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TRITERPENOID SAPONINS FROM *CENTIPEDA MINIMA*

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Key Word Index—*Centipeda minima*; Compositae, triterpenoid saponins, ursane and oleanane derivatives

Abstract—Four new triterpenoid saponins were isolated from the whole plant of *Centipeda minima*. They were characterized as 1α,3β,19α, 23-tetrahydroxyurs-12-en-28-oic acid-28-*O*-β-D-xylopyranoside, 1β,2α,3β,19α,23-pentahydroxyurs-12-en-28-oic acid-28-*O*-β-D-xylopyranoside, 3α,21α,22α,28-tetrahydroxyolean-12-en-28-*O*-β-D-xylopyranoside and 3α,16α,21α,22α,28-pentahydroxyolean-12-en-28-*O*-β-D-xylopyranoside Structures of all compounds were elucidated by spectroscopic methods with emphasis on mass spectra, ^{1}H and ^{13}C NMR spectra

INTRODUCTION

Centipeda minima is an annual herbaceous plant found in moist places throughout the plains of India and Ceylon It is also found in Afghanistan, Eastern tropical Asia, Australia and the Pacific Islands [1]. *Centipeda* is well known for its medicinal properties [2, 3] In the earlier work *Centipeda minima* was shown to yield a number of terpenoids [4] In this paper, we wish to report isolation and structure elucidation of four new triterpenoid saponins isolated from the water-soluble fraction of an ethanolic extract of *Centipeda minima*.

RESULTS AND DISCUSSION

Dried and crushed defatted plant material was extracted with ethanol. The water-soluble portion of the ethanol extract, after column chromatography and preparative TLC, afforded four new glycosides (**1**–**4**) Homogeneity and purity of these compounds was established by chromatography

Compound **1**, m p. 139° showed hydroxyl, ester carbonyl and tertiary hydroxyl absorptions at 3300, 1735 and 3500 cm^{-1} and on hydrolysis gave aglycone **1a** and the sugar D-xylose. The aglycone (**1a**) responded positively to Liebermann–Burchard, TCA and TMN tests Its IR spectrum showed absorptions due to hydroxyl, carboxyl, trisubstituted double bond and *gem* dimethyl groups. This suggested that the aglycone is a pentacyclic unsaturated triterpenoid acid.

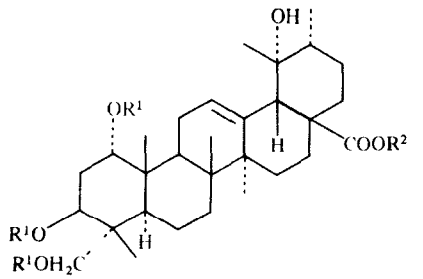
Compound **1a** on acetylation gave a monohydroxy triacetate (**1b**) and further methylation with diazomethane provided its monomethyl ester These results showed the presence of four hydroxyl groups (one tertiary) and one acidic group in **1a**. The ^{1}H NMR spectrum of **1a** showed a characteristic broad singlet at δ 2.59 (1H, 18β-H) together with tertiary methyl, secondary methyl (δ 0 98, *d*, J = 6.5 Hz) and vinylic proton (δ 5.17, *t*, J = 3.5 Hz, 12-H) signals, all of which suggested a 19α-hydroxyurs-12-en type of triterpenoid The ^{1}H NMR spectrum also showed an AB system at δ 3 15 and 3 55 (each 1H, each doublet J = 12 Hz) due to a hydroxymethyl group in **1a** and at δ 3.67 and 3.90 (each 1 H each doublet, J = 12 Hz) due to acetoxy methyl group in **1b**. The ^{1}H NMR spectrum had signals at δ 3.70 (1H, *dd*, J_1 = J_2 = 3.3 Hz, 1β-H) and 4 25 (1H, *dd*, J_1 = 10 Hz, J_2 = 7 Hz, 3α-H) due to hydroxymethine protons The splitting pattern of these protons suggested that one hydroxyl group was axial and one was equatorial.

