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QUASSINOIDS FROM *QUASSIA AMARA*

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Key Word Index—*Quassia amara*; Simarubaceae; wood; quassinoid glycosides; quassinoids.

Abstract—Three new quassinoids, 11-α-*O*-(β-D-glucopyranosyl)-16-α-*O*-methylneoquassin, 1-α-*O*-methylquassin and 12-α-hydroxy-13,18-dehydroparain have been isolated and identified from *Quassia amara* wood. In addition, 16-α-*O*-methylneoquassin and 11-acetylparain were isolated for the first time from a natural source. The structures of all compounds were determined by spectroscopical methods as well as by chemical correlations. The reaction products of the $NaBH_4$ reduction and of the alkaline isomerization of parain were also investigated.

INTRODUCTION

Quassia amara wood is still widely used in traditional medicine and recently some quassinoids and quassinoid glycosides isolated from plants of the Simarubaceae have received renewed attention due to their biological activity as potential antitumour agents [1–6] as well as antiulcer agents [7]. Stimulated by these findings we continued our research [8–10] on the minor components from *Quassia* wood.

This paper describes the isolation and identification of a new quassinoid glycoside, namely 11-α-*O*-(β-D-glucopyranosyl)-16-α-*O*-methylneoquassin (**1**) besides the known methylglucoside **4** from methanol extracts of the wood, and of the two new quassinoids, 1-α-*O*-methylquassin (**10**) and 12-α-hydroxy-13,18-dehydroparain (**19**) from chloroform extracts. Moreover, 16-α-*O*-methylneoquassin (**6**) and 11-acetylparain (**12**) have been isolated for the first time as natural products. The chemical and stereochemical structures of all the compounds have been assigned by means of spectral studies (IR, ^1H and ^{13}C NMR) and by chemical correlations. (Schemes 1–4). The products of the alkaline isomerization of parain (**11**) and of its reduction with $NaBH_4$, carried out in the presence and in the absence of NaOH, have been also investigated. (Scheme 3).

RESULTS AND DISCUSSION

The ^1H NMR (400 MHz) and ^{13}C NMR (50.32) spectra of **1** and its acetylderivative **1a** suggested the presence of β-D-glucopyranose and of a trimethoxylated quassinoid aglycone moiety, in which the relative configuration at C-16 (β-H) followed by comparison with the known compound **21** [8] (Tables 1–3). Acid hydrolysis of **1** [11], followed by CrO_3 oxidation, afforded 16-α-*O*-methylneoquassin which proved to be identical with the natural product **6** (Scheme 1, Tables 1 and 3). Moreover, the structure of **6** was proved by its identity with the methylated derivative (**7a**) of neoquassin (**7**) (TLC and ^1H NMR). Thus, the spectroscopical and chemical evidences provided a corroboration for the nature of **1** as an unusual 11-α-*O*-glucoside of 11-α-hydroxy-16-α-*O*-methylneoquassin (**2**) [12, 14].

The structure and stereochemistry of the new quassinoid 1-α-*O*-methylquassin (**10**) was assigned by means of ^1H and ^{13}C NMR spectroscopy (Tables 1 and 3), as well as by chemical correlation with an authentic sample of quassin (**8**) (Scheme 2). Analogously the structure and the stereochemistry of the new quassinoid 12-α-hydroxy-13,18-dehydroparain **19** has been achieved by ^1H and ^{13}C NMR examination and through its acetonide derivative **20** (Scheme 4, Tables 2 and 3).

The structures of the natural products 11-acetylparain (**12**) and 16-α-*O*-methylneoquassin (**6**) also resulted from spectroscopic evidence (^1H and ^{13}C NMR spectra in Tables 2 and 3) and from the chemical transformations reported in Schemes 1 and 3.

The reaction of parain **11** with $NaBH_4$/NaOH (Scheme 3) led, as expected [15], to the regioselective reduction of the carbonyl groups at C(1) and C(12). In fact, only one compound was isolated which was identified as 1-hydroxy-12-α-hydroxyparain (**14**) successively converted into the triacetylated derivative **14a**.

