nitrite as above for 50 hr. Vpc-pure cyclohexanone is obtained in 67% yield (9.75 g), bp 154–155°, n^{20}D 1.4505.

5-Nitro-2-hexanone into 2,5-hexanedione.—The nitro ketone (21.78 g, 0.15 mol), sodium nitrite (51.75 g, 0.75 mol), *n*-propyl nitrite (26.73 g, 0.30 mol), and 300 ml of DMSO are employed as above (reaction time 20 hr). Since the dione is water soluble, relatively large amounts of methylene chloride and relatively small amounts of water are used in the work-up. Also, the crude dione is chromatographed on silica gel prior to distillation. There is obtained 13.25 g (76% yield) of vpc-pure 2,5-hexanedione, bp 58–60° at 5 mm, n^{20}D 1.4253. The nmr spectrum consists of a singlet at δ 2.1 (6 H) and a singlet at 2.6 (4 H).

5-Nitro-2-octanone into 2,5-Octanedione.—Using 25.97 g (0.15 mol) of this nitro ketone the reaction is carried out as in the preceding experiment (reaction time 52 hr). The vpc-pure dione (bp 44–45° at 0.47 mm) is obtained in 71% yield (15.13 g), n^{20}D 1.4313 (lit.[17] n^{23}D 1.4317).

Anal. Calcd for $C_8H_{14}O_2$: C, 67.57; H, 9.93. Found: C, 67.49; H, 10.14.

2-Nitropropane into Acetone.—To facilitate isolation of the product hexamethylphosphoramide (HMPA) was employed as the solvent and *n*-octyl nitrite as the nitrosating agent. Under nitrogen, a mixture of 55.20 g (0.80 mol) of dry sodium nitrite, 35.99 g (0.40 mol) of 2-nitropropane, and 79.61 g (0.50 mol) of *n*-octyl nitrite in 800 ml of HMPA is stirred at 25–28° (subdued light) for 4.5 hr. The acetone is removed directly from the reaction mixture at room temperature *in vacuo* and collected at −80°. Distillation from Drierite gives 16.10 g (70% yield) of pure acetone, bp 56–58°, n^{20}D 1.3588.

Identification of Nitrous Oxide.—A colorless gas is evolved in all of these transformations. A sample of the gas produced in the conversion of 2-nitropropane to acetone (*vide supra*) was collected after passing through a −80° trap. Mass spectroscopy reveals, in addition to N_2 and O_2 peaks at *m/e* 28 and 32, peaks at *m/e* 44 (100%) for N_2O and *m/e* 30 (40%) presumed to be NO^+ derived from N_2O. A high resolution peak to peak comparison of the *m/e* 44 peak with the *m/e* 44 peak of CO_2 confirms the presence of nitrous oxide.[18] Calcd for N_2O: *m/e* 44.0011. Found: *m/e* 44.0003.

Registry No.—Sodium nitrite, 7632-00-00; α-phenylethyl bromide, 585-71-7.

Acknowledgment.—We thank the National Science Foundation and Eli Lilly and Co. for generous support.

(17) R. L. Huang, *J. Chem. Soc.*, 1749 (1956).

(18) We are indebted to Mr. W. Perry of the Purdue Mass Spectrometry Center for determining these spectra.

Datiscacin, a Novel Cytotoxic Cucurbitacin 20-Acetate from *Datisca glomerata*[1a,b]

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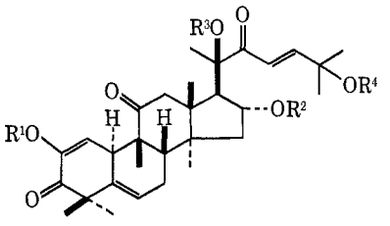
In the course of a continuing search for tumor inhibitors of plant origin, a chloroform extract of *Datisca glomerata* Baill. (Cucurbitaceae) was found to show significant activity against human carcinoma of the nasopharynx (KB) carried in cell culture.[2] A number of tumor-inhibitory principles have been isolated from this plant and the structure elucidation of one of these, datiscoside, has already been reported.[3] We report herein the structure elucidation of another cytotoxic principle, datiscacin (**1**), the first recognized cucurbitacin 20-acetate ester derivative.