The electron impact mass spectra of **1a** showed characteristic fragment peaks (*m/z* 264, 246, 219, 201) due to retro-Diels–Alder cleavage of urs-12-en-28-oic acid derivatives bearing one hydroxyl group in the D- or E-ring [5]

The ^{13}C NMR spectrum of compound **1a** was the same as that of 19α-hydroxyursolic acid [6] with regards to signals assignable to C-12, C-13, C-18, C-19 and C-28. An AB system was attributed to a primary hydroxyl on C-23, thus showing the presence of a hydroxymethyl group at C-4. This was further confirmed by the ^{1}H NMR spectra of **1a** and **1b** An AB system in **1b** had a chemical shift [7] close to that expected for an equatorial autoxymethyl group (*ca* δ 3 8) rather than that for an axial group (*ca* δ 4 2)

Apart from the resonances of the ring A and C-9 carbons in the ^{13}C NMR spectrum the resonances of the ring B and C carbons in **1a** agreed with corresponding assignments in 2α,3β,23-trihydroxy olean-12-en-28-oic acid [8] which restricts the positioning of the two hydroxyl function to ring A. On biogentic grounds, it is reasonable to assume that one must be equatorial at C-3 (J_1 = 10 Hz J_2 = 7 Hz) whereas the other axially oriented hydroxyl substituent must be attached at C-1. These positions of the hydroxyl groups were further supported by ^{1}H NMR spectroscopy The presence of a hydroxy function at C-2 would reduce the ^{1}H NMR signal for H-3 from a double doublet to a doublet. Finally ^{13}C NMR spectral signals of C-5 and C-9 were both strongly shielded relative to the equivalent signals in erythrodiol [9]. This shielding is due to typical γ-gauche interactions between these carbons and the axial hydroxyl substituent at C-1 [10] Accordingly, **1a** must be 1α,3β,19α,23-tetrahydroxy-urs-12-en-28-oic acid

The attachment of D-xylose to the carboxylic group



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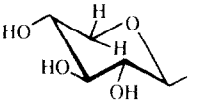
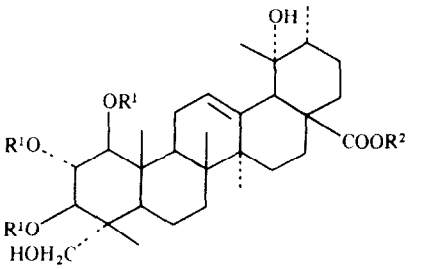
R^2 = 

1a R^1 = H, R^2 = H

1b R^1 = Ac, R^2 = H

1c R^1 = Ac, R^2 = Me



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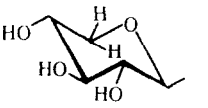
R^2 = 

2a R^1 = H, R^2 = H

2b R^1 = Ac, R^2 = H

2c R^1 = Ac, R^2 = Me

was confirmed by the IR spectrum and alkaline hydrolysis of the glycoside The ^{1}H NMR spectrum of **1** also showed an anomeric proton signal of D-xylose as a doublet (J = 7 6 Hz) indicating its β-linkage with the aglycone Thus **1** is 1α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid-28-*O*-β-D-xylopyranoside

Compound **2**, $C_{35}H_{56}O_{11}$, mp 210° was also a glycoside and on acid hydrolysis it gave aglycone **2a** and the sugar D-xylose The aglycone gave all colour reactions characteristic of triterpenoids. The IR spectrum of **2** showed peaks for hydroxyl, tertiary hydroxyl and ester carbonyl linkage.

Compound **2a** on acetylation gave a monohydroxy tetraacetate and further methylation with diazomethane provided its monomethyl ester These results showed the presence of five hydroxyl groups and one acidic group in the aglycone

The ^{1}H NMR spectrum of **2** showed a broad singlet at δ 2 59 similar to that in the spectrum **1** As the other signals of the ^{1}H and ^{13}C NMR spectra and the mass fragmentation pattern of **2a** were quite similar to **1a**, compound **2a** was considered to poossess the same skeleton as **1a** However notable differences between **1a** and **2a** could be seen with regard to the following

