Acknowledgment. This work was supported by the Air Force Office of Scientific Research under Grant No. AFOSR-71-1958.

(14) NDEA Fellow, 1969–present.
(15) (a) Alfred P. Sloan Foundation Fellow, 1970–1972; (b) Camille and Henry Dreyfus Foundation, Teacher–Scholar Grant Awardee, 1970–1975.

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Received October 20, 1971

Datiscoside, a Novel Antileukemic Cucurbitacin Glycoside from *Datisca glomerata*[1,2]

Sir:

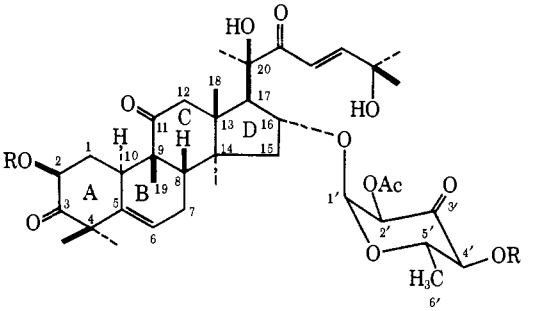
We wish to report on the isolation and structural elucidation of datiscoside (**1**), a novel antileukemic[3] principle from *Datisca glomerata* Baill. The structure of datiscoside was determined by X-ray crystallographic analysis of the di-*p*-iodobenzoate **3**, thereby establishing for the first time unambiguously the configurations of the cucurbitacins at C-20, assigned previously on biogenetic grounds, and at C-2, for which contradictory arguments have been presented in the literature.[4,5]

Alcoholic extracts of the roots of *D. glomerata*[6] showed significant inhibitory activity *in vivo* against Walker 256 intramuscular carcinosarcoma in the rat and the P-388 lymphocytic leukemia in the mouse and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB). Fractionation of the alcoholic extract was guided by the 9KB assay. Successive solvent partitions and chromatography on SilicAR CC-7 yielded fractions from which were crystallized datiscoside (**1**), $C_{38}H_{54}O_{12}$[7] (mp 174–175°; $[\alpha]^{23}D$ +26° (*c* 1.04, $CHCl_3$); uv max (MeOH) 231 nm (ϵ 11,600); ir (KBr) 2.90, 5.73, 5.80, 5.92, 6.17, 8.10, and 9.30 μ; nmr ($CDCl_3$) τ 2.96 (1 H, d, J = 15 Hz), 3.52 (1 H, d, J = 15 Hz), 4.24 (1 H, m), 4.75 (2 H, s), 5.51 (1 H, s), 7.89 (3 H, s), 8.47–8.80 (21 H, 7 × CH_3), 8.91 (3 H, s), and 8.99 (3 H, s); *m/e* 624, 498, 481, 458, 455, 403, 385, 369, 219, 144, 127, 126, 112, 111, 105, 100, and 96) and cucurbitacin D (**2**), identified by comparison of its properties with those reported in the literature.[8]

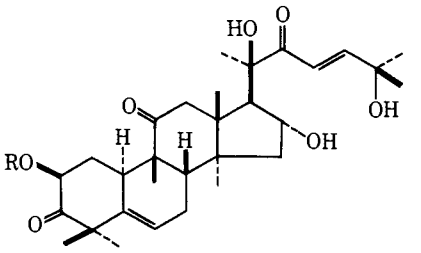
(1) Tumor Inhibitors. LXXII. Part LXXI: C. H. Smith, J. Larner, A. M. Thomas, and S. M. Kupchan, submitted for publication.
(2) Supported by grants from the National Cancer Institute (CA-11718 and CA-11760) and the American Cancer Society (T-275 and T-541), and a contract with Chemotherapy, National Cancer Institute (NIH 71-2099).
(3) Datiscoside showed confirmed *in vivo* activity against P-388 leukemia and WM-256 intramuscular carcinosarcoma and cytotoxicity (ED_{50} = 0.16 μg/ml) against cells derived from the human carcinoma of the nasopharynx (KB). Cytotoxicity (KB) and *in vivo* activity were assayed by the procedures described in *Cancer Chemother. Rep.*, **25**, 1 (1962).
(4) D. Lavie and B. S. Benjaminov, *J. Org. Chem.*, **30**, 607 (1965).
(5) G. Snatzke, P. R. Enslin, C. W. Holzapfel, and K. B. Norton, *J. Chem. Soc. C*, 972 (1967); *cf.* D. H. R. Barton, C. F. Garbers, D. Giacopello, R. G. Harvey, J. Lessard, and D. R. Taylor, *ibid.*, 1050 (1969); J. R. Bull and P. R. Enslin, *Tetrahedron*, **26**, 1525 (1970).
(6) The roots were collected in California in July 1962. The authors acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., U.S.D.A., in accordance with the program developed by Chemotherapy, National Cancer Institute.
(7) Elemental formulas were confirmed by concordant elemental analyses.
(8) P. R. Enslin, *J. Sci. Food Agr.*, **5**, 410 (1954); P. R. Enslin, R. Rehm, and D. E. A. Rivett, *ibid.*, **8**, 673 (1957).

