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FURTHER TRITERPENOIDS AND ^{13}C NMR SPECTRA OF OLEANANE DERIVATIVES FROM *PHYTOLACCA ACINOSA*

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Key Word Index—*Phytolacca acinosa*; Phytolaccaceae; berries; pentacyclic triterpenoids; phytolaccagenin A; acinosolic acid A; acinosolic acid B.

Abstract—In addition to five known triterpenoids, namely acinosolic acid, phytolaccagenin, phytolaccagenic acid, esculentic acid and jaligonic acid, three new oleanane derivatives, designated as phytolaccagenin A, acinosolic acid A and acinosolic acid B, have been isolated and characterized from the defatted berries of *Phytolacca acinosa*. The new compounds have been identified as 3β-acetoxy-30β-methyloleanate-12-en-2β,23α-diol-28β-oic acid, 3β-acetoxy-28β-methyloleanate-12-en-2β-ol-30β-oic acid and 2β-acetoxy-28β-methyloleanate-12-en-3β-ol-30β-oic acid, respectively.

INTRODUCTION

In continuation of our earlier studies [1, 2] on constituents of the defatted berries of *Phytolacca acinosa*, three more new triterpenoids, designated as phytolaccagenin A (**1**), acinosolic acid A (**2**) and acinosolic acid B (**3**), and five known oleanane derivatives have been isolated and characterized. A description of the ^{13}C NMR spectra of the triterpenoids isolated so far from these berries, as well as their semisynthetic derivatives, is also presented.

RESULTS AND DISCUSSION

From their positive response towards the Liebermann–Burchard, TCA and TNM tests, compounds **1–8** were found to be unsaturated pentacyclic triterpenoids. The compounds were transparent to UV light. Their IR spectra indicated the presence of carboxyl, ester, hydroxyl and geminal dimethyl groups and a trisubstituted double bond. The spectra of compounds **1–3** contained additional absorption bands for an acetoxyl function.

The ^1H NMR spectrum of **1** ($[M]^+$ at m/z 574, $C_{33}H_{50}O_8$) revealed the presence of five tertiary methyls at δ 1.06–1.54, an acetoxyl at 2.06, a carbomethoxyl at 3.70, a vinylic proton at 5.6 and an allylic proton, assignable to H-18 [3], at 2.9 (*d*, J = 13 Hz). The compound, on treatment with diazomethane, afforded a dimethyl ester **9** ($[M]^+$ at m/z 588, $C_{34}H_{52}O_8$) whose ^1H NMR spectrum displayed signals at δ 2.12 (OCOMe), 3.67 and 3.80 (3H each, *s*, 2 × CO_2Me), indicating the presence of a carboxyl in **1**.

A two-proton resonance signal, exchangeable with deuterium oxide, at δ 4.54 in the ^1H NMR spectrum of **1** indicated that the compound carried two hydroxyls, a fact also indicated by its transformation, on refluxing with acetic anhydride–pyridine, to **10** ($[M]^+$ at m/z 658, $C_{37}H_{54}O_{10}$). The ^1H NMR spectrum of **10** displayed signals for the protons of three acetoxyls at δ 2.0 (3H, *s*) and 2.1 (6H, *d*) and a carbomethoxyl at 3.70.

The high-resolution mass spectrum of **1** indicated that by typical retro-Diels–Alder fragmentation of ring C, compound **1** produced fragment **X** at m/z 292.1687, $C_{17}H_{24}O_4$, and a fragment **Y** at m/z 282 (Scheme 1). In the mass spectrum of compound **9**, fragment **X** was observed at m/z 306; fragment **Y** appeared at m/z 366 in the mass spectrum of **10**. The presence of only five tertiary methyls, a carboxyl and a carbomethoxyl in **1** together with the chemical shift and multiplicity of H-18 [3] and the mass spectral fragmentation [4] confirmed that the compound was an olean-12-ene derivative carrying two hydroxyls and an acetoxyl group in ring A/B, with two of its tertiary methyls, in ring D/E, transformed into a carboxyl and a carbomethoxyl function.

One of the hydroxyls in ring A/B was shown to be primary in nature by the resonance signals, due to the hydroxymethylene protons at δ 3.40 and 3.80 (1H each, poorly defined AB*q*, J = 10 Hz), which were shifted to 3.89 (2H, *q*, J = 10 Hz, CH_2OAc) in the ^1H NMR spectrum of **10**. These data were in agreement with the presence of a primary hydroxyl at an unhindered equatorial position attached to a tertiary carbon [2]. The upfield chemical shift of the acetoxymethylene protons [5] and the hydroxymethylene protons was also in accord with the presence of a 23α-hydroxyl function.

