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QUINOVIC ACID GLYCOSIDES FROM *GUETTARDA PLATYPODA*

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Key Word Index *Guettarda platypoda*; Rubiaceae; glucopyranosyl esters of quinovic acid.

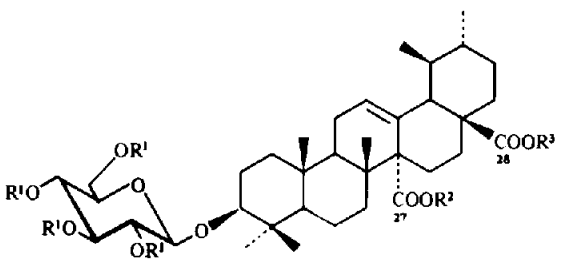
Abstract—A new triterpene glycoside, quinovic acid-3β-*O*-β-D-glucopyranosyl-(27→1)-β-D-glucopyranosyl ester, and two known glycosides, quinovic acid-3β-*O*-β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester and quinovic acid-3β-*O*-β-D-glucopyranoside, were isolated from the roots of *Guettarda platypoda*. Their structures were elucidated by chemical and spectral analysis of the non-derivatized glycosides.

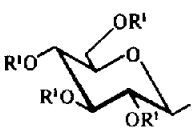
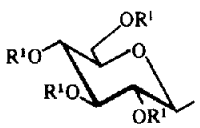
INTRODUCTION

Guettarda platypoda DC., a plant used in folk-medicine as a febrifuge, has recently been shown to contain the iridoids, morronoside and sweroside, and the triterpenoids quinovic acid and its 3-*O*-fucoside [1, 2]. These are the only reported phytochemical investigations on this species. We have re-examined the methanolic extract of the roots and obtained a new triterpene glycoside (**1**) and two known glycosides (**2** and **3**).

RESULTS AND DISCUSSION

The methanol extract of the roots of *G. platypoda* was chromatographed on a Sephadex LH-20 column and the glycoside containing fractions were further purified by DCCC (droplet counter-current chromatography) and HPLC to obtain the glycosides (**1–3**).



	R¹	R²	R³
1	H		H
6	Ac		H
7	Ac		Me
2	H	H	
4	Ac	H	
5	Ac	Me	
3	H	H	H

The glycoside **1** is a new natural compound; the glycosides **2** and **3** were isolated previously from *Guettarda angelica* [3] but the isolation and spectral analysis were reported only for the peracetyl methyl ester derivatives.

Acid methanolysis yielded methyl glucosides from **1**, **2** and **3** which were analysed by GLC. The molecular formulae $C_{42}H_{66}O_{15}$ for **1** and **2** and $C_{36}H_{56}O_{10}$ for **3** were determined by DEPT ^{13}C NMR studies (Table 1) and FAB mass spectral (Table 2) analysis in the negative ion mode. The FAB mass spectra of **1** and **2** gave the same fragmentation patterns and showed a quasi-molecular ion at *m/z* 809 $[M-H]^-$ and two peaks at *m/z* 647 and 631 which were interpreted as the cleavage of a glucose moiety with and without the glycosidic oygen. The facile decarboxylation from the *m/z* 647 and 631 fragments led to the peaks at *m/z* 603 and 587. Other fragments were observed at *m/z* 441 and 425 corresponding to the loss of a further glucose unit. The FAB mass spectrum of **3** gave ion species at *m/z* 647 $[M-H]^-$ (quasi-molecular ion) and 603 $[(M-H)-COO]$. The *m/z* 485 peak (aglycone ion) corresponded to the loss of a glucose unit from the *m/z* 647 in, whereas the peaks at *m/z* 441 and 425 corresponded to the loss of a glucose unit with and without the glycosidic oxygen from the fragment at *m/z* 603. The aglycone (molecular formula $C_{30}H_{46}O_5$) for the glycosides (**1–3**) was identified as quinovic acid on the basis of ^{1}H (Table 3), ^{13}C and DEPT ^{13}C NMR (Table 1) and FAB mass spectral (Table 3) data.

The β-D-pyranosyl form of glucose in compounds **1–3** was deduced from the δ and J values of the anomeric protons and carbons (see Tables 1 and 3) [4, 5].

