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QUINOVIC ACID GLYCOSIDES FROM *GUETTARDA ANGELICA*

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Key Word Index—*Guettarda angelica*; Rubiaceae; roots; quinovic acid glycosides; quinovic acid; ^{13}C NMR.

Abstract—Two new triterpene glycosides isolated from the root bark of *Guettarda angelica* were proven to be quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside] and quinovic acid-3β-*O*-β-D-glucopyranosyl-(28 → 1)-β-D-glucopyranosyl ester. In addition quinovic acid and two known glycoside derivatives (quinovic acid-3β-*O*-β-D-glucopyranoside and quinovic acid-3β-*O*-α-L-rhamnopyranoside) were isolated. The structures were elucidated by spectroscopic analysis of the peracetyl methyl ester derivatives.

INTRODUCTION

Many interesting glycosides of quinovic acid have been obtained from the Rubiaceae family [1–5]. We have previously reported [6] the isolation of quinovic acid (**4**) and its glucoside quinovic acid 3β-*O*-β-D-glucopyranoside (**1**), rotundic acid, hederagenin and a new 3β,23-dihydroxyurs-12-en-28-oic acid from *Guettarda angelica*. We have now re-examined more material of *G. angelica* that afforded in addition two new triterpene glycosides **2** and **3**, two known glycosides **1** and **5** and again quinovic acid (**4**).

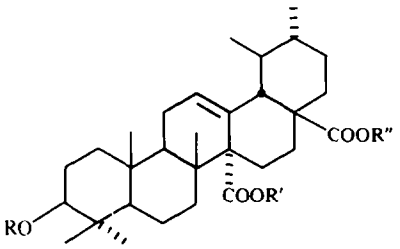
RESULTS AND DISCUSSION

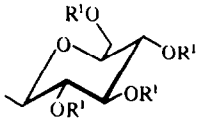
The ethanol extract from the root bark of *G. angelica* by alkaline extraction furnished a red-brown mixture. This material was acetylated and chromatographed on a silica gel column and the major fractions were methylated. Repetitive chromatographic separations yielded four peracetyl methyl ester glycosides **1a**, **2a**, **3a** and **5a**, and the quinovic acid derivative **4a**. The glycosides **1** and **5** were isolated previously from *Cinchona calisaya* [1] and from *C. pubescens* [2], respectively. In the present study the ^{13}C NMR spectrum, previously unreported for these known glycosides (Table 1), permitted the assignments of all carbons including the sugar moieties of the two new glycosides **2a** and **3a**. This was made possible by prior identification of the sugar moieties by application of the procedures used for determination of rhamnose and glucose in glycosides [2, 7]. Acid hydrolysis of the glycosides **1a**, **2a**, **3a** and **5a** with trifluoroacetic acid gave D-glucose and L-rhamnose, identified by paper chromatography and comparison with the authentic samples. The melting points of the peracetyl ester derivatives, **1a** and **5a**, are not cited in the literature. In the ^{1}H NMR spectrum of **1a**, **2a**, **3a** and **5a** the signals at δ5.68, 5.62, 5.68 and 5.63 for the H-12 olefinic proton are in accordance with those described earlier for acetyl dimethyl quinovate [5]. The ^{13}C NMR signals of C-12 and C-13 are similar to those found in methyl 3β,28-dihydroxyurs-12-en-14-oate [8]. The presence of the COOMe group at C-14 is justified by the shifts of C-7, C-8, C-14, C-15 and C-16 in its vicinity (Table 1), which differ from the model acetyl methyl ursolate [9]. On the basis of these results, we propose the structure of quinovic acid for the aglycone from **1a**, **2a**, **3a** and **5a** glycosides. In the ^{1}H NMR spectrum of **1a** and **5a** signals at δ4.56 (*d*, J = 8 Hz) and 4.76 (*br*) were identified as anomeric protons of β-D-glucose and α-L-rhamnose respectively. The rhamnose residue of **5a** also gave rise to a methyl doublet (J = 6 Hz) at δ1.14, absent from the spectrum of **1a**.

