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MAQUIROSIDE A, A NEW CYTOTOXIC CARDIAC GLYCOSIDE FROM *MAQUIRA CALOPHYLLA*

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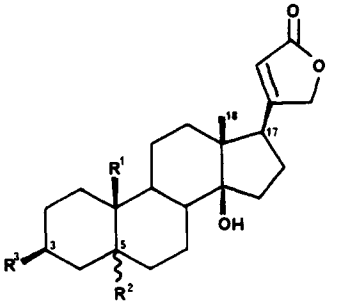
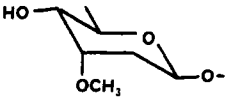
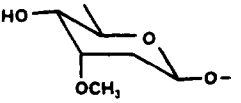
ABSTRACT.—Maquiroside A [**1**], a new cardiac glycoside, was isolated from the Peruvian plant *Maquira calophylla*. The structure was established using spectroscopic methods, and the aglycone was found to be cannogenol. The sugar portion was identified as D-cymarose. Maquiroside A [**1**] demonstrated activity against the KB cell culture.

In accord with our continuing interest in plant-derived natural products with potential chemotherapeutic value, we have investigated extracts of *Maquira calophylla* (P.& E.) C.C. Berg (Moraceae) for compounds that exhibit possible antineoplastic activity. Although many members of the Moraceae family have been examined phytochemically, there were no published examinations of *Maquira* species prior to our investigations. Original extracts of *M. calophylla* exhibited activity in vivo against the P-388 lymphocytic leukemia in mice and cytotoxicity against the KB cell culture system (1). Fractionation of ethanolic extracts of the stem bark of *M. calophylla*, guided by bioassays against the KB cell culture, resulted in the isolation of three coumarins (marmesin, oxypeucedanin hydrate, and pranferol) from non-cytotoxic fractions (2). However, isolation and characterization of the cytotoxic constituents of the stem bark were made difficult by the polarity of the constituents and the propensity of the major components to decompose. We now report the isolation and structure elucidation of the major cytotoxic constituent of *M. calophylla*, maquiroside A [**1**], a new cardiac glycoside.

RESULTS AND DISCUSSION

The ethanolic extract of the stem bark of *M. calophylla* was subjected to a standard



	R^1	R^2	R^3
1	$-CH_2OH$	β-H	
2	$-CH_2OH$	α-H	-OH
3	$-CH_2OH$	β-H	-OH
4	-CHO	β-OH	

partitioning scheme (3). The cytotoxic, chloroform-soluble material from this scheme was subjected to extensive column chromatography and preparative tlc over silica gel guided by bioassays against the KB cell culture to give an amorphous solid that retained the cytotoxicity. This solid was triturated with CH_2Cl_2/hexanes to yield maquiroside A [**1**] as a white powder, mp 123-125°.

Chromatograms of **1** yielded purple when sprayed with Kedde reagent (4), implying that maquiroside A [**1**] was a cardiac glycoside. Examination of the ^{1}H-nmr spectrum of **1** confirmed the steroidal nature of maquiroside and the presence of the characteristic α, β-unsaturated lactone of a cardenolide. The latter group was represented by a broad singlet at δ 5.88 (1H, 22-H) and an AB quartet further split by the 22-H at δ 4.88 (2H, 21-H_2). The steroid ring system was suggested by the broad series of resonances between δ 0.87 and δ 2.25, a multiplet at δ 2.76 (1H, 17-H), and a broad singlet at δ 4.02 (1H, 3-H). Only one of the two commonly observed methyl groups was observed as a singlet at δ 0.87 (3H). This resonance was assigned to the methyl group at C-13 by comparison with literature values (5,6), which meant that the methyl group at C-10 was substituted in some way. No aldehyde proton was present in the spectrum.

