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TRITERPENOID PROSAPONINS FROM LEAVES OF *MAESA CHISIA* VAR. *ANGUSTIFOLIA*

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Key Word Index—*Maesa chisia* var. *angustifolia*; triterpenoid; acylated sapogenins; camelliagenin A.

Abstract—Acid hydrolysis of the saponin fraction of the leaves of *Maesa chisia* var. *angustifolia* yielded a monoglucoside fraction besides camelliagenin A as a minor constituent. The glucose moiety of the former could be removed by hydrolysis by Smith degradation to yield two new acylated triterpenoids characterised as 16α-*O*-acetyl-22α-*O*-angeloyl-camelliagenin A and 16α-*O*-acetyl-22α-*O*-(2′-methylbutyroyl)-camelliagenin A as well as camelliagenin A and its 22α,28-glycolaldehyde acetal. The possibility of the later acetal derivative being an artefact could not, however, be ruled out.

INTRODUCTION

In view of the anti-inflammatory activity and analgesic, anti-pyretic and tranquilosedative properties (B. J. Ray Ghatak, A. Gomes and R. M. Sharma, personal communication) of the water soluble fraction of the methanol extract of the leaves of *Maesa chisia* D. Don var. *angustifolia* Hook f. and Th., we undertook the chemical investigation of the active fraction containing saponins.

RESULTS AND DISCUSSION

Reverse phase HPLC of the saponin containing fraction on a RP-8 column using the solvent system acetonitrile–water (1:1) exhibited eight peaks having very close retention times alongwith some shoulders. All attempts to separate the individual constituents were unsuccessful. Therefore we sought to characterise the aglycone parts. Accordingly, the crude saponin mixture was hydrolysed with 10% HCl in methanol to obtain a minor amount of camelliagenin A (**1**) and a major amorphous monoglucoside fraction.

Camelliagenin A (**1**), $C_{30}H_{50}O_4$, (*m/z* 474 $[M]^+$), mp 290° (272–274°), $[\alpha]_D$ + 21.2° (MeOH; *c*1.1) was identified from its spectral analyses (mass, ^1H and ^{13}C NMR spectra) and confirmed by direct comparison with an authentic specimen [1].

The amorphous monoglucoside fraction which could not be purified was acetylated with acetic anhydride–pyridine at room temperature to get a crystalline hexaacetate **2**, $C_{53}H_{78}O_{16}$ (*m/z* 970 $[M]^+$ from both positive and negative CIMS), mp 214–216°. Hydrolysis with 10% potassium hydroxide, **2** furnished the glucoside **3**, $C_{36}H_{60}O_9$ (*m/z* 636 $[M]^+$), mp 210–214°. However, the latter, on attempted re-acetylation under identical conditions did not regenerate the original acetate **2** but instead afforded a new hexaacetate **4**, $C_{48}H_{72}O_{15}$, mp 150–153°, *m/z* 888 $[M]^+$, while at 100° a heptaacetate **5**, $C_{50}H_{74}O_{16}$, mp 170–173°, *m/z* 930 $[M]^+$, was obtained. The foregoing observations clearly indicated the elimination of some *O*-acyl group(s) present in the original monoglucoside during alkali hydrolysis of **2**.

Compound **3** on hydrolysis with Kiliani mixture (AcOH–HCl–H_2O, 1.5:3.5:5) in a sealed tube at 100° followed by paper chromatography indicated the presence of D-glucose.

That the non-sugar part of **3** is camelliagenin A (**1**) became evident from its isolation after Smith degradation [2] of the glucose moiety of **3**, though another compound characterised (*vide infra*) as the 22α,28-glycolaldehyde acetal of **1** (**6**) was also isolated as a minor product.

A comparison of the ^{13}C NMR spectrum of **3** with that of **1** (Table 1) showed that the C-28 signal of **3** was shifted downfield by ~ 7 ppm indicating the attachment of the glucose moiety in **3** through the C-28 oxygen function of **1**. It was also supported by the fact that neither of the acetates **4** and **5** of **3** displayed any acetylation shift of the 28-H_2 proton resonance frequency as observed in the case of 3,22,28-tri-*O*-acetyl-camelliagenin A (**7**). Moreover, the very close ^{13}C NMR chemical shifts of the glucose moiety of **3** to those of methyl-β-D-glucoside [3] as well as the coupling constant of 7 Hz of the anomeric proton signal in the ^1H NMR spectra of the acetates **4** and **5** indicated a β-linkage of the glucose unit.