The ^1H NMR spectrum of **1** contained signals for two carbinylic protons at δ 4.91 (*d*, J = 4.5 Hz) and 4.41 (*d*, J = 5 Hz), which were displayed at δ 5.45 and 4.95, respectively, in the spectrum of **10**. The upfield carbinylic proton resonance signal in **1** was assigned to the proton geminal to the hydroxyl group and the downfield signal was due to the acetoxyl carbinylic proton. This confirmed the secondary nature of the second hydroxyl and the acetoxyl function in ring A/B. Since compound **1** failed to form an acetonide with acetone–potassium carbonate, as expected for 3β,23α-hydroxyls or 2β,3β-hydroxyls [6], the presence

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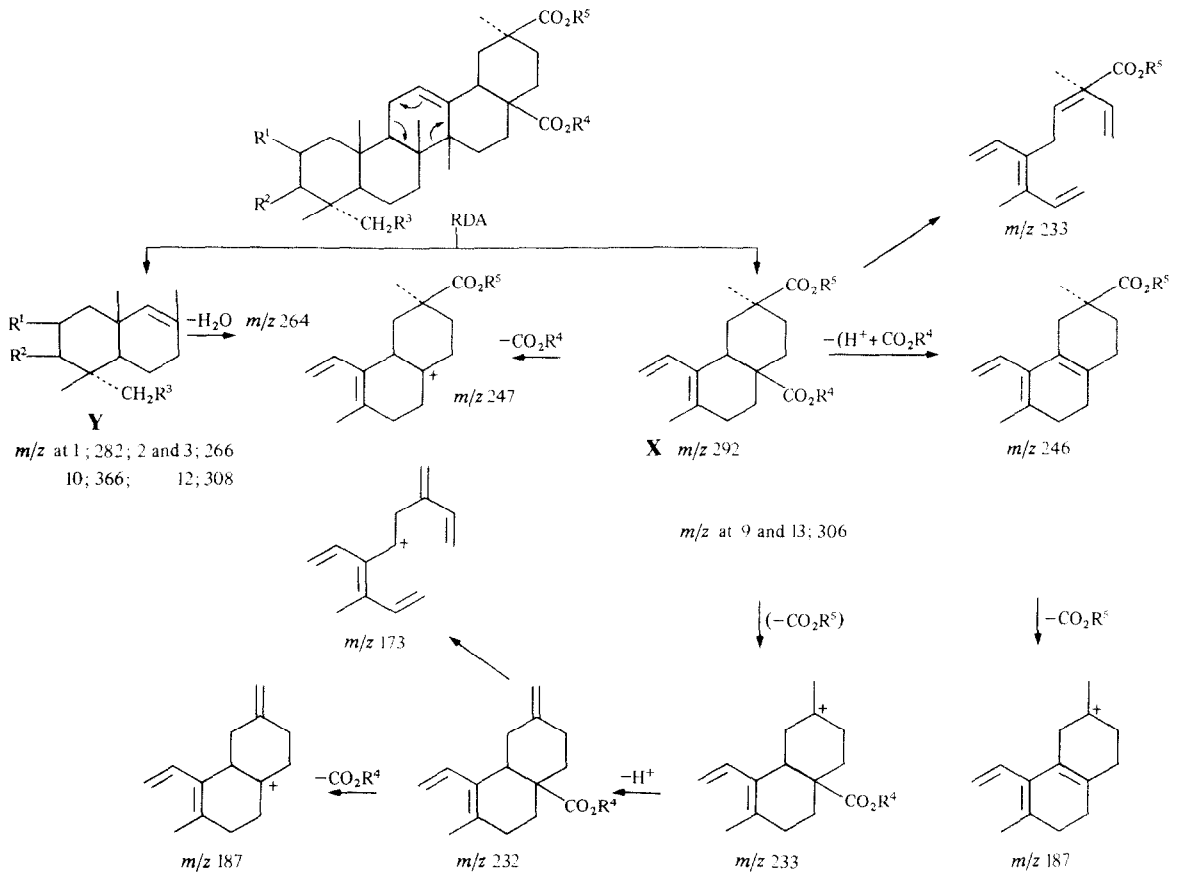


Scheme 1. Mass spectral fragmentation of triterpenoids.

of a free hydroxyl at C-3 was ruled out. Therefore, the acetoxyl function was placed at the C-3 position. Furthermore, the ^{1}H NMR spectra of **1** and **10** revealed that two tertiary methyls had shifted downfield, indicating that the secondary hydroxyl function was present at C-2, which is in a 1,3-diaxial relationship with the C-24 and C-25 methyls. The multiplicity and coupling constant of the carbinylic protons suggested that they underwent axial–equatorial coupling, confirming that both the hydroxyl and the acetoxyl functions were β-oriented.

