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TRITERPENE GLYCOSIDES RELATED TO QUADRANGULOSIDE FROM *PASSIFLORA QUADRANGULARIS*

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Key Word Index—*Passiflora quadrangularis*; Passifloraceae; cyclopropane triterpene glycosides; quadranguloside; oleanolic acid-3-sophoroside.

Abstract—Two new cyclopropane triterpene glycosides were isolated from the methanol extracts of leaves of *Passiflora quadrangularis*. On the basis of hydrolysis, spectral evidence and comparison with quadranguloside, these compounds were assigned the structures 9,19-cyclolanosta-22,25-epoxy-3β-21,22(*R*)-triol-3β-*O*-gentiobioside and 9,19-cyclolanosta-21,24-epoxy-3β-25,26-triol-3β-*O*-gentiobioside, respectively. Oleanolic acid-3-sophoroside was also isolated for the first time from a natural source.

INTRODUCTION

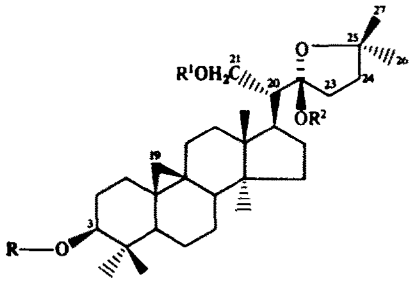
We have recently reported [1] the isolation and structure of a new triterpene glycoside, quadranguloside, from *Passiflora quadrangularis* L. From the leaves of this plant two new related triterpenes, **1** and **2**, were isolated together with oleanolic acid-3-sophoroside (**3**), a known compound but not previously isolated from a natural source [2]. A preliminary report of the structure of **1** has been published [2]. In this paper, evidence leading to the assignment of the structures **1** and **2** is reported together with a full characterization of compound **3**.

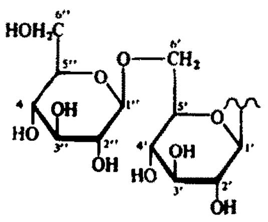
RESULTS AND DISCUSSION

Compound **1**, $C_{42}H_{70}O_{14}$, had a mp of 167–168°; enzymatic hydrolysis with fresh *Helix pomatia* digestive juice [3] afforded the aglycone (**4**), $C_{30}H_{50}O_4$. The ^1H NMR spectrum of **4** in $CDCl_3$ showed six singlet methyl signals, a pair of one-proton doublets characteristic of geminal cyclopropane protons, a hydroxymethine signal and a hydroxymethylene group as an ABX system. The signals corresponding to three hydroxyl groups disappeared on treatment with D_2O.

Acetylation of compound **4** with acetic anhydride in pyridine at room temperature gave a diacetate (**5**) which showed a free tertiary hydroxyl group. Further acetylation gave a tricetate (**6**).

By comparison of the respective ^{13}C NMR spectra, the aglycone **4** differs from the aglycone of quadranguloside only in the side chain. Two singlets at δ98.2 and 74.9 were assigned to C-22 and C-25, respectively; C-21 gave a triplet at δ65.2.



1 R =


R¹ = H; R² = H

4 R = H; R¹ = H; R² = H

5 R = Ac; R¹ = Ac; R² = H

6 R = Ac; R¹ = Ac; R² = Ac

Mass spectral fragmentation of compound **4** showed that three oxygen atoms are located in the side chain of a cyclopropane triterpene [1]. The most indicative ions are due to loss of water and (i) cleavage of the side chain (*m/z* 297), (ii) cleavage of ring D (*m/z* 255), and (iii) the side chain (*m/z* 159), as shown in Fig. 1.

The 1,3-relationship between the hydroxyl groups at C-21 and C-22 was confirmed by treatment of the aglycone **4** with phenyl boronic acid to give a cyclic boronate (**7a** or **7b**). The deshielding effect of the phenyl ring exercised on one of the two methyl groups at C-25 supports the diastereoisomer **7a** rather than **7b**. From these data the aglycone **4** was deduced to be 9,19-cyclolanosta-22,25-epoxy-3β,21,22(*R*)-triol.