The high resolution cims of **1** exhibited an $M^+ + 1$ ion at *m/z* 535.3353 suggesting a molecular formula of $C_{30}H_{46}O_8$. Cleavage of the glycosidic linkage with concomitant transfer of a hydrogen to the C-3 oxygen (7,8) resulted in ions for the aglycone at *m/z* 391.2483 (21.6%) and the sugar at *m/z* 145.0876 (100%). The aglycone ion corresponded to a molecular formula of $C_{23}H_{34}O_5$ for the aglycone. The major route of fragmentation of the aglycone involved two successive losses of 18 mass units to give ions at *m/z* 373.2379 and *m/z* 355.2274 followed by loss of CH_2OH to give an ion at *m/z* 323.2005. The latter loss, in conjunction with the lack of a methyl singlet for C-19, suggested that C-19 was present as a hydroxymethylene group.

The ^{13}C-nmr spectrum of maquiroside A [**1**] in C_5D_5N (Table 1) was similar to spectra of cardenolides reported in the literature (9-12), confirming the basic steroidal skeleton of the molecule. In place of a resonance for the C-19 methyl carbon at ca. 12 ppm, however, there was a resonance at 65.5 ppm in pyridine-d_5 (66.5 ppm in $CDCl_3$), which appeared as a triplet in the SFORD spectrum. This confirmed the placement of an hydroxyl group at C-19.

A search of the literature revealed three possible structures for the aglycone, coroglaucigenin [**2**] (13), 17βH-coroglaucigenin (14), and cannogenol [**3**] (15). Acid-catalyzed methanolysis of maquiroside A [**1**] gave the aglycone as an amorphous solid. The ^{1}H-nmr spectrum of the aglycone exhibited the resonances expected of a cardenolide skeleton. However, only one methyl singlet (δ 0.88) was again evident, that of the 18-methyl. The 19-CH_2 appeared as an AB quartet (J=11 Hz) at δ 3.50 and δ 3.88, as is typical of other cardenolides with this substitution (16,17). The low resolution cims exhibited a parent ion at *m/z* 391 (M^+ + H), followed by ions at *m/z* 373 (M^++H-H_2O) and *m/z* 355 (M^++H-2H_2O). This confirmed the formula of the aglycone as $C_{23}H_{34}O_5$. The ^{13}C-nmr spectrum of the aglycone (Table 1) was similar to, but did not match, the published data for coroglaucigenin [**2**] (9), and the aglycone was not identical with an authentic sample of **2** by tlc. Published ^{13}C-nmr data established that C-12 is shielded to the extent of ca. 9 ppm in spectra of 17βH-cardenolides (9,10) and appears at ca. 30 ppm. In maquiroside A [**1**] and its aglycone, the C-12 resonance appears at 40.5 and 40.2 ppm, respectively. Thus, the genin of maquiroside A [**1**] does not have the 17-βH configuration. The most likely genin of **1** was, therefore, determined to be cannogenol [**3**], a relatively rare structure for which scant published data are available.

This assignment was validated by further comparison of the ^{13}C-nmr data for

TABLE 1. ^{13}C-nmr Resonances of Maquiroside A [**1**], Cannogenol [**3**], Coroglaucigen, [**2**] and Cymarin [**4**] in Pyridine-d_5 (ppm)

Carbon	**1**	**3**	**2** (Ref. 9)	**4**
1	30.8t	24.3t	33.0	24.7t
2	27.2t	28.7t	32.7	25.6t
3	71.0d	66.1d	70.7	73.5d
4	30.8t	29.6t	39.8	35.7t
5	35.9d	35.8d	45.2	75.0s
6	27.2t	27.3t	28.8	37.0t
7	24.8t	21.9t	28.1	22.5t
8	42.0d	42.0d	42.4	41.9d
9	30.0d	34.6d	50.8	39.6d
10	39.9s	40.2s	40.6	55.3s
11	21.9t	21.9t	23.4	18.5t
12	40.5t	40.5t	39.8	39.6t
13	50.2s	50.3s	50.2	49.8s
14	84.9s	84.9s	84.8	84.3s
15	33.0t	33.0t	32.1	32.1t
16	27.4t	27.3t	27.1	27.1t
17	51.6d	51.6d	51.6	51.0d
18	16.3q	16.4q	16.3	15.9q
19	65.5t	65.6t	59.2	208.3s
20	175.9s	175.9s	176.2	175.4s
21	73.7t	73.7t	73.4	73.6t
22	117.7d	117.7d	117.6	117.7d
23	174.5s	174.3s	174.6	174.5s
1′	96.6d	—	—	97.6d
2′	36.2t	—	—	36.3t
3′	79.0d	—	—	78.7d
4′	74.3d	—	—	73.9d
5′	73.2d	—	—	71.1d
6′	19.1q	—	—	18.9q
-OCH_3	58.0q	—	—	58.0q

