Pergamon

0031-9422(95)00884-5

Phytochemistry, Vol. 42, No. 1, pp. 163–167, 1996

0031-9422/96 $15.00 + 0.00

ARYL AND TRITERPENIC GLYCOSIDES FROM *MARGYRICARPUS SETOSUS*

NUNZIATINA DE TOMMASI,* LUCA RASTRELLI, JATIVA CUMANDA,† GIOVANNA SPERANZA‡ and COSIMO PIZZA

Centro Interdipartimentale di Chimica, Biologia e Tecnologia Farmaceutica, Università di Salerno, Pla. V. Emanuele 9 84084, Penta di Fisciano, Italy; †Escuela Superior Politecnica de Chimborazo, Riobamba, Ecuador; ‡Dipartimento di Chimica Organica e Industriale, Universitá di Milano, Via Venezian 21, 20133 Milano, Italy

(*Received in revised form* 4 *October* 1995)

Key Word Index—*Margyricarpus setosus*; Rosaceae; piqui yoyo; aerial parts; aryl glycosides; triterpenoid saponins; tormentic acid glycosides.

Abstract—Investigation of aerial parts of *Margyricarpus setosus* afforded three new aryl glycosides, β-hydroxy-phenylethyl-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, benzyl-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, 4-methoxybenzyl-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, and three new triterpene glycosides, tormentic acid 3β-*O*-β-D-quinovopyranoside, tormentic acid 3β-*O*-β-D-fucopyranoside and tormentic acid 3β-*O*-α-L-rhamnopyranoside. Twelve known compounds were also found, among them β-hydroxy-3′,4′-dimethoxyphenylethyl glucoside and β-hydroxy-3′,4′-dimethoxyphenylethyl rutinoside, which were obtained previously by partial synthesis, were isolated for the first time from a natural source. The structures of the new compounds were elucidated on the basis of chemical and spectral data.

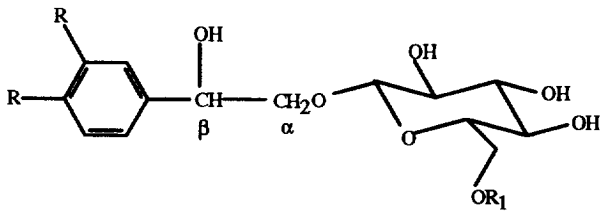
INTRODUCTION

Margyricarpus setosus R. et P. is a plant of the Andean area [1], commonly known as 'piqui yoyo'. The aerial parts are widely used in folk medicine for their anti-inflammatory and antiviral properties. No work has been done on the constituents of this plant. In continuation of our investigation of the active metabolites from the Rosaceae [2–5], we describe here the isolation and identification of three new aryl glycosides and three new tormentic acid glycosides from the aerial parts of *M. setosus*.

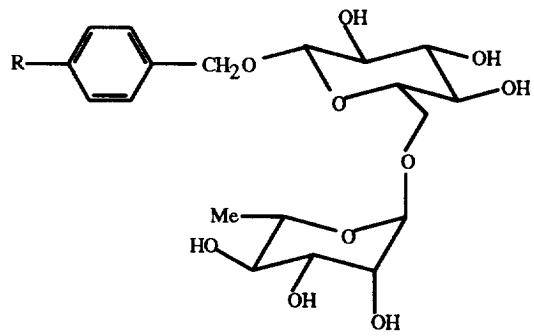
RESULTS AND DISCUSSION

The methanolic extract of the aerial parts of *M. setosus* afforded 18 compounds. The known compounds were identified as benzyl-β-D-glucoside [6], β-hydroxy-3′,4′-dimethoxyphenylethyl glucoside (**7**) [7], β-hydroxy-3′,4′-dimethoxyphenylethyl rutinoside (**8**) [8], epicatechin [9], catechin [9], quercetin 3-*O*-(6″-*p*-coumaroyl)-β-D-glucoside [10], tormentic acid [11], 6β-hydroxytormentic acid [11], tormentic acid ester glucoside [12], 23-hydroxytormentic acid ester glucoside [13], tormentic acid 3,28-*O*-bisglucoside [14] and 23-hydroxytormentic acid 3,28-*O*-bisglucoside [15] by spectral data and direct comparison of their physical properties with those reported previously for these compounds. Furthermore, compounds **7** and **8**, which had been obtained previously by partial synthesis [7, 8], were found for the first time in a plant. Six new compounds, **1**–**6**, were also isolated.

