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Triterpenoid saponins from *Acorus calamus*

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Two new triterpenoid saponins have been isolated from the rhizome of plant *Acorus calamus*. They are characterized as 1β, 2α, 3β, 19α-tetrahydroxyurs-12-en-28-oic acid-28-O-{-β-D-glucopyranosyl (1→2)}-β-D-galactopyranoside **1** and 3β, 22α, 24, 29-tetrahydroxyolean-12-en-3-O-{-β-D-arabinosyl (1→3)}-β-D-arabinopyranoside **2** and their structures elucidated by spectral and chemical studies.

Acorus calamus Linn. (Araceae) commonly called "Butch" is a useful medicinal plant found throughout India and in Ceylon in marshes, wild or cultivated ascending the Himalayas upto 6,000 ft. in Sikkim. Plentiful in the marshy tracts of Kashmir and Sirmon in Manipur and Nagahills[1]. It is well known for its medicinal properties[2] such as insecticidal against bed bugs, moths, lice, emetic stomach in dyspepsy, colic, remittet fever, nerve tonic in broncht, dysentry of children, insectifuge in snake bite.

In continuation of our research on the chemical investigation of medicinal plants we report herein an isolation and structure elucidation of two new triterpenoids.

The dried and crushed herbaceous plant was extracted with ethanol. Two compounds were isolated from ethanolic extract by flash column chromatography and TLC. Homogeneity and purity of these compounds were established by TLC.

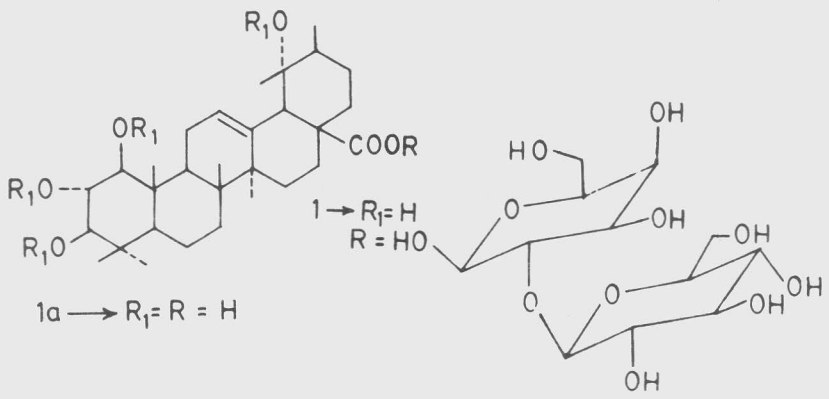
Compound **1**, $C_{42}H_{68}O_{16}$ (M^+ 828), m.p. 94°, non-reducing glycoside showed absorptions in the IR spectra for hydroxyl group and ester linkage at 3400 and 1715-1710 cm^{-1} respectively. On acid hydrolysis with 7% H_2SO_4 it gave an aglycone **1a** and two sugar moieties. Both the sugars were identified as D-galactose and D-glucose on the basis of co-Paper chromatography with an authentic sample.

The aglycone **1a**, $C_{30}H_{48}O_6$ (M^+504) responded positively to Leibermann-Burchard, TCA and TNM tests. On acetylation, the aglycone formed monohydroxy triacetate showing that the compound contains four hydroxyl groups and out of four -OH groups, one was tertiary in nature. The above facts suggested that the aglycone was a pentacyclic unsaturated triterpenoid. The ^{1}H NMR spectrum of **1a** showed a characteristic broad signal at δ 2.57 (1H, 18β-H) together with secondary methyl (δ 0.95, d, 3H, *J*=6.5Hz) and olefinic proton (δ5.35, t, 1H, *J*=3.6 Hz), all of which suggested a 19α-hydroxy-urs-12-en type of triterpenoid.

The mass spectrum of **1a** revealed diagnostically important peaks at m/z 264,246,218,201 and 165 which are characteristic of urs-12-en derivative.

^{1}H NMR spectrum of aglycone showed signals for six tertiary methyl groups at δ0.68(s,3H), 0.83(s,3H), 1.02(s, 6H), 1.22(s, 6H) and at δ0.95 (d, 3H, *J*=6.5 Hz) for one secondary methyl group. A triplet appeared at δ5.35(1H) for C-12 olefinic proton, the position of double bond at 12:13 of amyrin nucleus was also confirmed by positive colour test with Ruzicka reaction. The ^{13}C NMR spectrum of aglycone resembled with 2-epitormentic acid methyl ether[3] with regards to signals assignable for C-12, C-13, C-18, C-19 and C-28 (cf. Table I). These data suggested that the aglycone had a tertiary or hindered -OH group in the ring D/E.