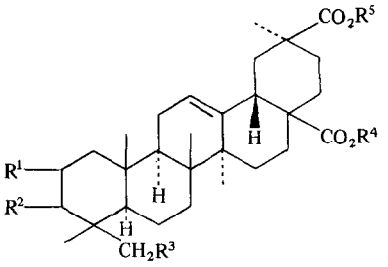
On treatment with bromine–methanol, compound **1** formed the bromo-γ-lactone **11** ([M]$^+$ at *m/z* 654, 652, $C_{33}H_{49}O_8Br$) as expected from a C-12 double bond and C-17 carboxyl group [7]. This demonstrated the presence of a 28β-carboxyl function. Close inspection of the mass spectral fragmentation of fragment **X** revealed its close resemblance to that of the similar fragment derived from sperjulagenic acid A [2] and dimethylsperjulagenate [8], both of which carry a 30β-carbomethoxyl function. Thus the structure of phytolaccagenin A (**1**) was established as 3β-acetoxy-30β-methyloleanate-12-en-2β,23α-diol-28β-oic acid. The identity of this compound was finally confirmed by deacetylation of **1** with sulphuric acid to yield **5**, identical in all respects to an authentic sample.

Compounds **2** and **3** were found to be isomers. Compound **2** had [M]$^+$ at *m/z* 558, $C_{33}H_{50}O_7$, and its ^{1}H NMR spectrum revealed the presence of six tertiary methyls at δ0.7–1.2, an acetoxyl at 2.08 (3H, *s*) and a carbomethoxyl function at 3.70, besides showing the presence of a vinylic function at 5.35.

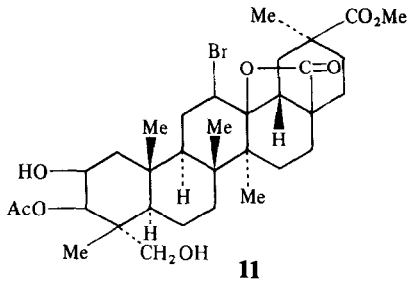
Compound **3** had [M]$^+$ at *m/z* 558, $C_{33}H_{50}O_7$, while its ^{1}H NMR spectrum exhibited resonance signals due to six tertiary methyls at δ0.75–1.2, an acetoxyl at 2.1 (3H, *s*), a carbomethoxyl at 3.6 and a vinylic function at 5.2. The ^{1}H NMR spectra of both compounds revealed the presence of a single deuterium oxide exchangeable proton, indicating the presence of a hydroxyl function.

On treatment with acetic anhydride–pyridine, both **2** and **3** were transformed into **12** with [M]$^+$ at *m/z* 600, $C_{35}H_{52}O_8$. However, their rates of reaction differed considerably. Compound **2** could be acetylated only under drastic conditions while compound **3** was acetylated at room temperature. The IR spectrum of **12** lacked the absorption due to a hydroxyl group and its ^{1}H NMR spectrum contained signals due to two acetoxyl functions at δ2.09 (6H, *s*, 2 × OCOMe) and a carbomethoxyl function at 3.70. When treated with freshly prepared diazomethane, compound **12** afforded the dimethyl ester **13** ([M]$^+$ at *m/z* 614, $C_{36}H_{54}O_8$), whose ^{1}H NMR spectrum revealed the presence of two carbomethoxyls at δ3.57 and 3.69.

The high-resolution mass spectra of **12** and **13** revealed that the double bond triggered the typical retro-Diels–Alder fragmentation of ring C. This resulted in fragments at *m/z* 292 (**X**) and 308 (**Y**) for compound **12** and fragments at *m/z* 306 (**X**) and 308 (**Y**) in the mass spectrum of **13**. These data, together with the ^{1}H NMR chemical shift, multiplicity and coupling constant of H-18 [3] as well as the presence of only six tertiary methyls in **2** and **3**, indicated that both compounds were olean-12-ene-



	R^1	R^2	R^3	R^4	R^5
1	βOH	βOAc	OH	H	Me
2	βOH	βOAc	H	Me	H
3	βOAc	βOH	H	Me	H
4	βOH	βOH	H	Me	H
5	βOH	βOH	OH	H	Me
6	H	βOH	OH	H	Me
7	H	βOH	OH	H	H
8	βOH	βOH	OH	H	H
9	βOH	βOAc	OH	Me	Me
10	βOAc	βOAc	OAc	H	Me
12	βOAc	βOAc	H	Me	H
13	βOAc	βOAc	H	Me	Me
14	βOAc	βOAc	OAc	Me	Me
16	H	βOAc	OAc	H	Me
17	H	βOAc	OAc	Me	Me
18	βOAc	βOAc	OAc	Me	H
19	H	βOAc	OAc	Me	H
20	H	βOH	H	H	Me
21	H	βOAc	H	H	Me



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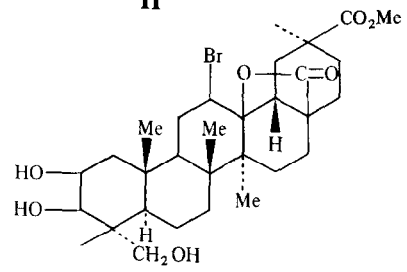

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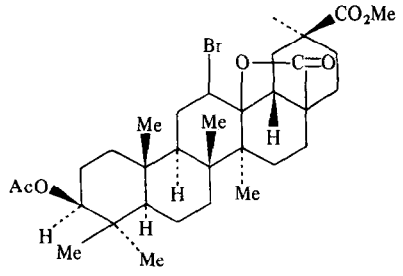

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derivatives carrying an acetoxyl and a hydroxyl in ring A/B and two of their tertiary methyls in ring D/E were transformed into a carboxyl and a carbomethoxyl function. Both **2** and **3** failed to form a bromo-γ-lactone. Also, further fragmentation of the ion **X** resembled that of the similar fragment in isophytolaccagenin A [2], thereby establishing the presence of C-30β-COOH and C-28β-CO_2Me in compounds **2** and **3**.

