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NEW QUINOVIC ACID GLYCOSIDES FROM *UNCARIA TOMENTOSA*[1]

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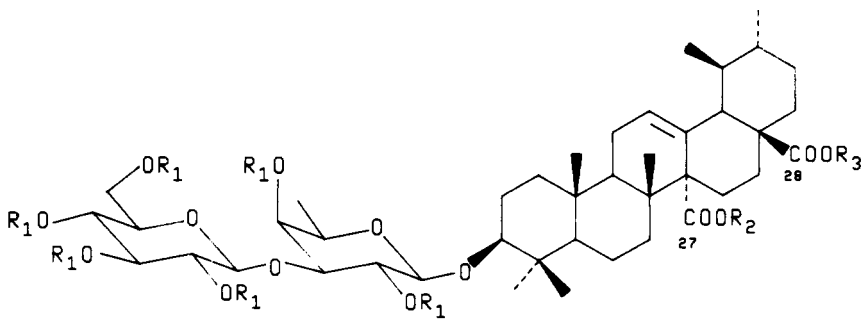
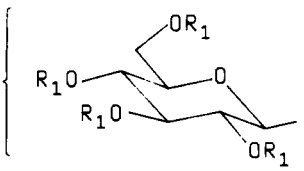
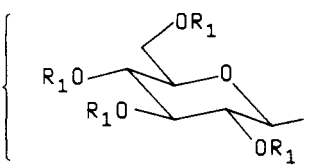
ABSTRACT.—From the bark of *Uncaria tomentosa*, three new quinovic acid glycosides have been isolated. The structures have been established as **1**, **2**, and **3** through spectral and chemical studies.

Uncaria tomentosa (Willd.) DC. (Rubiaceae), the Peruvian flora commonly known as "uña de gato," is widely used in local medicine for the treatment of cancer, arthritis, gastritis and certain epidermic diseases. Previous pharmacological work with the aqueous or EtOH extracts of this plant showed cytostatic, contraceptive, and anti-inflammatory activity (1). Phytochemical studies led to the isolation of a number of alkaloids (2) displaying a pronounced enhancement of phagocytosis. So far, no evidence is available in the literature regarding the non-alkaloidal constituents.

In the course of our investigation of metabolites from the Rubiaceae (3), we have isolated and identified three new quinovic acid glycosides, **1**, **2**, and **3**, from the bark of *U. tomentosa*. The glycoside **3** (4) includes a quinovic acid moiety and a disaccharide



	R_1	R_2	R_3
1	H		H
1a	Ac		H
1b	Ac		Me
2	H	H	
2a	Ac	H	
2b	Ac	Me	
3	H	H	H

[1]Part 9 of the series Plant Metabolites; for part 8 see Aquino *et al.* (3).

portion attached at C-3 of the aglycone; glycoside **2** has an additional glucose unit at C-28; the glycoside **1** has a glucose unit linked with an ester bond at C-27. The C-27 substitution has been found only in one other plant, *Guettarda platypoda* (3), of the same family, Rubiaceae.

RESULTS AND DISCUSSION

The glycoside mixture obtained from the $CHCl_3$-MeOH (9:1) extract of the bark of *U. tomentosa* was purified by Sephadex LH-20 cc and fractionated by hplc to yield **1**, **2**, and **3**.

The structures of **1–3** were determined by negative fabms spectrometry, ^{1}H- and ^{13}C-nmr measurements, and sugar analysis. On acid methanolysis, methylglucoside and methylfucoside were obtained in the ratio 2:1 from **1** and **2** and in the ratio 1:1 from **3**.

The fabms spectrum of **3** showed a molecular anion at *m/z* 793 $[M-H]^-$ and fragments at *m/z* 631 $[(M-H)-162]^-$ and 615 $[(M-H)-178]^-$ resulting from the cleavage of a glucose unit without and with the glycosidic oxygen and peaks at *m/z* 587 and 571 corresponding to the loss of a carboxylic group (44 mass units) from the 631 and 615 peaks, respectively. Other signals at *m/z* 441 and 425 corresponded to the subsequent loss of a fucose unit (146 mass units) and showed that this sugar is attached at the aglycone moiety.

The fabms spectrum of **2** showed a quasi-molecular anion at *m/z* 955 $[M-H]^-$ shifted 162 mass units relative to **3** and fragments at *m/z* 793 $[(M-H)-162]^-$ and 777 $[(M-H)-178]^-$ indicating the presence of an extra glucose moiety with respect to **3**. The facile decarboxylation from these two peaks led to the 749 and 733 fragments. The anion peaks at 587 (749−162) and 571 (733−162) showed clearly the further loss of a glucose unit while a fucose remained attached at the decarboxylated aglycone. The peaks at *m/z* 441 (587−146) and 425 (571−146), also present in the spectrum of **3**, corresponded to the subsequent loss of a fucose unit.