A singlet at δ2.57 in the ^{1}H NMR spectrum of the aglycone also suggested the presence of a 19α-hydroxy group. The compound gave positive Zimmermann test suggesting the C-3 position of hydroxyl which was also biogenetically favoured. The position of remaining two hydroxyl groups in ring A or B were assigned by spectral studies of



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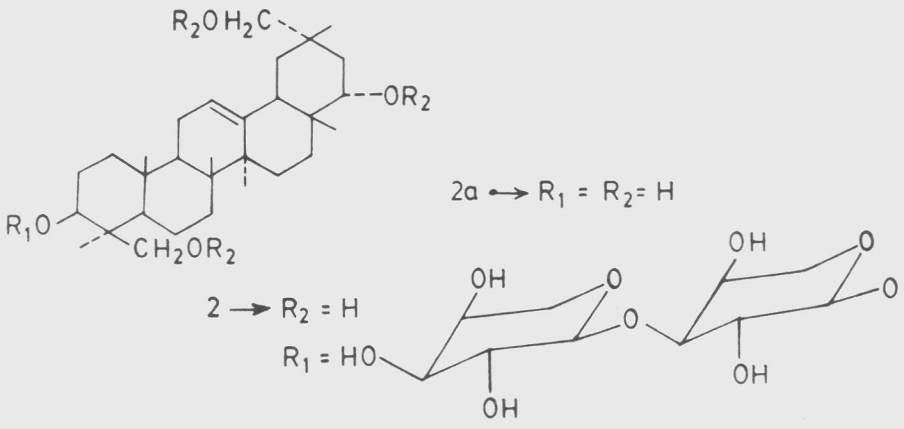

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acetyl derivative of **1a**. The ^{13}C NMR of compound on comparing with 2-epitormentic acid showed the presence of three -OH groups at adjacent positions, C-1, C-2 and C-3. The spectral values indicated that the protons at C-3 (C-1) and the protons at C-2 are *trans*-diaxial correlated protons. Axial protons at C-3 (C-1) and C-2 led to assign equitorial positions to these -OH groups[5].

The position of -COOH group was established by comparing the ^{13}C NMR spectrum of the compound with α-amyrin[6] (Table I). The C-18 carbon resonated at δ 52.4 ppm in the aglycone and at δ 58.9 ppm in α-amyrin. This difference (upto 6.5 ppm) resulted in an upfield effect due to C-28 carboxylic group in the aglycone[7]. Similarly, C-22 resonated at δ 38.2 in aglycone and at δ 41.5 in α-amyrin for the same reason. The carbon of carbomethoxy group (C-28) resonated at δ 180.9. Finally, the position of -COOH group at C-17 in the aglycone was confirmed by a signal at δ 3.6 ppm in ^1H NMR of its methyl ester. Hence, **1a** must be 1β, 2α, 3β,19α-tetrahydroxy-urs-12-en-28-oic acid.

The attachment of two sugars to the carboxyl group was confirmed by the IR spectra and alkaline hydrolysis of the glycoside. Hydrolysis with 7% H_2SO_4 gave an aglycone and two sugar moieties. The sugars were identified as D-galactose and D-glucose. ^1H NMR spectrum of **1** also showed two anomeric proton signals of D-galactose at δ4.88 (d, 1H, *J*=5.4 Hz) and D-glucose at δ5.74 (d, 1H, *J*=7.5 Hz) which indicated β-linkage with the aglycone.

Hydrolysis of **1** with β-glucosidase gave D-glucose but not the galactose. This confirmed that glycosidic linkage of glucose was ether linkage and the glycosidic linkage of galactose was ester linkage which was not hydrolysed by the enzyme. This confirmed that glucose was a terminal sugar