Close inspection of the mass spectra of **2** and **3** revealed that the intensities of the peak at *m/z* 266 and its daughter ions at *m/z* 248 $[266-H_2O]^+$ and 206 $[266-MeCOO+H]^+$ were different, indicating that compounds **2** and **3** were isomeric and must differ in the positions of the hydroxyl and acetoxyl in ring A/B. Based on biogenetic grounds, one of the groups was assigned to position C-3.

The exact positions of the acetoxyl and hydroxyl functions in **2** and **3** were decided on the basis of the chemical shifts of the carbinylic protons. The ^1H NMR spectrum of **2** revealed the signals due to two carbinylic protons as doublets at δ4.37 and 4.50 (J = 5 Hz). The former signal was assigned to the proton geminal to the hydroxyl function while the latter was assigned to the proton on the carbon carrying the acetoxyl function. The corresponding signals in **3** were observed at δ4.18 and 4.80 (each d, J = 5 Hz). The coupling constant of the carbinylic protons indicated that they underwent axial–equatorial coupling, showing that the functional groups at C-2 and C-3 were both β-oriented.

Furthermore, since compound **2** was acetylated only under drastic conditions, it was established that the hydroxyl in **2** must be axially oriented at C-2. If the hydroxyl function had been at C-3, the compound would have undergone facile acetylation at room temperature, as was observed in the case of compound **3**.

On deacetylation with 7% sulphuric acid, compounds **2** and **3** formed compound **14** ($[M]^+$ at *m/z* 516, $C_{31}H_{48}O_6$), which was identical to **4** by co-TLC and mmp. Compound **14** formed an acetonide, confirming that **4** contained two hydroxyls at C-2 and C-3.

From these observations, acinosolic acids A and B were assigned the structures 3β-acetoxy-28β-methyloleanate-12-ene-2β-ol-30β-oic acid and 2β-acetoxy-28β-methyloleanate-12-ene-3β-ol-30β-oic acid, respectively.

Compounds **4**, **5**, **6**, **7** and **8** were known from the chemical literature and their identities were determined by spectral data, chemical transformation, co-TLC and determination of mmp. Their structures were established as acinosolic acid (**4**) [9], phytolaccagenin (**5**) [10], phytolaccagenic acid (**6**) [11], esculentic acid (**7**) [12] and jaligonic acid (**8**) [13].

Assignments of the carbon chemical shifts in the ^{13}C NMR spectra were made by the use of the single-frequency off-resonance decoupling technique [14, 15], application of known chemical shift rules due to hydroxyl and acetoxyl substitutions, and steric γ- and δ-effects [14, 16–18], as well as by comparison with ^{13}C NMR spectral data of the known oleanane derivatives [19]. The ^{13}C NMR chemical shifts of the compounds (Table 1)

Table 1. ^{13}C NMR chemical shifts of oleanane derivatives from *P. acinosa* (δ_C, ppm, $CDCl_3$)

