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QUADRANGULOSIDE, A CYCLOARTANE TRITERPENE GLYCOSIDE FROM *PASSIFLORA QUADRANGULARIS*

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Key Word Index – *Passiflora quadrangularis*; Passifloraceae; cyclopropane triterpene glycoside; quadrangloside.

Abstract—Quadrangloside, a new cyclopropane triterpene glycoside was isolated from the methanolic extract of the leaves of *Passiflora quadrangularis*. Its structure has been established as 9,19-cyclolanost-24*Z*-en-3β,21,26-triol-3,26-di-*O*-gentiobioside on the basis of hydrolysis and spectral evidence.

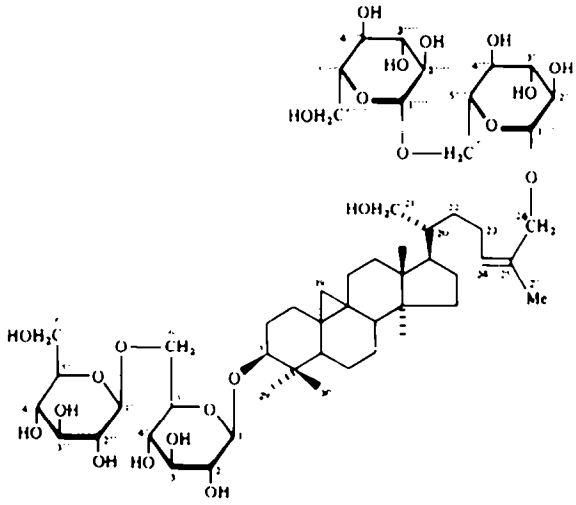
INTRODUCTION

The structure of a cyclopropane triterpene glycoside, passiflorine, obtained from the leaves of a number of *Passiflora* species, was reported in a previous paper [1]. Further investigation on *Passiflora quadrangularis* L. has led us to the isolation of a new cyclopropane triterpene glycoside which we named quadrangloside (**1**).

RESULTS AND DISCUSSION

A new glycoside, $C_{54}H_{90}O_{23}$, quadrangloside (**1**), was obtained as an amorphous powder by methanol extraction of *Passiflora quadrangularis*, subsequent fractionation by Sephadex LH 20 CC and final purification by DCCC.

The IR spectrum of quadrangloside exhibited hydroxyl absorption bands. Acid hydrolysis of quadrangloside yielded glucose and a complex mixture of unidentified compounds, whereas enzymatic hydrolysis with fresh *Helix Pomatia* digestive juice [2] afforded the aglycone **2**, $C_{30}H_{50}O_3$. Acetylation of **2** with acetic anhydride in pyridine gave a triacetate (**3**). The IR spectrum of **3** did not show any absorption bands of the hydroxyl groups.



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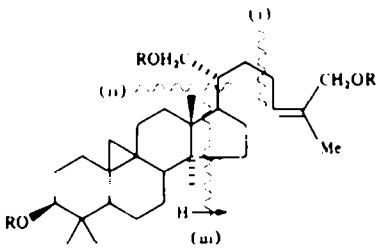
The ^{1}H NMR spectrum of **2** in $CDCl_3$ showed four singlet methyl signals, an olefinic methyl group, a pair of one-proton doublets characteristic of geminal cyclopropane protons, a triplet signal of an olefinic proton, an hydroxymethine signal, an hydroxymethylene group as an ABX system and an hydroxymethylene group as an AB system. The ^{1}H NMR spectrum of **2** in DMSO-d_6 confirmed the presence of a secondary hydroxyl group and two primary hydroxyl groups; the corresponding signals disappeared on treatment with D_2O. The mass spectral fragmentation of **1** showed that the two primary hydroxyl groups and the double bond are located in the side chain of a cyclopropane triterpene [3]. The three most indicative ions are due to (i) allylic cleavage, (ii) cleavage of the side chain and (iii) cleavage of the ring D, as shown in Fig. 1.



1 R = gentiobiose
2 R = H
3 R = Ac

Fig. 1. Mass spectral fragmentation pattern of quadrangloside (**1**) and the derived aglycone (**2**) or aglycone acetate (**3**).