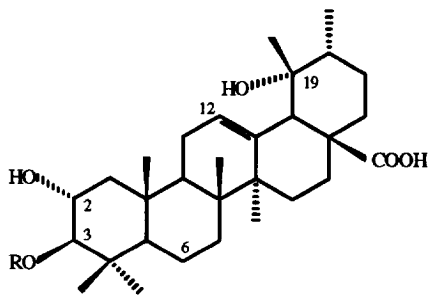
The molecular formulae of **1**–**3** ($C_{20}H_{30}O_{11}$ for **1** and **3**, $C_{19}H_{28}O_{10}$ for **2**) were determined from the ^{13}C and ^{13}C DEPT NMR data and the negative ion FAB-mass spectra. The ^{1}H NMR spectrum of **1** exhibited signals at δ 1.28 (3H, *d*, *J* = 6.5 Hz) due to the methyl protons of the sugar moiety, at δ 4.30 (1H, *d*, *J* = 7.5 Hz) and 4.80 (1H, *d*, *J* = 1.5 Hz) due to the anomeric protons of the sugar moiety, and at δ 7.11–7.43 due to the aromatic protons. A benzylic proton signal, the methine proton of =CHOH=, was readily located, giving a signal at δ 4.75 as a doublet of a doublet with *J* = 9 and 3 Hz. These values reflect a preferential conformation of the hydroxyl opposite (exo) to the glycosyl moiety. Irradiation of these protons allowed the recognition of the two protons of the α-CH_2 at δ 3.84 (*m*). In the ^{13}C NMR spectrum of **1**, 20 signals, including 12 signals due to a glucopyranosyl and a rhamnopyranosyl unit, were observed. Acidic hydrolysis of **1** gave an aglycone which was identified by ^{1}H and ^{13}C NMR as β-hydroxy-phenylacetaldehyde [8]. Acid methanolysis yielded methylglucoside and methylrhamnoside (analysed by GC) in the ratio of 1:1. The structure of the sugar moiety was determined as α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside by assignment of the ^{1}H and ^{13}C NMR signals with the aid of ^{1}H–^{1}H COSY and HETCOR experiments (Table 1).



1 R = H, R_1 = α-L-rhamnopyranosyl
7 R = OMe, R_1 = H
8 R = OMe, R_1 = α-L-rhamnopyranosyl



2 R = H
3 R = OMe



4 R = β-D-quinovopyranosyl
5 R = β-D-fucopyranosyl
6 R = α-L-rhamnopyranosyl

The ^{1}H NMR spectrum of **2** exhibited signals at δ 1.28 (3H, *d*, *J* = 6.5 Hz) due to the methyl protons of the sugar moiety, at δ 4.35 (1H, *d*, *J* = 7.5 Hz) due to the H-1 β-glucoside proton, at δ 4.82 (1H, *d*, *J* = 1.5 Hz) due to the H-1 α-rhamnosyl proton, at δ 4.65 and 4.90 (1H, *d*, *J* = 12.5 Hz) due to a benzylic methylene group and multiplet proton signals due to a phenyl group at δ 7.30, 7.43 and 7.95 (5H). On acid hydrolysis, compound **2** afforded benzyl alcohol as an aglycone. Acid methanolysis yielded methylglucoside and methylrhamnoside (GC) in the ratio 1 : 1. In the ^{13}C NMR spectrum of **2** the sp^2 carbon signals were similar to those of benzyl glucoside [6], while C-6 of glucose was shifted downfield at δ 68.50 as expected for a 6-O-substitution. Rhamnose was thus attached to the C-6 of glucose. The complete assignments of all proton and carbon resonances were based on ^{1}H–^{1}H COSY and HETCOR studies (Table 1). The structure of **2** was concluded to be benzyl-*O*-α-L-rhamnopyranosyl (1 → 6)-β-D-glucopyranoside.

The ^{1}H NMR spectrum of **3** exhibited signals at δ the H-1 β-glucoside proton, at δ 4.82 (1H, *d*, *J* = 1.5 Hz) due to the H-1 α-rhamnosyl proton and, at δ 4.60 and 4.85 (1H, *d*, *J* = 12.5 Hz) due to a benzylic methylene group. The signals of a two proton AB-quartet at δ 6.90 and 8.20 (*J* = 8 Hz) indicated the presence of a *p*-substituted benzene ring in the molecule (Table 1). Acid methanolysis of **3** yielded methylglucoside and methylrhamnoside (analysed by GC) in the ratio 1 : 1. In the ^{13}C NMR spectrum, the carbon signals due to the sugar moiety exhibited a good coincidence with those of **2** (Table 1). The structure of **3** was therefore concluded to be 4-methoxybenzyl-*O*-α-L-rhamnopyranosyl (1 → 6)-β-D-glucopyranoside.