Compound **2a** showed the presence of five hydroxyl groups On acetylation it showed signals for three hydroxymethine protons in the ^{1}H NMR spectrum at δ 3.22 and 3 41 (each 1H, doublet, J = 10 7 and 9 5 Hz, respectively, H-1 and H-3), 4 03 (1H, double doublet J_1 = 9 5 Hz, J_2 = 10 7 Hz, H-2) and three acetoxymethine protons in **2b** at δ4.80, (*d*, J = 10 5 Hz, 1H), 4 88 (1H, *d*, J = 9 3 Hz) and 5.20 (*dd*, 1H, J_1 = 10 5 Hz, J_2 = 9 3 Hz) and were assignable to C-1 (or 3) C-3 (or 1) and C-2, respectively As the J value of these signals indicated *trans*-diaxial correlated protons, the three hydroxyl groups must be equitorial It is concluded that compound **2a** is 1β,2α,3β,19α,23-pentahydroxyurs-12-en-28-oicacid This structure was supported by its ^{13}C NMR spectrum. The attachment of D-xylose to the carboxylic group was confirmed by IR and hydrolysis with base A doublet (J = 7 5 Hz) for the anomeric proton signal of D-xylose suggested its β-linkage with the aglycone Thus compound **2** was established as 1β,2α,3β,19α,23-pentahydroxyurs-12-en-28-oic acid-28-*O*-β-D-xylopyranoside.

Compound **3**, $C_{35}H_{58}O_8$, mp 126°, was also a glycoside It showed IR absorption for a hydroxyl group at 3450 (-OH) On acid hydrolysis, it gave the aglycone **3a** and the sugar D-xylose Compound **3a** gave all colour reactions and the IR spectrum characteristic of unsaturated pentacyclic triterpenoids On acetylation **3a** formed the tetraacetate (**3b**) showing the presence of four hydroxyl groups in **3a**

The ^{1}H NMR spectrum for **3** showed resonance signals for seven tertiary methyls, an AB system (δ 3 95 and 4 30, 1H each, a pair of doublets, J_{AB} = 10 Hz) due to hydroxy methyl group and a triplet (δ 5 39 J = 3 3 Hz) due to an olefinic proton The mass spectrum of **3a** revealed a pair of diagnostically important mass peaks at *m/z* 207 and 189 [207–H_2O] typical of retro-Diels Alder fragmentation in ring C of an olean-12-en-derivative containing a hydroxyl function in ring A/B [11] From the biogenetic point of view this hydroxyl is assumed to be located at C-3 and a signal at δ 3 44 (*dd*, J = 4 2, 2 7 Hz) corresponded to 3β-H*eq*

Furthermore, the mass spectrum of compound **3a** showed peaks at *m/z* 266, 248 and 199 derived from ring D and E showing that the remaining hydroxyl groups are present in ring D and E Also, the fragment at *m/z* 266 looses the hydroxymethyl group located at C-17 thus giving rise to the base peak at *m/z* 235 Compound **3** rapidly consumed one mole of periodic acid and afforded a diosphenol on oxidation with chromium trioxide–pyridine indicating the presence of a vicinal *trans*-diol system [12] Peaks at *m/z* 235, 217 and 199 showed that two vicinal hydroxyls are present at C-21 and C-22 This was confirmed by ^{13}C NMR spectroscopy which showed shielding of C-18 & C-19 due to γ-gauche interaction The signals in the ^{1}H NMR spectrum at δ4.07 (*d*, 1H, J=3.8 Hz, CHOH) and 3.79 (*d*, 1H, J =3 8 Hz, CHOH) were coupled with each other. This indicated that two secondary hydroxyl groups should be placed at C-21 and C-22, respectively, in a *trans*-diaxial configuration. From these data compound **3a** was assigned as 3α,21α,22α,28-tetrahydroxyolean-12-en [13] The ^{1}H NMR spectrum of **3** showed a signal at δ 5 05 (*d*, 1H, J = 7 Hz) for the anomeric proton of xylose showing its β-linkage with the aglycone **3a**

The site of glycosidation was found to be C-28 on the basis of ^{13}C NMR spectral data of **3** and **3a**, and this location is favoured biogenetically From the above evidence the structure of compound **3** was determined as 3α,21α,22α,28-tetrahydroxyolean-12-en-28-*O*-β-D-xylopyranoside.