In order to ascertain the nature and position of attachment of the acyl group(s) in the original monoglucoside, the amorphous material, obtained by acid hydrolysis of the crude saponin mixture without further purification, was subjected to Smith degradation. The product was resolved into four distinct compounds: (i) camelliagenin A (**1**), (ii) 16α-*O*-acetyl-22α-*O*-angeloyl-camelliagenin A (**8**), (iii) 16α-*O*-acetyl-22α-(2′-mrthylbutyroyl)-camelliagenin A (**9**) and (iv) the 22α,28-glycolaldehyde acetal derivative (**6**) of camelliagenin A. Compounds **1** and **6** were minor components.

The M_r of compound **8**, $C_{37}H_{58}O_6$, mp 140–142°, $[\alpha]_D$ −28.4° ($CHCl_3$; *c*1.3) was determined from both EI as well as positive and negative CI mass spectrometry to be

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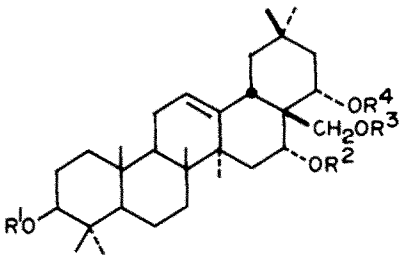


1 $R^1 = R^2 = R^3 = R^4 = H$

2 $R^1 = R^2 =$ Ac, $R^3 =$ Acetylated β-glucosyl, $R^4 =$ Angeloyl

3 $R^1 = R^2 = R^4 = H$, $R^3 = \beta$-Glucosyl

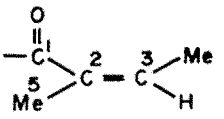
4 $R^1 = R^4 =$ Ac, $R^2 = H$, $R^3 =$ Acetylated β-glucosyl

5 $R^1 = R^2 = R^4 =$ Ac, $R^3 =$ Acetylated β-glucosyl

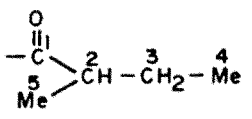
7 $R^1 = R^3 = R^4 =$ Ac, $R^2 = H$

8 $R^1 = R^3 = H$, $R^2 =$ Ac, $R^4 =$ Angeloyl

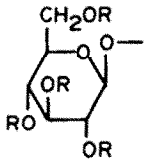
9 $R^1 = R^3 = H$, $R^2 =$ Ac, $R^4 =$ 2-Methylbutyroyl



Angeloyl

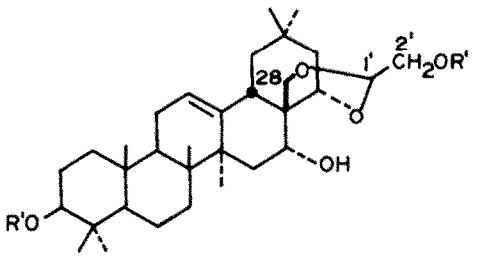


2-Methylbutyroyl



R = H, β-Glucosyl

R = Ac, Acetylated β-glucosyl



6 R' = H

10 R' = Ac

598 which was 124 mass units higher than that of **1**. Its IR spectrum exhibited strong absorption bands at 1730 and 1705 cm^{-1} for saturated and $\alpha\beta$-unsaturated ester functions respectively.

Its ^1H NMR spectrum showed, besides the singlets for methyl protons of an olean-12-ene skeleton, one three-proton singlet at ∂2.09 assignable to an acetyl grouping. Moreover, two three-proton signals at ∂1.84 (*d*, J = 1.5 Hz) and 1.97 (*qq*, J = 7, 1.5 Hz) and a one-proton quartet of a quartet at ∂6.10 (J = 7, 1.5 Hz) indicated [4] the presence of an angeloyl group in the molecule.

The ^{13}C NMR spectrum also corroborated the presence of the above mentioned acyl groups in **8**. Thus, of the seven additional signals observed in the spectrum of **8** compared to that of **1**, five signals at ∂15.7 (*q*), 20.4 (*q*), 127.3 (*s*), 139.4 (*d*) and 168.4 (*s*), and two signals at 21.7 (*q*) and 169.2 (*s*) could reasonably be assigned to angeloyl [5] and acetyl carbons respectively.