The stereochemistry at C-11 and C-12 in **14** was assigned on the basis of ^1H NMR evidence (coupling constants of H-9, H-11 and H-12 are shown in Table 2) and chemical correlations. Firstly, **14** was acetonylated to

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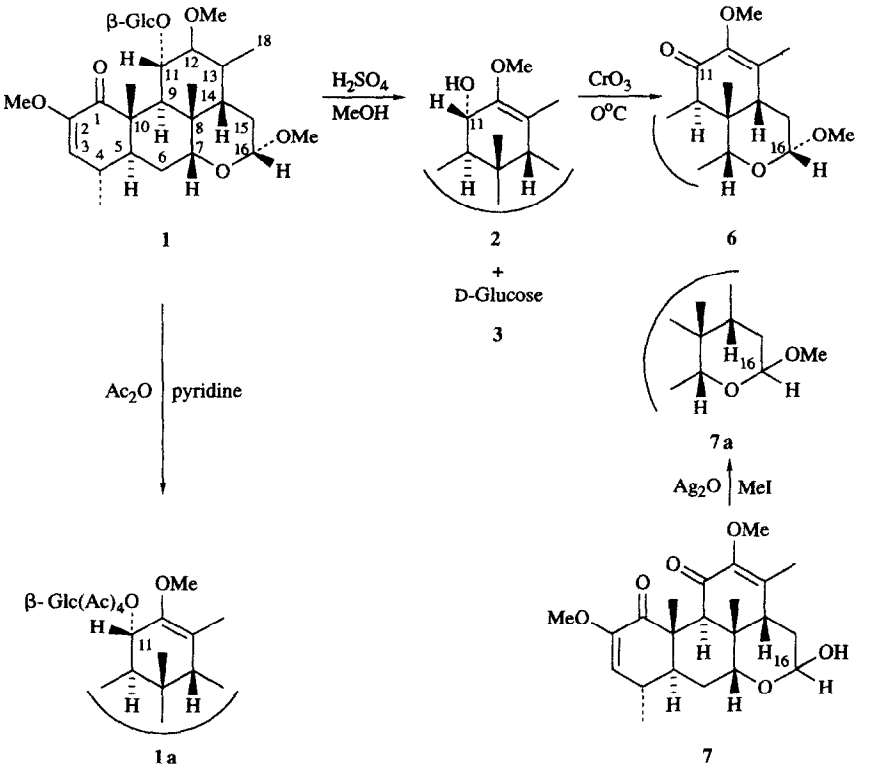
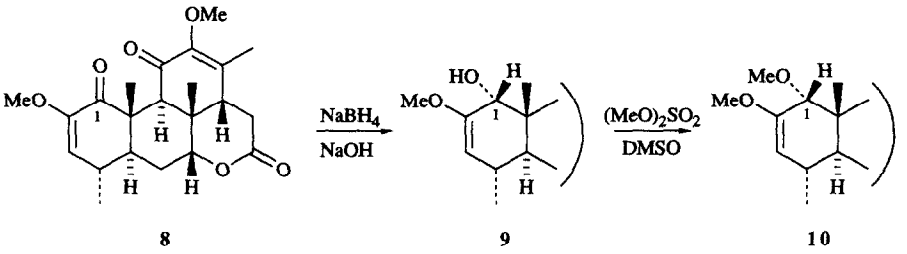


Scheme 1.



Scheme 2.

give **15** which was then treated with CrO_3 to yield **16**. The same compound **16** was obtained from paraine (**11**) in three steps, viz: by reduction with $NaBH_4$, followed by acetonylation and further Ag_2O oxidation (Scheme 3). In fact, the treatment of **11** with $NaBH_4$ in the absence of alkali reduced both the carbonyl group at C-12 and the lactone group, whereas the α,β-unsaturated ketone remained unchanged.

