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OLEANEN AND STIGMASTEROL DERIVATIVES FROM *AMBROMA AUGUSTA*

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Key Word Index—*Ambroma augusta*; Sterculiaceae; augustic acid; olean-12-en-2β,3β-diol-28-oic acid; augustoside; stigmast-5,22-dien-3-*O*-α-D-glucopyranoside.

Abstract—Augustic acid, a new oleanane derivative, and a stigmasterol glycoside were isolated from the roots of *Ambroma augusta*. Their structures were established as olean-12-en-2β,3β-diol-28-oic acid and stigmast-5,22-dien-3-*O*-α-D-glucopyranoside respectively, on the basis of full spectral analyses and chemical methods.

INTRODUCTION

Ambroma augusta Linn.f. (Sterculiaceae), commonly known as 'Ulatkambal', is a large, spreading, quick-growing, hairy shrub found both wild and cultivated throughout the hot and moister parts of India from Uttar Pradesh to Sikkim, Khasia Hills, Assam and Bengal. The plant drug possesses uterine tonic and emmenagogue properties and is used for the treatment of dysmenorrhoea, amenorrhoea sterility and other menstrual disorders [1]. The roots of *A. augusta* act as an abortifacient and antifertility agent [2, 3]. We describe herein the isolation and structural elucidation of augustic acid (**1**) and stigmasterol glucoside (**2**) from the roots of *A. augusta*.

RESULTS AND DISCUSSION

Compound **1** responded positively to the Liebermann–Burchard test and showed characteristic IR absorption bands at 3436 (OH), 1696 (CO_2H) and 1512 (C=C) cm^{-1}. It had a molecular ion peak at *m/z* 472 corresponding to the molecular formula $C_{30}H_{48}O_4$ and thereby indicated seven double bond equivalents. The mass spectrum of **1** exhibited a pair of diagnostically important mass peaks at *m/z* 248 and 203 [248-CO_2H] typical of the retro-Diels–Alder fragmentation in ring C of an olean-12-en derivative containing a carboxyl function in either ring D or E [4]. Acetylation did not alter the values of these two fragments whereas conversion to the methyl ester caused a mass increment of 14 amu in one of the fragments (*m/z* 262). Other important ions were observed at 454 $[M - H_2O]^+$, 428 $[M - CO_2]^+$, 426 $[M - HCO_2H]^+$, 408 $[426 - H_2O]^+$ and 189 $[204 - Me]^+$.

The ^1H NMR spectrum of **1** displayed a one-proton broad singlet at δ5.166 assigned to H-12 and another two-protons broad singlet at δ3.417 ascribable to hydroxymethines, one placed at C-3 on the basis of biogenetic analogy and another at C-2. The seven methyl signals, all located on saturated carbons, resonated at δ0.885 (Me-23), 0.689 (Me-24 and Me-26), 0.905 (Me-25) 1.075 (Me-27) and 0.858 (Me-29 and Me-30).

More compelling evidence for the structure was provided by a study of its ^{13}C NMR spectrum (Table 1), which showed the presence of 30 carbon atoms. The assignments of the carbon chemical shifts were made by comparison with the δ values of the corresponding carbon atoms in structurally similar compounds [5–9], namely, methyl oleanolate, hederagenin methyl ester, gypsogenin methyl ester, methyl maslinate and imberbic acid. The difference in melting points of compound **1** and its methyl ester derivative from that of maslinic acid and its methyl ester derivative suggested that they were different compounds. An upfield shift of C-2 (δ67.15) from that of methyl maslinate (δ68.8) [8] also suggested a difference in the stereo structure at C-2. Furthermore, apart from the resonances of the carbons of rings of A, B and C, the resonances of the ring D and E carbons in **1** agreed with the corresponding assignments in methyl maslinate, which restricted the positioning of two hydroxyl functions to ring A and a carboxyl group at C-17. The degree of protonation of each carbon atom was determined by DEPT experiments and by the ^1H–^{13}C COSY spectrum. The quaternary carbon atoms were determined by substracting DEPT from a broad band ^{13}C NMR spectrum. The connectivities of structure **1** were confirmed by ^1H–^{13}C long range experiments. The stereochemistry of

