Phytochemistry, Vol. 28, No. 1, pp. 199–201, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

FURTHER QUINOVIC ACID GLYCOSIDES FROM *GUETTARDA PLATYPODA*

RITA AQUINO, FRANCESCO DE SIMONE, COSIMO PIZZA and JOSÉ F. DE MELLO

Dipartimento di Chimica delle Sostanze Naturali, dell'Università di Napoli, Via Domenico Montesano, 49, 80131 Naples, Italy; Instituto dos Antibioticos, Universidade Federal de Pernambuco, 50000 Recife, Brazil

(*Received* 22 *March* 1988)

Key Word Index—*Guettarda platypoda*; Rubiaceae; root bark; quinovic acid glycosides; structural determination.

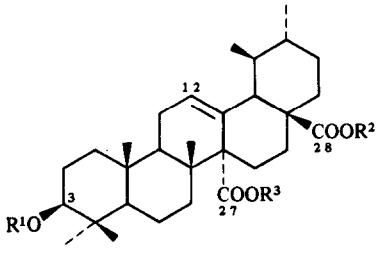
Abstract—Reinvestigation of the root bark of *Guettarda platypoda* afforded, in addition to known compounds, four new quinovic acid glycosides. Their structures were elucidated by NMR and FAB mass spectroscopy.

INTRODUCTION

In an earlier paper [1] we reported the isolation of the major triterpene glycosides from *Guettarda platypoda*, **1** representing the first quinovic acid glycoside with a 3,27-glycosylation pattern, **2** having a 3,28-glycosylation pattern and **3** having glucose attached at C-3 of the aglycone. In addition to these three compounds we report now the isolation of four new quinovic acid glycosides (**4**–**7**) from the bark of *G. platypoda*. The structure elucidations are discussed in this paper and are a continuation of our investigations of the active metabolites from the Rubiaceae [1–3].

RESULTS AND DISCUSSION

The methanol extract from the root bark of *G. platypoda* gave, in addition to the major quinovic acid glycosides (**1**–**3**) [1], yielded four new quinovic acid glycosides (**4**–**7**) in very small amounts.



	R^1	R^2	R^3
3	glucosyl	H	H
2	glucosyl	glucosyl	H
1	glucosyl	H	glucosyl
4	H	glucosyl	H
5	H	fucosyl	H
6	glucosyl-(1→3)-rhamnosyl	glucosyl	H
7	fucosyl~glucosyl	H	H

Acid methanolysis of **6** yielded methylglucoside and methylrhamnoside, analysed by GC, in the ratio 2:1, respectively. The molecular formula $C_{48}H_{76}O_{19}$ and the aglycone formula $C_{30}H_{46}O_5$ were determined by DEPT ^{13}C NMR (Table 1) and FAB mass spectral analysis in the negative ion mode (see Experimental).

The FAB spectrum of **6** showed a quasi-molecular anion at *m/z* 955 and two peaks at *m/z* 793 and 777 for the cleavage of glucose with or without the glycosidic oxygen (178 or 162 mu, respectively). The facile decarboxylation of these fragments, observed also in the FAB spectra of other quinovic acid glycosides previously isolated by us [1, 2], led in **6** to peaks at *m/z* 749 and 733 (loss of 44 mu). The cleavage of a further glucose unit, starting from the two last peaks, led to fragments at *m/z* 587 and 571. Two peaks at *m/z* 441 and 425 clearly showed the subsequent loss of a rhamnose unit (146 mu) from the *m/z* 587 and 571 peaks and indicated that rhamnose was attached at the aglycone moiety. The ^{1}H and ^{13}C NMR data for **6** (Tables 1 and 2) suggested the identity of the aglycone moiety as quinovic acid [1, 2].