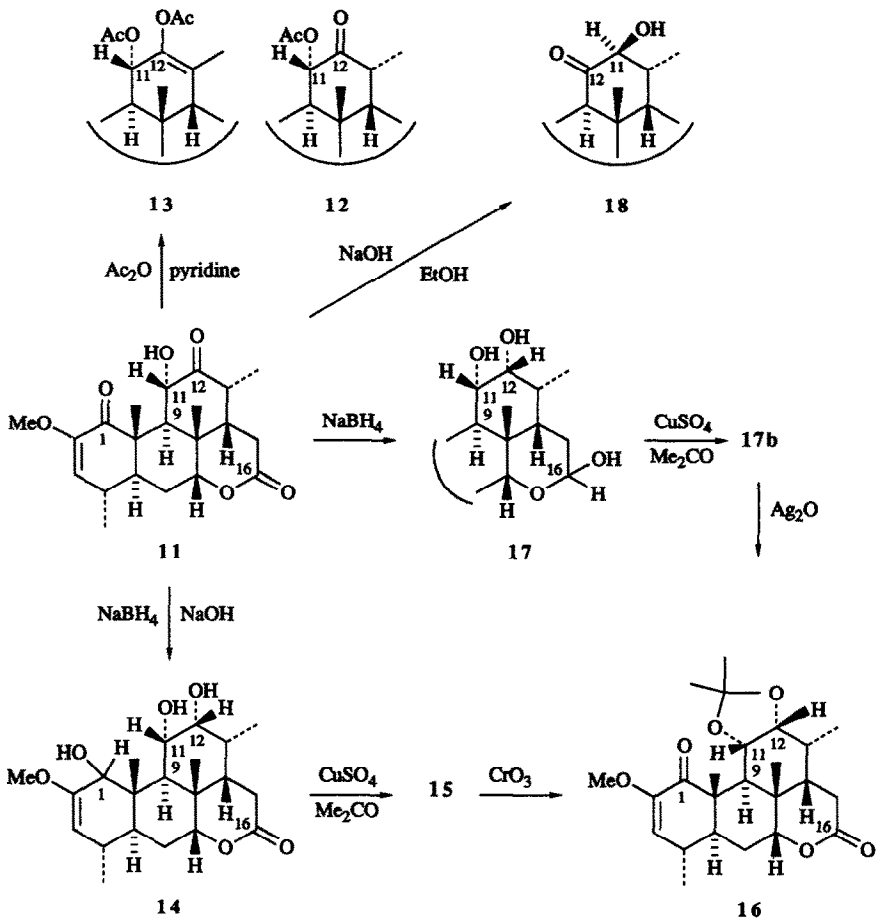
Unlike the case reported for quassin (**8**) [15], reduction of the C-1 carbonyl of **11** with $NaBH_4$/NaOH gave **14** which furnished ^1H and ^{13}C NMR data which were not diagnostic for the C-1 stereochemistry (Tables 2 and 3), probably because of the absence of a carbonyl in C-11, while reduction of the C-12 carbonyl group of **11** occurred with α-hydroxystereoselectivity (**14** and **17**) in the two different reaction conditions (Schemes 2 and 3).

Finally, to correlate natural isoparain (**18**) with parain (**11**), we have submitted **11** to alkaline isomerization with NaOH/EtOH obtaining **18** as the major reaction product. The further acetylation of **18**, either natural or semisynthetic, gave the same monoacetyl derivative **18a** (Scheme 3).

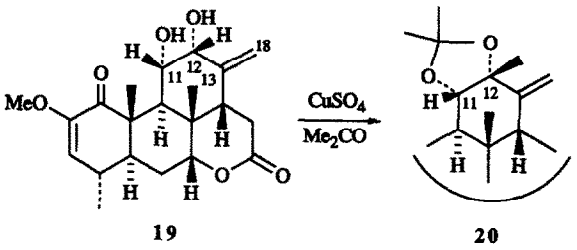
EXPERIMENTAL

General. Mps: uncorr. IR: $CHCl_3$ or KBr. UV: MeOH soln. ^1H NMR were recorded at 400 and 200 MHz using TMS as int. standard. ^{13}C NMR were recorded at 50 MHz. Merck DC-Alufolien Kieselgel 60 F_{254} and Merck Kieselgel 60 were used for TLC and CC. A voucher specimen of *Quassia amara* L. had been deposited at the Herbarium of the Institute of Vegetal Biology, University of Perugia, Italy [8].

Extraction. Wood chips were exhaustively extracted with MeOH at room temp. and the extract evapd *in vacuo* as described in ref. [8]. H_2O and MeOH were then added



Scheme 3.



Scheme 4.

to the residue and the mixt. extracted with $CHCl_3$. The aq. alcohol sol. fr. was evapd to dryness yielding a crude powdered residue which was repeatedly treated with MeOH. The MeOH extract was then evapd *in vacuo* and the residue (125 g) was repeatedly flash-chromato graphed on silica gel columns. Elution with solvents of increasing polarity $CHCl_3 \rightarrow CHCl_3$–MeOH (4:1), $CHCl_3 \rightarrow CHCl_3$–MeOH–$H_2O$ (21:21:8) yielded *inter alia*, methyl-D-glucoside (**4**) (320 mg) and 11-α-*O*-(β-D-glucopyranosyl)-16-α-*O*-methylneoquassin (**1**) (72 mg).

The $CHCl_3$ sol. fr. was evapd under red. pres. and the residue extracted with petrol as described in ref. [8]. The solvent was evapd to give 240 g of an oily residue, 9 g of which was dissolved in $CHCl_3$ and repeatedly flash-chromatographed on silica gel columns using eluents of increasing polarity $CHCl_3 \rightarrow CHCl_3$–MeOH (9:1). Quassin (**8**) (205 mg), 11-α-acetylparain (**12**) (40 mg), parain (**11**) (120 mg), isoparain (**19**) (20 mg), [8], 11-dihydro-12-norneoquassin (**21**) (30 mg) [8], 16,α-O-methylneoquassin (**6**) (25 mg) and neoquassin (**7**) (38 mg) were isolated and identified.