Table 1. ^1H and ^{13}C NMR chemical shifts of compound **1**

Position	^1H NMR α	^1H NMR β	^{13}C NMR
1	1.748 *br s*	0.775 *br s*	46.52
2	3.417 *br s*	—	67.15
3	3.417 *br s*	—	82.23
4	—	—	41.44
5	0.748 *dd* (14.12, 12.09)	—	54.84
6	1.456 *m*	1.456 *m*	18.11
7	1.229 *br s*	1.229 *br s*	32.41
8	—	—	37.72
9	1.507 *br s*	—	47.14
10	—	—	37.72
11	1.456 *m*	1.456 *m*	22.66
12	5.166 *br s*	—	121.54
13	—	—	143.95
14	—	—	41.44
15	0.987 *br s*	0.987 *br s*	27.44
16	1.806 *m*	1.889 *m*	23.08
17	—	—	45.76
18	—	2.718 *d* (9.16)	40.88
19	1.585 *m*	1.132 *br s*	45.52
20	—	—	30.44
21	1.316 *br s*	1.212 *br s*	33.40
22	1.585 *m*	1.400 *m*	32.17
23	0.885 *br s*	—	28.85
24	0.689 *br s*	—	16.94
25	0.905 *br s*	—	16.34
26	0.689 *br s*	—	17.14
27	1.075 *br s*	—	25.69
28	0.905 *br s*	—	178.51
29	0.858 *br s*	—	32.89
30	0.858 *br s*	—	23.42

Multiplicity for the protons and the coupling constant(s) in Hertz are given in parentheses.

1 was also elucidated on the basis of ^1H–^1H COSY analysis. Treatment of **1** with acetic anhydride and pyridine afforded a diacetyl derivative (**1a**), $C_{34}H_{52}O_6$, (*m/z* 556, ν_{max} 1735 and 1725 cm^{-1}), indicating the presence of two hydroxyl groups. Esterification of **1** with diazomethane formed a methyl ester (**1b**). On the basis of these findings, augustic acid (**1**) was identified as olean-12-en-2β,3β diol-28-oic acid.

Compound **2** gave a positive Liebermann–Burchard test and a negative Ehrlich reaction. The IR spectrum exhibited strong bands at 3476 and 1062 cm^{-1} characteristic of a glycoside. The FAB-mass spectrum of **2** gave a quasimolecular ion at *m/z* 597 $[M + Na]^+$, which suggested the M_r to be 574. The EI mass spectrum gave a fragment at *m/z* 396 due to the loss of glucose from the molecular ion peak.

The ^1H NMR spectrum of **2** showed the signals of two tertiary methyls at δ0.640 (Me-18) and 1.005 (Me-19), four secondary methyls at δ0.978 (Me-21), 0.885 (Me-26), 0.810 (Me-27) and 0.822 (Me-29), one trisubstituted olefinic proton at δ5.317 (H-6), two disubstituted olefinic protons at δ5.149 (H-22) and 5.006 (H-23) and one anomeric proton at δ4.212. The ^{13}C NMR spectrum (Table 2) showed the existence of 35 carbon atoms in the molecule. An anomeric signal at δ100.76 indicated the presence of a single monosaccharide moiety. The degree of protonation of each carbon atom was determined by DEPT experiments. The ^{13}C DEPT NMR spectrum revealed the presence of six methyl, 10 methylene and 16 methine carbon atoms. The four methine resonances at δ70.08, 76.74, 73.42 and 76.69 and one methylene resonance at δ61.0 were due to C-2′, C-3′, C-4′, C-5′ and C-6′, respectively, of the α-D-glucopyranoside. The olefinic resonances at δ121.13, 137.95 and 128.79 corresponded to the C-6, C-22 and C-23 methine carbons, and a signal at δ140.41 corresponded to the C-5 quaternary carbon of the sterol moiety.