The presence in **6** of a β-D-glucopyranose linked at a carboxyl group (C-27 or C-28) of the aglycone was deduced by the ^{1}H NMR signal at δ5.41 (H-1‴, 1H, *d*, *J* = 7.5 Hz) and by the ^{13}C NMR resonance at 95.8 ppm (C-1‴), both in full agreement with the corresponding signals which were previously observed in the NMR spectra of **1** and **2** [1, 2]. The glycosyl ester linkage was proposed to be at C-28 of the aglycone on the basis of the resonances of the C-12, C-13, C-14 (129.6, 134.8, 59.5 ppm, respectively) and of the 27 (179.6 ppm) and 28 (178.5 ppm) carboxyl groups. All these values matched well with those found in the 28 glycosyl ester (**2**) and usually observed in an urs-12-en-27,28-dioic acid with a C-27 unsubstituted carboxyl group [1, 2, 4].

In accordance with a C-28 glycosylated quinovic acid structure [1, 2] also the ^{1}H NMR spectrum of **6** showed the H-12 (δ5.58, 1H, *m*) and the Me-26 (δ0.90, 3H, *s*) resonances almost coincident with (**2**) but different from **1** (H-12, δ5.62, 1H, *m*; Me-26, δ0.92, 3H, *s*) [1]. The C-3 of the aglycone as the ether glycosidation site was deduced from the ^{13}C NMR signal at 90.5 ppm (C-3, CH by DEPT) [1, 2].

Table 2. ^{1}H NMR data for compounds **4**–**7** in δ (CD_3OD)

Aglycone	**4**	**5**	**6**	**7**
Me-23 (3H, *s*)	0.78	0.78	0.83	0.86
Me-26 (3H, *s*)	0.89	0.89	0.90	—
Me-29 and -30 (6H, *d*, sharp)	0.95	0.95	0.96	0.95
Me-25 (3H, *s*)	0.98	0.98	1.01	0.99
Me-24 (3H, *s*)	submerged by Me-25 signal		1.02	1.04
H-12 (1H, *m*)	5.57	5.57	5.58	5.58
Sugar proton				
H-1′ (1H, *d*)	5.40*	5.42†	4.77‡	4.35*
H-1″ (1H, *d*)	—	—	4.52*	4.28†
H-1‴ (1H, *d*)	—	—	5.41*	—
Fu-Me (3H, *d*, $J = 6$ Hz)	—	1.42	—	1.39
Rha-Me (3H, *d*, $J = 6$ Hz)	—	—	1.28	—

*$J = 7.5$ Hz; †$J = 7.0$ Hz; ‡$J = 1.5$ Hz.

Table 1. ^{13}C NMR data for compound **6** (CD_3OD)

Aglycone carbons	PPM	DEPT	Sugar carbons*	PPM	DEPT
1	39.7	CH_2	Rha-1′	102.9	CH
2	27.1	CH_2	Rha-2′	72.2	CH
3	90.5	CH	Rha-3′	83.1	CH
4	40.1	C	Rha-4′	73.5	CH
5	56.6	CH	Rha-5′	69.8	CH
6	19.4	CH_2	Rha-6′	18.0	CH_3
7	37.9	CH_2			
8	40.7	C	G-1″	105.8	CH
9	48.0	CH	G-2″	75.5	CH
10	37.9	C	G-3″	78.4	CH
11	24.1	CH_2	G-4″	71.9	CH
12	129.6	CH	G-5″	77.8	CH
13	134.8	C	G-6″	63.0	CH_2
14	59.5	C			
15	26.2	CH_2	G-1‴	95.8	CH
16	27.1	CH_2	G-2‴	74.1	CH
17	48.3	C	G-3‴	78.5	CH
18	55.7	CH	G-4‴	71.6	CH
19	40.4	CH	G-5‴	77.9	CH
20	38.0	CH	G-6‴	62.9	CH_2
21	31.3	CH_2			
22	37.3	CH_2			
23	19.3	CH_3			
24	28.6	CH_3			
25	16.9	CH_3			
26	18.3	CH_3			
27	179.6	C			
28	178.5	C			
29	17.1	CH_3			
30	21.3	CH_3			

*Rha = rhamnose, G = glucose.