11-α-O-(β-*glucopyranosyl*)-16-α-O-*Methylneoquassin* (**1**). Isolated from MeOH extract, yield 52 mg, powder from EtOH, mp < 200° dec. IR ν_{max}^{KBr} cm^{-1}: 3500–3300, 2980, 1430, 1070–1050. ^1H NMR: Table 1; ^{13}C NMR: Table 3.

Acetylation of compound **1**. Compound **1** (10 mg) was acetylated in Ac_2O–pyridine at room temp. Usual work-up and chromatographic purification yielded 8 mg of the pure tetra-acetylated derivative **1a**. ^1H NMR: Table 1.

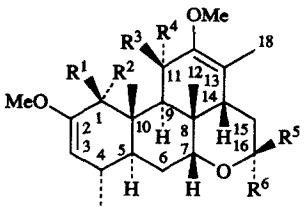
Acid hydrolysis of compound **1**. A soln of **1** (40 mg) in 1.5 M aq. alcoholic H_2SO_4 (15 ml) was refluxed for 6 hr and then poured into H_2O (15 ml) [16]. After concn under red. pres. the aq. residue was extd with $CHCl_3$. The combined $CHCl_3$ exts were dried over Na_2SO_4, evapd and chromatographed to give 23 mg 11-α-OH-16-α-*O*-methylneoquassin (**2**). ^1H and ^{13}C NMR: (Tables 1 and 3).

Oxidation of compound **2**. Compound **2** (20 mg) in Me_2CO was treated at 0° with Jones reagent. Usual work-up of the reaction mixt. and chromatographic

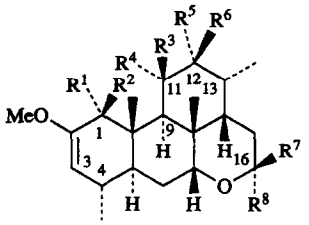
Table 1. ^{1}H NMR spectral data for compounds **1–10** (200 MHz, 400 MHz*, $CDCl_3$, pyridine-d_5†)

H	1*†‡	1a†§	2†	6*†	7	7a	8	9	10
1	—	—	—	—	—	—	—	4.55 *s*	4.48 *s*
3	5.25 *d* (5)	5.20 *d* (6)	5.25 *d* (5)	5.30 *d* (5)	5.35 *d* (5)	5.30 *d* (5)	5.30 *d* (3)	4.65 *d* (3)	4.68 *d* (3)
7	3.62 *m*	3.60 *m*	3.70 *m*	3.62 *m*	3.50 *m*	3.55 *m*	3.60 *m*	4.29 *m*	4.30 *m*
9	3.05 *d* (13)	3.00 *d* (12)	3.00 *d* (13)	3.21 *s*	3.20 *s*	3.20 *s*	3.15 *s*	3.38 *s*	3.25 *s*
11	5.05 *d* (13)	5.09 *d* (12)	4.27 *d* (13)		—	—	—	—	—
16	4.80 *d* (3)	4.80 *d* (3)	4.82 *d* (3)	4.80 *d* (3)	4.90 *d* (3)	4.85 *m*	—	—	—
OMe-2	3.60 *s*	3.60 *s*	3.60 *s*	3.61 *s*	3.63 *s*	3.65 *s*	3.62 *s*	3.55 *s*	3.58 *s*
OMe-12	3.65 *s*	3.68 *s*	3.65 *s*	3.67 *s*	3.70 *s*	3.70 *s*	3.69 *s*	3.60 *s*	3.65 *s*
OMe-16	3.40 *s*	3.42 *s*	3.45 *s*	3.39 *s*	—	—	—	—	—
Me-4	1.15 *d* (3)	1.15 *d* (2)	1.14 *d* (3)	1.13 *d* (3)	1.10 *d* (6)	1.12 *d* (6)	1.12 *d* (5)	1.00 *d* (3)	1.08 *d* (3)
Me-8	1.28 *s*	1.28 *s*	1.26 *s*	1.30 *s*	1.20 *s*	1.19 *s*	1.25 *s*	1.23 *s*	1.22 *s*
Me-10	1.58 *s*	1.60 *s*	1.58 *s*	1.57 *s*	1.56 *s*	1.56 *s*	1.57 *s*	1.10 *s*	1.12 *s*
Me-13	1.82 *s*	1.80 *s*	1.80 *s*	1.85 *s*	1.85 *s*	1.85 *s*	1.85 *s*	1.88 *s*	1.86 *s*

‡ Besides 4.95 (1 ×, *d*, J = 42 Hz) H-anom and 3.55–4.00 (6H, *m*) H-Glc.
§ Besides 4.98 (1H, *d*, J = 12 Hz) H-anom and 4.20–4.55 (6H, *m*)H-Glc.