The COSY and relayed COSY experiments reflected that the anomeric doublet at δ4.212 belonged to one glucose residue. In the COSY spectra all coupling constants were large and corresponded to the equatorial proton of one α-D-glucopyranose residue. The ^{13}C–^1H COSY-90° spectrum allowed complete correlation of the protonated carbon resonances with those of the ^1H spectrum. The ^1H–^1H COSY measurements also confirmed the assignments. The COSY-45° spectrum showed strong coupling interaction between the H-3 methine and H-4 methylene.

Acetylation of **2** with acetic anhydride–pyridine afforded a tetraacetyl derivative (**2a**) (ν_{max}^{KBr} 1760 cm^{-1}). The positive FAB-mass spectrum of **2a** gave a peak at *m/z* 765 $[M + Na]^+$. Acetylation deshielded all the methine protons alpha to acetates in the δ5.035–3.455 region. The homonuclear ^1H–^1H COSY study of **2a** not only correlated each proton in the spin system, but also revealed several interesting correlations via long range coupling. The assignment of carbon resonances of **2a** was achieved by the heteronuclear ^1H–^{13}C COSY spectrum including both one-bond and long-range coupling.

Acid hydrolysis of **2** yielded D-glucose and an aglycone (**2b**) which was identified as stigmasterol by spectral data as well as by direct comparison with the authentic samples (co-TLC, mmp). On the basis of these findings, **2** was identified as stigmast-5,22-dien-3-*O*-α-D-glucopyranoside.

EXPERIMENTAL

Mps: uncorr. IR: JASCO FT/IR 5000, KBr; UV: Beckmann DU-64, MeOH; ^1H (600 MHz) and ^{13}C (150 MHz) NMR: JEOL A-600, DMSO-d_6 with TMS as int. standard; MS: Hitachi M-80; CC: silica gel (Qualigen, 60–120 mesh); TLC: Silica gel 60 F_{254} pre-coated plates. The spots were visualized by exposure to I_2vapour and by spraying with vanillin–H_2SO_4.

Plant material. The roots of *A. augusta* were purchased from Herba Indica, Chandigarh, and identified in our Botany Department by Dr M. P. Sharma.

Isolation of constituents. Air dried and crushed roots of *A. augusta* (3 kg) were exhaustively extracted (Soxhlet)

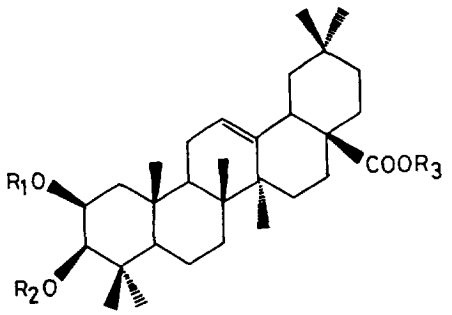
Table 2. ^1H and ^{13}C NMR chemical shifts of compounds **2** and **2a**