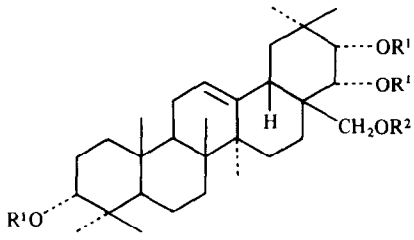
Another triterpenoid glycoside, compound **4**, had the molecular formula $C_{35}H_{58}O_9$ Compound **4** was acid hydrolysed to give D-xylose and aglycone **4a**. Compound **4a** had the molecular formula $C_{30}H_{50}O_5$ Its ^{1}H NMR, ^{13}C NMR and mass spectra suggested that it must be a β-amyrin type of triterpenoid whose one primary and three hydroxyl groups are located in ring D/E and one hydroxyl group in ring A/B The ^{1}H and ^{13}C NMR spectra of compound **4a** are almost similar to those of **3a** However, notable differences between **3a** and **4a** could be seen with regard to the following points. Compound **4a** formed a pentaacetate (**4b**) on acetylation. The ^{1}H NMR spectrum of **4a** showed a close similarity of six tertiary methyl signals to those in the spectrum of **3a**. In particular there was a low-field signal at δ 1.35 suggesting that the remaining one hydroxyl group should be present in a 1,3 diaxial relationship with this methyl group, so position C-16 was likely This was confirmed by the ^{13}C NMR spectrum Compound **4** also showed a signal at δ3.71 (*dd*, J=2.6, 4.7 Hz) for 16-H These results clearly establish the structure of compound **4a** as 3α,16α,21α,22α,28-pentahydroxyolean-12-ene

The ^{1}H NMR spectrum of **4** showed an anomeric proton signal of D-xylose as a doublet (J=7 Hz) indicating its β-linkage with the aglycone. The site of glycosidation was found to be C-28 on the basis of the ^{13}C NMR spectra of **4** and **4a** Thus glycoside **6** was identified as 3α,16α,21α,22α,28-pentahydroxyolean-12-en-28-*O*-β-D-xylopyranoside

EXPERIMENTAL

Plant material was collected in March from Allahabad, U P India (a herbarium specimen of the plant is on file in Botanical survey of India, sheet no =15459) Mps uncorr TLC was carried out on silica gel G (Merck 7731) with solvent systems (unless otherwise stated) as follows (a) $CHCl_3$–MeOH–H_2O (70 30 3) (b) $CHCl_3$–MeOH–H_2O (40 20 1) (c) $CHCl_3$–MeOH –H_2O (7 3 1) (d) $CHCl_3$–MeOH–H_2O (6 3 1) CC was performed on silica gel 60 (Merck 7734). IR spectra were run as KBr disks ^{1}H NMR spectra were recorded at 90 and 60 MHz in $CDCl_3$ soln and $(CD_3)_2CO$ soln unless otherwise specified using TMS as internal standard ^{13}C NMR spectra were taken at 25 05 MHz in C_5D_5N solution with TMS employing FT mode

Isolation of compounds The defatted plant was extracted in EtOH Concentrated extract was separated into water-soluble and insoluble portions The aq. portion was loaded onto a flash column and then eluted with different solvents of increasing polarity Elution with $CHCl_3$–MeOH–H_2O (14 5·1) yielded a fraction containing four compounds This fraction was subjected to prep TLC and yielded four glycosides (**1–4**) All compounds were crystallized in MeOH



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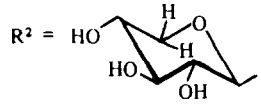
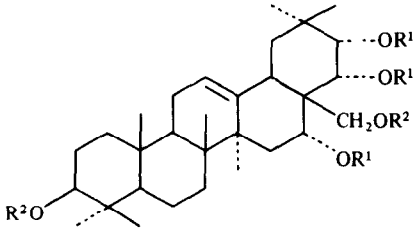
R² = 

3a R¹ = R² = H

3b R¹ = R² = Ac



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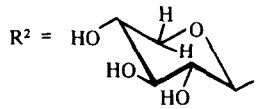
R² = 