The chloroform extract of the dried roots was subjected to successive solvent partitions and chromatographic separations, guided by the KB assay. Datiscacin[4] (**1**), $C_{32}H_{44}O_8$, mp 208–212°, $[\alpha]^{23}$D −18°, was crystallized from a cytotoxic fraction.

Elemental analysis and spectral data for datiscacin supported its formulation as a cucurbitacin monoacetate ester. Acetylation of datiscacin under mild conditions with acetic anhydride–pyridine yielded a triacetate (**2**), indicative of the location of the original acetate group on the C-17 side chain. That datiscacin contains a diosphenol in ring A was indicated by its positive ferric chloride test, absorption at 6.13 μ in the infrared and 268 nm in the ultraviolet, a one-proton doublet at τ 4.29 in the nmr spectrum,[5] and a peak at *m/e* 164 in the mass spectrum.[6] The known cucurbitacin E (**3**) contains a ring A diosphenol and a C-25 acetate ester, but its markedly different optical rotation ($[\alpha]^{26}$D −58°)[7] indicated that it differed from datiscacin. These considerations and the fact that there are but two hydroxyl groups in the cucurbitacin side chain led us to entertain the hypothesis that datiscacin is the C-20 acetate ester **1**.



1, $R^1 = R^2 = R^4 = H$; $R^3 = Ac$
2, $R^1 = R^2 = R^3 = Ac$; $R^4 = H$
3, $R^1 = R^2 = R^3 = H$; $R^4 = Ac$
4, $R^1 = R^2 = R^3 = R^4 = H$
5, $R^1 = R^2 = Ac$; $R^3 = R^4 = H$

Confirmation for the position of the acetate ester in **1** was derived from the results of periodate oxidation studies. Thus, datiscacin diacetate (**2**) was found to be unaffected by treatment with an excess of periodic acid, and the compound was recovered unchanged. In contrast, treatment of cucurbitacin I diacetate (**5**)[8] with periodic acid under the same conditions led to consumption of 1 molar equiv of the reagent, in accord with the expected sensitivity of the 20,22-ketol system.

Interrelation with a known cucurbitacin was deemed desirable to confirm the postulated structure and configuration of datiscacin. Hydrolysis of the tertiary C-20 acetate ester group to yield cucurbitacin I (**4**) was envisaged, but strong alkaline treatment was precluded by the known sensitivity of ring A diosphenols

(1) (a) Tumor Inhibitors. LXXXIII. Part LXXXII: S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.* **38**, 178 (1973). (b) This investigation was supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (IC-57H), and a contract with the National Cancer Institute (NIH-NCI-C-71-2099). (c) National Institutes of Health Postdoctoral Fellow, 1972–present.

(2) Cytotoxicity was assayed, under the auspices of the National Cancer Institute, by the procedure described in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(3) S. M. Kupchan, C. W. Sigel, L. J. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, **94**, 1353 (1972).

(4) Datiscacin showed significant cytotoxicity (ED_{50} = 2.9 × 10^{-2} μg/ml) against cells derived from the human carcinoma of the nasopharynx (KB).

(5) C. R. Noller, A. Melera, M. Gut, J. Shoolery, and L. F. Johnson, *Tetrahedron Lett.*, 15 (1960).

(6) H. Audier and B. Das, *ibid.*, 2205 (1966).

(7) S. M. Kupchan, A. H. Gray, and M. D. Grove, *J. Med. Chem.*, **10**, 337 (1967).

(8) D. Lavie and Y. Shvo, *J. Amer. Chem. Soc.*, **82**, 966 (1960).

to benzilic acid rearrangement and of the 23,24 bond of similar compounds to retro aldol cleavage.[9] In a parallel study, we recently found the alkaline solvolysis of the ester in a ketol acetate to be facilitated by the adjacent carbonyl group or its hemiketal adduct.[10] Accordingly, treatment of datiscacin (**1**) with sodium carbonate in aqueous methanol for 12 hr at room temperature effected a smooth solvolysis of the 20-acetate ester group, to yield cucurbitacin I (**4**). The interrelation completed the proof of the structure of datiscacin (**1**), the first recognized cucurbitacin 20-acetate ester derivative.