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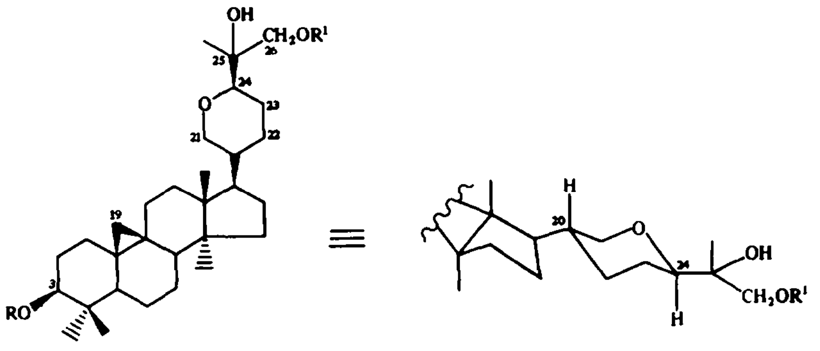

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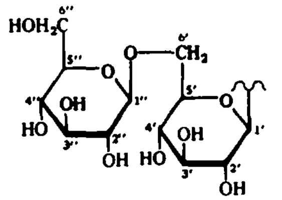
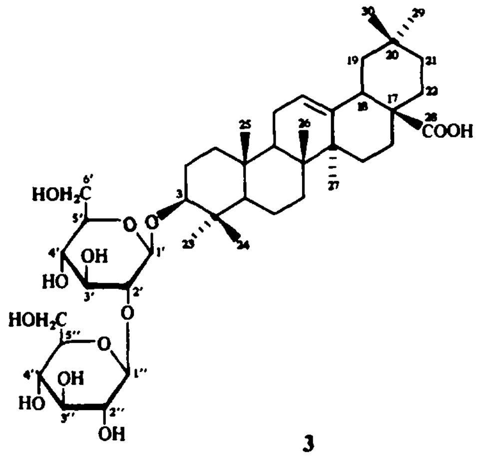


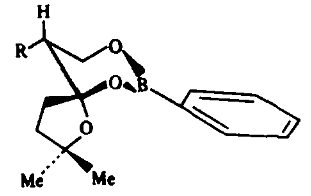
R¹ = H

8 R = H; R¹ = H

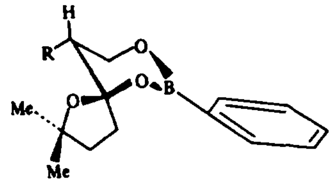
9 R = Ac; R¹ = Ac



3



7a



7b

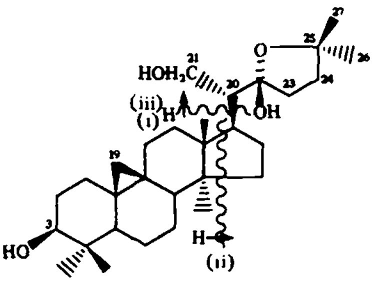


Fig. 1.

Acid hydrolysis of compound **1** yielded glucose. The ^{13}C NMR spectrum of compound **1** in pyridine-d_5 showed two anomeric carbon atoms; the remaining glycosyl carbon signals were in agreement with those reported for gentiobiose bonded by a glycosidic linkage [4].

The location of the glycosyl linkage to the aglycone (**4**) was deduced from a comparison of the ^{13}C NMR spectra of glycoside **1** and compound **4**. Glycosidation shifts were observed at C-3 (+10.6 ppm) and C-2 (−1.5 ppm). However, no glycosidation shift was observed at C-21 and C-22. The accumulated evidence described above led us to assign the structure 9,19-cyclolanosta-22,25-epoxy-3β,21,22(*R*)-triol-3β-*O*-gentiobioside to compound **1**.