4a R¹ = R² = H

4b R¹ = R² = Ac

Compound (**1**). Colourless needles, mp 139°, R_f 0.27 (solvent *a*). Found: C: 66.0; H: 9.0. Calculated for $C_{35}H_{56}O_{10}$ C: 66.1, H: 8.9%. IR ν_{max}^{KBr} cm^{-1}: 3500, 3300, 1735 (ester) 2900 (methylene) 1041, 890, 730. ^{1}H NMR: δ 0.71 (*s*, 3H), 0.85 (*s*, 3H), 0.89 (*s*, 3H), 1.02 (*s*, 3H), 1.23 (*s*, 3H), 0.98 (*d*, J = 6.5 Hz, 3H), 2.59 (*s*, H-18), 5.17 (*t*, 1H, J = 3.5 Hz, H-12), 3.15 and 3.55 (each 1H, a pair of AB doublet, J = 12 Hz, H-23), 3.70 (1H, *dd*, $J_1 = J_2 = 3.3$ Hz, 1-Hβ), 4.25 (1H, *dd*, J_1 = 10 Hz, J_2 = 7 Hz, 3-Hα), 5.15 (1H, *d*, J = 7.6 Hz, H-1 of xylose), 3.2–3.8 (*m*, sugar protons). EIMS *m/z*: (70 eV) 636 [M]$^+$, 264, 246, 219, 201. ^{13}C NMR: Table 1.

Acid hydrolysis of compound **1**. Compound **1** was hydrolysed with 7% H_2SO_4 to give aglycone **1a** and the sugar D-xylose (co-chromatography with authentic sample). Aglycone **1a**: mp 270–272°. IR ν_{max}^{KBr} cm^{-1}: 3400 (-OH), 1700 (COOH), 3200–2500 (*br*), 1680, 1380, 1360, 1020, 820. EIMS *m/z*: (70 eV) 504 [M]$^+$, 486, 464, 264, 246, 219, 201. ^{1}H NMR: δ 0.75–1.25 (6 × Me), 3.77 (1H, *dd*, $J_1 = J_2 = 3$ Hz, 1-Hβ), 4.29 (1H, *dd*, J_1 = 10 Hz, J_2 = 7 Hz, 3-Hα), 5.17 (1H, *d*, J = 3.6 Hz), 3.15 and 3.55 (each *d*, 1H, J = 12 Hz, H-23). ^{13}C NMR: Table 1. Acetylation of **1a** gave monohydroxy triacetate **1b** mp 225° as colourless needles. IR ν_{max}^{KBr} cm^{-1}: 3500, 1720, 1520, 1250, 1235, 1110, 1055, 1040, 995, 970, 950, 925. EIMS *m/z*: 630 [M]$^+$, 570, 525, 510, 264, 246, 219, 201. ^{1}H NMR: δ 0.70–1.34 (15H, 5 × Me), 1.06 (*d*, 3H, J = 6.5 Hz), 2.57 (*s*, 1H, 18β H), 5.30 (1H, *t*, J = 3.5 Hz, H-12), 3.67 and 3.90 (each *d*, 1H, J = 12 Hz, H-23), 4.72 (*dd*, 1H, $J_1 = J_2$ = 3 Hz, 1-Hβ), 4.87 (1H, *dd*, J_1 = 10 Hz, J_2 = 7 Hz, 3-Hα), 2.04, 2.06 and 2.10 (each *s*, 3H, 3 × OAc).

Table 1. ^{13}C NMR spectral data for compounds **1**, **1a**, **2** and **2a** (δc, ppm)

