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A BENZOIC ACID GLYCOSIDE FROM *GENIOSTOMA ANTHEROTRICHUM*

MOHAMMAD A. RASHID,* KIRK R. GUSTAFSON, JOHN H. CARDELLINA II and MICHAEL R. BOYD†

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bldg 1052, Rm. 121, Frederick, MD 21702-1201, U.S.A.

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Key Word Index—*Geniostoma antherotrichum*; Loganiaceae; benzoic acid glycoside; HIV-inhibitory tannins.

Abstract—Fractionation of an HIV-inhibitory organic extract of *Geniostoma antherotrichum* afforded a glycoside derivative, which has been characterized as 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid (**1**) on the basis of spectral analyses. The HIV-inhibitory activity of the extract was traced to polymeric tannins, while **1** was found to be inactive in the National Cancer Institute's primary anti-HIV screen.

INTRODUCTION

The genus *Geniostoma* (Loganiaceae) contains *Ca* 52 species and is distributed throughout the Indopacific region [1]. The only reference to *Geniostoma* we could find in the phytochemical literature was a report on the chemical screening of *G. borbonicum*, which suggested the presence of alkaloids, saponins and leucoanthocyanins in this species [2]. The observation of moderate HIV-inhibitory activity in an organic extract of *G. antherotrichum* in the U.S. National Cancer Institute's primary and anti-HIV screen [3] prompted us to investigate the chemistry of this plant. Here, we report the isolation and structural elucidation of a benzoic acid glycoside, 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid (**1**).

RESULTS AND DISCUSSION

A solvent–solvent partitioning protocol [4] concentrated the HIV-inhibitory activity of the *G. antherotrichum* organic extract into the water-soluble fraction. Gel permeation on Sephadex LH-20 afforded a total of eight fractions. Bioassays revealed that virtually all of the anti-HIV activity eluted in the first fraction. ^{1}H NMR analyses indicated that the active fraction contained a mixture of polymeric tannins; the anti-HIV activity of polyphenolics is now well known and of no further interest to our programme [5]. However, a late-eluting inactive fraction consisted of a pure glycoside derivative, **1**.

HRFAB mass spectrometry established the molecular formula $C_{13}H_{16}O_9$ for **1**. The ^{1}H and ^{13}C NMR spectra (Table 1) showed resonances indicative of a 1,2,3-trisubstituted benzene derivative. A broad IR absorption at 3330 cm^{-1} and a band at 1631 cm^{-1} could be assigned to a conjugated, hydrogen-bonded carboxylic acid moiety. This was supported by a ^{13}C NMR signal at δ175.5. ^{1}H NMR resonances for an anomeric proton, four oxymethine protons and an oxymethylene group revealed a pyranose subunit. Signal overlap in the ^{1}H NMR spectrum of **1** prevented complete coupling constant analysis; so, the peracetate derivative (**2**) was prepared. The well dispersed ^{1}H NMR resonances of **2** (Table 1) revealed characteristically large *trans* diaxial couplings (J = 7.7–9.5 Hz) between the pyranose ring protons, which identified the sugar as a β-linked glucose. The substitution pattern of the aromatic nucleus and the position of the glycosidic linkage were confirmed by HMBC correlations. The δ7.55 (H-6) proton exhibited ^3J correlations to an oxygenated aromatic carbon at δ152.9 and to the carboxyl carbon, which established H-6 as *ortho* to the carboxylic acid functionality. The δ7.25 (H-4) proton demonstrated two- and three-bond correlations to carbons at δ 146.8 (C-3) and 152.9 (C-2), respectively. The anomeric proton (H-1′) showed as a ^3J correlation to C-3, which confirmed the position of attachment of the glucose moiety. On this basis the metabolite was identified as 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid (**1**).

Compound **1** was previously described as a constituent of *Vinca minor* [6], based on the preparation and characterization of a methyl ester peracetate derivative. However, that report [6] did not provide any physical or spectral data for the natural product (**1**). The *in vitro* anti-HIV activity detected in the *G. antherotrichum* extract was traced to polymeric tannins; purified glycoside **1** was inactive.

*Permanent address: Department of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

†Author to whom correspondence should be addressed.