Table I—^{13}C NMR spectra of compounds **1**, **1a**, **2** and **2a**

C	1	1a	2	2a
1	74.5(d)	74.5(d)	38.6(t)	38.6(t)
2	74.9(d)	74.9(d)	27.0(t)	27.0(t)
3	73.3(d)	73.3(d)	92.0(d)	92.0(d)
4	40.6(s)	40.6(s)	43.0(s)	43.0(s)
5	53.3(d)	53.3(d)	57.0(d)	57.0(d)
6	17.8(t)	17.8(t)	19.0(t)	19.0(t)
7	32.6(t)	32.6(t)	33.9(t)	33.9(t)
8	41.2(s)	41.2(s)	40.1(s)	40.1(s)
9	48.0(d)	48.0(d)	48.6(s)	48.6(s)
10	37.4(s)	37.4(s)	36.9(s)	36.9(s)
11	24.4(t)	24.4(t)	24.6(t)	24.6(t)
12	129.5(d)	129.5(d)	123.0(d)	123.0(d)
13	137.2(s)	137.2(s)	145.6(s)	145.6(s)
14	41.4(s)	41.4(s)	42.0(s)	42.0(s)
15	29.8(t)	29.8(t)	26.0(t)	26.0(t)
16	26.0(t)	26.0(t)	29.3(t)	29.3(t)
17	48.6(s)	48.6(s)	37.5(s)	37.5(s)
18	52.4(d)	52.4(d)	44.5(d)	44.5(d)
19	73.4(s)	73.4(s)	41.9(t)	41.9(t)
20	42.6(d)	42.6(d)	36.8(s)	36.9(s)
21	27.2(t)	27.2(t)	37.2(t)	37.2(t)
22	38.2(t)	38.2(t)	76.2(d)	76.2(d)
23	28.3(q)	28.3(q)	23.2(q)	23.2(q)
24	16.2(q)	16.2(q)	74.2(t)	74.2(t)
25	11.5(q)	11.5(q)	16.2(q)	16.2(q)
26	16.8(q)	16.8(q)	17.5(q)	17.5(q)
27	25.6(q)	25.6(q)	26.8(q)	26.8(q)
28	180.9(s)	180.9(s)	21.1(q)	21.1(q)
29	27.2(q)	27.2(q)	72.9(t)	72.9(t)
30	17.0(q)	17.0(q)	24.8(q)	24.8(q)
31	98.2		103.8	
32	79.8		71.6	
33	71.9		75.0	
34	67.4		70.1	
35	74.4		67.5	
36	60.8		103.5	
37	102.2		71.2	
38	70.0		75.2	
39	73.8		70.0	
40	68.0		67.6	
41	77.1			
42	61.9			

unit and galactose was directly linked to aglycone. Thus, glycoside **1** was identified as 1β, 2α, 3β, 19α-tetrahydroxy-urs-12-en-28-oic acid-28-O-{-β-D-glucopyranosyl (1→2)}-β-D-galactopyranoside.

Compound **2**, $C_{40}H_{67}O_{12}$(M^+738), m.p. 270°, was also a non-reducing glycoside. On acid hydrolysis, it gave an aglycone **2a** and the two moieties of sugar arabinose. Compound **2a**, $C_{30}H_{50}O_4$ (M^+474), gave colour reactions characteristic of unsaturated pentacyclic triterpenoids. On acetylation **2a** formed the tetraacetate showing the presence of four hydroxyl groups in **2a**.

The ^1H NMR spectrum of **2a** showed singlets for six tertiary methyls at δ0.88-1.20 and a triplet at δ5.25 due to an olefinic proton. ^1H NMR and ^{13}C NMR spectra showed the presence of one hydroxyl (β-oriented) at C-3. This was also proved from Zimmermann test which was biogenetically favoured. The signals in ^{13}C NMR spectrum showed the presence of substituents/groups at C-3, C-22, C-24, C-29 by comparing their δ values with 3α, 16α, 21α, 22α, 28-pentahydroxyolean-12-en-28-O-β-D-xylopyranoside[8].

In ^{13}C NMR spectrum the δ value of C-4 of aglycone appeared at 43.0. The signal for C-4 appeared at δ38.7 in α-amyrin and at δ 43.2 in rotungenic acid. This clearly suggested that the substitution at C-4 was different from that of α-amyrin but identical to that of rotungenic acid which contained methyl and hydroxymethyl groups. In ^{13}C NMR spectra it was also found that C-23 appeared as quartet at δ23.2 and C-24 as triplet at δ74.2. The high δ value for C-24 was due to the presence of -OH group. This conclusively confirmed that C-24 was present in the form of hydroxymethyl at C-4 position.

C-24 having $-CH_2OH$, showed anisotropic effect corresponding to two protons at δ3.27 (d, 1H, J=12 Hz) and at δ4.12 (d, 1H, J=12 Hz) in different environment (Table I). From these data compound **2a** was assigned the structure as 3β, 22α, 24, 29-tetrahydroxyolean-12-en.