C	1	1a	2	2a
1	72.7 *d*	72.9 *d*	74.8 *d*	74.9 *d*
2	35.8 *t*	35.9 *t*	74.5 *d*	74.6 *d*
3	72.0 *d*	72.0 *d*	79.7 *d*	79.9 *d*
4	43.5 *s*	43.5 *s*	42.5 *s*	42.6 *s*
5	48.4 *d*	48.6 *d*	53.4 *d*	53.5 *d*
6	17.3 *t*	17.4 *t*	17.7 *t*	17.9 *t*
7	33.1 *t*	33.2 *t*	33.0 *t*	33.0 *t*
8	39.8 *s*	39.9 *s*	41.0 *s*	41.2 *s*
9	38.3 *d*	38.4 *d*	48.0 *d*	48.0 *d*
10	42.9 *s*	42.8 *s*	37.6 *s*	37.7 *s*
11	23.9 *t*	23.9 *t*	24.2 *t*	24.3 *t*
12	128.3 *d*	127.6 *d*	129.5 *d*	129.1 *d*
13	139.0 *s*	139.6 *s*	138.9 *s*	139.7 *s*
14	42.1 *s*	42.3 *s*	42.0 *s*	42.2 *s*
15	29.1 *t*	29.3 *t*	29.3 *t*	29.5 *t*
16	26.0 *t*	26.1 *t*	26.0 *t*	26.4 *t*
17	49.5 *s*	48.3 *s*	49.6 *s*	48.5 *s*
18	54.4 *d*	54.5 *d*	52.8 *d*	53.0 *d*
19	72.2 *s*	72.4 *s*	72.4 *s*	72.5 *s*
20	42.5 *d*	43.6 *d*	42.1 *d*	42.3 *d*
21	26.8 *t*	27.1 *t*	27.6 *t*	28.1 *t*
22	37.7 *t*	38.2 *t*	37.6 *t*	38.1 *t*
23	65.9 *t*	66.4 *t*	66.0 *t*	66.3 *t*
24	16.2 *q*	16.1 *q*	16.4 *q*	16.2 *q*
25	16.6 *q*	16.7 *q*	11.9 *q*	11.8 *q*
26	16.6 *q*	16.6 *q*	16.9 *q*	16.9 *q*
27	26.0 *q*	26.2 *q*	25.5 *q*	25.6 *q*
28	176.9 *s*	180.4 *s*	176.8 *s*	180.5 *s*
29	27.0 *q*	27.2 *q*	27.4 *q*	27.5 *q*
30	16.6 *q*	16.7 *q*	16.4 *q*	16.5 *q*
31	106.6 *d*		106.1 *d*	
32	74.8 *d*		75.1 *d*	
33	79.2 *d*		78.5 *d*	
34	70.9 *d*		70.9 *d*	
35	66.7 *t*		66.4 *t*	

Methylation of **1b** with CH_2N_2 afforded its monomethyl ester. ^{1}H NMR: δ 1.99, 2.04, 2.06 (each *s*, 3H, 3 × OAc), 3.62 (3H, *s*, COOMe), 0.83–1.18 (6C-methyls), 4.73 (1H, *dd*, $J_1 = J_2 = 3$ Hz, 1Hβ), 4.85 (1H, *dd*, J_1 = 10 Hz, J_2 = 7.5 Hz, 3'Hα), 5.25 (1H, *t*, J = 3.5 Hz). Compound **2**. White powder, mp 210°, R_f 0.31 (solvent *b*). Found C: 64.40, H: 8.58 calculated for $C_{35}H_{56}O_{11}$, C: 64.41, H: 8.59%. IR ν_{max}^{KBr} cm^{-1}: 3500, 3300, 1734, 2900, 1042, 890, 730, 720. ^{1}H NMR: δ 0.73–1.22 (each *s*, 5 × Me), 1.05 (*d*, 3H, J = 6.2 Hz), 2.59 (*s*, 1H, 18β-H), 5.30 (*t*, 1H, J = 3.4 Hz, H-12), 3.14 and 3.55 (each *d*, J = 12 Hz, H-23), 3.2–3.8 (sugar protons), 5.10 (1H, *d*, J = 7.6 Hz, H-1 of xylose), 3.22 and 3.41 (each 1H, *d*, J = 10.7 Hz, J = 9.5 Hz, C-1 and H-3), 4.03 (*dd*, 1H, J_1 = 9.5 Hz, J_2 = 10.7 Hz, H-2). EIMS: *m/z* 652 [M]$^+$ 264, 246, 219, 201. ^{13}C NMR: Table 1. Acid hydrolysis of compound **2** gave aglycone **2a** and the sugar D-xylose (co-chromatography).