To establish the sugar sequence and the relative sugar positions, **6** was subjected to alkaline hydrolysis to yield **6a**, previously described only as peracetyl dimethyl ester [5]. The ^{1}H and ^{13}C NMR spectra of **6a** showed no signals ascribable to a glucose ester linkage whereas the other sugar signals ascribable to a α-L-rhamnopyranosyl (H-1′, δ 4.77, 1H, *d*, $J = 1.5$ Hz, C-1′, 102.9 ppm) and to a β-D-glucopyranosyl (H-1″, δ 4.52, 1H, *d*, $J = 7.5$ Hz, C-1″, 105.8 ppm) remained almost unaffected with respect to **6**. This finding confirmed the presence in **6** and **6a** of a disaccharide chain linked at C-3 of the aglycone. The FAB mass spectrum of **6a** confirmed that rhamnose was attached at C-3 of the aglycone and glucose was the terminal sugar unit (see Experimental). The interglycosidic linkage between rhamnose and glucose was deduced from the ^{13}C NMR sugar signals which in **6** and **6a** were in agreement with a terminal glucose linked at the position C-3′ of the inner rhamnose [6]. In fact C-3′ of rhamnose resonated at 83.1 ppm, shifted downfield (β-effect) by 10.6 ppm, with respect to C-3′ of a methyl-α-L-rhamnopyranose model [7]. From these data the structure quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]-(28→1)-β-D-glucopyranosyl ester was assigned to **6**.

On acid methanolysis compound **4** liberated methyl-glucoside, compound **5** gave methylfucoside (GC). The FAB mass spectrum of **4**, in negative ion mode, showed a quasi-molecular anion at *m/z* 647 and major fragments at *m/z* 485 and 469 for the cleavage of a glucose unit. In the FAB spectrum of **5** were observed a quasi-molecular anion at *m/z* 631, which was shifted 16 mass units relative to **4**, and major fragments at *m/z* 485 and 469 suggesting the loss of a fucose unit. Other fragments for **4** and **5** at *m/z* 441 and 425 were due to the loss of a carboxyl group from the *m/z* 485 and 469 peaks. The quinovic acid structure and the substitution pattern of **4** and **5** followed from their ^{1}H NMR spectra. All aglycone proton signals (see Table 2) could be assigned by comparison with the ^{1}H NMR spectra of the related quinovic acid glycosides **1**–**3** and **6** [1]. The spectral patterns of **4** and **5** closely resembled that of **2** with regard to the Me-26, Me-29 and Me-30, Me-25 and H-12 resonances [1]. Clear differences were visible in the resonances of the Me-23 and Me-24 which were shielded by 0.07 ppm (from δ 0.85 in **2** to δ 0.78 in **4** and **5** and 0.06 ppm (from δ 0.85 in **2** to δ 0.78 in **4** and **5** and by 0.06 ppm (from δ 1.04 in **2** to δ 0.98 in **4** and **5**), respectively, indicating the presence of a free 3β-hydroxy group. Moreover, for the sugar protons the signal at δ 5.40 (1H, *d*, $J = 7.5$ Hz, anomeric hydrogen) in

the spectrum of **4** was ascribable to a β-D-glucopyranose linked at the C-28 carboxyl group, whereas in the spectrum of **5** the signals at δ 5.42 (1H, *d*, *J* = 7 Hz, anomeric hydrogen) and at δ 1.42 (3H, *d*, *J* = 6 Hz, methyl at C-5 of a 6-deoxy-galactosyl unit) were ascribable to a β-D-fucopyranose linked at the C-28 carboxyl group. As followed from these data **4** was determined to be quinovic acid-(28→1)-β-D-glucopyranosyl ester and **5**, quinovic acid-(28→1)-β-D-fucopyranosyl ester.

