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A NEW TERPENOID GLYCOSIDE FROM *NAUCLEA DIDERRICHII* BARK

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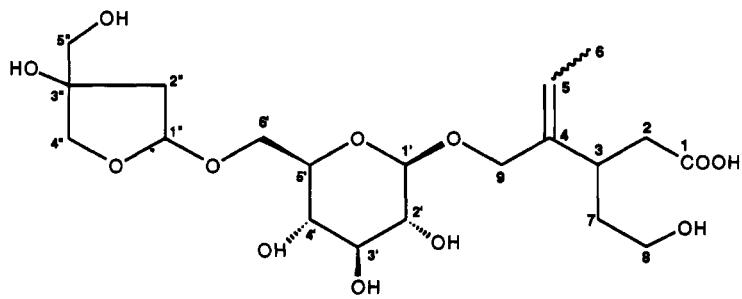
ABSTRACT.—A new terpenoid glycoside, marounoside [**1**], was isolated from the bark of *Nauclea diderrichii* and characterized by 1D and 2D nmr spectroscopy. The aglycone, a terpene with an unusual carbon skeleton, is linked to glucose, which, in turn, is connected to an apiofuranose residue.

Nauclea diderrichii (de Wild.) Merr. (Rubiaceae) is a plant used in traditional medicine, which grows in western and central Africa. Decoctions of the bark are used in folk medicine to eliminate parasites and treat other tropical diseases (1,2). Previous studies on the bark of *Nauclea* spp. have yielded several alkaloids (3,5) and secoiridoids (6). In the present paper, we report the isolation of a new terpenoid glycoside, marounoside [**1**]. The identity of the aglycone was established as 3-[2-hydroxyethyl]-4-hydroxymethylhex-4-enoic acid, on the basis of 1D and 2D nmr spectral data.

The molecular formula of **1** was found to be $C_{20}H_{33}O_{13}$ from the fabms spectrum [$(M-H)^-$ *m/z* 481]. Its ^{13}C-nmr spectrum exhibited twenty resolved resonances and the multiplicities of the individual signals, determined using the DEPT pulse sequence (7), consisted of ten methines, six methylenes, one methyl, and three quaternary carbons. Furthermore, the ^{1}H- and ^{13}C-nmr data suggested the occurrence of two sugar moieties which were shown to be glucose and apiofuranose. The coupling constants between the five ring protons of the glucose residue were more than 7.9 Hz, indicative of a trans-diaxial disposition for these atoms, while the ^{13}C-nmr chemical shifts of the apiofuranose unit were in good agreement with literature data (8).



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As a consequence of these observations, **1** was identified as a C-9 acyclic terpenoid glycoside.

The molecular framework and the complete ^{1}H- and ^{13}C-nmr chemical shift assignments for **1** were deduced from the combined application of homonuclear and both direct and long-range heteronuclear chemical-shift correlation nmr experiments. These 2D techniques established proton connectivities from the ^{1}H-^{1}H couplings (9,10) and correlated these resonances with the ^{13}C-nmr spectrum using the HMQC sequence (11). Assignments of the various CH_n groups were obtained from the HMBC diagram (12) using the long-range correlation responses over two or three bonds (^{2}J or ^{3}J couplings).

These 2D nmr data confirmed the occurrence of β-glucopyranose and apiofuranose, and were used to unambiguously identify the aglycone as 3-[2-hydroxyethyl]-4-hydroxymethylhex-4-enoic acid. Starting from the ethylenic ^{1}H-nmr signal [δ 5.67 (q, ^{3}J=6.9 Hz)], which was coupled to the methyl group at 1.70 ppm, we observed long-range couplings with methylene and methine carbons located, respectively, at δ 72.32 (CH_2-9) and 34.40 (CH-3). Using the COSY nmr spectrum, the methine proton H-3 showed a ^{3}J coupling with the methylene resonances H-2 and H-7. In turn, this latter signal was also coupled with the oxygen-bearing, methylene protons at H-8. Finally, the HMBC experiment revealed that the H-2 protons exhibited a correlation peak with the carboxylic carbon C-1 at 181.75 ppm. All of these results are presented in Figure 1.

It should be noted that the unusual skeleton (C-1 to C-9) of **1** has been found previously in gentiatibetine (13) and jasminidine (14). Finally, attachments of the furanose anomeric position to C-6′ of the glucose moiety and the glucose anomeric position to methylene C-3 were clearly indicated in the HMBC spectrum (Figure 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The fabms was obtained on a Nermag R10-10H mass spectrometer in the negative-ion mode in a thioglycerol matrix. Nmr spectra were performed on a Bruker AMX-400 nmr spectrometer using $CDCl_3$ solutions and TMS as internal standard (^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz). Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments (HMBC, HMQC, COSY). For other experimental details, see Faure *et al.* (15,16).