The molecular formulae ($C_{36}H_{58}O_9$) for compounds **4**, **5** and **6** were determined by the ^{13}C and ^{13}C DEPT-NMR data and the negative ion FAB-mass spectra. The ^{13}C NMR spectra of compounds **4–6** (Table 2) confirmed the presence of a sugar moiety and a triterpene aglycone with an ursolic-type skeleton (C-12 and C-13 at δ 129.5 and 139.5, respectively) [5]. The 2α, 3β-OH substitution of this skeleton was

Table 1. ^{1}H NMR and ^{13}C NMR chemical shifts for compounds **1–3** (in CD_3OD)*

	1		**2**		**3**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		133.30		133.10		125.01
2	7.11 *dd* (7.5, 1.5)	129.30	7.95 *dd* (7.5, 1.5)	129.30	6.90 *d* (8)	127.70
3	7.27 *m*	128.11	7.30 *m*	128.01	8.20 *d* (8)	115.50
4	7.43 *t* (7.5)	133.10	7.43 *t* (7.5)	132.40	8.20 *d* (8)	159.00
5	7.43 *m*	128.11	7.30 *m*	128.01	6.90 *d* (8)	115.50
6	7.11 *dd* (7.5, 1.5)	129.30	7.95 *dd* (7.5, 1.5)	129.30		127.30
α	3.84 *m*	78.10	4.65 *d* (12.5)	74.19	4.60 *d* (12.5)	74.22
			4.90 *d* (12.5)		4.85 *d* (12.5)	
β	4.75 *dd* (9, 3)	72.10				
OCH_3					3.43 *s*	56.50
Glucose						
1	4.30 *d* (7.5)	103.33	4.35 *d* (7.5)	103.30	4.42 *d* (7.5)	103.00
2	3.26 *dd* (7.5, 9.5)	75.22	3.28 *dd* (7.5, 9)	75.30	3.32 *dd* (7.5, 9)	75.00
3	3.08 *dd* (9.5, 9.5)	78.25	3.12 *dd* (9.5, 9.5)	78.30	3.18 *dd* (9.5, 9.5)	78.50
4	3.38 *dd* (9.5, 9.5)	71.90	3.41 *dd* (9.5, 9.5)	71.90	3.43 *dd* (9.5, 9.5)	71.10
5	3.98 *m*	77.05	3.98 *m*	77.00	3.98 *m*	77.00
6	3.81 *dd* (12, 3)	68.31	3.85 *dd* (12, 3)	68.50	3.86 *dd* (12, 3)	69.00
	3.70 *dd* (12, 5)		3.72 *dd* (12, 5)		3.72 *dd* (12, 5)	
Rhamnose						
1	4.80 *d* (1.5)	102.35	4.82 *d* (1.5)	102.41	4.82 *d* (1.5)	101.70
2	3.90 *dd* (1.5, 3)	72.61	3.90 *dd* (1.5, 3)	72.30	3.92 *dd* (1.5, 3)	72.10
3	3.68 *dd* (3, 9.5)	72.28	3.68 *dd* (3, 9.5)	72.61	3.70 *dd* (3, 9.5)	72.80
4	3.60 *dd* (9.5, 9.5)	74.90	3.60 *dd* (9.5, 9.5)	74.90	3.60 *dd* (9.5, 9.5)	74.90
5	4.05 *m*	69.96	4.05 *m*	70.00	4.08 *m*	69.50
6	1.28 *d* (6.5)	18.00	1.28 *d* (6.5)	18.20	1.30 *d* (6.5)	17.80

*Chemical shift values are in ppm from TMS, and *J* values in Hz presented in parentheses. Carbon multiplicities were determined using DEPT experiments. All signals were assigned by ^{1}H–^{1}H COSY and HETCOR studies.

confirmed by spin decoupling and COSY experiments, which showed a proton sequence H_a-1 (δ 1.78), H_b-1 (δ 1.00), H-2 (δ 3.64) and H-3 (δ 2.96). These spectral evidences led to the formulation of the aglycone of **4–6** as tormentic acid. On acid methanolysis, followed by GC analysis, methylquinovoside was obtained from **4**, methylfucoside from **5** and methylrhamnoside from **6**. The ether glycosidation site was shown to be at C-3 by the ^{13}C NMR absorptions of C-3, C-2 and C-4, which were in agreement with a model of tormentic acid substituted at C-3 [5]. The assignments of all resonances were based on COSY and HETCOR spectra. From these data the structures of glycosides was concluded to be tormentic acid 3β-*O*-β-D-quinovopyranoside (**4**), tormentic acid 3β-*O*-β-D-fucopyranoside (**5**) and tormentic acid 3β-*O*-β-D-rhamnopyranoside (**6**).