Aglycone (**2a**). IR ν_{max}^{KBr} cm^{-1}: 3410 (-OH) 1705 (COOH) EIMS (70 eV) *m/z*: 520 [M]$^+$, 264, 246, 219, 201. ^{13}C NMR: Table 1. The tetraacetate of compound **2** was prepared by treatment of Ac_2O–pyridine. IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1250, 1235, 1110, 995. EIMS *m/z*: 672 [M]$^+$, 264, 246, 219, 201. ^{1}H NMR: δ 0.71–1.25 (6-C-methyls), 1.99, 2.00, 2.04, 2.06 (each *s*, 3H, 4 × OAc), 2.52 (*s*, 1H, 18β-H), 4.80 and 4.88 (each *d*, 1H, J = 9.3 Hz, J = 10.5 Hz, H-1 and H-3), 5.20 (1H, *dd*, J_1 = 9.3 Hz, J_2 = 10.5 Hz, H-2), 5.30 (1H, *t*, J = 3.4 Hz, H-12), 3.57 and 3.84 (each 1H, a pair of AB *d*, J = 12 Hz). Methylation of **2b** with CH_2N_2 provided its monomethyl ester **2c**. ^{1}H NMR: δ 1.99, 2.01, 2.04, 2.06 (each *s*, 3H, 4 × OAc), 3.60 (*s*, 3H, COOMe). Compound **3**: mp 126°, R_f 0.34 (solvent *c*). Found C: 69.25, H: 9.53 calcd for $C_{35}H_{58}O_8$ C: 69.30; H: 9.57%. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1355, 1262, 1170, 1140, 1117, 1097, 1074, 1055, 1035, 995, 925, 875, 790, 730. ^{1}H NMR: δ 0.85–1.22 (7 × Me), 5.05 (*d*, 1H, J = 7 Hz, H-1 of xylose), 5.35 (1H, *t*, J = 3.4 Hz, H-12), 3.2–4.80 (sugar proton and H-28 protons, *m*), 3.93 and 4.30 (1H each pair of doublet, J = 10 Hz), 3.50 (1H, *d*, J = 3.8 Hz), 4.06 (1H, *d*, J = 3.8 Hz), 3.41 (1H, *dd*, J = 4.3, 2.7 Hz). EIMS *m/z*: 606 [M]$^+$. ^{13}C NMR: Table 2. Compound **3** on acid hydrolysis gave aglycone **3a** and sugar D-xylose (Authentic sample) mp 304°. IR ν_{max}^{KBr} cm^{-1}: 3430, 1660, 1340, 1100, 1050, 1005, 995, 730, 720. EIMS *m/z*: 474 [M]$^+$, 472, 266, 248, 235, 217, 199, 208, 207, 190, 175. ^{1}H NMR: δ 0.91–1.27 (7 × Me), 5.39 (1H, *t*, J = 3.3 Hz, H-12), 3.95 and 4.30 (each *d*, 1H, J = 10 Hz), 3.50 (1H, *d*, J = 3.8 Hz, H-22), 4.07 (1H, *d*, J = 3.8 Hz, H-21), 3.40 (1H, *dd*, J = 4.2, 2.7 Hz, H-3β). ^{13}C NMR: Table 2. The tetraacetate of compound **3** was prepared by treatment of **3** with boiling Ac_2O–pyridine. IR ν_{max}^{KBr} cm^{-1}: 1740, 1250, 1030, 970, 960, 940, 900, 875, 730. ^{1}H NMR: δ 0.88–1.18 (7 × Me), 1.98, 2.02, 2.04, 2.06 (each *s*, 3H, 4 × OAc), 4.90 and 5.30 (each *d*, 1H, J = 11 Hz), 5.30 (1H, *t*, J = 3.5 Hz, H-12), 2.80 (*dd* 1H, J = 13 Hz, J = 5 Hz), 4.75 (*d*, 1H, J = 3.7 Hz, H-21), 4.70 (*d*, 1H, J = 3.7 Hz, H-22), 4.00 (*dd*, J = 4.5, 2.8 Hz, H-3). Compound **4**: mp 145° R_f 0.35 (solvent d). Found C: 67.56; H: 9.35 calculated for $C_{35}H_{58}O_9$ C: 67.52 H: 9.32%. IR ν_{max}^{KBr} cm^{-1}: 3500, 1645, 1350, 1260, 1170, 1140, 1115, 1095, 1072, 1055, 1035, 998, 930, 875, 790, 730. ^{1}H NMR: δ 0.91–1.31 (21H, 7 × Me), 5.35 (*m*, 1H, H-12), 3.25–4.85 (complex *m*, sugar protons and H-28 protons), 3.71 (1H, *dd*, J = 4.7, 2.6 Hz, 16-H), 3.50 (*dd*, 1H, J = 4.2, 2.8 Hz, 3-H), 4.29 and 3.80 (each *d*, 1H, J = 3.0 Hz), 5.10 (1H, *d*, J = 7.6 Hz, H-1 of xylose). ^{13}C NMR: Table 2 EIMS *m/z*: 622 [M]$^+$.

