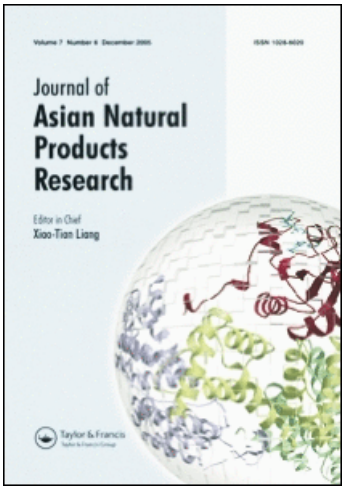

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ECLALBATIN, A TRITERPENE SAPONIN FROM *ECLIPTA ALBA*

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From the whole plant of *Eclipta alba*, a new triterpene saponin, named eclalbatin, together with α-amyrin, ursolic acid and oleanolic acid were isolated. The structure of eclalbatin has been established as 3-0-β-D-glucopyranosyl-3-β-hydroxy-olean-12-en-28-oic acid, 28-0-β-D-arabinopyranoside (**1**) on the basis of chemical and spectral data.

Keywords: *Eclipta alba*; Asteraceae; Whole plant; Triterpenoid saponin; Eclalbatin; α-Amyrin; Ursolic acid; Oleanolic acid

INTRODUCTION

Eclipta alba (L.) Hassk. (Asteraceae) known as "Bhringaraja" is distributed throughout India and mainly used in the treatment of liver diseases in Indian System of Medicine [1]. A number of compounds have earlier been reported from the plant [2, 3]. We report here the isolation of a new triterpene saponin designated eclalbatin together with α-amyrin, ursolic acid and oleanolic acid, from the methanolic fraction of the whole plant of *Eclipta alba*.

RESULTS AND DISCUSSION

The methanolic fraction of the whole plant of *Eclipta alba* yielded a saponin, eclabatin (**1**), m.p. 256–257°C (dec.). Its molecular formula was settled by

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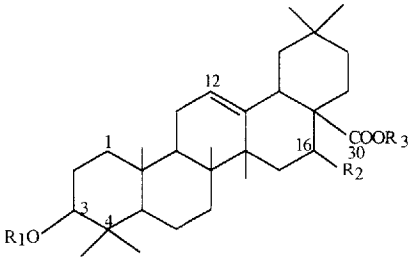
FAB-MS and elemental analysis as $C_{41}H_{66}O_{12}$. It was recognized as a triterpene saponin from its colour reaction in the Liebermann-Burchard test. Its IR spectrum showed absorption at 3380 cm^{-1} for a polyhydroxy system, at 1720 cm^{-1} for ester carbonyl and at 1640 and 895 cm^{-1} for a trisubstituted double bond. On acid hydrolysis, it gave glucose, arabinose and sapogenin (**2**). The sapogenin **2**, m.p. 305–307°C (dec.), $C_{30}H_{48}O_3$ (M^+, 456) was identified as oleanolic acid by spectral data (IR, ^1H NMR and MS) [4, 5] and direct comparison with authentic sample.

The presence of two sugar units in saponin **1** was proved by the appearance to two anomeric carbon signals at δ 96.2 (carboxylic ester) [6] and δ 105.5 ppm in its ^{13}C NMR spectrum. Based on this evidence and the fact that the parent saponin is an ester (ν_{max} 1720 cm^{-1}) and the sapogenin is an acid (ν_{max} 1695 cm^{-1}, bromothymol blue test), the attachment of one of the sugar units through an ester linkage was confirmed [6].



1 : R_1 = Glc, R_2 = H, R_3 = Ara
2 : R_1 = H, R_2 = H, R_3 = H
3 : R_1 = Glc, R_2 = H, R_3 = H
4 : R_1 = H, R_2 = OH, R_3 = H

Alkaline hydrolysis of **1** produced a prosapogenin (**3**) [7] $C_{36}H_{58}O_8$, amorphous powder, whose ^{13}C NMR spectrum (DMSO-d_6) showed signals due to a carboxylic group (δ 181.2) and one glucopyranosyl residue and *O*-glycosylated C-3 (δ 88.2) on oleanolic acid residue. The structure of **3** was thus elucidated as oleanolic acid -3-*O*-β-D-glucoside. Obviously the arabinose unit is attached at carboxylic unit in 1. The chemical shifts of anomeric carbons of glucosyl and arabinosyl residues corresponds with β- rather than α-configurations [8].