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The configuration of C-20 was assigned by comparison of the ^{1}H NMR spectrum of **3** in $CDCl_3$ with that of 24-methylenecycloartan-3β,21-diol diacetate (**4**) [3, 4]. The chemical shifts and the coupling constants are reported in Fig. 2.

The *Z*-stereochemistry of the Δ^{24}-double bond was defined by an NOE experiment carried out on the spectrum of compound **2** in $CDCl_3$. Irradiation of the olefinic proton at δ5.31 led to a $\sim$2% increase in the intensity of the peak due to the vinylic methyl group at δ1.77, while irradiation of the highest methyl group (δ1.77) led to a $\sim$7% increase in the intensity of the peak due to the hydrogen at C-24 (δ5.31). From these data, and biogenetic considerations, the aglycone **2** was assumed to be 9,19-cyclolanost-24-ene-3,21,26-triol (3β,24*Z*). The proposed structure was also supported by the ^{13}C NMR spectrum reported in Table 1 [5].

After elucidation of its aglycone, the structure of quadranguloside (**1**) was resolved by ^{13}C NMR spectroscopy. In fact its ^{13}C NMR spectrum in pyridine-d_5 showed four anomeric carbon atoms; the remaining glycosyl carbon signals are in agreement with those reported for gentiobiose bonded by a glycosidic linkage [4]. The location of the glycosyl linkage to the aglycone (**2**) was deduced from a comparison of the ^{13}C NMR spectra of quadranguloside (**1**) and of compound **2**. Glycosidation shifts were observed at C-3 (+10.7 ppm) and C-2 (−1.4 ppm); at C-26 (+6.7 ppm) and C-24 (+3.9 ppm) and C-25 (−1.4 ppm). However, no glycosidation shift was observed at C-21 and C-20.

The accumulated evidence described above led us to assign the structure **1** to the quadranguloside. Further work is in progress to determine the structure of minor constituents of the extract of *Passiflora quadrangularis*.

EXPERIMENTAL

Plant material was collected and identified by the Directoria de Parques e Pracas, Museu Botânico Municipal of the Prefeitura Municipal de Curitiba (Parana, Brasil).

Mps are uncorr. and were measured on a Buchi 510 Melting Point apparatus. Pre-coated Kieselgel 60 F_{254} plates (Merck) were used for TLC, eluting with the organic phase of the mixture $CHCl_3$–MeOH–*n*-PrOH–H_2O (5:6:1:4). Spots were detected by spraying with H_2SO_4–MeOH (1:9) followed by heating. Kieselgel 60 (70–230 mesh, Merck) was employed for column chromatography. Optical rotations were measured at 25° using 1 dm cell on a Perkin–Elmer 241 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WP80, on a Bruker CXP300, on a Varian XL200, on a Varian XL100 spectrometers;

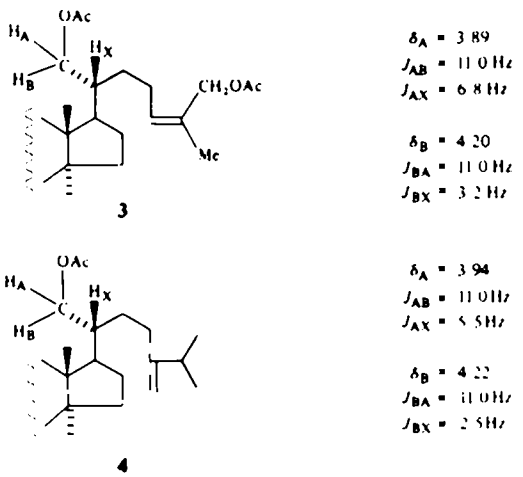

Fig. 2. ^{1}H NMR spectral data for compounds **3** and **4**.