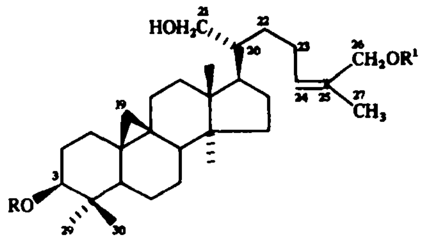
Compound **2**, $C_{42}H_{70}O_{14}$, had a mp of 189–191°; enzymatic hydrolysis with fresh *Helix pomatia* digestive juice afforded the aglycone **8**, $C_{30}H_{50}O_4$. The IR spectrum of **8** did not show any absorption bands due to a carbonyl group. The ^{1}H NMR spectrum of **8** in $CDCl_3$ showed five singlet methyl signals, a pair of one-proton doublets of geminal cyclopropane protons, a hydroxymethine signal as a double doublet, an oxymethylene group as an ABX system, a hydroxymethylene group as an AB system and an oxymethine signal as a broad doublet.

Acetylation of **8** with acetic anhydride in pyridine gave a diacetate (**9**). The ^{1}H NMR spectrum in $CDCl_3$ showed a downfield shift for the signals of the hydroxymethine and hydroxymethylene AB system whereas no shift was observed for the oxymethylene group (<u>AB</u>X system) and the oxymethine signal (AB<u>X</u> system). The signal corresponding to the remaining tertiary hydroxyl group disappeared on treatment with D_2O. The proton at C-24 gave rise to a double doublet, the larger coupling constant being 12 Hz. This value agreed with an axial proton opposite to the hydrogen at C-20.

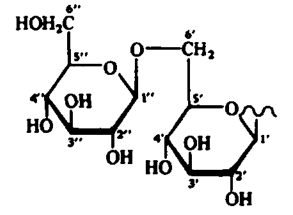
The small amount of compound **2** available was insufficient to produce a well-resolved ^{13}C NMR spectrum, hence the incomplete data in Table 1. The spectrum of compound **2** matches well, in part, the spectrum of quadranguloside. Signals that are clearly visible support the identity of the pentacyclic part in **2** and in quadranguloside and the presence in **2** of a gentiobiose moiety which must be linked to the hydroxyl group at C-3. Furthermore, the mass spectral fragmentation of diacetate **9** showed two indicative ions due to cleavage of the C-17/C-20 bond (*m/z* 357) and of the C-24/C-25 bond (*m/z* 441). On the basis of the above data, compound **2** is assigned the structure 9,19-cyclolanosta-21,24-epoxy-3β,25,26-triol-3β-*O*-gentiobioside.

In our previous publication [2], we reported the presence in the crude extract of the leaves of *Passiflora quadrangularis* L. of a 'fifth component', also detectable in TLC as a plum-coloured spot of R_f 0.18. Separation of this component was achieved by a sequence involving GFCC, CTLC and DCCC. This fifth component consisted mainly of a non-resolvable mixture of the two glycosides **10** and **11**.

The proposed structures were consistent with the following facts. The mass spectrum of the mixture showed that **10** and **11** have the same molecular formula, $C_{48}H_{80}O_{18}$. The NMR spectral data of the mixture were nearly identical with those of quadranguloside, and enzymatic hydrolysis with *Helix pomatia* gave an aglycone identical with the aglycone obtained from quadranguloside.

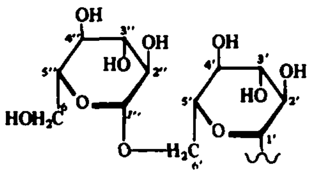


10 R =



R¹ = β-Glu

11 R = β-Glu; R¹ =



EXPERIMENTAL

Plant material was collected and identified by the Directoria de Parques e Pracas, Museu Botanico Municipal of the Prefeitura Municipal de Curitiba (Parana, Brazil). Mps are uncorr. and were measured on a Büchi 510 melting point apparatus. Pre-coated Kieselgel 60 F_{254} RP 18 and RP 8 (Merck) were used for TLC. Spots were detected by spraying with H_2SO_4–MeOH (1:9), followed by heating. Gel filtration column chromatography (GFCC), reversed-phase high-performance liquid chromatography (RP-HPLC), droplet counter-current chromatography (DCCC) and centrifugal thin-layer chromatography (CTLC) conditions have been described in our previous paper [2].