The presence of a β-glucose unit linked at the aglycone with an ether bond in compounds (**1–3**) was suggested by the ^{1}H NMR (δ4.35, 1H, *d*, J=7.5 Hz, H-1′) and by the ^{13}C NMR (δ106.6, C-1′) anomeric signals [4, 5]. The presence in **1** and **2** of a further β-glucose unit linked at a carboxyl roup (C-27 or C-28) of the aglycone was consistent with the ^{1}H NMR anomeric signal at δ5.40 (H-1″, 1H, *d*, J=7.5 Hz) and in full agreement with the observed carbon resonance of C-1″ at δ95.8 [4, 6]. The ether glycosidation site was shown to be at C-3 of quinovic acid by the ^{13}C NMR absorptions of C-3 (δ90.9),

Table 1. ^{13}C NMR spectral data for compounds **1–3**

C	1	2	3	DEPT		1 and 2	3	DEPT
1	40.0	39.8	40.0	CH_2		glucose at C-3		
2	27.1	27.1	27.1	CH_2	G-1′	106.6	106.5	CH
3	90.9	90.9	90.9	CH	G-2′	75.8	75.4	CH
4	40.1	40.1	40.1	C	G-3′	78.6	78.4	CH
5	56.9	56.6	56.8	CH	G-4′	71.9	71.9	CH
6	19.4	19.4	19.4	CH_2	G-5′	77.7	77.6	CH
7	37.9	37.9	37.9	CH_2	G-6′	63.0	63.1	CH
8	40.9	40.7	40.8	C		glucose at C-27	or C-28	
9	48.0*	47.9*	47.9*	CH	G-1″	95.8		CH
10	38.0	37.9	37.9	C	G-2″	74.1		CH
11	24.0	24.1	23.9	CH_2	G-3″	78.4		CH
12	130.7	129.7	130.0	CH	G-4″	71.6		CH
14	133.7	135.0	134.5	C	G-5″	78.4		CH
14	57.8	59.5	59.3	C	G-6″	62.9		CH_2
15	25.9	26.2	26.1	CH_2				
16	26.7	27.1	26.9	CH_2				
17	48.1*	48.3*	48.3*	C				
18	55.5	55.7	55.1	CH				
19	40.3	40.4	40.3	CH				
20	38.3	38.1	38.5	CH				
21	31.2	31.3	31.4	CH_2				
22	37.1	37.3	37.9	CH_2				
23	19.3	19.4	19.2	Me				
24	28.6	28.6	28.6	Me				
25	16.9	16.9	16.9	Me				
26	18.2	18.4	18.2	Me				
27	178.1	179.5	179.5	X				
28	182.0	178.5	182.0	C				
29	17.1	17.1	17.1	Me				
30	21.4	21.3	21.5	Me				

* Under CD_3OD signal.

Table 2. FABMS spectral data for compounds **1–3**

m/z	1 and 2	*m/z*	3
809	$[M-H]^-$	647	$[M-H]^-$
647	$[(M-H)-162]^-$	603	$[(M-H)-44]^-$
631	$[(M-H)-178]^-$	485	$[(M-H)-162]^-$
603	$[(M-H-44)-162]^-$	441	$[(M-H-44)-162]^-$
587	$[(M-H-44)-178]^-$	425	$[(M-H-44)-178]^-$
441	$[(M-H-44)-(2\times 162)]^-$		
425	$[(M-H-44)-(162+178)]^-$		

The mass units lost corresponded to the following fragments: *m/z* 162, glucose without glycosidic oxygen; 178, glucose with glycosidic oxygen; 44, carboxyl group.

C-2 (27.1) and C-4 (40.1) which were in agreement with a model of oleanolic acid substituted at C-3 in CD_3OD [4].

The glycosyl ester linkage was proposed to be at C-28 in **2** on the basis of the ^{13}C and ^1H NMR spectral data. In compound (**2**) C-12 and C-13 resonated at δ129.7 and 135.0, respectively. These values were similar to those found in **3** and diagnostic for the presence of an unsubstituted carboxyl group at C-14 in a urs-12-en-27,28-dioic acid model [7]. The C-28 esterified carboxyl group appeared at δ178.5, shifted upfield by 3.5 ppm (glycosidation shift) in respect to the C-28 (δ182.0) in **3**. The C-27 free carboxyl group resonated at δ179.5 as did the C-27 in **3**, in agreement with the literature data [4]. In addition, in the ^1H NMR spectra the H-12 resonance at δ5.58 (1H, *m*) was coincident for compounds **2** and **3**.