Elemental analysis and spectral data for datiscoside (**1**) supported assignment of a cucurbitacin-like nucleus to which a highly oxygenated substituent was attached. A glycoside structure appeared likely, but the nmr spectrum and relatively nonpolar characteristics were not indicative of a common sugar derivative. Initial attempts at acid hydrolysis led to extensive decomposition,[9] but treatment of **1** with 2 *N* H_2SO_4 at 70° for 11 hr did afford cucurbitacin D (**2**) in low yield. This result served to interrelate datiscoside (**1**) with other known cucurbitacins as well, since cucurbitacin D had been correlated with cucurbitacins B, E, and I.[10]

Unequivocal proof of the structure, stereochemistry, and absolute configuration of datiscoside was achieved by X-ray crystallographic analysis of datiscoside di-*p*-iodobenzoate (**3**), mp 215–216°. Crystals of the di-*p*-



1, R = H
3, R = *p*-iodobenzoate



2

iodobenzoate are orthorhombic with space group $P2_12_12_1$ and a = 19.609 (7), b = 31.485 (17), and c = 8.743 (3) Å, Z = 4. The asymmetric unit contains, in addition, two molecules of water of hydration. The calculated density is 1.475 g cm^{-3}, in reasonable agreement with the observed value of 1.49 (1) g cm^{-3}.

Intensity data were collected by counter diffractometry using monochromatic Cu Kα radiation. The iodine atoms were located from a three-dimensional Patterson synthesis, and the carbon and oxygen atoms were found from three successive three-dimensional electron-density syntheses calculated using the heavy atom method of phase determination. The atomic parameters were refined by the block-diagonal least-squares method using anisotropic thermal parameters for the iodine atoms only and isotropic parameters for the light atoms. Taking into account the anomalous dispersion terms for the iodine atoms ($\Delta f'$ = −1.03, $\Delta f''$ = 7.0), the parameters for the absolute configuration shown in Figure 1 yielded R = 0.100 for the 1627 independent significant reflections measured. A structure factor calculation with coordinates appropriate to

(9) *Cf.* D. Lavie, D. Willner, and Z. Merenlender, *Phytochemistry*, **3**, 51 (1964).
(10) D. Lavie, Y. Shvo, D. Willner, P. R. Enslin, J. M. Hugo, and K. B. Norton, *Chem. Ind. (London)*, 951 (1959).

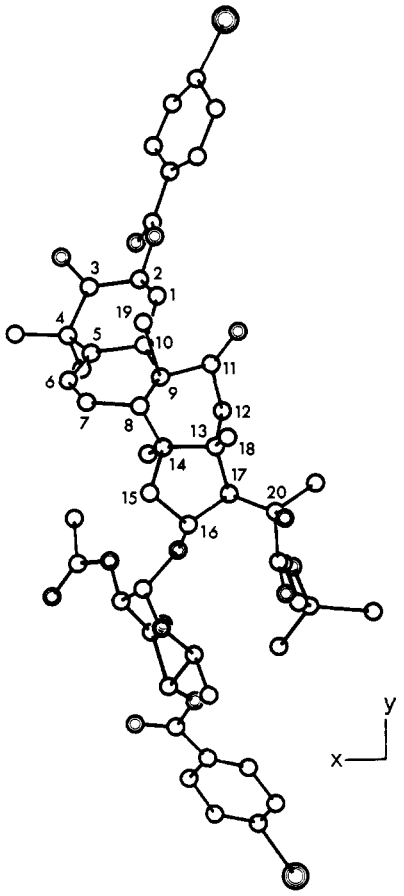


Figure 1. Molecular structure of the di-*p*-iodobenzoate of datiscoside as found in the crystal. Oxygen atoms are denoted by the double circles, the iodine atoms by the large circles. The axial system shown is right-handed and the molecule is shown with its correct absolute configuration.

