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TRITERPENOID AND PRENYLATED PHENOL GLYCOSIDES FROM *BLUMEA LACERA*

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Abstract—Two new glycosides, the triterpenoid glycoside 19α-hydroxyurs-12-ene-24,28-dioate 3-*O*-β-D-xylopyranoside and the phenol glycoside 2-isoprenyl-5-isopropylphenol 4-*O*-β-D-xylopyranoside have been isolated from the whole plant of *Blumea lacera* and their structures elucidated by means of spectral and chemical studies.

INTRODUCTION

Blumea lacera (Burm.f.) DC. [1] is a commonly occurring herb, distributed throughout the plains of India, Ceylon, China, Malaya, Australia and tropical Africa. It is known to cure bronchitis, blood diseases and fevers, and to alleviate burning sensations [2]. Since very little chemical work has been reported on this plant, we undertook the investigation of its constituents. So far, the presence of only small amounts of acetylinic compounds, a thiophene derivative, a diester [3], campesterol [4] and three flavonoids [5] have been reported from this plant. This paper describes the isolation and structural elucidation of two new glycosides.

RESULTS AND DISCUSSION

Glycosides **1** and **2**, were isolated from the petrol extract of the whole plant of *B. lacera*.

Compound **1** on acid hydrolysis gave the aglycone **1a** and a sugar, whereas on enzyme hydrolysis it gave the aglycone **1b** and a sugar. The sugar in both cases was identified as D-xylose on the basis of co-paper chromatography with an authentic sample.

The aglycone **1b** responded positively to Liebermann–Burchard [6], TCA [7] and TNM [8] tests. Its IR spectrum showed absorptions owing to hydroxyl (3460 cm^{-1}) and carbomethoxyl (1725, 1710 cm^{-1}) groups and a trisubstituted double bond (1630 cm^{-1}). This suggested that the aglycone was a pentacyclic unsaturated triterpenoid. In **1a**, as expected, no peaks were present at 1725 and 1710 cm^{-1}.

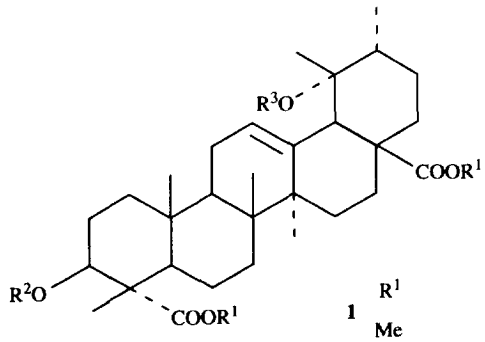
The ^{13}C NMR values for **1a** and **1b** were found to be identical except for those of C-24 and C-28. This was owing to the presence of two -CO_2Me groups in **1** which were hydrolysed to carboxyl groups in the formation of **1a** but not **1b**. On acetylation, **1a** and **1b** both formed diacetates and thus established the presence of two hydroxyl groups.

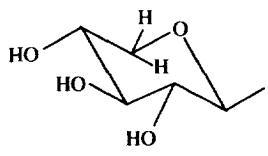
The ^1H NMR signals between δ0.71 and 1.28 (each *s*, 3H) and at δ0.95 (*d*, 3H, $J = 7.0$ Hz) were from five tertiary and one secondary methyl group, respectively. The signal at δ5.34 (*t*, 1H), owing to a vinylic proton, showed that the compound was an urs-12-ene derivative.

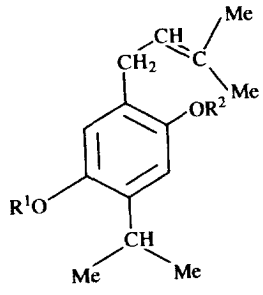
The mass spectrum of **1b** revealed a pair of diagnostically important mass peaks at *m/z* 278 and 260 [278 − H_2O] typical of retro-Diels–Alder fragmentation of ring C of an urs-12-ene derivative. It also gave a positive Zimmermann test [9] indicating a C-3 position for the hydroxyl function; this location was also biogenetically favoured. A double doublet at δ3.80 in the ^1H NMR spectrum [$J = 2.7$ Hz (diequatorial coupling) [10] and $J = 5.7$ Hz (equatorial axial coupling [11] corresponding to H-β (eq) of C-3)] showed the axial orientation of hydroxyl group at C-3, since equatorial (β) orientation of the hydroxyl group would increase the value of the coupling constant owing to diaxial coupling [12].