maquiroside A [**1**] and its aglycone (Table 1) with the published data, in particular, data for the C-3, C-5, and C-19 resonances. In 5α-cardenolides,[1] the C-5 resonance is more deshielded by 8-10 ppm than in 5β-cardenolides[2] (43-45 ppm vs. 32-37 ppm). The C-3 resonance is also deshielded more in 5α-cardenolides than in 5β-cardenolides by 3-4 ppm (75-76 ppm vs. 71-72 ppm in the glycosides, 70-71 ppm vs. 66-67 ppm in the aglycones). The C-19 resonance, on the other hand, is more shielded in the 5α-cardenolides than in the 5β-cardenolides (when C-19 is a methyl, 12-15 ppm vs. 23-24 ppm). The resonances for C-5, C-3, and C-19 of coroglaucigenin [**2**] (in C_5D_5N) appear at 45.2, 70.7, and 59.2 ppm, respectively. The resonances for the aglycone of maquiroside A [**1**] appear at 35.8, 66.1, and 65.6 ppm (C-5, C-3, and C-19, respectively), confirming that the aglycone has the 5β configuration and is, as a consequence, cannogenol [**3**]. The analogous resonances for **1** appear at 35.9, 71.0, and 65.5 ppm, further supporting this assignment.

[1]Published data from the following 5α-cardenolides or aglycones were examined: uzarigenin (9), mallogenin (9), coroglaucigenin (9), uzarigenin acetate (9), 17βH-uzarigenin (9), odorside B (18), and uzarigenin α-L-rhamnoside (19).

[2]Published data from the following 5β-cardenolides or aglycones were examined: thevetin A (11), thevetin B (11), digitoxigenin α-L-oleandroside (18), digitoxin (12), digitoxigenin (11), digoxin (12), neriifolin (12), cerbertin (12), and digitoxigenin α-L-rhamnoside (12).

From the molecular weight of maquiroside A [**1**] (534), it was apparent that **1** contained only one sugar. The ^{1}H-nmr spectrum of **1** contained a doublet at δ 1.28 (3H, J=6Hz) and a singlet at δ 3.43 (3H), which were not present in the ^{1}H-nmr spectrum of the aglycone. These resonances are typical of a 6-deoxy sugar bearing a methoxyl group. The ion in the high resolution cims due to the sugar portion of maquiroside A [**1**] occurred at *m/z* 145.0876 (100%), suggesting a molecular formula for the sugar of $C_7H_{14}O_4$. This confirmed that the sugar was a dideoxy sugar. Several dideoxy sugars, particularly cymarose, have been found in other cardenolides, and there are ^{13}C-nmr and mass spectral data available in the literature (7,18). The high resolution cims of **1** also contained ions at *m/z* 117.0692, *m/z* 74.0366, *m/z* 100.0523, and *m/z* 86.0372 that are consistent with the fragmentation path for cymarose published by Pettit *et al.* (7). However, these ions could also be derived from other 2, 6-dideoxy-3-methoxy sugars such as sarmentose. Comparison of the ^{13}C-nmr data for **1** with published data for cardiac glycosides containing oleandrose, diginose, cymarose, or sarmentose (18) showed that the best agreement of the sugar resonances was with cymarose (as the sugar portion of cymarin [**4**]). Furthermore, comparison of the ^{1}H-nmr spectra of **1** with the ^{1}H-nmr spectra of a sample of **4** (Table 2) showed that the resonances due to the sugar occurred at essentially identical chemical shifts in each case. Finally, acid hydrolysis of both **4** and **1**, followed by paper chromatography of the resulting sugars, showed that the sugars were identical, i.e., both sugars were D-cymarose.