Carbon	1*	9	10	12	4*	18	19	20	21	22
C-1	43.3	43.8	41.6	41.9	37.8	41.9	37.8	38.5	38.2	36.7
C-2	70.9	71.2	69.6	69.6	70.4	69.7	23.1	27.2	23.6	23.5
C-3	79.1	72.7	72.0	78.0	77.0	72.1	74.7	79.1	81.0	80.6
C-4	42.2	41.7	40.1	37.3	36.5	40.2	40.7	38.8	37.8	37.9
C-5	47.7	48.4	48.2	55.3	54.9	48.3	48.1	55.4	55.5	55.4
C-6	17.6	18.1	17.7	18.0	17.8	17.7	18.0	18.3	18.3	17.7
C-7	32.2	32.5	32.3	32.5	29.8	32.7	32.4	32.7	32.7	30.7
C-8	40.7	39.5	39.4	39.1	41.4	39.4	39.4	39.4	39.4	38.1
C-9	47.4	48.4	47.8	48.1	47.5	47.8	47.8	47.7	47.7	45.6
C-10	36.4	36.7	36.7	36.8	32.5	36.8	36.9	37.2	37.1	36.6
C-11	22.8	23.4	23.0	23.0	22.8	23.0	23.0	23.2	23.2	29.0
C-12	122.1	123.2	123.1	123.2	122.1	123.1	123.3	123.4	123.4	55.7
C-13	143.7	143.3	143.1	143.1	143.6	143.6	143.1	143.0	143.0	91.3
C-14	40.7	41.2	41.6	41.8	41.6	41.6	41.5	41.5	41.6	42.5
C-15	27.2	27.7	27.5	27.6	23.0	27.6	27.7	27.8	27.8	29.2
C-16	23.1	23.5	23.4	23.4	22.8	23.4	23.4	23.4	23.4	21.7
C-17	45.1	46.2	45.9	45.9	45.0	45.9	46.0	46.0	46.0	44.9
C-18	41.7	42.7	42.3	42.3	43.3	42.3	42.4	42.3	42.4	53.5
C-19	41.3	42.2	42.0	42.0	42.2	42.0	42.1	42.1	42.2	42.5
C-20	43.8	43.9	43.7	43.7	43.9	43.7	43.8	43.7	43.8	43.5
C-21	29.8	30.5	30.3	30.3	29.6	30.5	30.4	30.4	30.5	30.5
C-22	33.3	33.5	33.5	33.6	33.3	33.7	33.6	33.6	33.6	34.7
C-23	66.3	68.5	65.6	28.3	27.1	65.7	65.6	28.1	28.1	27.9
C-24	13.5	13.4	13.7	16.0	16.9	13.8	13.0	15.4	16.7	17.0
C-25	16.5	16.8	16.5	17.3	16.0	16.6	15.8	15.5	15.5	16.5
C-26	16.7	16.8	17.2	17.6	17.5	17.2	17.1	17.2	17.2	19.1
C-27	25.4	25.9	25.8	26.0	25.6	25.9	25.8	25.9	29.9	21.1
C-28	178.8	177.6	182.8	176.9	176.5	176.9	176.9	183.1	183.1	178.09
C-29	27.9	28.4	28.3	29.1	27.9	28.3	28.4	28.4	28.4	28.2
C-30	176.5	177.0	176.9	183.2	178.3	182.7	183.0	176.9	170.0	176.4
C-3 OAc	170.4	171.0	170.0	170.6	—	170.7	170.7	—	171.0	170.8
	20.7	20.9	20.7	20.8	—	20.7	21.1	—	21.3	21.2
C-2 OAc	—	—	170.3	170.1	—	170.0	170.9	—	—	—
			20.8	21.2		21.7	20.8			
C-23 OAc	—	—	170.7			170.2				
			21.2			20.8				
COOMe	51.7	51.7	51.7	51.7	51.6	51.7	51.7	51.8	51.8	51.9
COOMe		51.5								

*Solvent DMSO-d_6.

were found to be similar but with predictable differences. The low-field quaternary carbon signals at δ_C 46.0 and 43.8 were assigned to C-17 and C-20, respectively, irrespective of the presence of carboxyl or a carbomethoxyl at these carbons. These values are in agreement with those of oleanolic acid and 3-epikatonic acid [20]. Two carbon resonance signals due to C-27 and C-29 methyls appeared at low field: 29.9–21.1 and 29.1–27.9. The latter signal confirmed the presence of an axial carboxyl or carbomethoxyl group at C-20, in all these compounds. The presence of an equatorial oxygenated function at this carbon would have resulted in an upfield chemical shift, δ_C 19.5, of the geminal methyl carbon [21]. From a comparison of the chemical shifts of the E-ring carbons with the literature values [22], an inference to the effect that the oxygenated functions at C-17 and C-20 deshielded β-carbons and shielded γ-carbons could be drawn. Relative to 3β-hydroxy-olean-12-en-29-oic acid [21], the shielding effect of the γ-carbons was found to be more pronounced with C-17 oxygenated functions. This was indicated by the upfield shift by 4 ppm of C-18 in the compounds as compared to those carrying a C-17 methyl group. The *cis*-fusion of rings D and E in acetyldimethylserajinate, whose structure corresponds to the methyl ester of **21**, was confirmed by studying the ^{13}C NMR spectrum of its 11-keto derivative and comparison of the E-long range interactions of the C-14 and C-19 hydrogens with those of methyl glycyrrhetate [23]. The chemical shifts of these carbons in the substances reported here were very close to those of serajinic acid.

The acetylation of the C-3 hydroxyl in ring A of *Phytolacca* triterpenoids as well as the introduction of each acetoxyl function at C-23 and C-2 caused significant shifts in the C-1, C-2, C-4, C-5, C-24 and C-25 carbon resonance signals. The differences observed in the chemical shifts could be rationalized by considering the β and γ-effects of the acetoxyl functions. The C-23 acetoxyl group causes, by E-long range eclipsed interactions, a shielding effect on C-1 and C-5; the effect being more pronounced (7 ppm) at C-5.

With the bromo-γ-lactone **22**, the carbon chemical shifts varied considerably in comparison to olean-12-ene derivatives. With **22** the signals due to C-12 and C-13 appeared at δ_C 55.7 and 91.3, respectively, and the signal due to C-18 was shifted downfield to 53.5. As a consequence of the formation of the bromo-γ-lactone, the C-15 signal was shifted downfield by 1.4 ppm while the C-16 signal was shifted upfield by 1.7 ppm as compared to the parent compound **21**. Considerable shifts were also observed in the resonance signals of C-1, C-27, C-9, C-8, C-11, C-16 and C-25. These shifts can be attributed to the configuration of the lactone ring, bromo-function and their long range interactions. The chemical shift of C-12, δ_C 55.7, confirms the axial, and not the equatorial [24], configuration of the bromo function. In the latter case, the C-12 resonance signal would have appeared upfield by 4 ppm.