Table 1. ^{13}C NMR chemical shifts of compounds **1** and **2**

	1		**2**	
Carbon	DMSO-d_6	pyridine-d_5	DMSO-d_6	pyridine-d_5
1	31.3 *t*	32.2 *t*	32.2 *t*	32.3 *t*
2	29.2 *t*	29.8 *t*	30.9 *t*	31.2 *t*
3	87.5 *d*	88.6 *d*	77.3 *d*	77.9 *d*
4	40.3 *s*	41.1 *s*	41.3 *s*	41.0 *s*
5	46.8 *d*	47.4 *d*	47.4 *d*	47.4 *d*
6	20.4 *t*	21.3 *t*	21.4 *t*	21.4 *t*
7	29.0 *t*	27.7 *t*	27.6 *t*	27.7 *t*
8	47.3 *d*	47.9 *d*	48.1 *d*	48.1 *d*
9	19.4 *s*	19.9 *s*	20.0 *s*	20.0 *s*
10	25.7 *s*	26.2 *s*	26.4 *s*	26.3 *s*
11	25.7 *t*•	26.2 *t*•	26.6 *t*•	26.6 *t*•
12	35.1 *t*	35.7 *t*	35.8 *t*	35.8 *t*
13	44.7 *s*	45.4 *s*	45.4 *s*	45.5 *s*
14	48.3 *s*	48.9 *s*	49.0 *s*	49.0 *s*
15	31.5 *t*	32.2 *t*	32.2 *t*	32.3 *t*
16	25.9 *t*•	26.5 *t*•	26.6 *t*•	26.7 *t*•
17	41.9 *d*	42.8 *d*	42.7 *d*	43.1 *d*
18	18.8 *q*	18.5 *q*	18.8 *q*	18.6 *q*
19	29.0 *t*	29.7 *t*	29.7 *t*	29.9 *t*
20	45.6 *d*	46.6 *d*	46.3 *d*	46.7 *d*
21	60.7 *t*	61.7 *t*	61.2 *t*	61.9 *t*
22	29.8 *t*	30.7 *t*	29.9 *t*	30.9 *t*
23	24.1 *t*	25.0 *t*	24.4 *t*	24.9 *t*
24	129.9 *d*	131.5 *d*	127.3 *d*	127.6 *d*
25	131.0 *s*	134.4 *s*	135.5 *s*	135.8 *s*
26	66.4 *t*	67.5 *t*	60.1 *t*	60.8 *t*
27	21.4 *q*	22.0 *q*	21.9 *q*	21.8 *q*
28	19.3 *q*	19.7 *q*	19.9 *q*	19.7 *q*
29	25.1 *q*	25.6 *q*	26.3 *q*	26.1 *q*
30	14.9 *q*	15.3 *q*	14.9 *q*	14.8 *q*
β-Gentiobiose (values in pyridine-d_5)				
1′-1‴		103.0 *d*	106.3 *d*	
2′-2‴		74.6 *d*	74.6 *d*•	
3′-3‴		78.1 *d*	78.1 *d*	
4′-4‴		71.4 *d*	71.4 *d*	
5′-5‴		76.8 *d*	76.8 *d*	
6′-6‴		69.9 *t*	69.7 *t*	
1″-1⁗		104.9 *d*	104.9 *d*	
2″-2⁗		74.8 *d*	74.8 *d*•	
3″-3⁗		78.1 *d*	78.1 *d*	
4″-4⁗		71.4 *d*	71.4 *d*	
5″-5⁗		78.1 *d*	78.1 *d*	
6″-6⁗		62.6 *t*	62.6 *t*	

^{13}C NMR were recorded in DMSO-d_6 at 300 MHz and in pyridine-d_5 at 100 MHz. The chemical shifts are expressed in δ values relative to TMS used as internal standard.

•These values are interchangeable within their respective columns.

samples were dissolved in $CDCl_3$, DMSO-d_6 or C_5D_5N and TMS was used as internal standard. EIMS were recorded at 70 eV on a Varian Mat 112 spectrometer. Fast Atom Bombardment (FAB) mass spectra were obtained on a VG 7070 EQ mass spectrometer, equipped with a VG FAB source. The sample was 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. Dried, powdered leaves (100 g) were exhaustively extracted by percolation with MeOH at room temp. The concd extract was taken up with H_2O (150 ml) and Me_2CO (100 ml) and washed with *n*-hexane (3 × 100 ml), $CHCl_3$ (3 × 100 ml) and EtOAc (3 × 100 ml). The aq. soln was concd *in vacuo* to yield the crude residue (5.0 g).