	R^1	R^2	R^3	R^4	R^5	R^6
1	O	O	O-Glc	H	H	OMe
2	O	O	OH	H	H	OMe
6	O	O	O	O	H	OMe
7	O	O	O	O	H,OH	H,OH
8	O	O	O	O	O	O
9	OH	H	O	O	O	O
10	OMe	H	O	O	O	O
13	O	O	OAc	H	O	O



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
11	O	O	H	OH	O	O	O	O
12	O	O	H	OAc	O	O	O	O
14	H	OH	H	OH	OH	H	O	O
14a	H	OAc	H	OAc	OAc	H	O	O
15	H	OH	H	O-C(Me)$_2$-O		H	O	O
16	O	O	H	O-C(Me)$_2$-O		H	O	O
17	O	O	H	OH	OH	H	H,OH	H,OH
18	O	O	O	O	H	OH	O	O
18a	O	O	O	O	H	OAc	O	O
19*	O	O	H	OH	OH	H	O	O
20	O	O	H	O-C(Me)$_2$-O		H	O	O
21	O	O	H	OH	O	O	OH	H

* 13,18 - Dehydro

purification yielded 14 mg of a pure product which proved to be 16-α-*O*-methylneoquassin (**6**) identical by TLC, ^{1}H and ^{13}C NMR (Tables 1 and 3), with the natural product **6** isolated from the $CHCl_3$, extract.

Methylation of compound **3**. The aq. layer obtained from acid hydrolysis of **1** was chromatographed through a short column of anion-exchange resin (Amberlite RA 400, OH-form), treated with EtOH and evapd *in vacuo* to give an amorphous residue which was dried in the presence of P_2O_5 for 7 hr under red. pres. at 45°. The powdered product **3** (5 mg) obtained was identified as D-glucose by TLC comparison with an authentic sample [17]. Compound **3** was then suspended in dry MeOH (5 ml), previously satd, with gaseous HCl, and kept for 16 hr at 70° in a sealed tube. After evapn of solvent, the solid residue was suspended in H_2O, extd with EtOAc and the combined organic exts, dried over Na_2SO_4, were evapd at room temp. under N_2. The residue (3 mg) was identical by TLC (silica gel, $CHCl_3$–MeOH, 9:1 and EtOAc–C_6H_6, 1:1) and by ^{1}H NMR, to a synthetic mixture of methyl α,β-D-glucoside prepared from an authentic sample of D-glucose. It was also identical to the natural compound **4** (5 mg) isolated from the MeOH-sol. extract.

Acetylation of compound **4**. Three samples (10 mg each) of **4**, the natural one, the one obtained from the methylation of **3** and a synthetic mixt. of methyl-α,β-D-glucoside were acetylated in Ac_2O–pyridine to yield the same tetra-acetyl-α,β-D-glucoside (**5**).

Methylation of compound **7**. To a soln. of 50 mg of neoquassin (**7**) in 8 ml of DMFA were added 70 mg MeI and 70 mg Ag_2O and the reaction mixt. kept in a sealed tube for 17 hr at 85°. The reaction mixt. was then poured into H_2O (30 ml) and the soln extracted with $CHCl_3$. The combined $CHCl_3$ exts were repeatedly washed with H_2O, dried over Na_2SO_4 and evapd *in vacuo*. CC purification afforded 28 mg of 16-*O*-methylneoquassin (**7a**), which was identical, by TLC with natural product **6** and with **6** obtained from **1** as shown in Scheme 1. ^{1}H NMR: Table 1. ^{13}C NMR: Table 3.