EXPERIMENTAL

IR spectra were recorded in KBr. ^{1}H NMR spectra were run at 60 and 250 MHz. ^{13}C NMR spectra were recorded at 25.2 MHz and MS at 70 eV. Mps are uncorr.

Extraction and isolation. Extraction of the plant material has been described in ref. [1]. Development of the column with C_6H_6–EtOAc (1:1) afforded compound **1**, mp 165°; compound **2**, mp 310°; and compound **3**, mp 345°. Further development of the column with C_6H_6–EtOAc (1:2) afforded a mixture of **4** and **5**, which were separated further by CC on silica gel to give **4**, mp 200°, and **5**, mp 317°. Development of the column with EtOAc afforded **6**, **7** and **8**, which were purified by repeated CC and crystallization; **6**, mp 309°; **7**, mp 360°; **8**, mp 318°.

Identification of **1**. $[M]^+$ at m/z 574, $C_{33}H_{50}O_8$, (required $[M]^+$ at m/z 574.3504). IR ν_{max} cm^{-1}: 3400–2990 (br), 1720, 1640, 1380, 1360, 1560, 1220, 1140, 1110 and 800. MS m/z: 574 $[M]^+$, 528, 514, 292, 282, 247, 246, 233, 222, 187, 173.

Methylation of **1**. Compound **1** (70 mg) in Et_2O was treated with CH_2N_2. After usual work-up, **9** (65 mg), mp 120°, was recovered. $[M]^+$ at m/z 588, $C_{34}H_{52}O_8$ (required for $C_{34}H_{52}O_8$: 588.3662). IR ν_{max} cm^{-1}: 3420 (OH), 1720, 1245, 1680, 1365, 1380 and 820. ^{1}H NMR ($CDCl_3$): δ0.77 (3H), 1.04 (3H), 1.16 (6H), 1.29 (3H), 2.12 (3H, *s*, OCOMe), 2.5 (2H, D_2O exchangeable protons), 2.73 (1H, *d*, J = 10 Hz, H-18), 3.51 (2H, poorly defined ABq, J = 10 Hz, C$\underline{H}_2$OH), 3.67 (3H, *s*, COOMe), 3.80 (3H, *s*, COOMe), 4.96 (1H, *s*, H-3), 4.19 (1H, *d*, J = 5 Hz, H-2) and 5.45 (1H, *t*, J = 3.8, 3 Hz, H-12). MS m/z (rel. int.): 588 $[M]^+$, 528, 482, 306, 292, 282, 264, 247, 246, 222, 187 (100).

Acetylation of **1**. Compound **1** (70 mg) was treated with Ac_2O–pyridine and refluxed for 4 hr. After the reaction was complete, the product was purified to give **10**, mp 139; $[M]^+$ at m/z 658, $C_{37}H_{54}O_{10}$ (required $[M]^+$ at m/z 658.3718). IR ν_{max} cm^{-1}: 3200–2500 (br), 1735, 1720, 1640, 1385, 1360, 1240, 1045 and 820. ^{1}H NMR ($CDCl_3$): δ0.79 (3H), 1.05 (3H), 1.15 (6H), 1.25 (3H), 2.0 (3H, *s*, OCOMe), 2.1 (6H, *s*, 2 × OCOMe), 2.70 (1H, *d*, J = 15 Hz, H-18), 3.70 (3H, *s*, CO_2Me), 3.89 (2H, *q*, J = 10 Hz, CH_2OAc), 4.95 (1H, *d*, J = 4 Hz, H-3), 5.35 (1H, *br s*, H-12) and 5.45 (1H, *d*, J = 4 Hz, H-2). MS m/z (rel. int.): 658 $[M]^+$, 612, 598, 556, 510, 494, 366, 307, 306, 292, 248, 246, 187 (100).

Bromination of **1**. Compound **1** (35 mg) was dissolved in MeOH (6 ml) and treated with Br_2 (20 mg) in MeOH (4 ml). After 30 min, the soln was cooled in an ice bath to give colourless needles of **11**, mp 210°, $[M]^+$ at m/z 654, 652, $C_{33}H_{49}O_8$ Br, required $[M]^+$ at m/z 654.3427). IR ν_{max} cm^{-1}: 3400 (OH), 1767, 1720, 1245, 1680, 1380, 1375 and 820. ^{1}H NMR ($CDCl_3$): δ0.76 (3H, *s*), 1.00 (6H, *s*), 1.25 (6H, *s*), 2.1 (3H, *s*, $OCOCH_3$), 4.2 (1H, *d*, J = 4 Hz, H-2), 4.89 (1H, *d*, J = 5 Hz, H-3).