Tlc analysis of the compounds was performed on precoated Si gel plates (Kieselgel 60 F254; 0.25 mm Merck) using the following systems: (a) *n*-BuOH-HOAc-H_2O (4:1:5); (b) EtOAc-HCOOH-HOAc-H_2O (100:11:11:27). The plates were visualized by spraying with H_2SO_4, followed by heating at 110°.

PLANT MATERIAL.—The bark of *Nauclea diderrichii* was collected in the vicinity of Libreville, Gabon, in August 1992. A voucher specimen has been deposited in the Department of

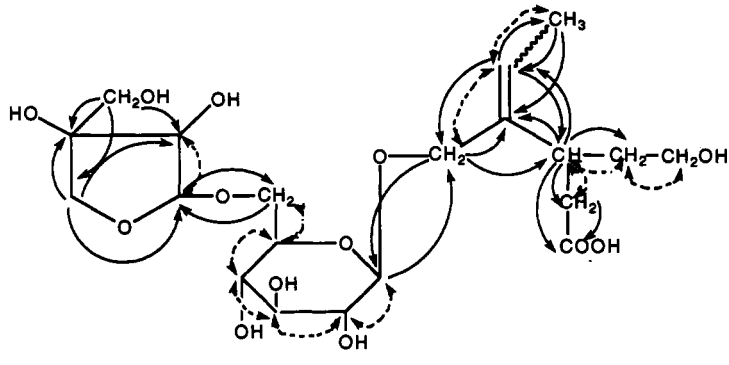

FIGURE 1. Proton-proton (···→) and long-range proton-carbon (→) connectivities derived, respectively, from COSY and HMBC nmr experiments for marounoside [**1**].

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EXTRACTION AND ISOLATION.—Marounoside [**1**] was extracted from *N. diderrichii* bark (500 g) with a mixture of H_2O-CH_3OH (20:80). After concentration, the H_2O layer was freeze-dried. The residue (20 g) was solubilized in MeOH and chromatographed on a column of activated charcoal. The MeOH extract was evaporated to dryness (12 g). From this extract marounoside [**1**] (65 mg) was obtained by prep. lpc (Jobin-Yvon) on RP-8 in H_2O.

Marounoside [**1**].—Obtained as a yellow powder; fabms *m/z* $[M-H]^-$ 481, calcd for $C_{20}H_{33}O_{13}$; ^1H nmr ($CDCl_3$, 400.13 MHz) δ 2.35 (1H, dd, J=13.7 Hz and 8.0 Hz, H-2a), 2.29 (1H, d, J=7.2 Hz, H-2b), 3.21 (1H, d, J=7.2 Hz, H-3), 5.67 (1H, d, J=6.9 Hz, H-5), 1.70 (1H, s, H-6), 1.75 (1H, d, J=7.2 Hz, H-7), 3.51 (1H, s, H-8), 4.33 (1H, d, J=11.8 Hz, H-9a), 4.05 (1H, s, H-9b), 4.32 (1H, d, J=7.9 Hz, H-1′), 3.16 (1H, d, J=9.2 Hz, H-2′), 3.34 (1H, d, J=9.0 Hz, H-3′), 3.22 (1H, d, J=9.7 Hz, H-4′), 3.37 (1H, dd, J=1.9 and 6.6 Hz, H-5′), 3.98 (1H, d, J=11.3 Hz, H-6′a), 3.58 (1H, s, H-6′b), 5.02 (1H, d, J=2.4 Hz, H-1″), 3.90 (1H, H-2″), 3.97 (1H, d, J=9.7 Hz, H-4″a), 3.76 (1H, s, H-4″b), 3.57 (1H, s, H-5″); ^{13}C nmr ($CDCl_3$, 100.61 MHz) δ 181.75 (s, C-1), 43.62 (t, C-2), 34.40 (d, C-3), 138.63 (s, C-4), 127.82 (d, C-5), 13.62 (q, C-6), 37.30 (t, C-7), 61.60 (t, C-8), 72.32 (t, C-9), 102.61 (d, C-1′), 75.22 (d, C-2′), 78.06 (d, C-3′), 72.00 (d, C-4′), 77.0 (d, C-5′), 68.77 (d, C-6′), 111.07 (d, C-1″), 78.24 (d, C-2″), 80.57 (s, C-3″), 75.22 (t, C-4″), 65.64 (t, C-5″).

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