	17.0	1.06	40.1 (C-4)		16.8	1.00 s
25	16.0	0.96		44.2 (C-25)	15.5	1.01 s
26	17.7	0.96			17.3	1.05 s
27	27.3	0.97	47.6 (C-19)		27.0	1.17 s
28	181.1	-			175.7	
29	33.4	0.86	31.3 (C-20)		33.0	1.36 s
30	28.5	1.37			24.4	1.84 s
1’	106.6	4.33 (1H, d, J=7.9)	75.6 (C-2’)	90.8 (C-3)	106.6	4.87 m
2’	75.6	3.19 (1H, t, J=7.9)	106.6 (C-1’)		75.4	
3’	78.2	3.29 (1H, m)			79.0	
4’	71.7	3.19 (1H, m)			71.4	
5’	77.6	3.28 (1H, m)	62.8 (C-6’), 71.6 (C-4’)		78.3	
6’	62.8	3.67 (1H, dd, J=11.8, 5.0) 3.84 (1H, br.d)			62.6	
1’’					95.6	
2’’					73.8	
3’’					78.4	
4’’					70.7	
5’’					77.9	
6’’					61.9	

* ^{13}C and ^1H data acquired at 100 and 400 MHz, respectively.



	R_1	R_2
1 :		H
2:		
3:	H	H
4:		H

demonstrated some key correlations (Table 1) to substantiate the structure. The correlation between the anomeric proton at H-1' and the carbon at δ 90.8 indicated the linking of the sugar moiety at C-3 of the aglycone, echinocystic acid (**3**). The olefinic proton at C-12 showed ^{3}J interactions with the methine at δ 42.6 (C-18) and δ 42.6 (C-14) and ^{2}J correlation at δ 24.4 (C-11). The C-16 proton showed a ^{2}J correlation

to the quaternary carbon at δ 48.4 (C-17) and a ^{3}J correlation to the methine at δ 42.6 (C-18). On the other hand, H-18 showed a connectivity with the carboxylic acid group carbon at δ 181.1. This allowed to place the COOH group R at C-17. The proton at C-18 demonstrated the highest number of correlations over two and three bonds at δ 181.4 (C-28), 145.0 (C-13), 123.4 (C-12), 75.2 (C-16), 48.4 (C-17), 47.6 (C-19) and 42.6 (C-14). On this basis, compound **1** was unambiguously characterized as eclalbasaponin II, which has previously been reported from *E. alba.* The structure of **1** was resolved by analyses of the ^{1}H and ^{13}C NMR data of the hydrolyzed product.[8] However, the use of 2D NMR including HSQC and HMBC experiments allowed complete assignment of the ^{13}C resonances of the parent eclalbasaponin II (**1**) for the first time.

The mass spectrum of compound **2** also showed pseudomolecular ion peaks at *m/z* 819 $[M+Na]^{+}$ and 795 $[M-H]^{+}$ in the positive and negative mode FAB-MS, respectively. These mass spectral data revealed a molecular formula of $C_{42}H_{68}O_{14}$ for compound **2**. This was substantiated by the presence of 42 carbons resonances in the ^{13}C NMR of spectrum compound **2**. The molecular composition suggested that compound **2** could be one of the isomeric saponins, eclalbasaponin I **(2)** or eclalbasaponin IV **(4)**, both of which have the same molecular composition $C_{42}H_{68}O_{14}$.

Both eclalbasaponin I and IV reported from *Eclipta alba*[8] contain the same aglycone, echinocystic acid **(3)** and two glucose residues. In eclalbasaponin IV (**4**), both of the glucose moieties are linked in an apparently disaccharide form, while in eclalbasaponin I (**2**), the glucose units are attached to C-3 and C-28 of the aglycone skeleton. The ^{13}C NMR spectrum of compound **2** (Table 1) showed two anomeric carbon at δ 96.0 and 106.6 which were identical to those found in eclalbasaponin I (with δc 95.6 and 106.3). On the other hand, in eclalbasaponin IV (**4**) the anomeric carbon appeared at δ 104.9 and 105.8. The remaining carbon resonances and the ^{1}H NMR data were found to be consistent with eclalbasaponin I (Table 1). On this basis, compound 2 was identified as eclalbasaponin I (**2**).

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