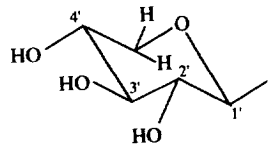
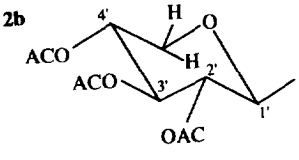
The ^{13}C NMR spectrum of the compound resembled that of methyl ursolate [13], showing that the compound was a methyl ursolate derivative. The ^1H NMR spectrum showed a characteristic broad singlet at δ2.60 (1H, H-18β), in accordance with the presence of a 19α hydroxyl group. This was further confirmed by comparing the ^{13}C NMR spectrum of the compound with the methyl ester of myriaboric acid [14].

The ^1H NMR spectra exhibited two peaks at δ3.60 and 3.76 corresponding to two carbomethoxyl groups. The high δvalue of C-4 in the compound as compared to that of α-amyrin was a result of deshielding by the -CO_2Me group at C-4. The mass ion peak at *m/z* 219 [278 − CO_2Me], owing to ready loss of -CO_2Me groups, showed that the second -CO_2Me group was at C-17. The ^{13}C NMR signals for C-24 at δ177.1 and for C-28 at δ178.2 were from the carbonyl functions and confirmed the -CO_2Me groups were at C-4 and C-17. The IR spectrum of the compound showed three intense bands at 1160, 1185 and 1235 cm^{-1} characteristic of di- and



	R^1	R^2	R^3
1	Me		H
1a	H	H	H
1b	Me	H	H
1c	Me	AC	AC



	R^1	R^2
2		H
2a	H	H
2b		AC

triterpenes carrying-CO_2Me groups in axial orientation. Thus, the aglycone (**1b**) was identified as 3β,19α-dihydroxyurs-12-ene-24,28-methyl dioate.

The ^{1}H NMR spectrum of the glycone showed a signal at δ5.15 (*d*, 1H, J = 7.5 Hz) for the anomeric proton of xylose and established its β-linkage with the aglycone. The downfield signal for C-3 at δ82.2 which appeared at δ73.3 in the aglycone revealed C-3 as the site of glycosidation. This was further confirmed by the ^{13}C NMR spectrum (Table 1). Consequently, **1** was 19α-hydroxyurs-12-ene-24,28-dioate 3-*O*-β-D-xylopyranoside.

Compound **2** was assigned the molecular formula $C_{19}H_{28}O_6$. On acid hydrolysis it gave an aglycone (**2a**) and D-xylose. The aglycone gave a blue-green colour with alcoholic $FeCl_3$ solution, indicating its phenolic nature. IR absorption bands at 3300 cm^{-1} owing to hydroxyl groups and at 1500–1600 cm^{-1} owing to an aromatic ring, further confirmed the phenolic hydroxyl nature of the aglycone. On acetylation, it formed a diacetate (**2b**), thus confirming the presence of two hydroxyl groups.

Furthermore, on the basis of standard colour reactions and spectral studies, it was found to have a *p*-hydroquin-

Table 1. ^{13}C NMR spectral data for **1**, **1a** and **1b**

C	1	1a	1b
1	41.2	41.3	41.3
2	28.0	27.5	27.5
3	82.2	73.1	73.2
4	48.6	48.0	48.0
5	53.1	53.0	53.0
6	21.0	21.0	21.0
7	33.4	33.1	33.2
8	41.3	41.3	41.3
9	47.1	47.0	47.1
10	41.6	41.5	41.5
11	25.2	25.0	25.0
12	129.2	129.2	129.2
13	138.6	138.6	138.6
14	41.3	41.2	141.2
15	28.6	28.6	28.6
16	25.5	25.5	25.5
17	48.6	48.3	48.3
18	53.8	53.6	53.6
19	77.4	77.5	77.5
20	41.0	41.1	41.1
21	25.6	25.1	25.7
22	37.8	37.4	37.4
23	25.8	25.5	25.5
24	177.0	180.0	177.1
25	15.6	15.8	15.8
26	17.2	17.3	17.3
27	26.4	26.1	26.1
28	178.4	180.4	178.2
29	27.4	27.4	27.4
30	16.0	16.0	16.1
	106.0		
	106.0		
	74.6		
	79.4		
	71.1		
	68.5		
	52.1	52.0	
CO_2Me	51.6	51.4	CO_2Me

one nucleus [15] containing isoprenyl and isopropyl groups at positions C-2 and C-5, respectively.