Table 2. ^{1}H NMR spectral data for compounds **11–21** (200 MHz, $CDCl_3$)

H	11	12	13	14	14a	15	16	17	17a	18	18a	19	20	21
1	—	—	—	4.05 *s*	5.03 *s*	4.03 *s*	—	4.03 *s*	5.0 *s*	—	—	—	—	—
3	5.40 *d* (3)	5.21 *d* (3)	5.28 *d* (3)	4.81 *d* (4)	4.80 *d* (4)	4.80 *d* (4)	5.25 *d* (4)	4.80 *d* (4)	5.40 *d* (4)	5.30 *d* (5)	5.35 *d* (5)	5.38 *d* (3)	5.35 *d* (3)	5.38 *d* (2)
7	4.30 *m*	4.32 *m*	4.22 *m*	4.30 *m*	4.30 *m*	4.30 *m*	4.00 *m*	4.35 *m*	4.30 *m*	3.90 *m*	3.80 *m*	3.98 *m*	3.85 *m*	4.35 *d* (3) *d* (2)
9	2.48 *d* (12)	2.81 *d* (12)	2.79 *d* (12)	2.62 *d* (12)	2.80 *d* (13)	2.65 *d* (13)	2.85 *d* (13)	2.70 *d* (12)	2.75 *d* (13)	3.0 *s*	3.06 *s*	2.97 *d* (12)	2.98 *d* (13)	2.66 *d* (12)
11	4.37 *d* (12)	5.31 *d* (12)	5.70 (12)	3.36 *d* (12) *d* (4)	4.35 *d* (13) *d* (3)	3.50 *d* (13) *d* (2)	3.52 *d* (13) *d* (2)	3.40 *d* (12) *d* (4)	4.45 *d* (13) *d* (13)	—	—	3.55 *d* (12) *d* (4)	3.60 *d* (13) *d* (2)	4.35 *d* (12)
12	—	—	—	3.57 *d* (4) *d* (2)	4.58 *d* (3) *d* (3)	3.66 *d* (2) *d* (2)	3.68 *d* (2) *d* (2)	3.55 *d* (4) *d* (2)	4.52 *d* (3) *d* (2)	3.52 *d* (2)	4.50 *d* (2)	3.41 *d* (4)	3.48 *d* (2)	—
16	—	—	—	—	—	—	—	4.96 *d* (3)	5.61 *d* (3)	—	—	—	—	4.75 *d* (10) *d*(7) *d* (3)
18	1.83 *d* (6)	1.85 *d* (6)	1.62 *s*	1.85 *d* (6)	1.79 *d* (6)	1.87 *d* (6)	1.80 *d* (6)	1.80 *d* (6)	1.78 *d* (6)	1.85 *d* (6)	1.82 *d* (5)	5.02 *s* 4.97 *s*	5.00 *s* 4.89 *s*	— 1.85 *d* (5)
OMe-2	3.58 *s*	3.58 *s*	3.57 *s*	3.35 *s*	3.40 *s*	3.38 *s*	3.40 *s*	3.44 *s*	3.49 *s*	3.60 *s*	3.60 *s*	3.66 *s*	3.65 *s*	3.58 *s*
Me-4	1.04 *d* (6)	1.08 *d* (6)	1.14 *d* (6)	1.05 *d* (6)	1.08 *d* (4)	1.05 *d* (6)	1.06 *d* (5)	1.09 *d* (6)	1.10 *d* (6)	1.12 *d* (5)	1.09 *d* (6)	1.21 *d* (5)	1.20 *d* (6)	1.09 *d* (4)
Me-8	1.32 *s*	1.37 *s*	1.36 *s*[a]	1.19 *s*	1.16 *s*	1.28 *s*	1.25 *s*	1.28 *s*	1.20 *s*[b]	1.32 *s*	1.30 *s*	1.30 *s*	1.32 *s*	1.38 *s*
Me-10	1.52 *s*	1.53 *s*	1.38 *s*[a]	1.09 *s*	1.02 *s*	1.14 *s*	1.58 *s*	1.06 *s*	1.15 *s*[b]	1.66 *s*	1.58 *s*	1.54 *s*	1.58 *s*	1.50 *s*
O_2C Me_2	—	—	—	—	—	1.36 *s* 1.59 *s*	1.35 *s* 1.60 *s*	—	—	—	—	—	1.39 *s* 1.62 *s*	— —
OAc	—	2.10 *s*	2.12 *s* 2.09 *s*	—	2.08 *s* 2.10 *s* 2.11 *s*	—	—	—	2.06 *s* 2.12 *s*	—	2.13 *s*	—	—	—

[a,b] Values may be interchanged.