The fabms of **1** showed the same fragmentation pattern observed in **2** and indicated that **1** was isomeric to **2**. The molecular formula $C_{48}H_{76}O_{19}$ for **1** and **2**, $C_{42}H_{66}O_{14}$ for **3**, and the aglycone formula $C_{30}H_{46}O_5$ were deduced by fabms and DEPT ^{13}C nmr.

Analysis of ^{1}H- and ^{13}C-nmr data (Tables 1,2) suggested the identity of the aglycone moiety as quinovic acid (3–5).

The ^{1}H- and ^{13}C-nmr anomeric signals of a fucose (H-1′, δ 4.28, d, J=7.0 Hz; C-1′, 106.9 ppm) and of a glucose (H-1″, δ 4.58, d, J=7.5 Hz; C-1″, 106.0 ppm) confirmed that the two sugars, both in the β-D-pyranosyl form, were occurring in **3** as a disaccharide attached to the aglycone through a β-fucoside linkage. In fact, the other ^{13}C-nmr sugar signals (Table 1) were in agreement with a terminal glucose unit (6) linked at C-3 of an inner fucose unit. Compared with methyl-β-D-fucopyranoside (6), the C-3 of the fucose was shifted downfield by 7.0 ppm (from 75.2 to 82.4) as expected for a glycosidation shift. Furthermore, the aglycone signal at 90.9 ppm (CH, by DEPT) was superimposable on the same signal of oleanolic acid model glycosidate at C-3, in CD_3OD (7), and indicated that this carbon supported the sugar chain. From these data the structure quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside] was assigned to **3**.

A detailed comparison of nmr spectral data showed that the disaccharide chain located at C-3 of quinovic acid is identical for compounds **1**, **2**, and **3** (Tables 1,2).

The presence in **1** and **2**, with respect to **3**, of an additional glucose unit linked at a carboxyl group (C-27 or C-28) of quinovic acid was confirmed by an extra anomeric signal (^{1}H nmr H-1‴, δ 5.42, d, J=7.5 Hz; ^{13}C nmr C-1‴, 95.8 ppm).

The glycosyl ester linkage was proposed to be at C-28 in **2** and at C-27 in **1** on the

TABLE 1. ^{13}C-nmr Spectral Data for Compounds **1**, **2**, and **3**.

Aglycone carbon	Compound			DEPT	Sugar carbon	Compound		DEPT
	1	**2**	**3**			**1** and **2**	**3**	
1	40.0	39.9	39.9	CH_2				
2	27.3	27.1	27.1	CH_2	Fu[b]-1′	106.9	106.9	CH
3	90.8	90.9	90.9	CH	Fu-2′	71.9	71.9	CH
4	40.3	40.3	40.2	C	Fu-3′	82.4	82.4	CH
5	57.0	56.8	56.8	CH	Fu-4′	73.7	73.7	CH
6	19.4	19.4	19.6	CH_2	Fu-5′	71.3	71.3	CH
7	37.9	37.9	38.0	CH_2	Fu-6′	17.4	17.4	CH_3
8	40.9	40.7	40.8	C				
9	48.0[a]	47.9[a]	47.9[a]	CH	G[c]-1″	106.0	106.0	CH
10	38.0	37.9	38.1	C	G-2″	76.0	76.1	CH
11	24.0	24.1	24.0	CH_2	G-3″	78.6	78.4	CH
12	130.8	129.9	129.0	CH	G-4″	71.9	71.9	CH
13	133.7	134.7	135.5	C	G-5″	78.1	78.1	CH
14	57.7	59.2	59.1	C	G-6″	63.1	63.2	CH_2
15	26.7	26.9	27.1	CH_2				
16	25.9	26.2	26.4	CH_2				
17	48.1[a]	48.1[a]	48.1[a]	C				
18	55.5	55.7	56.3	CH	Glucose at			
19	40.1	40.1	40.2	CH	C-27 or			
20	38.3	38.2	38.6	CH	C-28			
21	31.2	31.3	31.8	CH_2	G-1‴	95.8		CH
22	37.1	37.3	38.0	CH_2	G-2‴	74.1		CH
23	19.3	19.4	19.6	CH_3	G-3‴	78.5		CH
24	28.6	28.6	28.7	CH_3	G-4‴	71.7		CH
25	16.9	16.9	16.9	CH_3	G-5‴	78.5		CH
26	18.1	18.4	18.6	CH_3	G-6‴	62.9		CH_2
27	178.1	179.5	179.5	C				
28	182.0	178.5	182.0	C				
29	17.1	17.1	17.1	CH_3				
30	21.3	21.3	21.6	CH_3				

[a]Under CD_3OD signal.
[b]Fucose.
[c]Glucose.

basis both of the significantly different resonances of C-12, C-13, C-14, and of -COOH groups in the ^{13}C-nmr spectra, and of the Me-26 and H-12 resonances in the ^{1}H-nmr spectra.