Identification of **2**. $[M]^+$ at m/z 558, $C_{33}H_{50}O_7$ (required: $[M]^+$ at m/z 558.3556). IR ν_{max} cm^{-1}: 3480, 1720, 1245, 1730, 2500–3900, 1680, 820, 1380 and 1360. ^{1}H NMR ($CDCl_3$): δ0.7 (3H, *s*), 0.9 (3H, *s*), 1.0 (3H, *s*), 1.05 (3H, *s*), 1.2 (6H, *s*), 2.08 (3H, *s*, OCOMe), 2.70 (1H, *d*, J = 13 Hz, H-18), 3.70 (3H, *s*, COOMe), 4.37 (1H, *d*, J = 5 Hz, H-2), 4.50 (1H, *d*, J = 5 Hz, H-3), 5.35 (1H, *br s*, H-12) MS m/z: 558, 540, 512, 292, 266, 248, 246, 187.

Identification of **3**. $[M]^+$ at m/z 558, $C_{33}H_{50}O_7$ (required $[M]^+$ at m/z 558.3557). IR ν_{max} cm^{-1}: 3450, 2500–3000 (br), 1730, 1720, 1245, 1680, and 820. ^{1}H NMR ($CDCl_3$): δ0.75 (3H, *s*), 0.95 (3H, *s*), 1.1 (3H, *s*), 1.15 (3H, *s*), 1.2 (6H, *s*), 2.1 (3H, *s*, OCOMe), 2.69 (1H, *br d*, J = 12.5 Hz, H-18), 3.6 (3H, *s*, CO_2Me), 4.18 (1H, *d*, J = 5 Hz, H-3), 4.80 (1H, *d*, J = 5 Hz, H-2) and 5.2 (1H, *br s*, H-12).

Acetylation of **2** *and* **3**. Compounds **2** and **3** (70 mg of each) were acetylated with Ac_2O and pyridine. Compound **2** formed an acetate only on refluxing, while **3** was transformed into its acetate at room temp. Both **2** and **3** afforded the diacetate **12**, mp 272°.

Identification of **12**. $[M]^+$ at m/z 600, $C_{35}H_{52}O_8$ (required $[M]^+$ at m/z 600.3662). IR ν_{max} cm^{-1}: 3200–2500 (br), 1720, 1640, 1365, 1380, 1245, 1025 and 820. ^{1}H NMR ($CDCl_3$): δ0.79 (3H, *s*), 0.90 (3H, *s*), 1.09 (3H, *s*), 1.20 (6H, *s*), 1.25 (3H, *s*), 2.09 (6H, *d*, 2 × OCOMe), 2.7 (1H, *br d*, J = 15.5 Hz, H-18), 3.70 (3H, *s*, CO_2Me), 4.65 (1H, *d*, J = 5 Hz, H-3), 5.35 (2H, *br d*, J = 4 Hz, H-12 and H-2). MS m/z: 600 $[M]^+$, 554, 526, 452, 317, 308, 248, 246, 189, 187.

Methylation of **12**. Compound **12** (50 mg) was methylated in the usual manner to give **13**, mp 205°, $[M]^+$ at m/z 614, $C_{36}H_{54}O_8$ (required $[M]^+$ at m/z 614.3818). IR ν_{max} cm^{-1}: 1730, 1240, 1640, 1015 and 840. ^{1}H NMR ($CDCl_3$): δ0.75 (3H, *s*), 0.90 (3H, *s*), 1.05 (3H, *s*), 1.14 (3H, *s*), 1.15 (3H, *s*), 1.19 (3H, *s*), 2.02 (3H, *s*, OCOMe), 2.04 (3H, *s*, OCOMe), 2.7 (1H, *br d*, J = 15.9 Hz, H-18), 3.57 (3H, *s*, CO_2Me), 3.69 (3H, *s*, CO_2Me), 4.64 (1H, *d*, J = 4 Hz, H-3), 5.35 (2H, *d*, J = 4 Hz, H-12 and H-2). MS m/z: 614 $[M]^+$, 554, 495, 494, 308, 306, 248, 246, 187, 186.

Identification of **4**. $[M]^+$ at m/z 516, $C_{31}H_{48}O_6$ (required $[M]^+$ at m/z 516.3450). IR ν_{max} cm^{-1}: 3340, 2900–3200, 1720, 1680, 1375, 1360 and 820. ^{1}H NMR (DMSO-d_6): δ0.7 (3H, *s*), 0.9 (6H, *s*), 1.08 (6H, *s*), 1.2 (3H, *s*), 2.5 (1H, *br d*, J = 13.4 Hz, H-18), 2.8 (1H, D_2O exchangeable OH), 3.19 (1H, D_2O-exchangeable OH), 3.42 (3H, *s*, COOMe), 3.9 (1H, *d*, J = 4 Hz, H-3), 4.2 (1H, *d*, J = 4 Hz, H-2), 5.2 (1H, *br s*, H-12). MS m/z (rel. int.): 516 $[M]^+$, 498, 476, 292 (100), 246, 224, 206, 187, 173, 133.