EXPERIMENTAL

NMR: CD_3OD, 250 or 500 MHz; negative ion FAB-MS, DEPT, COSY and HETCOR experiments were performed as described earlier [16].

Plant material. The aerial parts of *M. setosus* was collected at Riobamba in the Chimborazo Region in *M. setosus* (450 kg) was defatted with petrol and $CHCl_3$ in a Soxhlet apparatus and then extracted at room temp. with $CHCl_3$–MeOH (9:1) and MeOH to give 24 g of residue. Part of the MeOH extract (12 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (7.0 g), which was subjected to CC on Sephadex LH-20 using MeOH as eluent and collecting frs of 8 ml. Frs 19–27 (525 mg), 31–41 (602 mg) and 50–62 (360 mg) were combined according to TLC (silica gel, *n*-BuOH–HOAc–H_2O, 12:3:5). Frs 31–41 contained the resolved glycosides **1–3**, **7** and **8**; frs 19–27 the glycosides **4–6**. Fractionation was achieved by HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 ml min^{-1}) using MeOH–H_2O (9:11) to yield **1** (15.0 mg), **2** (17.7 mg), **3** (20.8 mg), **7** (25.5 mg) and **8** (27.8 mg) from frs 31–41 and MeOH–H_2O (7:3) to yield **4** (14.2 mg), **5** (21.5 mg) and **6** (19.4 mg) from frs 19–27.

Acid hydrolysis. Glycosides **1** and **2** (3.0 mg) in 6% HCl (3.5 ml) was refluxed for 2 hr. The reaction mixt. was diluted with H_2O and then extracted with EtOAc. The resulting aglycones were identified by their ^{1}H and ^{13}C NMR spectra.

Acid methanolysis and GC analysis. A soln of each

Table 2. ^{13}C NMR chemical shift assignments* (δ in CD_3OD) of compounds **4–6**

C	DEPT	4	5	6
Aglycone				
1	CH_2	48.91	48.41	48.91
2	CH	70.17	69.91	70.16
3	CH	86.16	85.96	86.18
4	C	39.40	39.40	39.42
5	CH	54.86	54.74	54.90
6	CH_2	21.67	21.10	21.67
7	CH_2	34.55	34.24	34.58
8	C	41.00	41.00	41.00
9	CH	47.70	47.68	47.76
10	C	38.48	38.44	38.52
11	CH_2	26.90	26.88	26.94
12	CH	139.50	139.46	139.50
13	C	129.50	129.52	129.52
14	C	48.10	48.10	48.12
15	CH_2	29.50	29.46	29.70
16	CH_2	24.89	24.84	24.94
17	C	48.00	48.06	48.04
18	CH	54.90	54.86	54.92
19	C	73.00	72.88	73.00
20	CH	42.56	42.34	42.58
21	CH_2	26.50	26.45	26.50
22	CH_2	38.16	38.10	38.16
23	Me	19.15	19.13	19.14
24	Me	27.90	27.76	27.91
25	Me	18.00	17.80	18.02
26	Me	17.90	17.46	17.94
27	Me	25.00	25.00	25.01
28	Me	182.10	182.08	182.12
29	Me	25.75	25.74	25.75
30	Me	15.93	15.90	15.98
Sugar		Quinovose	Fucose	Rhamnose
1′	CH	106.60	105.10	102.31
2′	CH	76.00	72.80	72.43
3′	CH	78.10	76.01	72.30
4′	CH	72.00	73.20	74.08
5′	CH	77.10	71.00	69.72
6′	Me	18.02	17.02	18.01

*All signals were assigned by ^1H–^1H COSY and HETCOR studies.

β-Hydroxy-phenylethyl-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**1**). HPLC R_t 15 min; FAB-MS, *m/z*: 445 $[M-H]^-$, 299 $[(M-H)-146]^{-1}$, 137 $[(M-H)-(146+162)]^-$, $[\alpha]_D^{25} = -35°$ (MeOH; *c* 1).

Benzyl-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**2**). HPLC R_t 8 min; FAB-MS *m/z*: 415 $[M-H]^-$, 269 $[(M-H)-146]^-$, 107 $[(M-H)-(146+162)]^-$, 91 $[(M-H)-(146+178)]^-$; $[\alpha]_D^{25} = -50°$ (MeOH; *c* 1).