The ^{1}H NMR spectrum of **7** (Table 2) was similar to that of **3** for the aglycone signals and indicated a quinovic acid derivative with free C-27 and C-28 carboxyl groups and a 3-*O*-glycosylated group [1]. On acid methanolysis, **7** liberated methylglucoside and methylfucoside in the ratio 1:1 (GC). The sugar sequence in **7** was supported by the FAB mass spectrum which showed a quasi-molecular anion at *m/z* 793, fragments at *m/z* 647 and 631 due to the loss of a fucosyl unit with or without the glycosidic oxygen and at *m/z* 603 [647−44] and 587 [631−44] due to the cleavage of a carboxyl group. Starting from the *m/z* 603 and 587 fragments, the subsequent loss of a glucosyl unit led to peaks at *m/z* 441 and 425 showing that glucose was attached at the aglycone. The ^{1}H NMR spectrum of **7** showed sugar signals at δ4.35 (1H, *d*, *J* = 7.5 Hz, H-1′) ascribable to an inner β-D-glucopyranosyl unit linked at C-3 of the aglycone, and at δ 4.28 (1H, *d*, *J* = 7 Hz, H-1″) and at δ 1.39 (3H, *d*, *J* = 6 Hz, Me at C-5 of 6-deoxygalactose) [2] ascribable to a terminal β-D-fucopyranose. The lack of material prevented further experiments to establish the position of the interglycosidic linkage which remained undefined. From the above findings the structure quinovic acid-3β-*O*-[β-D-fucopyranosyl-β-D-glucopyranoside] was assigned to **7**.

EXPERIMENTAL

Negative ion FABMS and DEPT experiments were obtained as described earlier [1].

Extraction and isolation. Air-dried root bark of *G. platypoda* DC (1 kg, collected near Itamaraca, Recife, Brazil, voucher deposited at the Herbarium of the Instituto dos Antibioticos, Universidade Federal de Pernambuco, Recife, Brazil) was extd × 3 with MeOH. The residue (7.2 g) was separated firstly on a Sephadex LH-20 column and further by DCCC ($CHCl_3$–MeOH–H_2O 7:3:8, ascending mode) as reported previously [1]. Fractions were monitored by TLC ($CHCl_3$–MeOH–H_2O, 40:9:1), and similar fractions were combined and evapd to dryness. DCCC fractions 32–37 (90 mg), 38–46 (280 mg), 141–160 (22 mg), 161–179 (24 mg) and 180–210 (60 mg) were unresolved mixts. They were submitted to semi-prep. HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm id; flow ratc: 3 ml/min) to give **6** (10 mg) from fractions 32–37 (MeOH–H_2O, 13:12); **1** (90 mg) and **2** (47 mg) from fr. 38–46 (MeOH–H_2O, 13:12; **5** (2 mg) and **4** (3 mg) from frs 144–160 (MeOH–H_2O, 7:3); **7** (2 mg) and **1** (2 mg) from frs 161–179 (MeOH–H_2O, 7:3); **1** (20 mg) from frs 180–210 (MeOH–H_2O, 7:3).

Acidic methanolysis. Glycosides **4**–**7** (each 0.5 mg) were subjected to acidic methanolysis as described in ref. [7].

Alkaline hydrolysis. Glycoside **6** (6 mg) was subjected to alkaline hydrolysis [1] to give compound **6a** identified by ^{1}H and ^{13}C NMR [5].

Quinovic acid-(28→1)-β-D-glucopyranosyl ester (**4**). HPLC R_t 10 min; FABMS: *m/z* 647 $[M-H]^-$, 485 $[(M-H)-162]^-$, 469 $[(M-H)-178]^-$, 441 $[(M-H-44)-162]^-$, 425 $[(M-H-44)-178]^-$; ^{1}H NMR (CD_3OD, 250 MHz) see Table 1; $[\alpha]_D^{25} = 58°$ (MeOH; *c* 1).