Identification of **5**. $[M]^+$ at m/z 532, $C_{31}H_{48}O_7$ (required $[M]^+$ at m/z 532.3400). IR ν_{max} cm^{-1}: 3400, 3200–2900 (br), 1730, 1680, 1380, 1360 and 820. ^{1}H NMR (DMSO-d_6): δ0.6–1.10 (5 × tert. Me's), 2.50 (1H, *br d*, J = 13.2 Hz, H-18), 3.5 (3H, *s*, COOMe), 3.60 (2 × OH, D_2O-exchangeable), 3.85 (2H, *q*, CH_2OH), 4.25 (1H, *br s*, $W_{1/2}$ = 5 Hz, H-3), 4.5 (1H, *d*, J = 4 Hz, H-2), 5.15 (1H, *br s*, H-12). MS m/z: 532 $[M]^+$, 514, 486, 292, 247, 246, 239, 232, 221, 187, 173, 133.

Acetylation of **5**. Compound **5** (50 mg) was acetylated in the usual manner whereupon it afforded a compound which was identical (co-TLC and mmp) to **10**.

Methylation of **10**. Compound **10** (70 mg) in Et_2O was treated with freshly prepared CH_2N_2 to give **14**, mp 100°.

Identification of **14**. $[M]^+$ at m/z 672.3890, $C_{38}H_{56}O_{10}$ (required $[M]^+$ at m/z 672.3874). IR ν_{max} cm^{-1}: 1740, 1730, 1640, 1380, 1360, 1240, 1042 and 820. ^{1}H NMR ($CDCl_3$): δ0.72–1.19 (5 × tert. Me's), 2.05–2.08 (3 × OCOMe), 3.5 and 3.7 (3H each, *s*, CO_2Me), 3.8 (2H, poorly resolved *q*, CH_2OAc), 4.9 (1H, *d*, J = 4 Hz, H-3), 5.36 (1H, *br s*, H-12), 5.4 (1H, *d*, J = 3 Hz, H-2). MS m/z (rel. int.): 672 $[M]^+$, 657, 612, 553, 552, 433, 306, 246, 187 (100).

Bromination of **5**. Compound **5** (30 mg) was treated with Br_2–MeOH in the usual manner to give **15**, mp 225°.

IR ν_{max} cm^{-1}: 3429, 1767, 1730, 1240, 1640, 820, 1050, 1365 and 1360.

Identification of **6**. $[M]^+$ at *m/z* 516.3355, $C_{31}H_{48}O_6$. IR ν_{max} cm^{-1}: 3400, 2900–3300 (br), 1730, 1640, 1380, 1365 and 820. ^{1}H NMR (DMSO-d_6): δ0.6–1.00 (5 × tert. Me's), 2.52 (1H, *br d*, J = 13.5 Hz, H-18), 3.45 (3H, *s*, COOMe), 4.3 (1H, *t*, J = 9, 4.5 Hz, H-3), 3.85 (2H, poorly resolved *q*, CH_2OH), 5.05 (1H, *br s*, H-12), MS *m/z*: 516 $[M]^+$, 496, 470, 292, 233, 223, 247, 246, 232, 205, 187, 173, 133.

Acetylation of **6**. Compound **6** (80 mg) was acetylated in the usual manner to give **16**, mp 280°, $[M]^+$ at *m/z* 600, $C_{35}H_{52}O_8$ (required *m/z* at 600.3663). IR ν_{max} cm^{-1}: 2500–3200 (br, COOH), 1720, 1245, 1640, 820, 1365 and 1360. ^{1}H NMR ($CDCl_3$): δ0.7–1.13 (5 × tert. Me's), 2.02 (6H, *s*, OCOMe), 3.68 (3H, *s*, CO_2Me), 4.2 (2H, poorly resolved *q*, CH_2OAc), 4.5 (1H, *br s*, H-3), 5, 6 (1H, *br s*, H-12). MS *m/z* (rel. int.): 600 $[M]^+$, 540, 308, 292, 246, 187 (100).

Methylation of **16**. Compound **16** (50 mg) in Et_2O was methylated in the usual manner to give **17**, mp 105°. IR ν_{max} cm^{-1}: 1730, 1720, 1240, 1680, 1365, 1380 and 820. ^{1}H NMR ($CDCl_3$): δ0.7–1.2 (5 × tert. Me's), 2.06 (6H, *s*, 2 × OCOMe), 2.73 (1H, *br d*, J = 13 Hz, H-18), 3.6 and 3.7 (3H each, *s*, OCOMe), 4.19 (2H, poorly resolved *q*, CH_2OAc), 4.79 (1H, *br s*, H-3), 5.2 (1H, *br s*, H-12).

Identification of **7** *and* **8**. Compound **7**, mp 360°, and compound **8**, mp 318, had spectral data superimposable with authentic samples. Their mmps and co-TLC confirmed their identities.

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