4-Methoxybenzyl-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**3**). HPLC R_t 12 min; FAB-MS, *m/z*: 445 $[M-H]^-$, 299 $[(M-H)-146]^-$, 137 $[(M-H)-(146+162)]^-$; $[\alpha]_D^{25} = -40°$ (MeOH; *c* 1).

nals superimpossible on lit. values, quinovose: δ 4.22 (1H, *d*, $J = 7.5$ Hz, H-1′), 3.58 (1H, H-2′), 3.42 (1H, overlapped, H-3′), 3.42 (1H, overlapped, H-4′), 3.60 (1H, H-5′), 1.30 (3H, *d*, $J = 6$ Hz, Me-6′); $[\alpha]_D^{25} = +18°$ (MeOH; *c* 1).

Tormentic acid 3β-O-β-D-fucopyranoside (**5**). HPLC R_t 20 min; FAB-MS, *m/z*: 633 $[M-H]^-$, 487 $[(M-H)-146]^-$; ^1H NMR (CD_3OD): fucose: δ 4.40 (1H, *d*, $J = 7.0$ Hz, H-1′), 3.45 (1H, *dd*, $J = 9$, 7 Hz, H-2′), 3.50 (1H, *dd*, $J = 9$, 4 Hz, H-3′), 3.62 (1H, *dd*, $J = 4$, 1.5 Hz, H-4′), 3.70 (1H, *m*, H-5′), 1.32 (3H, *d*, $J = 6.5$ Hz, Me-6′); $[\alpha]_D^{25} = +26$ (MeOH; *c* 1).

Tormentic acid 3β-O-α-L-rhamnopyranoside (**6**). HPLC R_t 19 min; FAB-MS, *m/z*: 633 $[M-H]^-$, 487 $[(M-H)-146]^-$; ^1H NMR (CD_3OD): rhamnose: δ

J = 6.5, 9.5 Hz, H-5′), 1.28 (3H, *d*, J = 6.5 Hz, Me-6′); $[\alpha]_D^{25}$ = +11 (MeOH; *c* 1).

REFERENCES

1. Velasco, J. (1946) '*Historia del Rein de Quito*' *La Historia Natural* Vol. 1. Empresa Editoria El Comercio, Quito, Ecuador.
2. De Tommasi, N., De Simone, F., Aquino R., Pizza, C. and Liang, Z. Z. (1990) *J. Nat. Prod.* **53**, 810.
3. De Tommasi, N., De Simone F., Cirino, G., Cicala, C. and Pizza, C. (1991) *Planta Med.* **57**, 413.
4. De Tommasi, N., Aquino R., De Simone, F. and Pizza, C. (1992) *J. Nat. Prod.* **55**, 1025.
5. De Tommasi, N., De Simone, F., Pizza, C., Mahmood, N., Moore, P., Conti, C., Orsi, N. and Stein, M. (1992) *J. Nat. Prod.* **55**, 1067.
6. Miyase, T., Ueno, A., Takizawa, N., Kobayashi, H. and Karasawa, H. (1987) *Chem. Pharm. Bull.* **35**, 1109.
7. Andary, C., Privat, G., Chevallet, P., Orzalesi, H., Serrano, J. J. and Boucard, M. (1980) *Farmaco, Ed. Sci.* **35**, 3.
8. Nishibe, S., Okabe, K., Tsukamoto, H., Sakushima, A., Hisada, S., Baba, H. and Akisada, T. (1982) *Chem. Pharm. Bull.* **30**, 4548.
9. Porter, L. J., Newman, R. H., Foo, L. Y. and Wong, H. (1982) *J. Chem. Soc., Perkin Trans. I* 1217.
10. Higuchi, R. and Donelly, D. (1978) *Phytochemistry* **17**, 787.
11. Gopalsamy, N., Vargas, D., Gueho, J., Ricaud, C. and Hostettmann, K. (1988) *Phytochemistry* **27**, 3593.
12. Du, H. Q., Zhao, X., Zhao, T. Z., Wang, M. T., Zhang, Z. W, Yao, M. and Yu, S. Z. (1983) *Yaoxue Xuebao* **18**, 314.
13. Shigenaga, S., Kouno, I. and Kawano, N. (1985) *Phytochemistry* **24**, 115.
14. Jia, Z., Liu, X. and Liu, Z. (1992) *Phytochemistry* **32**, 155.
15. Higuchi, R., Kawasaki, M., Biswas, M., Pandey, V. B. and Dasgupta, B. (1982) *Phytochemistry* **21**, 907.
16. De Tommasi, N., Piacente, S., De Simone, F., Pizza, C. and Liang, Z. Z. (1993) *J. Nat. Prod.* **56**, 1669.