In compound **2**, C-12 and C-13 resonated at 129.9 and 134.7 ppm, respectively. These values were similar to those found in **3** and usually observed in a urs-12-en-27,28-dioic acid with the unsubstituted COOH group at C-14 (4,8).

In compound **1** were observed a downfield shift, with respect to **2**, of C-12 by 0.9 ppm (130.8 ppm) and upfield shifts of C-13 by 1.0 ppm (133.7 ppm) and of C-14 by 1.5 ppm (γ effect, from 59.2 to 57.7). The esterification of the C-27 carboxyl group caused a large deviation of C-12, C-13, and C-14 as previously described in quinovic acid and dimethylquinovate (8). Furthermore, in compound **2** a free carboxyl group resonated at 179.5 ppm as did the C-27 in compound **3**, and a glycosylated carboxyl group resonated at 178.5 ppm (C-28), whereas in **3** C-28 resonance appeared at 182.0 ppm in agreement with an oleanolic acid model (7).

In compound **1** the glycosylation at C-27 shifted this signal to higher field (178.1), while the C-28 signal (182.0 ppm) was diagnostic of a 28 free carboxyl group.

TABLE 2. ^{1}H-nmr Data for Compounds **1**, **2**, and **3**.

Aglycone proton	Compound		
	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 Me-30 signal
Me-29 and Me-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-12 (1H, m)	5.64	5.59	5.59
Sugar proton[a]			
Fu[b]-CH_3 (3H, d, J=6.0 Hz)	1.33	1.33	1.33
H-1′ (1H, d, J=7.0 Hz)	4.28	4.28	4.28
H-1″ (1H, d, J=7.5 Hz)	4.60	4.59	4.58
H-1‴ (1H, d, J=7.5 Hz)	5.42	5.42	

[a]Other signals were overlapped in the region δ 3.0–4.0.
[b]Fucose.

In accordance with a C-27 glycosylated quinovic acid structure, the ^{1}H-nmr spectrum of **1** showed the Me-26 signal deshielded by 0.03 ppm [from δ0.89 in **2** to δ0.92 in **1**] and the H-12 olefinic proton deshielded by 0.05 ppm (from δ 5.59 in **2** and **3** to δ 5.64 in **1**), while the other aglycone signals were identical in **1** and **2**.

On basic hydrolysis, both compounds **1** and **2** gave glycoside **3**; on acetylation **1** and **2** gave the peracetyl derivatives **1a** and **2a** which could be readily methylated with CH_2N_2 to yield the peracetyl monomethyl ester derivatives **1b** and **2b**. The proton spectrum of **1a** ($CDCl_3$, see Experimental) showed the Me-26 and H-12 signals shifted downfield compared with **2a**, as observed for the corresponding proton resonances in **1** and **2**.

In the ^{1}H-nmr spectra of **1b** and **2b** ($CDCl_3$) appeared a -$COOCH_3$ signal at δ 3.64 (3H, s) and at δ 3.63 (3H, s), respectively, while the other proton signals were superimposable. This observation finally confirmed that **2a** contained a 28 free carboxyl group and **1a** contained a 27 free carboxyl group.

These data led to the assignment of the structure quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1↦3)-β-D-fucopyranosyl]-(27↦1)-β-D-glucopyranosyl ester to **1** and quinovic acid-3β-*O*-[β-D-glucopyranosyl-(1↦3)-β-D-fucopyranosyl]-(28↦1)-β-D-glucopyranosyl ester to **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker MW-250 Spectrospin; ms, Kratos MS 902 spectrometer equipped with Kratos fab source; hplc, Waters Model 6000 A pump equipped with a U6K injector and a model 401 refractive index detector; glc, Perkin-Elmer Sigma 115 Instrument with an SE-30 20 m column; optical rotation, Perkin-Elmer 241 polarimeter. The fabms spectra were obtained, in negative ion mode, by dissolving the samples in a glycerol-thioglycerol 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 the CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz.

PLANT MATERIAL.—The bark of *U. tomentosa* was collected at Lima, Peru, and identified by Edmundo Szeliga of the Instituto Peruano de Investigacion Fitoterapica Andina. A voucher sample of the plant is deposited at the Herbarium of this Institute.