The 16α- and 22α-hydroxyl groups of **1** are acylated in **8** as is evident from the ^1H NMR spectrum of **8** which displayed downfield shifts of the 16β-H and 22β-H signals at ∂5.04 *m* ($W_{1/2}$ = 8 Hz) and 5.41 *dd* (J = 12, 6 Hz) compared to ∂4.68 *m* ($W_{1/2}$ = 9 Hz) and 4.04 *dd* (J = 12, 6 Hz), respectively, in the spectrum of **1** (in $CDCl_3$). As expected, the signals of the 3α-H and 28-H_2 protons did not exhibit any acylation shift.

That compound **9**, $C_{37}H_{60}O_6$, amorphous, mp 116–120°, $[\alpha]_D$ −33.3° ($CHCl_3$; *c*0.3), *m/z* 600 $[M]^+$, is also a 16α-*O*-22α-*O*-diacyl derivative of **1** was evident from the following observations. Its IR spectrum exhibited a broad absorption band at 1710–1730 cm^{-1} for ester function(s). The appearance of a new set of signals at ∂11.6

Table 1. ^{13}C NMR chemical shifts*

Carbon	1†	2‡	3§	4‡	5‡	6†	8‡	9‡	10‡
1	39.2	38.2	39.6	38.2	38.2	39.2	38.6	38.6	38.1
2	28.1	23.2	28.3	23.4	23.3	27.9	26.9	27.1	23.2
3	78.0	80.5	78.6	80.7	80.6	78.1	78.6	78.7	80.6
4	39.3	37.5	39.6	37.6	37.6	39.4	38.6	38.7	37.5
5	55.8	55.1	56.2	55.1	55.2	55.8	55.2	55.2	55.1
6	18.8	17.9	19.1	18.1	18.0	18.8	18.1	18.1	18.8
7	33.2	32.6	33.6	32.8	32.7	33.2	32.6	32.7	32.5
8	40.1	39.7	40.6	39.7	39.7	40.4	39.7	39.8	39.8
9	47.1	46.3	47.6	46.4	46.4	47.1	46.5	46.6	46.3
10	37.2	36.6	37.7	36.7	36.6	37.2	36.7	36.8	36.6
11	23.9	23.2	24.2	23.4	23.3	23.8	23.3	23.4	23.3
12	122.6	124.5	123.3	123.6	124.6	123.0	124.6	124.7	122.8
13	144.1	139.9	144.1	141.3	140.0	143.1	140.4	140.5	141.5
14	42.2	40.7	42.4	41.0	40.7	42.5	40.8	40.8	41.8
15	44.6	30.5	34.8	33.8	30.6	35.1	30.4	30.7	34.7
16	68.3	70.7	69.1	69.5	71.0	68.5	70.6	70.7	69.3
17	44.7	42.3	45.2	43.1	42.2	40.4	43.6	43.6	39.8
18	42.5	40.0	42.9	40.4	40.2	43.7	39.9	39.9	42.9
19	47.8	46.3	48.1	46.6	46.4	46.6	46.5	46.6	45.9
20	31.8	31.2	31.8	31.2	31.1	32.0	31.3	31.3	31.5
21	45.8	41.1	45.8	42.0	41.3	40.4	41.5	41.5	39.8
22	74.3	71.6	73.3	73.0	71.5	83.5	72.4	72.0	83.2
23	28.7	27.8	29.0	27.9	27.9	28.8	27.9	28.0	27.8
24	16.6	16.5	16.7	16.6	16.6	16.6	15.5	15.5	16.5
25	15.9	15.4	16.2	15.5	15.5	15.8	15.5	15.5	15.4
26	17.0	16.5	17.5	16.6	16.6	17.2	16.6	16.6	16.8
27	27.5	26.5	27.7	26.8	26.5	27.9	26.7	26.7	27.4
28	70.2	69.9	77.0	71.3	71.0	76.9	63.6	63.8	76.7
29	33.7	33.1	33.9	32.8	33.1	33.7	33.1	33.2	33.1
30	25.5	24.2	25.8	24.2	24.2	26.5	24.5	24.5	25.7
1′	—	99.7	105.1	100.1	100.1	104.2	—	—	99.8
2′	—	70.5	75.1	70.8	70.6	64.5	—	—	64.7
3′	—	72.6	78.6	72.7	72.6	—	—	—	—
4′ Glc	—	68.2	72.0	68.5	68.3	—	—	—	—
5′	—	71.3	78.2	71.3	71.5	—	—	—	—
6′	—	61.8	63.1	61.9	61.9	—	—	—	—
1 Angeloyl	—	166.4	—	—	—	—	168.4	177.7	—
2	—	128.0	—	—	—	—	127.3	41.6	—
3	—	137.4	—	—	—	—	139.4	26.5	—
4 *or* 2-Me-butyroyl	—	20.3	—	—	—	—	20.4	11.6	—
5	—	15.4	—	—	—	—	15.7	16.6	—
COMe	—	169.0, 169.3, 169.8, 170.1, 170.6	—	169.3, 169.6 170.0, 170.3, 170.7	169.1, 169.4, 169.9, 170.3, 170.7	—	169.2	169.2	170.3, 170.7
COMe		20.3, 20.4, 21.0, 21.7	—	20.4, 20.5, 21.1	20.3, 20.4, 20.5, 20.9 21.9	—	21.7	21.8	20.6, 21.9