The site of xylosidation was found to be C-4 on the basis of its ^{13}C NMR spectrum. The ^{13}C NMR data of acetate **2b** of the glycoside suggested that the acetoxy group must be located *ortho* to the isopropyl group because the isopropyl, methyl and methine carbons showed considerable downfield shifts (from δ23.2 to 24.1 and from δ27.8 to 29.9, respectively), upon acetylation of the phenolic hydroxyl group, whereas the isoprenyl $-CH_2$ ^{1}H NMR signal at δ3.30 showed no change. Therefore, the structure of the acetate **2b** was deduced to be 2-isoprenyl-4-xylosyloxy-5-isopropylphenol acetate. The ^{1}H NMR spectrum of **2** also showed an anomeric proton signal of D-xylose as a doublet at δ5.10 (1H, *d*, J = 7 Hz) indicating its β-linkage with the aglycone. Thus, **2** was identified as 2-isoprenyl-5-isopropylphenol 4-*O*-β-D-xylopyranoside.

EXPERIMENTAL

Plant material (at full leaf state) was collected in January from Allahabad, India. A herbarium specimen of the plant is on file at the Botanical Survey of India, sheet no. = 45466. Mps: uncorr.; TLC: silica gel G (Merck 7731) with solvent systems (a) C_6H_6–DCM (4:1) and (b) EtOAc–MeOH (4:1); CC: silica gel 60 (Merck 7734); IR: KBr disks; ^{1}H NMR: 90 MHz, $CDCl_3$ soln, using TMS as int. standard; ^{13}C NMR: 25.05 MHz, pyridine-d_5 soln, with TMS employing the FT mode.

Air-dried plant material was extracted in petrol. The concd extract was loaded onto a flash column and then eluted with different solvents of increasing polarity. Elution with C_6H_6 yielded a fr. containing a crystalline compound (**1**) and elution with EtOAc yielded a shiny compound (**2**).

Compound **1**. Homogeneous on TLC, R_f 0.39 (solvent a). Mp 147° (Found: C, 67.07; H, 8.02. Calc for $C_{37}H_{57}O_{10}$: C, 67.17; H, 8.62%). IR ν_{max}^{KBr} cm^{-1}: 3560, 3460, 1000, 930, 1725, 1630, 1160, 1050, 930, 1185, 1235; ^{1}H NMR [$CDCl_3$, 100 MHz]: δ2.60 (*br s*, 1H, -OH), 3.60 (*s*, 3H, $-CO_2Me$), 3.76 (*s*, 3H, $-CO_2Me$), 5.34 (*t*, 1H, H-12), 0.95 (*d*, 3H, J = 7 Hz, sec-Me), 5.15 (*d*, 1H, J = 7.5 Hz, H-1′), 3.2–3.8 (*m*, 5H, sugar protons), 0.71–1.28 (18H, 6 × Me), 3.8 (1H, *dd*, J = 2.7, 5.7 Hz); ^{13}C NMR: Table 1.

Acid hydrolysis (7% H_2SO_4) of compound **1** gave aglycone **1a** and the sugar xylose (co-chromatography with an authentic sample). The aglycone crystallized from DCM, mp 275° (R_f 0.60 solvent b). IR ν_{max}^{KBr} cm^{-1}: 3560, 3460, 1690 (CO_2H), 1630, 1000; ^{13}C NMR: Table 1.