Kieselgel 60 (70–230 mesh, Merck) was employed for CC. Optical rotations were measured at 25° using a 1 dm cell on a Perkin-Elmer 241 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker CXP 300, Varian XL 200 and Varian XL 100 spectrometers; samples were dissolved in $CDCl_3$ or C_5D_5N, and TMS was used as internal standard. EIMS spectra were recorded at 70 eV on a Varian Mat 112 mass spectrometer.

FAB MS spectra were obtained on a VG 7070 EQ mass spectrometer equipped with a VG FAB source. Samples were dissolved in a thioglycerol matrix and placed on a steel target prior to bombardment with Ar atoms of energy 7–8 kV.

Extraction and isolation has been described in our previous papers [1, 2].

Compound **1** was crystallized from EtOAc–EtOH (7:3), mp 167–168°, $[\alpha]_D^{25}$ +6° (*c* 0.57; MeOH); FAB MS *m/z* 838 $[M+Ca]^+$, 822 $[M+Mg]^+$, 781 $[M^+ - 17]$, 763 $[781 - 18]$. ^{1}H NMR (300 MHz, C_5D_5N): δ1.64 (3H, *s*, -Me), 1.61 (3H, *s* -Me), 1.32 (3H, *s*, -Me), 1.08 (3H, *s*, -Me), 0.97 (3H, *s*, -Me), 0.79 (3H, *s*, -Me), 0.41 (1H, *d*, *J* = 4.5 Hz, H-19B), 0.19 (1H, *d*, *J* = 4.5 Hz, H-19A). (Found: C, 61.62; H, 8.57. $C_{42}H_{70}O_{14} \cdot H_2O$ requires: C, 61.46; H, 8.78%.)

Enzymatic hydrolysis of compound **1**. The hepatopancreatic juice of three snails (*Helix pomatia*), diluted with H_2O (10 ml) and filtered, was added to **1** (75 mg). Stirring was continued at 28° for 8 days. After extraction (3 × 10 ml $CHCl_3$ and 3 × 10 ml EtOAc) and solvent evaporation, the crude material was purified by CC on silica gel eluting with $CHCl_3$–*i*-PrOH (97:3) (50 ml), affording 19 mg of the aglycone (**4**). Compound **4** was crystallized from diisopropyl ether–EtOAc (7:3), mp 184–185.5° (with decomposition), $[\alpha]_D^{25}$ +45° (*c* 0.14; MeOH); high resolution MS