*The chemical shifts are expressed on the δ scale with TMS as internal standard.
†Spectra were recorded in C_5D_5N; ‡ in $CDCl_3$; § in C_5D_5N at 70°.

(*q*), 16.6 (*q*), 26.5 (*t*), 41.6 (*d*) and 177.7 (*s*) in its ^{13}C NMR spectrum compared to that of **1** indicated [1] the presence of a 2-methylbutyroyl group in the molecule. The presence of an acetyl group was also evident from the three-proton ^{1}H NMR signal at ∂2.10 as well as two ^{13}C NMR signals at ∂21.8 (*q*) and 169.2 (*s*). The downfield shift of the 16β-H (∂5.06 *m*, $W_{1/2}$ = 8 Hz) and 22β-H (5.31 *dd*, *J* = 12, 6 Hz) signals in the ^{1}H NMR spectrum compared to those of **1** demonstrated that the acyl groups were attached to the oxygens at C-16 and C-22 as in **8**.

While the ^{1}H chemical shifts of the 16β-H were very close in **8** and **9**, those of the 22β-H differed by ~ 0.1 ppm,

compound **8** exhibiting a downfield signal indicating that the angeloyloxy group of **8** and the 2-methyl-butyroyloxy group of **9** were attached to C-22. The deshielding of the 22β-H signal in the spectrum of **8** would be attributed to the anisotropic effect of the double bond of the angeloyl group.

Based on the above observations, compounds **8** and **9** could be inferred to be 16α-*O*-acetyl-22α-*O*-angeloyl- and 16α-*O*-acetyl-22α-*O*-(2'-methylbutyroyl)-camelliagenin A, respectively.

Both EI and CI mass spectra of compound **6**, $C_{32}H_{52}O_5$, mp 276–280°, $[\alpha]_D$ +11.7° ($CHCl_3$, *c*1.2), exhibited its molecular ion at *m/z* 576 which is 42 mass units higher than that of **1**. It showed no IR absorption band for a carbonyl function and no ^1H NMR proton signal was seen for an acetyl group. On acetylation with acetic anhydride–pyridine at room temperature **6** yielded a diacetate (**10**).

Detailed ^1H (*Vide* experimental) and ^{13}C (Table 1) NMR spectral analyses of **6** and **10** led to the assignment of the 22α,28-glycolaldehyde acetal of camelliagenin A as the structure of compound **6**. The possibility of compound **6** being an artefact could not, however, be ruled out.

EXPERIMENTAL

Mps uncorr. Optical rotations were measured in a Perkin Elmer (Model 141) spectropolarimeter using a 1 dm cell. IR spectra were recorded in a Perkin Elmer 177 spectrometer. Unless otherwise stated, ^1H and ^{13}C NMR spectra were taken at 100 MHz and 25.05 MHz respectively in a Jeol FX-100 instrument employing the FT mode. EIMS were run on a direct insertion probe at 80 eV in a Hitachi RMU-6L instrument.

Plant Material. The plant was collected from Darjeeling district, West Bengal and identified by the Division of Botany, Central Drug Research Institute, Lucknow. A voucher specimen is available from there.

Extraction and Isolation. Defatted crushed leaf (1 kg) was extracted for 72 hr with MeOH (3 × 10 l) at room temp. The extract was concentrated *in vacuo* to 1.5 l, diluted with water (4 l) and extracted with EtOAc (3 × 1 l). The water soluble portion was carefully distilled under red. pres. to remove residual MeOH and the aq. part was lyophilized to yield 75 g of crude saponin mixture (7.5%).