TABLE 2. Comparison of the ^{1}H-nmr Resonances of the Sugar of Maquiroside A [**1**] with the Sugar of Cymarin [**4**] in $CDCl_3$

Proton	**1**[a]	**4**[b]
1′-H	4.72 dd (1.5,9)	4.77 dd (1.5,9)
2′-Hz	2.1 brd	not observable
3′-H	3.64 m	3.64 m
3′-OCH_3	3.43 s	3.43 s
4′-H	3.22 dd (3.4,9)	3.21 dd (3.4,9)
5′-H	3.5 m	3.5 m
6′-CH_3	1.28 d (6)	1.27 d (6)

[a]Recorded at 90 MHz and 360 MHz in $CDCl_3$.
[b]Recorded at 90 MHz in $CDCl_3$.

Thus, maquiroside A [**1**] was shown to be the cymaroside of the relatively rare genin, cannogenol [**3**]. Maquiroside A [**1**] demonstrated activity against the KB cell culture system with an ED_{50}=0.013 μg/ml. Three additional cardiac glycosides have now been isolated from *M. calophylla* and work on their structures is in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Ir spectra were measured on a Perkin-Elmer 283 instrument, and uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. ^{1}H-nmr spectra were recorded on a JEOL FX90Q spectrometer at 89.56 MHz or on a Nicolet 360 MHz spectrometer at the University of Virginia in $CDCl_3$ with TMS as an internal standard. ^{13}C-nmr spectra were recorded on a JEOL FX90Q spectrometer at 22.5 MHz in $CDCl_3$ or C_5D_5N with TMS as an internal standard. High and low resolution chemical ionization mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Center, Philadelphia, Pennsylvania, or at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska. Elemental analyses were carried out by Atlantic Microlab, Inc., Atlanta, Georgia. Specific rotations were measured on a Perkin-Elmer Model 141 Polarimeter. Cymarin was obtained from Aldrich Chemical Co. KB in vitro analyses were performed at Arthur D. Little, Inc., Cambridge, Massachusetts, or at the Southwest Foundation for Research and Education, San Antonio, Texas. All tlc was

carried out using precoated silica gel 60 plates (EM Labs), and all column chromatography was carried out on silica gel 60 (EM Labs).

PLANT MATERIAL.—Stem bark of *M. calophylla* (B805592, PR-46135) was collected in Peru in December 1975, and supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, where voucher specimens are preserved.

ISOLATION OF MAQUIROSIDE A [**1**].—The dried, ground stem bark (5.77 kg) of *M. calophylla* was extracted with 40 liters of 95% EtOH in a Soxhlet extractor for 24 h. The extract was evaporated in vacuo to give a dark gum (193 g) which was partitioned between H_2O (1 liter) and $CHCl_3$ (3×1 liter). The combined $CHCl_3$ layers were evaporated, and the residue was partitioned successively between 90% MeOH (1 liter) and petroleum ether (3×1 liter), 80% MeOH (1.12 liter) and CCl_4 (3×1 liter), and 60% MeOH (1.37 liter) and $CHCl_3$ (3×1 liter). The final $CHCl_3$ layers were combined and concentrated in vacuo to give a brown solid (22 g). This solid was subjected to column chromatography over silica gel 60 and eluted with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2. The fractions eluting with 4-5% MeOH in CH_2Cl_2 were collected, concentrated, and again subjected to column chromatography in the same manner. The fractions eluting with 4-5% MeOH in CH_2Cl_2 were concentrated and subjected to preparative tlc over silica gel 60 developed in EtOH-Et_2O-toluene (2:7:7) to yield **1** as an amorphous solid. Trituration of this material with CH_2Cl_2/hexanes yielded 410 mg of **1** as a white powder.