The foregoing data thus proved the structure of saponin **1** as 3-*O*-β-D-glucopyranosyl-3-β-hydroxyolean-12-en-28-oic acid, 28-*O*-β-D-arabinopyranoside, designated eclalbatin.

From the whole plant of *Eclipta alba*, six oleanane glycosides have earlier been reported having echinocystic acid (**4**) as the aglycone [2]. The

isolation of eclalbatin (**1**) is the new addition of oleanane glycoside from *Eclipta alba*.

EXPERIMENTAL SECTION

General Experimental Procedures

Mps. were determined on a Toshniwal apparatus and are uncorrected, IR were recorded on a Perkin-Elmer spectrophotometer model 221 in KBr pellet. ^1H and ^{13}C NMR were taken an 100 and 300 MHz NMR on Bruker HX 90 with TMS as internal standard. MS were performed on Karatos MS -50 mass spectrometer operation at 70 eV with evaporation of sample in the ion source at 200°; and $[\alpha]_D$ in MeOH at 20° was carried out on Perkin-Elmer polarimeter 141. CC: silica gel columns (BDH, 60–120 mesh); TLC: silical gel G (Merck); PC: Whatman paper No.1; solvents for TLC: $CHCl_3$–MeOH (8:1) (solvent A), $CHCl_3$–MeOH–H_2O (14:7:1) (solvent B), $CHCl_3$–MeOH–H_2O (65:35:10) (solvent C) and for PC: *n*-BuOH-HOAc-H_2O (4:1:5) (solvent D); paper chromatogram: developed with acetonic $AgNO_3$/5% alcoholic NaOH/$Na_2S_2O_3$/H_2O.

Plant Material

The plant material was collected from Varanasi District and identified by Dr. N. K. Dubey, Department of Botany, Banaras Hindu University, Varanasi. A specimen sample is kept in the department.

Extraction and Isolation

Dried whole plant (3 kg) was powdered and repeatedly extracted with MeOH by percolation at 30°C. The MeOH extract afforded a brown semisolid (42 g) which was fractionated into petroleum ether (60–80°), C_6H_6, EtOAc and MeOH fractions by passing through SiO_2 gel column. The C_6H_6 fraction was rechromatographed over SiO_2 gel column and the eluants from C_6H_6–$CHCl_3$ (9:1), (4:1) and (2:1) furnished respectively α-amyrin (24 mg), m.p. 184–186°C, ursolic acid (21 mg), m.p. 290–292°C and oleanolic acid (30 mg), m.p. 300–302°C. The MeOH fraction was further chromatographed over SiO_2 column and the eluants from EtOAc yielded eclalbatin (**1**) as colourless granules (48 mg), m.p. 256–257°C (dec.) (MeOH); $[\alpha]_D^{20}$ +1.5 (*c*, 1.25, MeOH); TLC single spot, R_f 0.38 (solvent

C); FAB-MS: $[M+K]^+$ at *m/z* 789; (Elemental analysis, Found: C, 66.26%, H, 8,8%, $C_{41}H_{66}O_{12}$ requires: C, 66.20%; H, 8.5%); IR (KBr) ν_{max}, cm $^{-1}$: 3380 (br), 1720, 1640, 895; ^{13}C NMR (DMSO-d_6) δ: 38.4 (C-1), 27.0 (C-2), 88.3 (C-3), 38.7 (C-4), 55.2 (C-5), 18.3 (C-6), 32.7 (C-7), 39.3 (C-8), 47.6 (C-9), 37.0 (C-10), 23.0 (C-11), 122.2 (C-12), 143.6 (C-13), 41.7 (C-14), 27.7 (C-15), 23.4 (C-16), 46.4 (C-17), 41.1 (C-18), 46.0 (C-19), 30.7 (C-20), 35.1 (C-21), 32.5 (C-22), 28.1 (C-23), 15.6 (C-24), 15.3 (C-25), 16.9 (C-26), 26.0 (C-27), 176.5 (C-28), 33.1 (C-29), 23.6 (C-30), 105.5 (C-1′), 75.1 (C-2′), 79.8 (C-3′) 71.7 (C-4′), 77.8 (C-5′), 61.5 (C-6′), 96.2 (C-1″), 72.1 (C-2″), 73.8 (C-3″), 69.7 (C-4″), 67.2 (C-5″).