Hydrolysis of the saponin mixture. Crude saponin (25 g) obtained above was refluxed for 8 hr with 10% methanolic HCl (500 ml). After removing MeOH under red. pres., the reaction mixture was diluted with H_2O (200 ml) and extracted with EtOAc to yield 10 g of crude product. CC over silica gel with $CHCl_3$–MeOH (99:1) afforded 0.6 g of camelliagenin A (**1**) crystallised from Me_2CO–light petrol as colourless prisms, mp 290°, $[\alpha]_D$ +21.24° (MeOH; *c*1.1). IR ν_{max} cm^{-1}: 3400. EIMS *m/z* (rel. int.): 474 $[M]^+$ (0.5), 456 (0.4), 441 (0.3), 438 (0.3), 425 (0.3), 407 (0.3), 389 (5), 248 (10), 217 (100), 199 (6), 173 (4). ^1H NMR ($CDCl_3$): δ0.78, 0.90 (3H each, *s*, Me × 2), 0.94 (9H, *s*, Me × 3), 1.00, 1.42 (3H each, *s*, Me × 2), 3.22 (1H, *t*, *J* = 8 Hz, 3-H), 3.30 and 3.61 (1H each, *d*, *J* = 11 Hz, 28-H_2), 4.04 (1H, *dd*, *J* = 6, 12 Hz, 22-H), 4.68 (1H, *m*, $W_{1/2}$ = 9 Hz, 16-H), 5.28 (1H, *m*, 12-H). ^1H NMR (C_5D_5N): δ0.44 (6H, *s*, Me × 2), 1.00 (6H, *s*, Me × 2), 1.12, 1.20, 1.79 (3H, each, *s*, Me × 3), 3.44 (1H, *t*, *J* = 8 Hz, 3-H), 3.62, 4.01 (1H each, *d*, *J* = 11 Hz, 28-H_2), 4.58 (1H, *dd*, *J* = 6, 12 Hz, 22-H), 5.09 (1H, *m*, $W_{1/2}$ = 8 Hz, 16-H), 5.40 (1H, *m*, 12-H). It was identified by direct comparison (IR) with an authentic sample.

Further elution with $CHCl_3$–MeOH (97:3) yielded an amorphous monoglucoside fraction (6.5 g) which could not be purified as such and was acetylated.

Acetylation of the amorphous monoglucoside. The amorphous solid (3 g) was acetylated at room temp. with Ac_2O–C_5H_5N for 24 hr. The product obtained after removal of the excess reagent under vacuum was submitted to CC over silica gel eluting with petrol–$CHCl_3$ (75:25) to $CHCl_3$–MeOH (95:5). This afforded a solid which was repeatedly crystallised from MeOH–$CHCl_3$, C_6H_6–petrol, EtOAc–petrol and Me_2CO–$CHCl_3$ to furnish colourless shining plates (1 g) of **2**, mp 214–216°, $[\alpha]_D$ −15.5° ($CHCl_3$, *c*1.02). IR ν_{max} cm^{-1}: 1750–1700 (*br*); EIMS: *m/z* 970 $[M]^+$; positive CIMS (NH_3) *m/z*: 988 $[M + NH_4]^+$ negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 1005 $[M + Cl]^-$, 969 $[M - 1]^-$: ^1H NMR (200 MHz, $CDCl_3$): δ0.86 (6H, *s*, Me × 2), 0.89 (3H, *s*, Me × 1), 0.95 (6H, *s*, Me × 2) 1.04, 1.29 (3H each, *s*, Me × 2), 1.83 (3H, *q*, *J* = 1.5 Hz, 5-Me of angeloyl), 1.96 (3H, *qd*, *J* = 1.5, 7 Hz, 4-Me of angeloyl), 1.99, 2.00, 2.05, 2.07, 2.08, 2.13 (3H each, *s*, Me × 6), 3.02, 3.62 (1H each, *d*, *J* = 9 Hz, 28-H_2), 3.62 (1H, 5'-H), 4.02 (1H, *dd*, *J* = 12, 3 Hz, 6'-H), 4.17 (1H, *dd*, *J* = 12, 4 Hz, 6'-H), 4.38 (1H, *d*, *J* = 8 Hz, 1'-H), 4.49 (1H, *t*, *J* = 8 Hz, 3-H), 4.92–5.18 (*m*, 2', 3' and 4'-H), 5.20 (1H, *m*, 16-H), 5.33 (1H, *dd*, *J* = 10, 7 Hz, 22-H), 6.01 (1H, *qq*, *J* = 7, 1.5 Hz, = CH- of angeloyl).