^1H NMR spectrum of **2** showed two signals at δ4.90 (d, 1H, J=5.2 Hz) and at δ5.78 (d, 1H, J=7.5 Hz) for the anomeric protons of two arabinose moieties showing its β-linkage with the aglycone **2a**.

The site of glycosidation was found to be C-3 on the basis of ^{13}C NMR spectral data of **2** and this location is biogenetically favoured.

From the above evidences, the structure of Compound **2** was determined as 3β, 22α, 24, 29-tetrahydroxyolean-12-3-O-{β-D-arabinosyl (1→3)}-β-D-arabinopyranoside.

Experimental Procedure

The plant material used in this study was collected in January from Jabalpur, M.P., India (a

herbarium specimen is in file in Botanical Survey of India, Allahabad, sheet No. 18881). All m.p.'s are uncorrected. TLC was carried out on silica gel G(Merck 17631) with solvent system, unless otherwise stated, as follows: (a) Benzene-$CHCl_3$ (2:8, v/v) (b) Benzene-$CHCl_3$(8:2, v/v). Column chromatography was done on silica gel 60 (Merck 24398). IR spectra were recorded in KBr pellets; ^1H NMR spectra at 200 MHz in CCl_4 unless otherwise specified using TMS as internal standard; and ^{13}C NMR spectra at 25 MHz in $CDCl_3$ solution with TMS as internal standard employing the FT mode.

Extraction and Isolation

The air dried plant Rhizome (3 Kg) was extracted first with ethanol and the concentrated extract chromatographed over silica gel column. Elution with Benzene -DCM (9:1, v/v) fraction contained compound **1** and elution with ethyl acetate-methanol (8:2, v/v) yielded compound **2**.

Compound **1**, m.p. 94° ($CHCl_3$), yield 400 mg, homogeneous on TLC, R_f 0.71 (C_6H_6-$CHCl_3$; 2:8 v/v) (Found: C, 61.0; H, 7.16. $C_{42}H_{68}O_{16}$ requires C, 61.09; H, 7.07%); IR (KBr): 3400, 1715, 1710, 1520 cm^{-1}; ^1H NMR (CCl_4, 200 MHz): δ 0.68 (s, 6H), 0.83(s, 3H), 0.95 (d, 3H, *J*=6.5 Hz), 1.02 (s, 6H), 1.22 (s, 6H), 2.57 (br.s, 1H), 3.0-3.6, (br.m, 15H), 5.35 (t, 1H, *J*=3.5 Hz), 4.88 (d, 1H, *J*=5.4 Hz), 5.74 (d, 1H, *J*=7.5 Hz); MS:m/z 504 (M^+), 264, 246, 218, 201, 165; ^{13}C NMR data are given in Table I.

Acid hydrolysis (7% H_2SO_4) of compound **1** Gave aglycone **1a** and two sugar moieties, D-galactose and D-glucose (co-chromatography with an authentic sample). The aglycone was crystallized from DCM, m.p. 98°; IR(KBr) : 3400, 1715, 1710, 1520 cm^{-1}; ^{13}C NMR (cf. Table I).

Compound **2**, m.p. 270° ($CHCl_3$), yield 350 mg, homogeneous on TLC, R_f 0.75 (C_6H_6-$CHCl_3$; 8:2 v/v) (Found: C, 63.5; H, 8.8. $C_{40}H_{67}O_{12}$ requires C, 63.6; H, 8.7%); ^1H NMR (CCl_4, 200 MHz): δ0.88 (s, 3H), 0.95 (s, 3H) 0.99 (s, 3H), 1.02 (s, 3H), 1.16 (s, 3H), 1.20 (s, 3H), 3.27 (d, 1H, *J*=12Hz), 3.28-3.40 (m, 3H), 3.46 (m, 1H), 4.12 (d, 1H, *J*=12 Hz), 5.25 (t, 1H), 4.90 (d, 1H, *J*=5.2 Hz), 5.78 (d, 1H, *J*=7.5 Hz); MS: m/z 474 (M^+).

Acid hydrolysis (7% H_2SO_4) of compound **2** gave aglycone **2a** and two sugar moieties of D-arabinose(co-chromatography with an authentic sample), m.p. 256° (Found : C, 75.6; H, 10.4, $C_{30}H_{50}O_4$ requires C, 76.0; H, 10%).

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