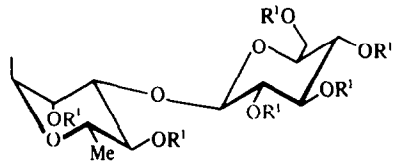
Confirmations of the structures **1a** and **5a** were made by ^{13}C NMR spectroscopy. On going from acetyl dimethyl ester quinovate **4a** to **1a** the signal due to C-3 was deshielded by 9.7 ppm in accordance with the results observed for bryonoside and its aglycone bryodulcosigenin [10]. The difference between **5a** and its aglycone **4a** was 8.7 ppm (Table 1). Comparison of the ^{13}C NMR spectra between the aglycone, quinovic acid, and its glycosides is seen in Table 1.

Quinovic acid-3β-O-[β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside] peracetyl dimethyl ester (**2a**)

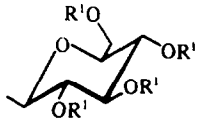
In the ^{1}H NMR spectrum of **2a**, the presence of signals due to six acetoxyl groups suggested that the aglycone was linked to a disaccharide moiety. The type of the linkage between the disaccharide and the aglycone which could be either an ether (C-3) or an ester (C-27 or C-28) was shown to be at C-3 by the ^{13}C absorption at δ89.0 in comparison with the absorption in the aglycone **4a** (80.6) demonstrating that this carbon supports an ether glycosidation. This observation is in agreement with the ^{1}H NMR absorption at δ3.64 that integrates for two methoxyl groups (C-27 and C-28). In the ^{1}H NMR spectrum the region corresponding to the glycosidic hydrogens, the doublet at δ1.12 (J = 6 Hz) associated with the presence of α-L-rhamnose and the ^{13}C signal at δ99.2 (C-1′) was evidence that this sugar was linked to the C-3 of the aglycone as in **5a**. The terminal sugar of the disaccharide part, which was identified as glucose, showed a doublet at δ4.62 (C-1″, J = 8 Hz). This constant value indicates a β-pyranoside



1 R =  R' = R'' = H **1a** R' = Ac; R'' = Me

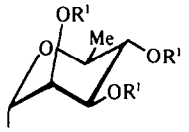
2 R =  **2a** R'' = Me; R' = Ac

R' = R'' = H

3 R = R'' =  **3a** R'' = Me ; R' = Ac

R' = R'' = H

4 R = R'' = R'' = H **4a** R'' = Me R = Ac

5 R =  **5a** R'' = Me; R' = Ac

R' = R'' = R'' = H

form to the glucose [11]. Comparison of the rhamnose carbon chemical shifts in the spectrum of **2a** and those of methyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside [12] showed that the linkage between rhamnose and glucose must occur at C-3', since the signal of this carbon is correspondingly at δ74.5. This is also consistent with the value 66.3 assigned to C-5' [13, 14]. From these spectral data the structure of **2** has been settled as quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside].

Quinovic acid-3β-O-[β-D-glucopyranosyl-(28 → 1)-β-D-glucopyranosyl] ester peracetyl methyl ester (**3a**)

The ^{1}H NMR spectrum of **3a** presented eight acetoxyl signals and only one carbomethoxyl absorption. These observations lead us to propose that this substance is a bisdesmoside. The signal at δ4.57 (*d*, *J* = 8 Hz) was attributed to the anomeric hydrogen at C-1' of glucose linked at C-3 of the aglycone part as in **1a** and the absorption at 5.65 (*d*, *J* = 8 Hz) was consistent with the anomeric hydrogen at C-1'' of another glucose molecule in ester linkage. The glycosyl ester linkage was proposed to be at C-28 in view of the ^{13}C NMR spectrum that showed only an absorption at δ175.2 indicating that glycosylation shifts the C-28 carbonyl absorption to higher field and therefore coincident with the C-27 absorption in this glycoside (Table 1).