Table 1. NMR spectral data for compounds 1 and 2

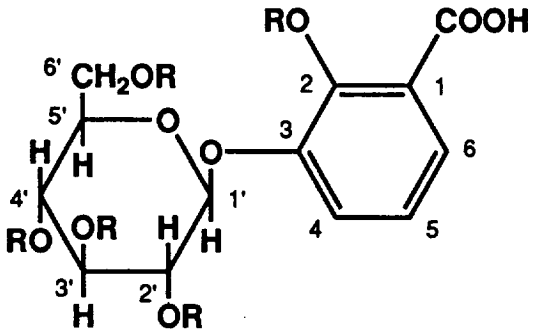
	1*		2†
Carbon	$\delta_{^{13}C}$	$\delta_{^1H}$‡	$\delta_{^1H}$‡
1	120.4		
2	152.9		
3	146.8		
4	121.3	7.25 *dd* (8.0, 1.5)	7.22 *dd* (8.0, 1.5)
5	118.5	6.72 *t* (8.0)	7.10 *t* (8.0)
6	125.1	7.55 *dd* (8.0, 1.5)	7.55 *dd* (8.0, 1.5)
1′	103.4	4.86 *d* (7.5)	5.34 *d* (7.7)
2′	74.2§	3.50‖*m*	5.20 *dd* (9.5, 7.7)
3′	77.0§	3.52‖*m*	5.38 *t* (9.5)
4′	70.9	3.43 *m*	5.10 *t* (9.5)
5′	78.0	3.43 *m*	4.23 *ddd* (9.5, 5.5, 2.2)
6′	61.5	3.73 *dd* (12.0, 5.0)	4.29 *dd* (12.1, 5.5)
		3.86 *dd* (12.0, 2.0)	4.18 *dd* (12.1, 2.2)
CO_2H	175.5		
OAc			1.92 *s*, 6H
			1.96 *s*, 3H
			2.01 *s*, 3H
			2.17 *s*, 3H

*CD_3OD; 125 MHz for ^{13}C; 500 MHz for ^1H; assignments aided by HMQC and HMBC correlations.

†Acetone-d_6, 500 MHz; assignments aided by COSY-45 correlations.

‡Multiplicity, *J* (Hz).

§‖Assignments within a column may be reversed.



1 R = H
2 R = Ac

EXPERIMENTAL

General. NMR spectra was recorded at 500 and 125 MHz, respectively, for ^1H and ^{13}C, and referenced to the residual solvent signal. The number of attached protons for ^{13}C signals was determined from a DEPT experiment. Proton detected heteronuclear correlations were measured using HMQC (optimized for ^1J CH = 140 Hz) and HMBC (optimized for ^nJ CH = 8.3 Hz) pulse sequences.

Collection, extraction and isolation. Samples of *G. antherotrichum* (Gilg and Bened.) were collected in the Morboe Province of Papua New Guinea in January 1989. Voucher specimens (Q6606921) are maintained at the Lae Herbarium, University of New Guinea. Air-dried twigs were ground into a coarse powder (370 g) and extracted successively with CH_2Cl_2–MeOH (1:1) and MeOH (100%). The combined extracts were evapd to dryness *in vacuo* to give 10.5 g brown gum. Solvent–solvent partioning [4] afforded 5.7 g H_2O-soluble material, which was purified by gel permeation on Sephadex LH-20 using MeOH–H_2O (9:1) to give 125 mg **1** as amorphous solid.

2-Hydroxy-3-O-β-D-glucopyranosyl-benzoic acid (**1**). $[\alpha]_D$ − 67.5° (MeOH; *c* 0.2); UV (MeOH) λ_{max} 210 (log ε = 4.43), 240 (3.81), 302 (3.66) nm; IR ν_{max} (film) cm^{-1}: 3327, 1631, 1586, 1468, 1393, 1254, 1070, 840; HRFABMS *m/z* 315.0697 ($[M - H]^-$, $C_{13}H_{15}O_9$, calc. 315.0716); EIMS *m/z* (rel. int.):316 $[M]^+$ (68), 272 $[M - CO_2]^+$ (100), 154 $[M - C_6H_{10}O_5]^+$ (10); ^1H and ^{13}C NMR: see Table 1.

Peracetate derivative (**2**). To 5 mg **1** in 0.3 ml pyridine were added 0.5 ml Ac_2O and a few crystals of dimethylaminopyridine. The reaction mixt. was stirred overnight at room temp. Evapn of the mixt. under N_2 gave **2**. ^1H NMR: see Table 1.

Anti-HIV assay. DMSO solns of the extract, chromatographic frs and pure compounds were diluted and tested in an *in vitro* XTT-based assay, as previously described [7].

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REFERENCES

1. Mabberley, D. J. (1987) *The Plant Book*, p. 238. Cambridge University Press, Cambridge, U.K.
2. Buffard, G., Desconclois, J. F., Jehanno, A., Provost, J., Tiberghien, R. and Touche, A. (1981) *Plant. Med. Phytother.* **15**, 80.
3. Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H. and Boyd, M. R. (1989) *J. Natl Cancer Inst.* **81**, 577.
4. Van Wagenen, B. C., Larsen, R., Cardellina, J. H., II, Randazzo, D., Lidert, Z. C. and Swithenbank, C. (1993) *J. Org. Chem.* **58**, 335.
5. Cardellina, J. H., II, Munro, M. H. G., Fuller, R. W., Manfredi, K. P., McKee, T. C., Tischler, M., Bokesch, H. R., Gustafson, K. R., Beutler, J. A. and Boyd, M. R. (1993) *J. Nat. Prod.* **56**, 1123.
6. King, F. E., Gilks, J. H. and Partridge, M. W. (1995) *J. Chem. Soc.* 4206.
7. Gulakowski, R. J., McMahon, J. B., Staley, P. G., Moran, R. A. and Boyd, M. R. (1991) *J. Virol. Meth.* **33**, 87.