the mirror image of **1** yielded $R = 0.124$ indicating a significant discrimination between the two enantiomers.[11] The correctness of the assignment of the absolute configuration was further confirmed by the measurement of a substantial number of Friedel pairs of reflections where, in all cases, the observed difference in intensity was in good agreement with theoretical expectation.[12]

The glycoside moiety at C-16 of datiscoside has thus been characterized as a novel 2′-*O*-acetyl-6′-deoxy-α-L-*gluco*-hexos-3′-ulopyranoside.[13] The β-equatorial configuration for the C-2 substituent of dihydrocucurbitacin D acetate had been correctly assigned by Lavie, *et al.*,[4] on the basis of the nmr signal for the C-2 proton [τ 4.4 (dd, J = 13.5, 5.1 Hz)]. A corresponding signal appeared in the nmr spectrum of datiscoside diacetate. The CD-based arguments which led to proposal of the opposite configuration require further study.[5,13a]

(11) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).

(12) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature (London)*, **168**, 271 (1951).

(13) See W. Pigman and D. Horton, Ed., "The Carbohydrates," 2nd ed, Vol. IIB, Academic Press, New York, N. Y., 1970, pp 809–833, for nomenclature.

(13a) NOTE ADDED IN PROOF. The present work confirms the 2β,3β-diol configuration in cucurbitacins O, P, and Q [S. M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970)], in view of the demonstrated cis configuration of their 2,3-diol system and of their interrelation with cucurbitacin B [S. M. Kupchan, R. M. Smith, Y. Aynehchi, and M. Maruyama, *J. Org. Chem.*, **35**, 2891 (1970)].

The observed antileukemic and tumor-inhibitory activity of datiscoside (**1**) confirms and extends an earlier report of antitumor activity of a cucurbitacin glycoside.[14] Investigations are in progress to determine the significance of the glycoside and of other structural features to the tumor-inhibitory activity of datiscoside.

(14) D. Lavie, D. Willner, M. Belkin, and W. G. Hardy, *Acta Unio. Int. Contra Cancrum*, **15**, 177 (1959); H. El Khadem and M. M. A. Abdel Rahman, *J. Chem. Soc.*, 4991 (1963).

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Maytansine, a Novel Antileukemic Ansa Macrolide from *Maytenus ovatus*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors from plant sources, we found that an alcoholic extract of *Maytenus ovatus* Loes.[3] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) and against five standard animal tumor systems.[4] We report herein the isolation and structural elucidation of maytansine (**1**), a novel antileukemic ansa macrolide tumor inhibitor[5] from *Maytenus ovatus*. Maytansine is the first ansa macrolide shown to contain carbinolamine, epoxide, or aryl halide functions and appears to be the first member of the series reported to show significant *in vivo* tumor inhibitory activity.

Fractionation of the alcohol extract, guided by assay against KB and P-388, revealed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition and in the methanol layer of a 10% aqueous methanol–petroleum ether partition. Column chromatography on SilicAR CC7 followed by treatment with acetic anhydride–pyridine[6] and extensive column chromatography and preparative tlc on alumina, silica gel, and SilicAR afforded a highly enriched concentrate (A, 1 mg/kg of plant). Attempts to prepare different derivatives in methanol solution yielded a common product, apparently a methyl derivative, **2**, whereas similar experiments in ethanol solution yielded a common ethyl derivative, **3**. Accordingly, concentrate A was treated at room temperature with 3-bromopropanol-

(1) Tumor Inhibitors. LXXIII. Part LXXII: S. M. Kupchan, C. W. Sigel, L. J. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, **94**, 1353 (1972).

(2) Supported by grants from the National Cancer Institute (NCI, CA-11718) and American Cancer Society (T-275 and T-541), and a contract with Chemotherapy, National Cancer Institute (NIH 71-2099).

(3) Fruits were collected in Ethiopia in Jan 1962. Roots and the wood of stems from Ethiopia and Kenya also yielded active extracts. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.

(4) Activity was noted against sarcoma 180, Lewis lung carcinoma, and L-1210 and P-388 leukemias in the mouse and Walker 256 intramuscular carcinosarcoma in the rat. Cytotoxicity and *in vivo* activity were assayed as in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(5) Maytansine showed significant antileukemic activity against P-388 lymphocytic leukemia over a 50–100-fold dosage range at the microgram per kilogram level, and cytotoxicity (ED_{50}) against KB cell culture at 10^{-4}–10^{-5} μg/ml.

(6) Pilot experiments revealed that acetylation facilitated the subsequent separation without affecting maytansine.