Experimental Section

Melting points were determined on a Mettler FP2 melting point apparatus. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Ultraviolet spectra were recorded on a Coleman Hitachi EPS-3T recording spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian HA-100 spectrometer using TMS as an internal reference. Mass spectra were recorded on either Hitachi Perkin-Elmer RMU-63 or AEI MS-902 spectrometers, equipped with direct insertion probes. High-pressure liquid chromatography was carried out on a Waters ALC-202/401 liquid chromatographic system. Analytical and preparative tlc were carried out on Brinkmann Silplates. Petroleum ether refers to the fraction of bp 60–68°. Evaporations were carried out at reduced pressure below 40°. Analyses were carried out by Spang Microanalytical Laboratories, Ann Arbor, Mich.

Extraction and Fractionation.—The dried ground roots of *Datisca glomerata* Baill. (10 kg)[11] were continuously extracted with chloroform for 20 hr. Evaporation gave crude extract A (270 g), which was partitioned between water (500 ml) and chloroform (two 1-l. portions). The chloroform solution was evaporated to give a viscous brown residue (C, 260 g). The aqueous solution was freeze-dried to yield fraction B (3 g). Fraction C was partitioned between aqueous methanol (1:9, 1 l.) and petroleum ether (three 1-l. portions). Evaporation of the aqueous methanol solution gave fraction D (230 g) and the combined petroleum ether extracts yielded residue E (25 g). Fraction D was partitioned between aqueous methanol (2:8, 1 l.) and carbon tetrachloride (two 1-l. portions). The aqueous methanol layer gave fraction F (154 g) and the carbon tetrachloride layer gave fraction G (70 g).

Isolation of Datiscacin.—Fraction G (70 g) was further fractionated by column chromatography on silica gel (1.2 kg, 70–325 mesh). Elution with chloroform followed by 5% methanol–chloroform yielded a fraction (H, 1.35 g) enriched in datiscacin. Fraction H was separated by repeated preparative tlc with diethyl ether and the main band was eluted with methanol to give a light-yellow gum. The gum was crystallized from ethanol to give datiscacin (**1**, 25 mg), $C_{32}H_{44}O_8$: mp 208–212°; $[\alpha]^{23}D$ −18° (*c* 0.87, $CHCl_3$); uv $\lambda_{max}^{CHCl_3}$ 231 nm (ε 9600), 268 (5400); ir (KBr) 2.79–2.88, 3.35, 3.41, 5.81, 5.95, 6.13, 7.14, 7.30, 7.89, 8.81, 8.89, 9.02, 10.1, and 12.6 μ; nmr ($CDCl_3$) τ 3.17 (1 H, d, J = 16 Hz), 3.75 (1 H, d, J = 16 Hz), 4.19 (1 H, s), 4.29 (1 H, d, J = 2.5 Hz), 4.47 (1 H, m), 5.90 (2 H, m), 8.19 (3 H, s), 8.62 (3 H, s), 8.63 (3 H, s), 8.74 (3 H, s), 8.76 (3 H, s), 8.82 (3 H, s), 8.92 (3 H, s), 9.12 (3 H, s), and 9.17 (3 H, s); mass spectrum *m/e* 496, 478, 401, 385, 383, 369, 367, 219, 164, 113, 96, and 43.

Anal. Calcd for $C_{32}H_{44}O_8 \cdot {}^1/_2H_2O$: C, 67.94; H, 8.02. Found: C, 67.81; H, 8.18.