Table 3. ^{13}C NMR spectral data for compounds **1**, **2**, **6**, **8**, **10–12**, **14**, **17–19** and **21** (50 MHz) ($CDCl_3$, *pyridine-d_5)

C	1*	2	6	8	10	11	12	14	17	18	19	21
1	193.1	193.6	197.8	197.5	70.3[e]	197.7	196.8	72.0[e]	209.4	208.8	208.2	211.3[f]
2	148.0	147.5	140.1	147.9	150.9	146.8	146.5	153.9	148.2	148.5	147.5[d]	148.7
3	116.5	116.4	116.1	116.3	104.4	114.0	113.9	101.6	116.5	116.2	115.8	117.7
4	31.3	30.9	30.8	30.7	30.9	31.6	32.0	31.8	30.9	32.3	30.8	41.3
5	45.8	44.4	42.9	43.0	44.9	43.1	45.0	42.9	44.2	45.0	43.2	42.7
6	25.1[a]	25.3[a]	25.4[a]	25.4[a]	25.2[a]	24.9[d]	25.2[a]	25.8[a]	26.0[a]	25.5[a]	25.9[a]	26.1[a]
7	68.1	68.2	68.4	81.5	82.8[e]	80.2[e]	80.8[e]	81.4[e]	78.9[e]	82.0[e]	82.6	75.2[e]
8	38.2	36.0	36.5	36.7	37.1	35.9	36.8	37.0	36.6	35.9	35.8	37.3
9	46.3[b]	44.1[b]	48.2[b]	45.6[b]	46.0[b]	42.9[b]	42.5[b]	42.8[b]	43.6[b]	43.1[b]	42.5[b]	42.9[b]
10	40.1	42.3	45.4	45.5	41.9	43.3	44.0	45.2	40 9	44.4	46.0	48.7
11	90.8	79.8	192.1	190.7	192.0	76.0[e]	80.1[e]	75.8[e]	75.9[e]	206.1	75.6	77.3[e]
12	139.9	140.0	139.8	138.0	139.8	204.8	205.6	76.4[e]	76.6[e]	77.5[e]	77.1	204.4[f]
13	148.2	148.5	147.4	147.6	147.5	39.6	42.9	43.0	43.2	46.0	146.1[d]	50.6
14	49.6[b]	48.6[b]	45.8[b]	45.9[b]	48.8[b]	44.9[b]	45.1[b]	44.3[b]	44.8[b]	44.6[b]	44.5[b]	44.4[b]
15	29.6[a]	30.2[a]	28.9[a]	31.9[a]	30.7[a]	30.9[a]	31.6[a]	31.1[a]	30.8[a]	31.5[a]	31.5[a]	31.2[a]
16	95.7	96.1	96.6	168.7	168.9	169.4	169.0	169.5	98.2	168.8	169.3	97.0
18	13.1[d]	13.0[d]	14.8[d]	14.9[d]	15.1[d]	12.3[d]	10.5[d]	10.7[d]	10.9[d]	11.3[d]	114.3	11.0[d]
19	12.7[d]	12.9[d]	12.4[d]	12.3[d]	12.7[d]	12.2[d]	12.7[d]	12.6[d]	12.5[d]	12.3[d]	12.5	12.3[d]
30	19.4[c]	18.9[c]	19.2[c]	19.0[c]	19.2[c]	18.9[c]	19.3[c]	18.8[c]	19.2[c]	19.0[c]	18.8[c]	19.2[c]
Me-4	19.3[c]	20.1[c]	21.5[c]	21.8[c]	19.0[c]	20.8[c]	21.1[c]	19.8[c]	20.4[c]	20.8[c]	20.9[c]	21.2[c]
OMe	54.8	55.1	54.3	54.6	54.4	54.5	54.6	54.3	55.0	54.8	55.2	54.9
	59.0	59.0	58.6	58.9	59.1	—	—	—	—	—	—	—
	55.8	55.9	53.8	—	58.6	—	—	—	—	—	—	—
OAc	—	—	—	—	—	—	168.8 21.3[c]	—	—	—	—	—
1′	106.4											
2′	78.6											
3′	77.4	—	—	—	—	—	—	—	—	—	—	—
4′	77.0											
5′	75.4											
6′	59.9											

Signals with the same letters in the same vertical column may be reversed.

16-α-O-*Methylneoquassin* (**6**). Isolated from $CHCl_3$ extract. Yield 75 mg, powder from MeOH, mp 173–176°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 1680, 1640. ^1H NMR: Table 1. ^{13}C NMR: Table 3. This compound was spectroscopically identical with the one obtained by acid hydrolysis of **1** followed by CrO_3 oxidation and it had the same TLC R_f value as the product of methylation (**7b**) of natural neoquassin (**7**).

1-*Dihydro-α-methoxyquassin* (**10**). Isolated from $CHCl_3$ extract. Yield 12 mg, powder from MeOH, mp 168–172°. ^1H NMR: Table 1. ^{13}C NMR: Table 3.