EXTRACTION AND ISOLATION.—The bark of the air-dried liane (500 g) was defatted in a Soxhlet with light petroleum ether and $CHCl_3$ and then extracted with $CHCl_3$-MeOH (9:1) to give 7.8 g of residue. Part of the residue (4.4 g) was chromatographed on a Sephadex LH-20 column (80 × 4 cm). Fractions (8 ml) were eluted with MeOH and checked by tlc [Si gel, *n*-BuOH-HOAc-H_2O (60:15:25)]. Fractions 22–24 I (218 mg) contained a mixture of the more polar glycosides **1** and **2**, fractions 25–27 II (311 mg) contained the less polar one **3**. Both mixtures were submitted to hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 3.5 ml/min) using MeOH-H_2O (55:45) as eluent to give pure **1** (10 mg) and **2** (25 mg) from mixture I and MeOH-H_2O (65:35) to obtain pure **3** (23 mg) from mixture II.

Acidic methanolysis.—Methanolysis of each glycoside (0.5–1 mg) was achieved as described earlier (9) to obtain methylglucoside and methylfucoside in the ratio 2:1 from **1** and **2** and in the ratio 1:1 from **3**.

Alkaline hydrolysis.—The glycosides **1** (5 mg) and **2** (10 mg) in 0.5 M KOH (1 ml) were separately heated at 110° in a stoppered reaction vial for 2 h. The reaction mixture was adjusted to pH 7 with HCl and then extracted with *n*-BuOH. The organic phase was evaporated to dryness. Both glycosides gave compound **3** identified by tlc and ^{1}H nmr.

Acetylation and methylation.—Glycosides **1** and **2** were acetylated as usual with Ac_2O/pyridine to give **1a** and **2a**, which were then methylated with CH_2N_2 to yield **1b** and **2b**.

Compound **1**.—Hplc retention time 26.3 min; $[\alpha]^{25}D$ +8° (*c* = 1, MeOH); fabms see text; ^{1}H and ^{13}C nmr (CD_3OD) see Tables 1,2.

Compound **1a**.—^{1}H nmr ($CDCl_3$) δ 0.71 (3H, s, Me-23), 0.83 (3H, s, Me-26), 0.87 and 0.92 (overlapping signals, 4 Me), 1.30 (3H, d, *J* = 6 Hz, 5-Me of fucose), 2.00–2.14 (10-Ac), 3.65 (2H, m, H-5′ and H-3′), 3.80–3.90 (2H, m, H-5″ and H-5‴), 4.10–4.30 (4H, m, H-6″ and H-6‴), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 4.54 (1H, d, *J* = 8 Hz, H-1″), 4.95–5.30 (7H, m, H-2′, 2″, 2‴, 3″, 3‴, 4″, 4‴), 5.46 (1H, m, H-4′), 5.62 (1H, d, *J* = 8 Hz, H-1‴), 5.75 (1H, m, H-12).

Compound **1b**.—^{1}H nmr ($CDCl_3$) δ 0.71, 0.83, 0.86, 0.91 (6 Me), 1.30 (3H, d, *J* = 6 Hz, 5-Me of fucose), 1.98–2.10 (10 Ac), 3.64 (3H, s, -COOMe), 4.46 (1H, d, *J* = 7.5 Hz, H-1′), 4.52 (1H, d, *J* = 8 Hz, H-1″), 5.60 (1H, d, *J* = 8 Hz, H-1‴), 5.73 (1H, m, H-12).

Compound **2**.—Hplc retention time 22.2 min; $[\alpha]^{25}D$ +9° (*c* = 1, MeOH); fabms see text; ^{1}H and ^{13}C nmr (CD_3OD) see Tables 1,2.

Compound **2a**.—^{1}H nmr ($CDCl_3$) δ 0.72 (3H, s, Me-23), 0.80 (3H, s, Me-26), 0.87 and 0.92 (overlapping signals, 4 Me), 1.31 (3H, d, *J* = 6 Hz, 5-Me of fucose), 2.00–2.14 (10 Ac), 4.48 (1H, d, *J* = 7.5 Hz, H-1′), 4.53 (1H, d, *J* = 8 Hz, H-1″), 5.61 (1H, d, *J* = 8 Hz, H-1‴), 5.71 (1H, m, H-12).

Compound **2b**.—^{1}H nmr ($CDCl_3$) superimposable on that of compound **1b**.

Compound **3**.—Hplc retention time 23.5 min; $[\alpha]^{25}D$ +17° (*c* = 1, MeOH); fabms see text; ^{1}H and ^{13}C nmr see Tables 1,2.

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