Crude residue (2.0 g) was dissolved in MeOH (5 ml) and applied to a column (3 × 70 cm) of Sephadex LH 20 (130 g). The column was eluted with MeOH (600 ml); fractions (2 ml) 63–89, containing mainly compound **1**, were collected, taken to dryness (566 mg) and submitted to Droplet Counter Current Chromatography (D.C.C.-A Tokyo Rikakikai Ltd.) with $CHCl_3$–MeOH–*n*-PrOH–H_2O (5:6:1:4) in the ascending mode (flow rate 0.25 ml/min, 300 tubes 400 × 2 mm). Fractions (2 ml) 19–24 were collected and taken to dryness to afford quadranguloside (**1**).

Quadranguloside (**1**). An amorphous powder mp 164–165° (with decomposition); $[\alpha]_D^{25}$ − 11° (*c* 0.89, MeOH); IR ν_{max}^{nujol} cm^{-1}: 3300 (OH); MS *m/z* (rel. int.): 441 (8), 424 (16), 409 (12), 407 (8), 381 (4), 355 (4), 297 (8), 255 (4), 145 (32), 133 (34), 121 (36), 95 (100); FAB MS *m/z*: 1129 [M + Na]; ^{1}H NMR (200 MHz, DMSO-d_6): δ5.34 (1H, *br t*, *J* = 5.5 Hz, H-24), 1.76 (3H, *br s*, H-27), 1.02 (3H, *s*, Me), 0.99 (3H, *s*, Me), 0.92 (3H, *s*, Me), 0.84 (3H, *s*, Me), 0.54 (1H, *d*, *J* = 4.0 Hz, H-19A), 0.34 (1H, *d*, *J* = 4.0 Hz, H-19B). (Found: C, 55.91; H, 8.12%. $C_{54}H_{90}O_{23}\cdot 2H_2O$ requires: C, 56.74; H, 8.38%.

Enzymatic hydrolysis of quadranguloside (**1**). The hepatopancreatic juice of five snails (*Helix pomatia*), diluted with H_2O (10 ml) and filtered, was added to quadranguloside (**1**, 350 mg). The clear soln rapidly became cloudy and stirring was continued at 28° for 3 hr. The mixture was extracted with $CHCl_3$ (3 × 5 ml). The extract was evaporated and purified by CC on silica gel eluting with $CHCl_3$–MeOH (99:1) to afford 41 mg of compound **2** which was crystallized from *n*-hexane–EtOAc (1:1), mp 170°; $[\alpha]_D^{25}$ + 43° (*c* 1.02, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ5.31 (1H, *t*, *J* = 6.6 Hz, H-24), 4.11 (1H, *d*, *J* = 11.8 Hz, H-26A), 4.08 (1H, *d*, *J* = 11.8 Hz, H-26B), 3.72 (1H, *dd*, *J* = 3.3 and 11.0 Hz, H-21A), 3.56 (1H, *dd*, *J* = 5.1 and 11.0 Hz, H-21B), 3.25 (1H, *dd*, *J* = 4.4 and 10.6 Hz, H-3α), 1.77 (3H, *s*, H-27), 0.96 (3H, *s*, Me), 0.94 (3H, *s*, Me), 0.88 (3H, *s*, Me), 0.78 (3H, *s*, Me), 0.54 (1H, *d*, *J* = 4.4 Hz, H-19A), 0.31 (1H, *d*, *J* = 4.4 Hz, H-19B). ^{1}H NMR (300 MHz, DMSO-d_6): δ5.12 (1H, *br t*, *J* = 6.6 Hz, H-24), 4.51 (1H, *t*, *J* = 5.5 Hz, CH_2OH), 4.33 (1H, *d*, *J* = 4.6 Hz, CHOH), 4.20 (1H, *t*, *J* = 5.0 Hz, CH_2OH), 3.90 (2H, *d*, *J* = 5.5 Hz, H-26), ~ 3.3 (2H, *m*, overlapped, H-21), 3.05 (1H, *m*, H-3α), 1.67 (3H, *br s*, H-27), 0.91 (3H, *s*, Me), 0.85 (3H, *s*, Me), 0.84 (3H, *s*, Me), 0.69 (3H, *s*, Me), 0.45 (1H, *d*, *J* = 4.4 Hz, H-19A), 0.28 (1H, *d*, *J* = 4.4 Hz, H-19B). Spin decoupling experiments allowed assignments of signals at δ4.51, 4.33 and 4.20. In fact irradiation at δ4.51 turned the doublet at δ3.90 to a singlet; at δ4.33 turned the multiplet at δ3.05 to a double doublets; on irradiation at δ4.20 the signal at δ ~ 3.3 is simplified loosing the coupling constant of 5.0 Hz. Irradiation respectively at δ3.90, ~ 3.3 and 3.05 turned the signals at δ4.51, 4.33 and 4.20 to three singlets. ^{13}C NMR: see Table 1. (Found: C, 78.38; H, 10.78%. $C_{30}H_{50}O_3$ requires: C, 78.60; H, 10.92%).