Hydrolysis of **2** *to give* **3**. Compound **2** (1.5 g) was hydrolysed with 10% KOH in aq. MeOH (20:80; 100 ml) for 4 hr under reflux. The reaction mixture was cooled and the separated solid was filtered, washed with water and dried. This on repeated crystallisations from MeOH–CH_3CN and Me_2CO–CH_3CN afforded **3** as transparent cubes (0.5 g), mp 210–214°, $[\alpha]_D$ −3.03° (MeOH; *c*0.99). Positive CIMS (NH_3) *m/z*: 637 $[M + H]^+$; negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 671 $[M + Cl]^-$, 635 $[M - 1]^-$. ^1H NMR (C_5D_5N at 70°): δ0.93 (6H, *s*, Me × 2), 0.95 (3H, *s*, Me × 1), 1.00 (6H, *s*, Me × 2), 1.13, 1.64 (3H each, *s*, Me × 2), 3.36 (1H, *t*, *J* = 8 Hz, 3-H), 3.68-4.12 (*m*, 28-H_2 and 2', 3', 4' and 5'-H), 4.22 (*m*, 6'-H_2), 4.50 (1H, *dd*, *J* = 12, 6 Hz, 22-H), 4.72 (1H, *d*, *J* = 7 Hz, 1'-H), 4.94 (1H, *m*, $W_{1/2}$ = 9 Hz, 16-H), 5.36 (1H, *m*, 12-H).

Acetylation of **3** *at room temp. to give* **4**. A soln. of **3** (0.1 g) in Ac_2O–pyridine was allowed to stand at room temp. for 48 hr. The crude oily product on CC over silica gel with $CHCl_3$–MeOH (9.9:0.1) and crystallisation from $CHCl_3$–petrol gave **4** (0.1 g) in fine needles, mp 150–153°, $[\alpha]_D$ −13.6° ($CHCl_3$; *c*1.2). IR ν_{max} cm^{-1}: 1745–1705 (*br*). EIMS *m/z*: 888 $[M]^+$. ^1H NMR ($CDCl_3$): δ0.88 (9H, *s*, Me × 3), 0.91, 0.96, 0.98, 1.42 (3H each, *s*, Me × 4), 1.98, 2.00 (3H each, *s*, OAc × 2), 2.04 (6H, *s*, OAc × 2), 2.08, 2.09 (3H each, *s*, OAc × 2), 2.95, 3.55 (1H each, *d*, *J* = 9 Hz, 28-H_2), 3.60 (1H, 5'-H), 4.12 (2H, *m*, 6'-H_2), 4.20 (1H, *m*, 16-H), 4.34 (1H, *d*, *J* = 7 Hz, 1'-H). 4.52 (1H, *t*, *J* = 8 Hz, 3-H), 5.28–5.48 (2H, *m*, 12-H and 22-H).

Acetylation of **3** *at* 100° *to give* **5**. Acetylation of **3** (0.1 g) with the above reagent at 100° for 5 hr yielded an oily product which on repeated crystallisations from $CHCl_3$–petrol furnished **5** (0.11 g), mp 170–173°, $[\alpha]_D$ −18.3° ($CHCl_3$; *c*1.3). IR ν_{max} cm^{-1}: 1750–1710 (*br*). EIMS *m/z*: 930 $[M]^+$. ^1H NMR ($CDCl_3$): δ0.85 (6H, *s*, Me × 2), 0.89, 0.93, 0.95, 0.98, 1.28, (3H each, *s*, Me × 5), 1.96, 1.98, 1.99, 2.03 (3H each, *s*, OAc × 4), 2.06 (6H, *s*, OAc × 2), 2.12 (3H, *s*, OAc × 1). 3.00, 3.60 (1H each, *d*, *J* = 9 Hz, 28-H_2), 3.60 (1H, 5'-H), 4.02 (1H, *dd*, *J* = 12, 3 Hz, 6'-H), 4.20 (1H, *dd*, *J* = 12, 4 Hz, 6'-H). 4.36 (1H, *d*, *J* = 7 Hz, 1'-H), 4.50 (1H, *t*, *J* = 8 Hz, 3-H), 5.22 (1H, *m*, 16-H), 5.28–5.48 (2H, *m*, 12-H and 22-H).