MAQUIROSIDE A [**1**].—Mp 123-125°; $[\alpha]^{25}D$+17.9° (*c* 0.07, $CHCl_3$); ir ($CHCl_3$) 3568, 3322, 2933, 2870, 1740, 1615, 1448, 1085 cm^{-1}; uv max (EtOH) 218 (ε 5628), 290 nm (ε 239); ^{1}H-nmr ($CDCl_3$) δ 0.87 (s, 3H, 18-CH_3), 1.23 (d, *J*=6 Hz, 3H, 6′-CH_3), 1.3-2.0 (m, ~25H), 2.07 (m, 1H, 2′-H), 2.76 (m, 1H, 17-H), 3.22 (dd, *J*=3.4,9 Hz, 1H, 4′-H), 3.43 (s, 3H, OCH_3), 3.5 (m, 1H,5′-H), 3.64 (m, 1H, 3′-H), 3.54, 3.80 (ABq, *J*=11 Hz, 2H, 19-CH_2), 4.02 (brs, 1H, 3-H), 4.72 (dd, *J*=1.5,9 Hz 1H, 1′-H), 4.89 (ABq, J_{AB}=6 Hz, $J_{21,22}$=1.5 Hz, 2H, 21-CH_2), 5.88 (s, 1H, 22-H); ^{13}C-nmr (C_5D_5N) see Table 1; hrcims (isobutane reagent gas) *m/z* 535.3353 (calcd for $C_{30}H_{46}O_8$+H, 535.3271); cims *m/z* 535 (M^++H, 2.3%), 391, (21.6), 389 (2.6), 373, (24.2), 355 (44.95), 219 (12.3), 179 (3.9), 165 (1.7), 162 (1.1), 147 (14.7) 145 (100), 144 (6.0), 117 (10), 111 (20.2), 100 (2.9), 87 (87.2), 86 (3.2), 74 (37.9).

Anal. calcd for $C_{30}H_{46}O_8$: C, 67.39; H, 8.67. Found: C, 67.38; H, 8.95.

PREPARATION OF CANNOGENOL [**3**].—Maquiroside A [**1**] (47.7 mg) was dissolved in 1 N methanolic HCl (5 ml) and stirred at room temperature for 24 h. The reaction mixture was diluted with H_2O (20 ml) and extracted with CH_2Cl_2 (3×25 ml). The combined CH_2Cl_2 layers were washed with 1N NH_4OH (2×40 ml), H_2O (2×40 ml), dried over anhydrous Na_2SO_4, and evaporated to a glassy solid. Preparative tlc of the residue yielded 7.9 mg of **3** as a white solid: ir ($CHCl_3$) 3450, 2940, 1740, 1620, 1030 cm^{-1}; uv max (EtOH) 218 (ε3613), 305 nm (ε204); ^{1}H-nmr ($CDCl_3$) δ 0.88 (s, 3H, 18-CH_3), 1.2-2.0 (m, ~24H), 2.05 (m, 1H, 17-H), 3.50, 3.88 (ABq *J*=11 Hz, 2H, 19-CH_2), 4.12 (brs, 1H, 3-H), 4.89 (ABq, J_{AB}=6 Hz, $J_{21,22}$=2Hz, 2H, 21-CH_2), 5.88 (s, 1H, 22-H); hrcims (isobutane reagent gas) *m/z* 373.2370 (M^++H-H_2O, calcd for $C_{23}H_{32}O_7$+H, 373.2379); cims *m/z* 373, 355, 325, 113, 111.

D-CYMAROSE FROM MAQUIROSIDE A [**1**] AND CYMARIN [**4**].—A solution of the cardiac glycoside (10 mg) in MeOH (1 ml) was heated at reflux with 0.1 N H_2SO_4 (1 ml) for 25 min under N_2. H_2O (1 ml) was added and, after removal of the MeOH in vacuo, the residue was extracted with $CHCl_3$ (2×5 ml). The combined $CHCl_3$ layers were washed with H_2O (5 ml) and the H_2O layers were combined. After neutralization of the H_2O layers with 0.1N NaOH, the H_2O was removed in vacuo to give ca. 2 mg of a dark yellow oil. This residue from each hydrolysis was subjected to paper chromatography (descending method) developed with 5% NH_4OH in *n*-BuOH. After 24 h, the paper was air-dried and visualized with 0.1N $AgNO_3$. The sugar obtained from hydrolysis of **1** was identical with that obtained from hydrolysis of **4**.

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