Acid hydrolysis of compound **1**. A soln of quadranguloside (**1**, 11 mg) in 5 ml of 2% HCl·MeOH was refluxed for 2 hr. The soln was neutralized ($NaHCO_3$) and extracted with $CHCl_3$. The aq. phase was recognized to contain glucose by comparison with a pure sample.

Acetylation of compound **2**. Compound **1** (19 mg) was treated with Ac_2O–pyridine (0.5:0.5 ml) for 12 hr at room temp. The crude product was purified by CC on silica gel eluting with *n*-hexane–EtOAc (9:1) to give triacetate **3** (19 mg), mp 72° (MeOH); $[\alpha]_D^{25}$ + 29° (*c* 0.24, $CHCl_3$); MS *m/z* (rel. int.): 524 [M − 60]$^+$ (7), 509 (4), 481 (3), 449 (5), 421 (2), 404 (2), 156 (2), 154 (3), 147 (24), 145 (7), 135 (24), 133 (13), 121 (33), 119 (7), 107 (31), 43 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ5.36 (1H, *dt*, *J* = 7.0 and 2.0 Hz, H-24), 4.55 (3H, *m*, H-26 + H-3α), 4.20 (1H, *dd*, *J* = 3.2 and 11.0 Hz, H-21A), 3.89 (1H, *dd*, *J* = 6.8 and 11.0 Hz, H-21B), 2.03 (6H, *s*, 2 × CO_2Me), 2.04 (3H, *s*, CO_2Me), 1.72 (3H, *d*, *J* = 2.0 Hz, H-27), 0.99 (3H, *s*, Me), 0.88 (3H, *s*, Me), 0.86 (3H, *s*, Me), 0.82 (3H, *s*, Me), 0.55 (1H, *d*, *J* = 4.5 Hz, H-19A), 0.32 (1H, *d*, *J* = 4.5 Hz, H-19B). ^{1}H NMR (300 MHz, C_6D_6): 5.37 (1H, *br t*, *J* = 6.8 Hz, H-24), 4.76 (1H, *dd*, *J* = 4.3 and 11.5 Hz, H-3α), 4.67 (2H, *s*, H-26), 4.42 (1H, *dd*, *J* = 3.2 and 11.0 Hz, H-21A), 4.00 (1H, *dd*, *J* = 6.8 and 11.0 Hz, H-21B), 1.83 (3H, *s*, CO_2Me), 1.78 (3H, *s*, CO_2Me), 1.74 (3H, *s*, H-27), 1.72 (3H, *s*, CO_2Me), 0.99 (3H, *s*, Me), 0.97 (3H, *s*, Me), 0.91 (3H, *s*, Me), 0.89 (3H, *s*, Me), 0.41 (1H, *d*, *J* = 4.5 Hz, H-19A), 0.12 (1H, *d*, *J* = 4.5 Hz, H-19B).

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