Reduction of quassin **8**. A soln of 100 mg of **8** in 4 ml of aq. NaOH (10%) was treated with 300 mg of $NaBH_4$ stirring at 80° for 30 min. The reaction mixt. was then cooled and quenched with excess Me_2CO. After 30 min the mixt. was acidified with aq. H_2SO_4 (1%) to pH 2 and extracted with $CHCl_3$ [15]. After usual work-up, CC purification yielded 45 mg of 1-dihydro-α-hydroxy-quassin (**9**), mp 197–200°. ^1H NMR: Table 1. ^{13}C NMR: Table 3, in agreement with those reported in the lit. [11].

Methylation of compound **9**. Compound **9** (10 mg) was methylated with Me_2SO_4 in DMSO at 50° for 15 hr according to the usual procedure. The product of the reaction, after CC purification, (25 mg) was identical by TLC, mp, ^1H and ^{13}C NMR (Tables 1 and 3) to the natural compound **10**.

11-*Dihydro-12-norneoquassin* **21**. Isolated from $CHCl_3$ extract. Yield 19 mg, identical to an authentic sample [8]. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Parain (**11**). Isolated from $CHCl_3$ extract. Yield 250 mg, identical to an authentic sample [8]. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

11-*Acetylparain* (**12**). Isolated from $CHCl_3$ extract. Yield 14 mg, ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Acetylation of parain (**11**). Parain (60 mg) was acetylated in Ac_2O–pyridine at room temp. After usual work-up, CC sepn afforded 40 mg of 11-acetylparain (**12**), identical to the isolated natural compound, and 5 mg of the diacetylated product **13**. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Reduction of parain (**11**). Parain (**11**) (120 mg) was reduced with $NaBH_4$–NaOH as described in ref. [15]. CC purification of the product of reaction yielded 45 mg of 1-hydroxy-12-α-hydroxyparain (**14**), mp 219–223°.

Powder from MeOH. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. Compound **14** was also acetylated yielding the triacetyl derivative **14a**.

Acetonylation and oxidation of compound **14**. Compound **14** (25 mg) was treated with dry $CuSO_4$ in Me_2CO at reflux temp. for 24 hr. After the usual work-up 20 mg of 12,13-acetonylderivative (**15**) was obtained. ^{1}H NMR: Table 2. Compound **15** was then oxidized by Jones reagent at 0° yielding product **16**.

Reduction of parain (**11**) *by* $NaBH_4$. Parain (**11**) (40 mg) was dissolved in 40 ml of EtOH and stirred with 120 mg of $NaBH_4$ at 60° for 1 hr. The reaction furnished 22 mg of 12-dihydro-α-hydroxyparain (**17**) identified by ^{1}H (Table 2) and ^{13}C NMR (Table 3) and by the formation of the triacetyl derivative (**17a**).

Acetonylation and oxidation of compound (**17**).Compound **17** (20 mg) was acetonylated as described above. The product of reaction was directly oxidized with Ag_2O in EtOH–H_2O [8] yielding, after CC purification, product **16** (10 mg) which was identical by TLC and ^{1}H NMR to the compound obtained by CrO_3 oxidation of **15** (Scheme 3 and Table 2).

Isoparain (**18**). Isolated from $CHCl_3$ extract. Yield 28 mg, needles from MeOH, mp 259–261°. ^{1}H NMR (Table 1) identical to those reported in lit. [8, 18]. ^{13}C NMR: Table 3.

Isomerization of parain (**11**). Parain (**11**) (20 mg) was poured into a mixt. of 10 ml of EtOH and 10 ml of 0.1 M aq. NaOH and the soln refluxed for 48 hr. After acidification with 0.1 M HCl and extraction with $CHCl_3$, usual work-up gave a residue which was chromatographed yielding 25 mg of isoparain (**18**) identical by TLC, ^{1}H and ^{13}C NMR (Tables 2 and 3) to the natural compound.

Acetylation of isoparain (**18**). A sample of **18** from alkaline isomerization of **11** (20 mg) and a sample of natural **18** (20 mg) were acetylated with Ac_2O–pyridine furnishing the same monoacetyl derivative (**18a**). ^{1}H NMR: Table 2.

12-α-*Hydroxy*-13,18-*dehydroparain* (**19**). Isolated from $CHCl_3$ extract. Yield 11 mg, powder from MeOH, mp 238–241°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 2980, 2950, 1730, 1690, 1630, 1450, 1400, 1130. ^{1}H HMR: Table 2. ^{13}C NMR: Table 3. Compound **19** was then acetonylated according to the procedure above described to give the acetonyl derivative **20**.

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