Smith degradation of the amorphous monoglucoside fraction. $NaIO_4$ (3 g) dissolved in H_2O (10 ml) was added to a soln. of the monoglucoside fraction (3.26 g) in EtOH (50 ml) and the mixture was stirred at room temp. for 6 hr. Excess $NaIO_4$ was decomposed by addition of ethylene glycol (2 ml) followed by further stirring for 0.5 hr. It was filtered and to the filtrate, $NaBH_4$ (4 g) was added and stirred for another 2 hr at room temp. The bulk of the alcohol was distilled off under red. pres., the

concentrate was diluted with H_2O (100 ml) and extracted with EtOAc. Evaporation of the solvent gave an amorphous solid which was treated with 2 N methanolic H_2SO_4 (50 ml) and allowed to stand at room temp. for 10 hr. The reaction mixture was diluted with H_2O (200 ml) and extracted with $CHCl_3$ to get the crude aglycone mixture. CC over silica gel with $CHCl_3$ yielded **6** crystallising out as fine needles (0.15 g) from MeOH–CH_3CN, mp 276–280°, $[\alpha]_D$ + 11.7° ($CHCl_3$; *c*1.2). EIMS *m/z* (rel. int.): 516 $[M]^+$ (0.3), 498 (0.3), 483 (0.5), 467 (0.3), 465 (0.1), 437 (1), 308 (30), 277 (68), 231 (53), 217 (57), 213 (100), 199 (77), positive CIMS (NH_3) *m/z*: 534 $[M + NH_4]^+$, negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 551 $[M + Cl]^-$. ^{1}H NMR ($CDCl_3$): δ0.76, 0.86, 0.90, 0.93, 0.97, 1.00, 1.39 (3H each, *s*, Me × 7), 3.17, 3.24 (1H each, *d*, *J* = 12 Hz, 28-H_2), 3.24 (1H each, *t*, *J* = 7 Hz, 3-H), 3.63 (2H, *d*, *J* = 4 Hz, > CH-C<u>H</u>$_2$OH), 3.72 (1H, 22-H), 4.74 (1H, *m*, 16-H), 4.81 (1H, *t*, *J* = 4 Hz, > C<u>H</u>-CH_2OH), 5.27 (1H, *m*, 12-H). Continued elution with $CHCl_3$ afforded an amorphous material (0.9 g) which was found to be an intimate mixture of **8** and **9** on TLC (C_6H_6–EtOAc, 9:1, triple run). Further elution with $CHCl_3$–MeOH (9.9:0.1) yielded **1** (0.15 g).

Separation of **8** and **9**. Careful repeated CC of the mixture obtained above over silica gel with C_6H_6–EtOAc (9.8:0.2) furnished homogenous **8** (0.2 g) crystallised as prisms from Me_2CO and **9** (0.1 g) as amorphous solid.

Compound **8**. Mp 140–142°, $[\alpha]_D$ −28.4° ($CHCl_3$; *c*1.3). IR ν_{max} cm^{-1}: 1730, 1705, EIMS *m/z* (rel. int.): 598 $[M]^+$ (0.6), 538 (0.3), 520 (0.4), 498 (0.7), 480 (0.9), 438 (6), 420 (3), 408 (8), 393 (6), 300 (4), 271 (3), 253 (4), 200 (100). Positive CIMS (NH_3) *m/z*: 616 $[M + NH_4]^+$, 599 $[M + 1]^+$; negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 633$[M + Cl]^-$. ^{1}H NMR (200 MHz, $CDCl_3$): δ0.78, 0.91, 0.93 (3H each, *s*, Me × 3), 0.98 (6H, *s*, Me × 2), 1.05, 1.30 (3H each, *s*, Me × 2), 1.84 (3H, *q*, *J* = 1.5 Hz, 5-Me of angeloyl), 1.97 (3H, *qd*, *J* = 1.5, 7 Hz, 4-Me of angeloyl), 2.09 (3H, *s*, OAc × 1). 3.02, 3.33 (1H each, *d*, *J* = 11 Hz, 28-H_2), 3.22 (1H, *dd*, *J* = 10, 6 Hz, 3-H), 5.04 (1H, *m*, $W_{1/2}$ = 8 Hz, 16-H), 5.41 (1H, *dd*, *J* = 12, 6 Hz, 22-H), 5.45 (1H, *m*, 12-H), 6.11 (1H, *qq*, *J* = 7, 1.5 Hz, = C<u>H</u>-of angeloyl).