Acid Hydrolysis of Eclalbatin (1)

Eclalbatin **1** (37 mg) was dissolved in MeOH (8 ml) and H_2O (2 ml) and refluxed with H_2SO_4 (1 ml) for 6 hrs. The reaction mixture was poured into H_2O, the MeOH removed and the mixture extracted with $CHCl_3$. The $CHCl_3$ extract yielded oleanolic acid (**2**) as granules (18 mg), m.p. 305–307°C (dec.), $C_{30}H_{48}O_3$ (M^+ 456), $[\alpha]_D^{20}+76.0$ (*c*, 0.50, MeOH); IR (KBr) ν_{max}, cm^{-1}: 3450 (br)) (OH), 1695 (COOH); ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, *s*, H-23), 0.73 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 0.87 (3H, *s*, H-26), 0.89 (3H, *s*, H-27), 0.94 (3H, *s*, H-29), 1.10 (3H, *s*, H-30), 3.18 (1H, *dd*, $J=3.5$ and 10.5 Hz, H-3), 5.24 (1H, *m*, H-12); MS: *m/z* 456 (M^+) 248, 240, 230, 214, 208, 203, 119. Acetylation of **2** with acetic anhydride and pyridine at room temperature gave an acetate, m.p. 260–262°C identical to oleanolic acid acetate. The hydrolysate showed two spots on PC which corresponded to glucose and arabinose (co-PC with authentic samples, solvent D).

Alkaline Hydrolysis of Eclalbatin (1)

A mixture of eclalbatin **1** (42 mg), MeOH (3 ml), H_2O (1 ml) and KOH (0.1 g) was heated under reflux for 4 hrs. The reaction mixture was poured into water, acidified by addition of hydrochloric acid and extracted with $CHCl_3$. The $CHCl_3$ layer gave after the usual work up, prosapogenin (**3**) (26 mg), m.p. 231–233°C; FAB-MS: *m/z* 618 $[C_{36}H_{58}O_8+K]^+$; ^{13}C NMR (DMSO-d_6) δ: 38.5 (C-1), 27.1 (C-2), 88.2 (C-3), 38.7 (C-4), 55.1 (C-5), 18.4 (C-6), 32.7 (C-7), 39.2 (C-8), 47.7 (C-9), 37.0 (C-10), 23.0 (C-11), 122.1 (C-12), 143.5 (C-13), 41.6 (C-14), 27.7 (C-15), 23.5 (C-16), 46.3 (C-17), 41.2 (C-18), 46.0 (C-19), 30.6 (C-20), 35.3 (C-21), 32.4 (C-22), 28.0 (C-23), 15.5 (C-24), 15.4 (C-25), 17.0 (C-26), 26.0 (C-27), 181.2 (C-28), 33.2 (C-29), 23.7 (C-30), 105.0 (C-1′), 74.2 (C-2′), 79.9 (C-3′), 71.5 (C-4′), 77.3 (C-5′), 61.4

(C-6′); Elemental analysis, Found: C, 66.03%, H, 9.54%; calcd. for $C_{36}H_{58}O_8$: C, 66.00% H, 9.5%.

Hydrolysis of Prosapogenin (3)

A mixture of prosapogenin (**3**) (20 mg), MeOH (2 ml), H_2O (0.5 ml) and H_2SO_4 (0.2 ml) was heated under reflux for 4 hrs. Water was added and the resulting mixture was extracted with $CHCl_3$. From the $CHCl_3$ layer, oleanolic acid (**2**) (8 mg), m.p. 305–307°C was obtained. It was identified by mmp., co-TLC and superimposible IR with authentic sample.

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