The nature of the sugars was confirmed by a comparative analysis of the ^{13}C NMR data of **3a** and that presented by 3β-*O*-[β-D-glucopyranosyl]-platicodigenin acetylated [15] and 1,2,3,4,6-penta-*O*-acetylglucopyranose [16]. In this way it was possible to verify the presence of two molecules of 2,3,4,6-tetra-*O*-acetyl-β-glucopyranose linked at C-3 and C-28 of the aglycone. The ester linkage was corroborated by the signal at δ91.4

Table 1. ^{13}C NMR spectral data for compounds **4a**, **1a**, **2a**, **3a** and **5a** (δ relative to TMS, 25.2 MHz, $CDCl_3$)

	Acetyl dimethyl esters				
aglycone carbons	quinovic acid **4a**	glucosyl **1a**	glucosyl **3a**	glucosyl rhamnosyl **2a**	rhamnosyl **5a**
1	38.5	38.7	38.7	38.8	38.8
2	23.6	25.8	25.8	26.3	25.1
3	80.6	90.3	90.2	89.0	89.3
4	37.6	39.6	39.7	39.5	39.5
5	55.5	55.6	55.6	55.4	55.5
6	18.1	18.2	18.3	18.2	18.2
7	37.0	36.9	37.0	36.9	36.9
8	37.6	37.1	37.0	37.0	36.6
9	47.2	47.2	47.1	47.2	47.2
10	36.9	36.7	36.6	36.6	36.6
11	22.8	22.8	22.7	22.7	22.7
12	129.0	129.2	129.9	129.0	129.1
13	131.9	131.9	131.0	132.0	131.9
14	56.1	56.1	56.0	56.1	56.1
15	24.7	24.6	24.6	24.7	24.6
16	25.1	25.2	25.1	25.2	25.1
17	48.4	48.5	48.4	48.4	48.4
18	53.7	53.7	53.4	53.7	53.7
19	39.4	38.7	38.7	38.8	39.5
20	38.8	38.7	38.7	38.8	38.8
21	29.8	29.9	29.6	29.8	29.7
22	36.0	36.0	36.6	36.0	36.0
23	28.0	27.7	27.7	28.1	28.1
24	16.8*	16.3*	16.3*	16.3*	16.3*
25	16.3*	16.4*	16.4*	16.6*	16.4*
26	18.3	18.3	18.3	18.2	18.2
27	175.2	175.3	175.2	175.2	175.2
28	177.6	177.7	175.2	177.7	177.6
29	17.1	17.0	17.0	17.0	17.0
30	21.0	21.1	21.0	21.0	21.0
COOMe	51.2	51.2	51.2	51.1	51.1
	51.4	51.4	—	51.4	51.4
		glucose	glucose	rhamnose	rhamnose
1′		102.8	102.8	99.2	99.4
2′		71.4	71.4	71.7	70.1
3′		72.8	72.7	74.5	69.1
4′		68.7	68.6	72.6	71.1
5′		71.6	71.5	66.3	66.3
6′		62.1	62.1	17.1	17.3
		ester sugar carbons glucose		Terminal sugar carbons glucose	
1″		91.4		100.7	
2″		69.9		71.1	
3″		72.4		72.6	
4″		67.9		68.1	
5″		72.7		71.7	
6″		61.5		61.4	

*These values are interchangeable.

(C-1″) and was in full agreement with the observed ^{1}H NMR signal at δ5.65 correlated with the anomeric proton while the signal at 102.8 ppm that was attributed to the C-1′ in an ether linkage also agrees with the absorption at δ4.57. All these considerations about **3a** led us to assign to 3 the structure quinovic acid-3β-*O*-β-D-glucopyranosyl-(28 → 1)-β-D-glycopyranosyl ester.

EXPERIMENTAL

The root bark of *G. angelica* (6.5 kg) was extracted with EtOH at room temp. The extract, concd to dryness *in vacuo*, furnished a brown mass (405 g). This material was washed with HCl (5% soln) and the alkaloid-containing fraction was set aside. The residual non-alkaloidal extract was extracted with Na_2CO_3 (5% soln) and, upon acidification, a ppt consisting mainly of triterpene glycosides (Liebermann–Burchard test) was separated. This material was acetylated, chromatographed on a silica gel column and the major fractions were methylated. After repetitive chromatographic separations **1a** (400 mg), **2a** (180 mg), **3a** (170 mg), **4a** (300 mg) and **5a** (170 mg) were obtained.