	2		**2a**	
Position	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1	2.344 *m* 2.093 *m*	38.27	0.991 *m* 1.817 *m*	37.20
2	1.306 *m*	33.30	0.902 *br s* 1.270 *m*	33.94
3	3.469 ($W_{1/2}$ = 22.35)	76.89	3.455 *br m* ($W_{1/4}$ = 22.71)	72.92
4	1.775 d (10.63) 1.142 (6.59)	36.79	1.887 *br s* 1.135 *m*	39.73
5	—	140.41	—	140.36
6	5.317 *br s*	121.13	5.331 *br s*	122.16
7	1.481 *m*	31.31	1.459 *m*	31.94
8	1.495 *br s*	31.38	1.270 *br s*	31.87
9	0.916 *br s*	49.56	0.818 *br s*	50.17
10	—	36.16	—	36.13
11	1.178 *m*	22.57	1.998 *br s* 1.981*br s*	21.20
12	1.138 *m* 1.957 *m*	39.63	0.964 *br s* 1.459 *m*	40.45
13	—	40.03	—	42.33
14	1.070 *m*	56.22	0.835 *br s*	56.75
15	1.033 *m*	24.79	1.034 *m*	24.29
16	1.792 *br s* 1.637 *br s*	29.21	1.637 *br s*	28.22
17	1.043 *m*	56.12	1.043 *m*	56.05
18	0.640 *s*	11.80	0.653 *s*	11.98
19	1.005 *s*	19.03	1.002 *s*	19.34
20	1.306 *m*	35.42	1.270 *m*	36.72
21	0.978 *d* (6.23)	18.78	0.991 *d* (6.60)	18.77
22	5.149 *m*	137.95	5.164 *m*	138.27
23	5.006 *m*	128.79	5.052 *m*	129.31
24	0.917 *br s*	45.11	1.550 *br s*	29.16
25	1.438 *m*	31.26	1.459 *m*	31.87
26	0.885 *d* (6.59)	19.64	0.891 *d* (6.59)	19.80
27	0.810 *d* (6.96)	18.88	0.818 *d* (6.96)	19.03
28	1.054 *br s*	23.80	1.196 *br s*	23.07
29	0.822 *d* (7.33)	11.62	0.835 *d* (7.32)	11.85
1'	4.212 *d* (9.69)	100.76	4.576 *d* (8.06)	99.64
2'	3.054 *m*	70.08	3.455 *m*	80.07
3'	3.403 *m*	76.74	3.482 *m*	71.51
4'	3.113 *m*	73.42	5.035 *m*	68.55
5'	3.070 *m*	76.69	4.919 *m*	71.55
6a'	4.406 *d* (5.5)	61.0	4.817 *d* (10.10)	62.10
6b'	4.212 *d* (7.69)		4.081 *d* (10.10)	
$COCH_3$	—	—	2.054 *s*	170.69, 21.20
			2.025 *s*	170.35, 21.04
			1.998 *s*	169.40, 20.76
			1.981 *s*	169.30, 20.71

Multiplicity for the protons and the coupling constant(s) in Hertz are given in parentheses.

with EtOH (95%) and the combined extracts concd to dryness under red. pres. to yield a brown semi-solid residue (100 g). The residue was fractionated between EtOAc and MeOH, and EtOAc solubles (25 gm) were subjected to CC on silica gel. Elution was carried out with petrol and petrol containing increasing amounts of EtOAc.

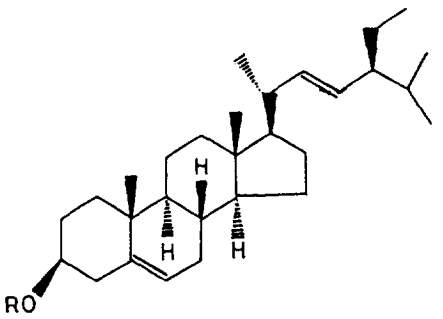
Augustic acid (**1**). Frs eluted with petrol–EtOAc (7:3) yielded a residue which was crystallized from petrol–Me_2CO as white crystals (60 mg), mp 256–258°. TLC R_f 0.33 (toluene–EtOAc, 1:1). IR ν_{max} cm^{-1}: 3436, 2948, 1696, 1512, 1464, 1390, 1282, 1180, 1110, 1050, 1034, 994, 962, 822. UV λ_{max} 216 (log ε 4.2). FAB-MS (rel. int. *m/z*: 495 $[M + Na]^+$ (14.8); EIMS (rel. int.) *m/z*: 472



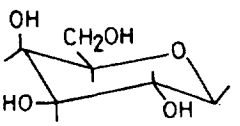
1 R_1 = H, R_2 = H, R_3 = H

1a R_1 = Ac, R_2 = Ac, R_3 = H

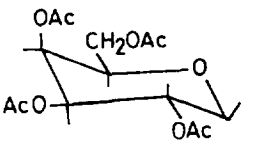
1b R_1 = H, R_2 = H, R_3 = Me



2 R =



2a R =



2b R = H

$[M]^+$ ($C_{30}H_{48}O_4$) (4.1), 454 (1.0), 436 (1.0), 428 (3.0), 426 (6.1), 408 (2.4), 393 (1.0), 248 (100), 233 (2.1), 219 (2.1), 206 (6.1), 204 (25.8), 203 (80.0), 191 (3.8), 189 (22.2), 175 (21.2), 161 (13.9), 147 (13.6), 133 (31.2), 119 (32.1), 105 (32.2), 91 (30.0), 80 (32.6), 69 (54.1), 55 (75.2), ^{1}H and ^{13}C NMR: Table 1.