Compound **2**, on basic hydrolysis, gave **3**. On acetylation it gave the peracetyl derivative (**4**) which upon subsequent treatment with diazomethane gave **5**, identified as quinovic acid-3β-*O*-[β-D-glucopyranosyl-(28→1)-

Table 3. ^{1}H NMR data for compounds **1–3***

H	1	2	3
Me-23 (3H, *s*)	0.85	0.85	0.85
Me-26 (3H, *s*)	0.92	0.89	submerged by Me-29 and -30 signal
Me-29 and -30 6H, *d*, sharp)	0.96	0.96	0.95
Me-25 (3H, *s*)	1.00	0.99	0.99
Me-24 (3H, *s*)	1.04	1.04	1.04
H-1′ (1H, *d*, J=7.5 Hz)	4.35	4.35	4.35
H-1″ (1H, *d*, J=7.5 Hz)	5.40	5.40	—
H-12 (1H, *m*)	5.62	5.58	5.58

* Other glucose signals were overlapped in the region 3.00–3.95.

β-D-glucopyranosyl ester] peracetyl methyl ester by literature data [3].

From these chemical and spectral data the structure of compound **3** is shown to be quinovic acid-3β-*O*-β-D-glycopyranoside and the structure of **2** is quinovic acid-3β-*O*-β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester.

Compound **1**, as well as compound **2**, formed **3** on basic hydrolysis. On acetylation they gave the peracetyl derivative **6** which upon subsequent treatment with diazomethane yielded the peracetyl monomethyl ester derivative **7**.

The ^{1}H and ^{13}C NMR sugar resonances of **1** were superimposable on those of **2**. The location of glucose in the ester linkage at C-27 was supported by the spectral differences between **1** and **2**. In the ^{1}H NMR spectra the most significant differences resided in the C-26 methyl group signal (δ0.92, 3H, *s*) deshielded by 0.03 ppm, and in the H-12 signal (δ5.62, 1H, *m*) deshielded by 0.04 ppm in compound **1** while the remaining aglycone protons showed small or no modifications. Also, the ^{13}C NMR spectrum of **1** showed significant changes if compared with that of **2**; C-13 was shifted upfield by 1.3 ppm from 135.0 to 133.7, C-12 was shifted downfield by 1.0 ppm from 129.7 to 130.7, C-14 was shielded by 1.7 ppm (γ-effect) from 59.5 to 57.8. Further small differences were observed for the carbons neighbouring C-14.

The observed chemical shift differences for the olefinic carbons (C-12 and C-13) in compounds **1** and **2** were in agreement with the models quinovic acid and its dimethyl ester derivative, reported in a recent paper [7] and confirmed the presence of a substitution at C-27 in **1**.

In addition the glycoside at C-27 in compound **1** shifted the C-27 absorption to higher field while the C-28 absorptions were coincident in the spectra of **1** and **3** (see Table 1).

Further evidence for the C-27 glycosylation in compound **1** was provided by a comparative analysis of the spectra of the peracetyl derivatives **4** and **6** and of the peracetyl monomethyl ester derivatives **5** and **7**. The ^{1}H NMR spectrum of compound **6** (in $CDCl_3$, see Experimental) showed the C-26 methyl and H-12 signals shifted downfield when compared with the spectrum of compounds **4**, as observed in the proton spectra of compounds **1** and **2**.

The ^{1}H NMR spectra (in $CDCl_3$) of compounds **5** and **7** showed a signal at δ3.64 (3H, *s*) in compound **5** and at δ3.63 (3H, *s*) in **7** due to a –COOMe group while the other proton signals were superimposable demonstrating that in compound **6** the C-28, and in **4** the C-27 carboxyl group were free.

From these data compound **1** was proven to be quinovic acid-3β-*O*-β-D-glucopyranosyl-(27→1)-β-D-glucopyranosyl ester. This is the first report of a quinovic acid derivative glycosylated at C-27.

EXPERIMENTAL

The FABMS spectra, in negative ion mode, were obtained by dissolving the samples in a glycerol-thyoglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of energy 2–6 kV.

The DEPT experiments were performed using polarization transfer pulses of 90 and 135°, respectively, to obtain in the first case only CH groups and in the other case positive signals for CH and Me and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz.

The NMR spectra were obtained CD_3OD for compounds **1–3** and in $CDCl_3$ for **4–7** at 250 MHz (^{1}H NMR) and at 69.5 MHz (^{13}C NMR) with TMS as int. standard.

Biological material. Roots of *G. platypoda* were collected at Itamaraca (Recife, Brazil). A voucher specimen of the plant is deposited at the Herbarium of the Instituto dos Antibioticos, Universidade Federal de Pernambuco, Recife, Brazil.