Glycosyl peracetyl dimethyl ester (**1a**). White powder, mp 100–105°; IR ν_{max} cm^{-1}: 2980, 2940, 1760, 1730, 1640, 1225. ^{1}H NMR (100 MHz, $CDCl_3$): δ0.73, 0.85, 0.90, 0.93, (*s*, 6Me), 2.00 (1-Ac), 2.04 (2-Ac), 2.10 (1-Ac), 3.65 (*s*, OMe), 3.67 (*s*, OMe), 3.80 (*m*, H-5′), 4.04–4.34 (*m*, 2H-6′), 4.56 (*d*, J = 8 Hz, H-1′), 4.98–5.26 (*m*, 3H-2′, 3′, 4′), 5.68 (*m*, H-12). ^{13}C NMR ($CDCl_3$): see Table 1.

Glycoside **2a**. White powder, mp 103–105°; IR ν_{max} cm^{-1}: 2980, 1760, 1730, 1640, 1230. ^{1}H NMR (100 MHz, $CDCl_3$): δ0.79, 0.82, 0.90, 0.94 (*s*, 6-Me), 1.12 (*d*, Me rhamnose), 1.98 (1-Ac), 2.02 (2-Ac), 2.08 (1-Ac), 2.10 (2-Ac), 3.64 (*s*, OMe), 3.80–3.98 (*m*, H-5′ and H-5″), 4.08–4.16 (*m*, 2H-6″), 4.62 (*d*, J = 8 Hz, H-1″), 4.78 (*s*, slightly broadened singlet, H-1′), 4.90–5.16 (*m*, 6H-2′, 2″, 3′, 3″, 4′, 4″), 5.62 (*m*, H-12). ^{13}C NMR ($CDCl_3$): see Table 1.

Glycoside **3a**. White powder, mp 204–206°; IR ν_{max} cm^{-1}: 2970, 1740, 1720 (shoulder), 1620, 1230. ^{1}H NMR (100 MHz, $CDCl_3$): δ0.73, 0.84, 0.92, 0.96 (*s*, 6-Me), 2.04 (4Ac), 2.07 (2-Ac), 2.09 (2-Ac), 3.66 (*s*, OMe), 3.74–3.88 (*m*, H-5′ and H-5″), 4.00–4.38 (*m*, H-6′ and H-6″), 4.57 (*d*, J = 8 Hz, H-1′), 4.90–5.26 (*m*, 6H-2′, 2″, 3′, 3″, 4′, 4″), 5.65 (*d*, J = 8 Hz, H-1″), 5.68 (*m*, H-12). ^{13}C NMR ($CDCl_3$): see Table 1.

Acetyl dimethyl ester **4a**. White powder, mp 207–211° (lit. [5, 17] 219–222°). ^{1}H NMR (60 MHz, $CDCl_3$): δ0.80, 0.91, 0.96 (*s*, 6Me), 2.03 (1-Ac), 3.63 (*s*, OMe), 4.50 (*m*, H-3), 5.67 (*m*, H-12). ^{13}C NMR: ($CDCl_3$): see Table 1.

Rhamnosyl peracetyl dimethyl ester **5a**. White powder, mp 208–211°; IR ν_{max} cm^{-1}: 2990, 1750, 1725, 1040, 1235. ^{1}H NMR (60 MHz, $CDCl_3$): δ0.83, 0.92 (*s*, 6Me), 1.14 (*d*, Me rhamnose), 2.0 (1-Ac), 2.05 (1-Ac), 2.14 (1-Ac), 3.63 (*s*, OMe), 3.98 (*m*, H-5′), 4.76 (*s*, slightly broadened singlet, H-1′), 5.0–5.30 (*m*, 3H-2′, 3′, 4′). ^{13}C NMR ($CDCl_3$): see Table 1.

Hydrolysis of glycosides **1a**, **2a**, **3a** *and* **5a**. Compounds (5 mg) in 4 N TFA (2.5 ml) were heated in a sealed tube for 2 hr. The acid soln was concd under red. pres. to dryness and redissolved in *iso*-PrOH. The sugars were identified by PC using an eluent EtOAc–pyridine–H_2O (20:5.5:3). Spots were visualized with acid aniline phthalate spray. Glucose and rhamnose were used as reference.

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