Quinovic acid-(28→1)-β-D-fucopyranosyl ester (**5**). HPLC R_t 14 min; FABMS: *m/z* 631 $[M-H]^-$, 485 $[(M-H)-146]^-$, 469 $[(M-H)-162]^-$, 441 $[(M-H-44)-146]^-$, 425 $[(M-H-44)-162]^-$; ^{1}H NMR (CD_3OD 250 MHz) see Table 1; $[\alpha]_D^{25} = 56°$ (MeOH; *c* 1).

Quinovic acid-3β-O-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]-(28→1)-β-D-glucopyranosyl ester (**6**). HPLC R_t 20 min; FABMS: *m/z* 955 $[M-H]^-$, 793 $[(M-H)-162]^-$, 777 $[(M-H)-178]^-$, 587 $[(M-H-44)-(2\times162)]^-$, 571 $[(M-H-44)-(162+178)]^-$, 441 $[587-146]^-$, 425 $[571-146]^-$; ^{1}H and ^{13}C NMR (CD_3OD) see Tables 1 and 2; $[\alpha]_D^{25} = 23°$ (MeOH; *c* 1).

Quinovic acid-3β-O-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside] (**6a**) [5]. FABMS: *m/z* 793 $[M-H]^-$, 631 $[(M-H)-162]^-$, 615 $[(M-H)-178]^-$, 587 $[(M-H-44)-162]^-$, 571 $[(M-H-44)-178]^-$, 441 $[(M-H-44)-(162+146)]^-$, 425 $[(M-H-44)-(178+146)]^-$; NMR (CD_3OD): the aglycone signals were superimposable on those of glycoside (**3**) [1]; sugar signals, ^{13}C NMR, Rha-1′–Rha-6′, C-1′–C-6′: 102.8 (CH), 72.1 (CH), 83.2 (CH), 73.3 (CH), 69.7 (CH), 17.9 (Me), G-1″–G-6″ 105.5 (CH), 75.3 (CH), 78.5 (CH), 71.6 (CH), 77.9 (CH), 62.9 (CH_2); ^{1}H NMR: δ4.78 (1H, *d*, *J* = 1.5 Hz), 4.50 (1H, *d*, *J* = 7.5 Hz), 1.27 (3H, *d*, *J* = 6 Hz).

Quinovic acid-3β-O-[β-D-fucopyranosyl-β-D-glucopyranoside] (**7**). HPLC R_t 12 min; FABMS: *m/z* 793 $[M-H]^-$, 647 $[M-H-146]^-$, 631 $[M-H-162]^-$, 603 $[(M-H-44)-146]^-$, 587 $[(M-H-44)-162]^-$, 441 $[603-162]^-$, 425 $[587-162]^-$; ^{1}H NMR (CD_3OD) see Table 1; $[\alpha]_D^{25} = 16°$ (MeOH; *c* 1).

REFERENCES

1. Aquino, R., De Simone, F., Pizza, C., Cerri, R. and De Mello, J. F. (1988) *Phytochemistry* **27**, 2927.
2. Aquino, R., De Simone, F., Cerri, R. and Pizza, C. (1988) *J. Nat. Prod.* **51**, 257.
3. Aquino, R., D'Agostino, M., De Simone, F. and Pizza, C. (1988) *Phytochemistry* **27**, 1827.
4. Miana, A. G. and Hassan Al-Hazimi, M. G. (1987) *Phytochemistry* **26**, 225.
5. Sousa, M. P., Matos, M. E. O., Machado, M. I. L., Braz Filho, R. (1986) *Phytochemistry* **25**, 1419.
6. Bruno, I., Minale, L., Pizza, C., Zollo, F., Riccio, R. and Mellon, F. M. (1984) *J. Chem. Soc. Perkin Trans I*, 1875.
7. Aquino, R., Behar, I., De Simone, F., D'Agostino, M. and Pizza, C. (1986) *J. Nat. Prod.* **49**, 1096.