Acetylation of compound **1.** Compound **1** (15 mg), dissolved in a 1: 1 mixt. of pyridine–Ac_2O, was warmed slightly. On standing overnight and on usual work-up it afforded a diacetyl derivative (**1a**) (12 mg), mp 148–150°, IR ν_{max} cm^{-1}: 3310, 1735, 1725, 1685, 1525, 1465, 1385, 1270, 1185, 1055, 1030, 995, 955, 825. EIMS (rel. int.) *m/z*: 556 $[M]^+$ (1.0), 496 (21.0), 451 (5.1), 436 (2.5), 248 (85.1), 233 (15.3), 187 (33), 43 (100).

Esterification of compound **1.** Compound **1** (15 mg) was treated with excess of ethereal CH_2N_2 at room temp. On evapn of the solvent, the Me ester (**1b**) was obtained, mp 175–176°; EIMS (rel. int.) *m/z*: 486 $[M]^+$ (3.1), 440 (23.1), 262 (100), 216 (3.5), 203 (8.5), 187 (5.1).

Compound **2** (stigmasterol glucoside). Frs eluted with petrol–EtOAc (1:1) yielded a residue which was crystallized from $CHCl_3$–MeOH as white crystals (60 mg), mp 268–70°. TLC R_f 0.5 ($CHCl_3$–MeOH, 9:1). IR ν_{max} cm^{-1}: 3476, 2944, 1646, 1556, 1370, 1340, 1214, 1168, 1114, 1062, 1026. UV λ_{max} 209 (log ε 2.9). FAB-MS (rel. int.) *m/z*: 597 $[M + Na]^+$ (9.2) 574 (14.2). EIMS (rel. int.) *m/z* 412 (82.5), 396 (91.8), 394 (34.0), 381 (24.4), 329 (3.2), 303 (5.3), 255 (6.6), 213 (5.7), 189 (4.0), 173 (5.8), 157 (10.0), 145 (13.2),131 (13.1), 117 (11.6), 105 (25.8), 93 (19.0), 81 (28.8), 69 (73.6), 55 (100). ^{1}H and ^{13}C NMR: Table 2.

Acetylation of compound **2.** Compound **2** (20 mg) was acetylated in the same manner as described above to give a tetraacetyl product (**2a**), mp 146–147°. IR ν_{max} cm^{-1}: 2990, 1760, 1641, 1458, 1385, 1258, 1220, 1176, 1110, 1080, 1042, 910. FAB-MS (rel. int.) *m/z*: 765 $[M + Na]^+$ (8.2), 743 $[M + H]^+$ (7.2). EIMS (rel. int.) *m/z*: 396 (10.3), 382 (3.2), 353 (1.1), 331 (5.0), 255 (1.1), 213 (5.1), 201 (2.2), 185 (3.0), 169 (25.1), 157 (8.9), 145 (15.2), 133 (7.2), 119 (9.5), 105 (18.6), 93 (23.8), 81 (30.3), 69 (65.3), 55 (100). ^{1}H and ^{13}C NMR: Table 2.

Acid hydrolysis of compound **2.** Compound **2** (10 mg) was refluxed with 2 N HCl in 80% MeOH (1:1, 10 ml) for 4 hr. After cooling, the reaction mixt. was poured into crushed ice, and the hydrolysate was then extracted with EtOAc to give the aglycone. The aglycone, mp 169°, was identified as stigmasterol by spectral and chromatographic comparison (co-TLC, mmp). The neutralized (Ag_2CO_3) and concd aq. hydrolysate showed the presence of glucose on comparison with authentic sugars on silica gel TLC, R_f 0.4 (EtOAc–HOAc–H_2O–MeOH, 6:1:1:2).

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