Table. 1. ^{13}C NMR chemical shifts of compounds **1**–**4**

Carbon	**1** (100 MHz, C_5D_5N)	**4** (300 MHz, C_5D_5N)	**2** (300 MHz, C_5D_5N)	**3** (100 MHz, C_5D_5N)
1	32.5 *t*	32.6 *t*	32.2 *t*	38.7 *t*
2	29.6 *t*	31.3 *t*	29.8 *t*	26.5 *t*
3	88.5 *d*	77.9 *d*	88.7 *d*	88.9 *d*
4	41.2 *s*	41.1 *s*	41.4 *s*	39.7 *s**
5	47.4 *d*	47.5 *d*	47.6 *d*	55.8 *d*
6	21.2 *t*	21.4 *t*	21.2 *t*	18.4 *t*
7	27.0 *t*	27.1 *t*	27.9 *t*	34.2 *t*
8	47.9 *d*	47.9 *d*	48.1 *d*	39.4 *s**
9	19.9 *s*	20.0 *s*	20.0 *s*	47.9 *d*
10	26.3 *s*	26.5 *s*	26.4 *s*	36.9 *s*
11	26.1 *t*	26.1 *t*	26.3 *t*	23.8 *t*
12	35.7 *t*	35.7 *t*	35.8 *t*	120.8 *d*
13	45.4 *s*	45.5 *s*	45.6 *s*	144.8 *s*
14	48.8 *s*	48.9 *s*	49.3 *s*	42.1 *s*
15	32.4 *t*	32.5 *t*	32.5 *t*	27.9 *t*
16	26.3 *t*	26.3 *t*	26.6 *t*	23.8 *t*
17	45.1 *d*	45.5 *d*	42.9 *d*	46.6 *s*
18	18.7 *q*	18.6 *q*	18.7 *q*	42.1 *d*
19	29.8 *t*	29.9 *t*	30.0 *t*	46.6 *t*
20	49.6 *d*	49.6 *d*	†	30.9 *s*
21	65.2 *t*	65.2 *t*	68.0 *t*	33.2 *t*
22	98.1 *s*	98.2 *s*	†	29.7 *t*
23	25.0 *t*	25.0 *t*	†	28.2 *q*
24	24.8 *t*	24.8 *t*	†	15.4 *q*
25	74.9 *s*	74.9 *s*	†	16.8 *q*
26	19.9 *q*	†	62.2 *t*	17.4 *q*
27	†	†	21.2 *q*	26.1 *q*
28	19.5 *q*	19.6 *q*	19.8 *q*	‡
29	25.7 *q*	26.1 *q*	27.8 *q*	33.2 *q*
30	15.4 *q*	14.7 *q*	15.5 *q*	23.8 *q*
1′	105.1 *d*		105.2 *d*	104.1 *d*
2′	75.1 *d**		75.2 *d**	83.2 d
3′	78.2 *d*		78.3 *d**	77.7 *d*
4′	71.7 *d*		71.9 *d*	71.6 *d**
5′	77.0 *d*		77.1 *d*	78.2 *d*
6′	70.2 *t*		70.3 *t*	62.7 *t*
1″	106.5 *d*		106.8 *d*	105.8 *d*
2″	75.5 *d**		75.6 *d**	76.8 *d*
3″	78.2 *d*		78.4 *d**	77.9 *d**
4″	71.7 *d*		71.9 *d*	71.5 *d**
5″	78.4 *d*		78.5 *d*	77.7 *d**
6″	62.7 *t*		62.9 *t*	62.7 *t*

*These values are interchangeable within their respective columns.

†These values cannot be unequivocally assigned.

‡This value was best determined as δ178.3 in the aglycone.

on $[M - H_2O]^+$, $C_{30}H_{48}O_3$ (theor. 456.36030) gave 456.36037. EIMS *m/z* (rel. int.): 456 $[M]^+$ (5), 297 (9), 255 (9), 159 (76); ^{1}H NMR (200 MHz, $CDCl_3$): δ3.78 (1H, *dd*, *J* = 11.0 and 3.0 Hz, H-21A), 3.52 (1H, *dd*, *J* = 11.0 and 5.5 Hz, H-21B), 3.26 (1H, *dd*, *J* = 10.5 and 4.5 Hz, H-3α), 1.22 (3H, *s*, -Me), 1.14 (3H, *s*, -Me), 0.96 (3H, *s*, -Me), 0.92 (3H, *s*, -Me), 0.83 (3H, *s*, -Me), 0.76 (3H, *s*, -Me), 0.55 (1H, *d*, *J* = 4.5 Hz, H-19B), 0.32 (1H, *d*, *J* = 4.5 Hz, H-19A); -OH resonances are at δ3.07, 2.20, 1.60 and disappear after D_2O treatment. (Found: C, 70.34; H, 10.44; $C_{30}H_{50}O_4 \cdot 2H_2O$ requires: C, 70.59; H, 10.59%.)

Acetylation of compound 4. Compound **4** (7 mg) was treated with 0.5 ml pyridine and 0.5 ml Ac_2O, at room temp. overnight. After work-up, two products were present. They were separated by CC on silica gel eluting with 50 ml petrol–EtOAc (4:1) to yield 4 mg of a diacetate (**5**) and 2.5 mg of a triacetate (**6**). Further acetylation of **5** (0.5 ml pyridine and 0.5 ml Ac_2O) and refluxing for 3 hr gave the triacetate (**6**). It was purified by prep. TLC eluting with petrol–EtOAc (3:1, double development).