Isolation. The roots of air-dried plant material (0.5 kg) were extracted with MeOH (X3) at room temp to yield 43 g of extract. A portion (3.6 g) of the residue was chromatographed on a Sephadex LH-20 column (80 × 4 cm, MeOH as eluent). Fractions (6 ml) were collected and analysed by TLC on silica gel in $CHCl_3$–MeOH–H_2O (40:9:1). Fractions 13–41 (1.2 g) containing the crude glycosidic mixture were further purified by DCCC with $CHCl_3$–MeOH–H_2O (7:13:8) (ascending mode, the lower phase was the stationary phase, flow 12 ml/hr, 4 ml fractions were collected) to give the unresolved glycosides **1** and **2** in the fractions 38–46 (150 mg) and **3** in the fractions 180–210 (31 mg). Fractionation was achieved by HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 3 ml/min) using MeOH–H_2O (13:12) as eluent to yield pure **1** (48 mg) and **2** (25 mg) from the more polar fractions and MeOH–H_2O (7:3) to yield pure **3** (12 mg) from the less polar ones.

Compounds **1** and **2** were acetylated as usual to give **4** and **6** respectively, which were then methylated with CH_2N_2 to give **5** and **7**, respectively.

Alkaline hydrolysis. Glycosides **1** (20 mg) and **2** (10 mg) in 0.5 M KOH (1 ml) were separately heated at 110° in a stoppered reaction vial for 2 hr. The reaction mixture was adjusted to pH 7 with dil. HCl and then extracted with *n*-BuOH. The organic phase was evaporated to dryness, dissolved in CD_3OD and analysed by ^1H NMR.

Acidic methanolysis. Methanolysis of each glycoside (0.5–1 mg) was achieved as described earlier [8].

Quinovic acid-3β-O-β-D-9-glucopyranosyl-(27→1)-β-D-glucopyranosyl ester (**1**). HPLC retention time 30 min; FAB MS, ^1H NMR and ^{13}C NMR: see Tables 1–3; $[\alpha]_D^{25} = +14°$ (MeOH; *c* 1). Alkaline hydrolysis of **1** afforded the glycoside (**3**) identified by comparison of ^1H NMR spectra. Acidic methanolysis of **1** afforded a methyl glucoside (×2) identified by GLC (SE-30, 20 m, 140°).

Quinovic acid-3β-O-[β-D-glucopyranosyl-(27→1)-β-D-glucopyranosyl ester] peracetylate (**6**). ^1H NMR ($CDCl_3$): δ0.71, 0.83, 0.87, 0.92 (*s*, 6-Me), 2.00–2.14 (8 Ac), 3.78–3.90 (*m*, H-5′ and H-5″), 4.00–4.35 (*m*. H-6′ and H-6″), 4.54 (*d*, *J* = 8 Hz, H-1′), 4.98–5.30 (*m*. 6H, H-2′,2″, 3′, 3″, 4′, 4″), 5.60 (*d*, *J* = 8 Hz, H-1″), 5.72 (*m*, 1H, H-12).

Quinovic acid-3β-O-[β-D-glucopyranosyl-(27→1)-β-D-glucopyranosyl ester] peracetyl monomethyl ester (**7**). ^1H NMR superimposable on those of compound **5**.

Quinovic acid-3β-O-D-glucopuranosyl-(28→1)-β-D-glucopyranosyl ester (**2**). HPLC retention time 26 min; FABMS, ^1H NMR and ^{13}C NMR: see Tables 1–3 $[\alpha]_D^{25} = +15°$ (MeOH; *c* = 1). Alkaline hydrolysis of **2** gave compound **3** identified by ^1H NMR data. Acidic methanolysis gave methyl glucoside (×2) identified by GLC (SE-30, 20 m, 140°).

Quinovic acid 3β-O-[β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester]peracetylate (**4**). ^1H NMR ($CDCl_3$): δ0.72, 0.83, 0.86, 0.92 (*s*, 6-Me), 2.00–2.14 (8 Ac), 3.78–3.90 (*m*, H-5′ and H-5″). 4.00–4.35 (*m*, H-6′ and H-6″), 4.54 (*d*, *J* = 8 Hz, H-1′), 4.95–5.32 (*m*, 6H, H-2′. H-2″, H-3′, H-3″, H-4′, H-4″), 5.60 (*d*, *J* = 8 Hz, H-1″), 5.70 (*m*. 1H, H-12).

Quinovic acid-3β-O-[β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester] peracetylmonomethyl ester (**5**). Identified by literature data [3].

Quinovic acid-3β-O-β-D-glucopyranoside (**3**). HPLC retention time 16 min: FABMS, ^1H and ^{13}C NMR: see Tables 1–3; $[\alpha]_D^{25} = +55°$ (MeOH: *c* 1).

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