Acetylation of Datiscacin (1) to Triacetate 2.—A solution of datiscacin (1, 10 mg) in anhydrous pyridine (0.5 ml) and acetic anhydride (0.5 ml) was stirred overnight at room temperature under nitrogen. The solution was evaporated *in vacuo* and the residue was dissolved in ethanol and reevaporated. The oily residue (10 mg) was separated by preparative tlc with diethyl ether. The product (8 mg) was crystallized from diethyl ether–hexane to give **2** (5 mg): mp 119–120°; $[\alpha]^{23}D$ −43° (*c* 1.40, $CHCl_3$); ir (KBr) 2.80, 3.35–3.52, 5.75, 5.92, 6.15, 6.90, 7.30, 8.00, 8.30, 9.65, and 13.4 μ; nmr ($CDCl_3$) τ 2.82 (1 H, d, J = 16 Hz), 3.58 (1 H, d, J = 16 Hz), 4.16 (1 H, m), 4.78 (1 H, m), 5.06 (1 H, s), 7.76 (3 H, s), 7.92 (3 H, s), 8.08 (3 H, s), 8.36 (6 H, s), 8.48 (6 H, s), 8.60 (6 H, s), and 8.88 (6 H, s); mass spectrum *m/e* 580, 538, 487, 485, 411, 409, 367, 351, 309, 111, 96, 79, 60, 45, and 43.

Anal. Calcd for $C_{36}H_{48}O_{10}$: C, 67.48; H, 7.55. Found: C, 67.10; H, 7.72.

Periodic Acid Titrations.—The titrations were performed essentially according to the procedure of Jackson.[12] A solution of substrate (13 mg) in 95% ethanol (3.00 ml) was treated with 0.043 *M* periodic acid (2.00 ml) in an erlenmeyer flask (25 ml). The flask was kept in the dark under nitrogen for 7 days. The solution was then treated with 0.056 *M* iodine solution (7.02 ml) and titrated with sodium arsenite (0.10 *M*, 3.06 ml) to the blue starch end point. Datiscacin diacetate (**2**) consumed no periodic acid and was recovered unchanged. Cucurbitacin I diacetate (**5**) consumed 1.1 molar equiv of periodic acid.[8]

Solvolysis of Datiscacin (1) to Cucurbitacin I (4).—A solution of datiscacin (**1**, 10 mg) in methanol (2 ml) was treated with aqueous sodium carbonate (0.1 *M*, 0.5 ml) and allowed to stand overnight at room temperature. The mixture was neutralized with acetic acid and extracted with ethyl acetate. Evaporation of the ethyl acetate solution gave a residue (8 mg) which was separated by preparative tlc with 7% methanol–chloroform. Elution of the major band followed by evaporation gave a crude product (4.5 mg) which was further separated by high-pressure liquid chromatography [column, Corasil II,[13] 3 ft × 0.375 in.; solvent, hexane–ether (3:7)]. The crystalline product (0.9 mg, from ether–petroleum ether) was characterized as cucurbitacin I (**4**) by mixture melting point, mass spectrum, tlc, and high pressure lc comparisons with an authentic sample.

Registry No.—**1**, 38308-89-3; **2**, 38308-90-6; **4**, 2222-07-3.

(9) *Cf.* D. Lavie, Y. Shvo, O. R. Gottlieb, and E. Glotter, *J. Org. Chem.*, **27**, 4546 (1962).

(10) S. M. Kupchan and G. Tsou, *ibid.*, **38**, 1055 (1973).

(11) 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. Department of Agriculture, in accordance with the program developed by the National Cancer Institute.

(12) E. L. Jackson, *Org. React.*, **2**, 341 (1944).

(13) From Waters Associates Inc., Framingham, Mass.

A Synthesis of Homoserine Phosphate and a Blocked Derivative Suitable for Peptide Synthesis

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In the course of our studies of oligopeptide transport in *E. coli* it became of interest to synthesize peptides containing the amino acid homoserine phosphate. A search of the literature revealed no suitable chemical synthesis for either homoserine phosphate or a blocked derivative thereof. Homoserine phosphate has been prepared enzymically with crude yeast homoserine kinase;[1] we found the method cumbersome and not appropriate for the production of the relatively large quantities of blocked derivatives required for peptide synthesis. We wish to report a simple synthesis leading to *O*-diphenylphosphorohomoserine benzyl ester tosylate in an overall yield of 17% starting with homoserine. The compound can either be introduced at the carboxyl end of a suitably blocked peptide or subjected to hydrogenolysis to yield homoserine phosphate in roughly 100% yield. The synthesis has been carried out starting with DL-homoserine and L-homoserine;

(1) Y. Watanabe and K. Shimura, *J. Biochem.*, **43**, 283 (1956).