Compound **5** showed ^{1}H NMR (200 MHz, $CDCl_3$): δ4.56 (1H, *dd*, *J* = 10.5 and 4.5 Hz, H-3α), 4.21 (1H, *dd*, *J* = 11.0 and 3.0 Hz, H-21A), 3.90 (1H, *dd*, *J* = 11.0 and 5.5 Hz, H-21B), 2.0 (6H, *s*, 2 × -OCOMe), 1.36 (6H, *s*, 2 × -Me), 0.94 (3H, *s*, -Me), 0.86 (3H, *s*, -Me), 0.84 (3H, *s*, -Me), 0.80 (3H, *s*, -Me), 0.52 (1H, *d*, *J* = 4.5 Hz, H-19B), 0.28 (1H, *d*, *J* = 4.5 Hz, H-19A); the -OH resonance is at δ3.70 and disappears after D_2O treatment.

Compound **6** showed ^{1}H NMR (200 MHz, $CDCl_3$): δ4.58 (1H, *dd*, J = 10.5 and 4.5 Hz, H-3α), 4.20 (1H, *dd*, J = 11.0 and 3.0 Hz, H-21A), 3.81 (1H, *dd*, J = 11.0 and 5.5 Hz, H-21B), 2.02 (3H, *s*, -OCOMe), 2.00 (6H, *s*, 2 × -OCOMe), 1.40 (6H, *s*, 2 × -Me), 0.92 (3H, *s*, -Me), 0.84 (3H, *s*, -Me), 0.81 (3H, *s*, -Me), 0.78 (3H, *s*, -Me), 0.52 (1H, *d*, J = 4.5 Hz, H-19B), 0.26 (1H, *d*, J = 4.5 Hz, H-19A).

Compound **2** was crystallized from EtOAc–EtOH (19:1), mp 189–191°, $[\alpha]_D^{25} + 5°$ (*c* 0.07; MeOH); FAB MS *m/z*: 838 $[M + Ca]^+$. (Found: C, 59.16; H, 9.29; $C_{42}H_{70}O_{14} \cdot 3H_2O$ requires: C, 59.15; H, 8.92%.)

Cyclic phenylboronate (**7**). To 5 mg **4**, dissolved in dry C_6H_6 (0.5 ml) phenylboronic acid was added, refluxing for 3 hr. After evaporation, excess phenylboronic acid was removed by crystallization from hexane. ^1H NMR (200 MHz, $CDCl_3$): δ8.1 (1H, *dd*, J = 10, 2 Hz), 7.84 (1H, *dd*, J = 10, 2 Hz), 7.8–7.45 (3H, aromatic protons), 3.86 (1H, *dd*, J = 11, 4.5 Hz), 3.62 (1H, *d*, J = 11, 4.5 Hz), 3.27 (1H, *dd*, J = 10, 4.5 Hz, H-3α), 1.36 (3H, *s*), 1.23 (3H, *s*), 1.02 (3H, *s*), 0.95 (3H, *s*), 0.9 (3H, *s*), 0.76 (3H, *s*), 0.55 (1H, *d*, J = 5 Hz, H-19B), 0.33 (1H, *d*, J = 5 Hz, H-19A). MS *m/z*: 561 $[M + H]^+$; 543 $[M - H_2O + H]^+$; 457 $[M - C_6H_5BO + H]^+$.

Enzymatic hydrolysis of compound **2**. Compound **2** (42 mg) was hydrolysed in the same manner as compound **1** at 30° for 16 hr. After extraction (3 × 5 ml $CHCl_3$ and 3 × 5 ml EtOAc), the crude material was purified on silica gel (2.5 g) eluting with $CHCl_3$-*i*-PrOH (97:3) to yield 5 mg of the aglycone (**8**). Compound **8** was crystallized from diisopropyl ether, mp 138–140°; $[\alpha]_D^{25} + 17.8°$ (*c* 0.08; MeOH); FAB *m/z* 474; ^1H NMR (200 MHz, $CDCl_3$): δ4.06 (1H, *d*, J = 12 Hz, H-24), 3.62 (1H, *d*, J = 11.6 Hz, H-26A), 3.5 (2H, *m*, H-21), 3.41 (1H, *d*, J = 11.6 Hz, H-26B), 3.26 (1H, *dd*, J = 10.5 and 4.5 Hz, H-3α), 1.2 (3H, s, Me), 1.05 (3H, s, Me), 0.96 (3H, *s*, Me), 0.93 (3H, *s*, Me), 0.80 (3H, *s*, Me), 0.56 (1H, *d*, J = 4.5 Hz, H-19A), 0.28 (1H, *d*, J = 4.5 Hz, H-19B).