Enzyme hydrolysis of glycoside **1** yielded the sugar xylose and aglycone **1b**. A mixture of the glycoside (0.1 g) in 50% aq. EtOH (20 ml) and emulsion soln (10 ml; prepd from almonds), was kept at 45° for 2 hr and then at room temp. for 4 days. The soln was extracted with EtOAc and the remaining hydrolysate was concd in a rotary evaporator. The syrup so obtained on paper chromatography gave one spot (R_f 0.21, solvent system BAW, spray AHP). Co-PC with an authentic sample gave only one spot. Mp 260° (R_f 0.52 solvent b). IR ν_{max}^{KBr} cm^{-1}: 3560, 3460, 1725, 1710, 1630, 1000; ^{13}C NMR: Table 1. Acetylation of **1b** gave the diacetate **1c** as needles, mp 205°.

Compound **2**. Mp 203° (from CH_2Cl_2). Homogeneous on TLC, R_f 0.51 (EtOAc–MeOH, 4:1) (Found: C, 69; H, 8.1. Calc. for $C_{19}H_{28}O_6$: C, 69.5; H, 8.53%). ^{1}H NMR ($CDCl_3$, 90 MHz): δ1.15 (6H, *d*, J = 7 Hz, $-CHMe_2$), 1.60 (3H, *s*, Me), 1.60 (3H, *s*, Me), 3.30 (2H, *d*, J = 6 Hz, $-CH_2-$), 5.06 (1H, *t*, J = 6 Hz, $-CH=$), 6.54 (1H, *s*), 6.96 (1H, *s*), 3.1–3.8 (*m*, sugar protons), 5.10 (1H, *d*, J = 7 Hz, C-1 of Xyl); ^{13}C NMR: δ149.5 (*s*), 147.5 (*s*), 123.2 (*s*), 118.8 (*s*), 134.6 (*d*, C-3), 113.7 (*d*, C-6), 27.8 (*d*, C-7), 23.2 (*q*, C-8), 23.2 (*q*, C-9), 28.8 (*t*, C-10), 27.0 (*d*, C-11), 29.0 (*s*, C-12), 24.4 (*q*, C-13), 24.4 (*q*, C-14), 106.0 (anomeric carbon), 74.5, 79.5, 71.2, 68.5.

Tetracetate of compound **2**. Compound **2** (0.05 g) was acetylated with Ac_2O (5 ml) and pyridine (1 ml) at room temp. for 48 hr. Crystallization from EtOAc gave a solid (Found: C, 60.8; H, 6.2. Calc. for $C_{27}H_{36}O_{14}$: C, 61.3; H,

6.81%). ^{1}H NMR ($CDCl_3$, 90 MHz): δ1.13 (3H, *d*, J = 7 Hz, $-CHMe_2$), 1.14 (3H, *d*, J = 7 Hz, $-CHMe_2$), 2.08 (9H, *s*, 3 × OAc), 2.10 (3H, *s*, OAc), 6.80 (1H, *s*), 6.90 (1H, *s*), 5.10 (1H, *d*, J = 7 Hz, C-1 of Xyl), 5.00–5.38 (*m*, sugar protons), 5.10 (1H, *d*, J = 7 Hz, $-CH<$), 1.64 (3H, *s*, Me), 3.30 (2H, *d*, J = 6 Hz, $-CH_2-$), 6.0 (1H, *t*, J = 6 Hz, $-CH=$); ^{13}C NMR: δ149.4 (*s*), 147.0 (*s*), 123.1 (*s*), 119.1 (*s*), 134.1 (*d*, C-3), 118.0 (*d*, C-6), 29.9 (*d*, C-7), 24.1 (*q*, C-8), 24.1 (*q*, C-9), 170.2, 170.4, 169.8, 169.0 (each *s*, CO).

Hydrolysis of glycoside. An EtOH soln of **2** was refluxed with 7% H_2SO_4 for 4 hr. The mixt. was then poured into ice-cold water. A yellowish white ppt. sepd and was crystallized from EtOAc to provide the aglycone **2a** (R_f 0.69 $CHCl_3$) (Found: C, 76.0; H, 8.1. Calc. for $C_{14}H_{19}O_2$: C, 76.7; H, 8.6%); ^{13}C NMR: δ149.5 (*s*), 148.0 (*s*), 132.5 (*s*), 123.0 (*s*), 118.8 (*d*), 113.7 (*d*), 27.8 (*d*), 23.2 (*q*), 23.2 (*q*), 28.8 (*d*), 27.0 (*d*), 29.0 (*d*), 24.2 (*q*). The sugar in the aq. layer was identified as D-xylose.

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