Acid hydrolysis of **4** to yield **4a**. The glycoside was hydrolysed to give aglycone **4a** and the sugar D-xylose (R_f 0.28, BuOH–AcOH–H_2O; 4:1:5) mp 315°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1340, 1100, 1050, 995, 730. ^{1}H NMR δ: 0.96–1.35 (21H, 7 × Me), 5.40 (1H, *t*, J = 3.5 Hz), 3.92 and 4.21 (each *d*, 1H, J = 12 Hz), 3.89

Table 2 ^{13}C NMR spectral data for compounds **3**, **3a**, **4** and **4a**

C	3	3a	4	4a
1	38 2 *t*	38 5 *t*	38 6 *t*	38 7 *t*
2	23 5 *t*	28 5 *t*	27 2 *t*	27 2 *t*
3	79 3 *d*	79 4 *d*	80 1 *d*	80 5 *d*
4	37 8 *s*	38 8 *s*	37 5 *s*	38 5 *s*
5	55.7 *d*	56 0 *d*	48 5 *d*	49 0 *d*
6	18 5 *t*	18 9 *t*	17 4 *t*	17 9 *t*
7	32 5 *t*	32 0 *t*	33 5 *t*	32 2 *t*
8	40 0 *s*	40 2 *s*	39 7 *s*	39 8 *s*
9	47 7 *d*	46 0 *d*	48 0 *d*	47 4 *d*
10	37 2 *s*	39 1 *s*	39 7 *s*	42 7 *s*
11	24 3 *t*	23 8 *t*	24 3 *t*	23 7 *t*
12	124 5 *d*	123 7 *d*	123 7 *d*	123 9 *d*
13	144 6 *s*	144 7 *s*	144 1 *s*	144 9 *s*
14	41 7 *s*	41 7 *s*	45 6 *s*	41 9 *s*
15	26 5 *t*	28 5 *t*	34 0 *d*	36 9 *d*
16	16 0 *t*	16 5 *t*	77 6 *t*	78 5 *t*
17	34 1 *s*	36 0 *s*	34 8 *s*	36 9 *s*
18	34 8 *d*	34 2 *d*	34 9 *d*	34 3 *d*
19	38 9 *t*	38 5 *t*	38 3 *t*	37 7 *t*
20	40 5 *s*	39 0 *s*	37 5 *s*	40 5 *s*
21	70 9 *d*	71 7 *d*	70 9 *d*	71 9 *d*
22	74 9 *d*	75 6 *d*	74 9 *d*	75 8 *d*
23	28 5 *q*	27 1 *q*	29 0 *q*	28 7 *q*
24	17 0 *q*	17 0 *q*	17 5 *q*	17 2 *q*
25	15 9 *q*	15 5 *q*	16 1 *q*	16 0 *q*
26	17 3 *q*	17 0 *q*	16 9 *q*	17 7 *q*
27	26 3 *q*	26 1 *q*	27 5 *q*	26 5 *q*
28	68 8 *t*	67 1 *t*	69 8 *t*	68 1 *t*
29	33 4 *q*	32 9 *q*	30 5 *q*	29 9 *q*
30	28 8 *q*	28 5 *q*	28 4 *q*	28 5 *q*
31	107 1 *d*		106 8 *d*	
32	74 8 *d*		74 9 *d*	
33	79 1 *d*		79 3 *d*	
34	73 7 *d*		73 7 *d*	
35	66 7 *t*		66 4 *t*	

(1H, *d*, $J = 3$ 1 Hz, H-21), 4 38 (1H, *d*, $J = 3$ 1 Hz, H-22), 3 49 (*dd*, 1H, $J = 4$ 3, 2 8 Hz, H-3), 3 70 (1H, *dd*, $J = 4$ 9, 2 7 Hz, H-16), 2 85 (*dd*, 1H, $J = 12$ Hz, $J = 5$ Hz, 18-βH) EIMS 490 $[M]^+$, 282, 264, 246, 233, 215, 197, 190, 175 ^{13}C NMR Table 2

Pentaacetate of **4** IR ν_{max}^{KBr} cm^{-1} 1750, 1250, 1235, 1110, 1050, 1040, 995, 970, 955 and 925 ^{1}H NMR δ1 96, 2 03, 2 06, 2 08 and 2 10 (each *s*, 3H) EIMS *m/z* 916 $[M]^+$

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