Acetylation of compound **8**. Compound **8** (3 mg) was acetylated as described previously at room temp. to yield 3 mg **9** which was purified by CC on silica gel eluting with $CHCl_3$-*i*-PrOH (99:1). EIMS *m/z* (rel. int.): 558 $[M]^+$ (3), 441 (14), 357 (19); ^1H NMR (200 MHz, $CDCl_3$): δ4.56 (1H, *dd*, J = 10.5, 4.5 Hz, H-3α), 4.06 (1H, *d*, J = 12 Hz, H-24), 4.04 (1H, *d*, J = 11.6 Hz, H-26A), 4.00 (1H, *d*, J = 11.6 Hz, H-26B), 3.45 (1H, *dd*, J = 11, 2.5 Hz, H-21A), 3.26 (1H, *dd*, J = 11, 5.5 Hz, H-21B), 2.60 (1H, *s*, disappears after treatment with D_2O), 2.04 (3H, *s*, COMe), 2.00 (3H, *s*, COMe), 1.20 (3H, *s*, Me), 1.08 (3H, *s*, Me), 0.88 (3H, *s*, Me), 0.82 (3H, *s*, Me), 0.78 (3H, *s*, Me), 0.50 (1H, *d*, J = 4.5 Hz, H-19a), 0.30 (1H, *d*, J = 4.5 Hz, H-19B).

Oleanolic acid-3-sophoroside (**3**). Crystallized from EtOAc–EtOH (9:1). Decomposed at 213°; $[\alpha]_D^{25} + 22°$ (*c* 0.57; MeOH). FAB MS *m/z*: 803 $[M + Na]^+$. EIMS *m/z* (rel. int.): 411 (0.5), 248 (0.4), 206 (0.3), 205 (0.5), 84 (100). Treatment of compound **3** with CH_2N_2 gave a methyl ester which was confirmed by a singlet (3H) at δ3.63 (^1H NMR 200 MHz, C_5D_5N) and by FAB MS *m/z*: 817 $[M + Na]^+$.

Enzymatic hydrolysis of compound **3**. The hepatopancreatic juice of three snails (*Helix pomatia*), diluted with H_2O (10 ml) and filtered, was added to **3** (120 mg). Stirring was continued at 28° for 31 days. The mixture was extracted with $CHCl_3$ (3 × 10 ml) and EtOAC (3 × 10 ml) (the emulsion produced was filtered on Celite under vacuum). The extract was taken to dryness and purified by CC on silica gel eluting with $CHCl_3$-*i*-PrOH (97:3) to afford 25 mg of the aglycone of **3**, which was recognized to be oleanolic acid by comparison (TLC, R_f and ^1H NMR, 200 MHz) with a pure sample and comparing its ^{13}C NMR spectrum (300 MHz, DMSO-d_6) with literature data [5].

Isolation of compounds **10** and **11**. The mixture (83 mg) with R_f 0.18 (three substances) was separated by CTLC (EtOAC–EtOH–H_2O, 8:2:1; flow rate 1 ml/min) to yield a mixture of **10** and **11** (51 mg) which was further purified by DCCC ($CHCl_3$–MeOH–H_2O, 13:7:2; ascending mode) to give 35 mg **10** and **11**, showing a single spot on TLC.

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REFERENCES

1. Orsini, F., Pelizzoni, F. and Verotta, L. (1986) *Phytochemistry* **25**, 191.
2. Orsini, F. and Verotta, L. (1985) *J. Chromatogr.* **349**, 69.
3. Canonica, L., Orsini, F. and Pelizzoni, F. (1976) *Gazz. Chim. Ital.* **106**, 889.
4. Usui, T., Yamaoka, N., Matsuda, K., Tuzimura, K., Sugiyama, H. and Seto, S. (1973) *J. Chem. Soc. Perkin Trans.* **1**, 2425.
5. Tori, K., Seto, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* **48**, 4227.