Compound **9**. Mp 116–120°, $[\alpha]_D$ −33.3° ($CHCl_3$; *c*0.3), IR ν_{max} cm^{-1}: 1710–1730 (broad). EIMS *m/z*: 600$[M]^+$; positive CIMS (NH_3) *m/z*: 618 $[M + NH_4]^+$, 601 $[M + 1]^+$; negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 635$[M + Cl]^-$. ^{1}H NMR (200 MHz, $CDCl_3$): δ0.78, 0.90, 0.93, 0.97, 0.98, 1.03 (3H each, *s*, Me × 6), 1.13 (3H, *d*, *J* = 7 Hz, CH_3-CH of 2-methylbutyroyl), 1.30 (3H, *s*, Me), 2.10 (3H, *s*, OAc), 3.02, 3.34 (1H each, *d*, *J* = 11 Hz, 28-H_2), 3.22 (1H, *dd*, *J* = 10, 6 Hz, 3-H), 5.06 (1H, *m*, $W_{1/2}$ = 8 Hz, 16-H), 5.31 (1H, *dd*, *J* = 12, 6 Hz, 22-H), 5.44 (1H, *m*, 12-H).

Triacetate **7** of **1**. Acetylation of **1** (0.1 g) at room temp. with Ac_2O–pyridine gave **7** (0.09 g) as an amorphous solid. EIMS *m/z*: 600$[M]^+$. ^{1}H NMR ($CDCl_3$): δ0.85, 0.87, 0.90, 0.93, 0.96, 1.02, 1.42 (3H each, *s*, Me × 7), 2.03 (3H, *s*, OAc × 1), 2.15 (6H each, *s*, OAc × 2), 3.66, 3.72 (1H each, *d*, *J* = 11 Hz, 28-H_2), 4.27 (1H, *m*, $W_{1/2}$ = 9 Hz, 16-H), 4.51 (1H, *dd*, *J* = 9, 7 Hz, 3-H), 5.20-5.44 (2H, *m*, 12-H, 22-H).

Diacetate **10** *of* **6**. Acetylation of **6** (0.15 g) at room temp. with Ac_2O–pyridine for 24 hr furnished **10** (0.12 g) crystallizing from MeOH as fine needles, mp 258–260°, IR ν_{max} cm^{-1}: 1742, 1715, EIMS *m/z*: 600$[M]^+$; positive CIMS (NH_3) *m/z*: 618 $[M + NH_4]^+$, 601 $[M + 1]^+$; negative CIMS ($NH_3 + NH_4Cl$) *m/z*: 635$[M + Cl]^-$. ^{1}H NMR ($CDCl_3$): δ0.87 (9H, *s*, Me × 3), 0.94 (6H, *s*, Me × 2), 1.02, 1.40 (3H each, *s*, Me × 2), 2.03, 2.08 (3H each, *s*, OAc × 2), 3.17, 3.90 (1H each, *d*, *J* = 11 Hz, 28-H_2), 3.71 (1H, *dd*, *J* = 12, 5 Hz, 22-H), 4.15 (2H, *dd*, *J* = 12, 4 Hz, > CH-C<u>H</u>$_2$OAc), 4.50 (1H, *dd*, *J* = 9, 7 Hz, 3-H), 4.80 (1H, *m*, $W_{1/2}$ = 8 Hz, 16-H), 4.94 (1H, *t*, *J* = 4 Hz, > C<u>H</u>-CH_2OAc), 5.29 (1H, *m*, 12-H).

Smith degradation of **3**. Following the same procedure as mentioned above, compound **3** (1.1 g) yielded crude product (0.6 g), CC of which over silica gel gave **6** (0.1 g) and **1** (0.35 g).

Identification of glucose in monoglucoside. The monoglucoside (2 mg) was heated in a sealed tube at 100° for 6 hr with Kiliani mixture (AcOH–HCl–H_2O, 1.5:3.5:5). The hydrolysate showed the presence of D-glucose on paper chromatography (ascending, Whatman No. 1 paper).

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