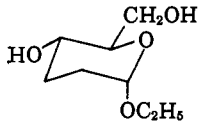
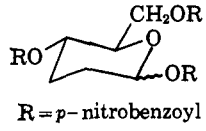
Further, in this work a 2,3-dideoxy sugar was directly converted in 44% yield to a single nucleoside which was proven to have the β configuration. The starting 2,3-dideoxy sugar was the previously reported VI, ethyl 2,3-dideoxy-α-D-*erythro*-hexanopyranoside.[6] Compound VI was hydrolyzed with 2 *N* hydrochloric acid and treated with excess *p*-nitrobenzoyl chloride in pyridine to give 72% of an anomeric mixture of VII, m.p. 113–146°, with correct elemental analyses for carbon, hydrogen, and nitrogen.

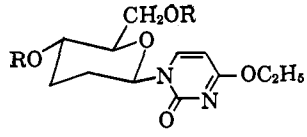


VI



R = *p*-nitrobenzoyl

VII



R = *p*-nitrobenzoyl

VIII

Compound VII was converted to the glycosyl chloride according to the procedure of Zorbach and Payne[7] using methylene chloride and dry hydrogen chloride, and the resulting glycosyl chloride was converted directly to the nucleoside using diethoxypyrimidine at 100°. Although the yield of VIII, m.p. 206–207°, $[\alpha]^{25.4}D$ +48.4° (*c* 1.3, $CHCl_3$), was only 44%, this yield represents two steps from VII and the nucleoside could be isolated by simple crystallization.

The configuration of the glycosidic linkage was proven to be β by conversion to V in 57% yield using ethoxide ion in ethanol followed by benzaldehyde and zinc chloride.

All new compounds had acceptable analyses.

Acknowledgment.—This investigation was aided by grant T-294 of the American Cancer Society.

(6) M. Bergmann, *Ann.*, **443**, 223 (1925); S. Laland, W. G. Overend, and M. Stacey, *J. Chem. Soc.*, 738 (1950); C. L. Stevens and P. Blumbergs, Abstracts, 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962, p. 9N.

(7) W. W. Zorbach and T. A. Payne, Jr., *J. Am. Chem. Soc.*, **80**, 5564 (1958); R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **76**, 1663 (1954).

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The Structure of Tubulosine, a Novel Alkaloid from *Pogonopus tubulosus* (DC.) Schumann[1,2]

Sir:

Pogonopus tubulosus (DC.) Schumann (fam. *Rubiaceae*) is a tree growing in the northern part of Argentina, and its bark extracts were claimed to be active against fever. A preliminary survey[3] indicated the presence of several amorphous alkaloids.

We have isolated from an extract of the bark a crystalline base [m.p. 259–261°, Kofler; $[\alpha]^{24}D$ −65.9° (*c* 2.0, pyridine) pK_{MCS} = 6.3 and 8.0], named tubulosine, for which the biogenetically novel structure I is proposed. The elementary and functional group analyses as well as the mass spectrum agree with the empirical formula $C_{29}H_{37}N_3O_3$, containing two methoxy and one C-methyl groups. The alkaloid gave a positive phenol test, thus elucidating the nature of the three oxygen atoms. The ultraviolet spectrum showed a maximum at 281 mμ (log ϵ 4.16) and a shoulder at 225 (4.55), while the infrared spectrum (potassium bromide) exhibited a sharp band at 2.95 (NH) as well as a shoulder at 2.70–2.80 μ.

Tubulosine gave positive indole tests with dimethylaminobenzaldehyde, Keller–Kiliani reagent, and vanilline–hydrochloric acid. All attempts to prepare crystalline salts failed, and treatment with diazomethane gave a noncrystalline product (II) with a negative phenol test.

Acetylation with acetic anhydride in methanol led to a monoacetyl derivative (m.p. 184–186°), while acetic anhydride–pyridine produced a diacetate (m.p. 149–151°) which could be transformed into the monoacetate by mild treatment with sodium hydrogen carbonate solution. Oxidation of tubulosine with potassium permanganate in slightly alkaline solution produced *m*-hemipinic acid which was identified as its methylimide.

The n.m.r. spectrum showed a sharp signal at 7.75 p.p.m. (δ-units) representing the six methoxy protons, in agreement with the Zeisel determination and the degradation to *m*-hemipinic acid. In the aromatic proton region a multiplet was present corresponding to five protons. Other signals were at 4.15 (NH), 8.4 (OH), and 10.4 (indole NH), while a broad one at 0.95 p.p.m. was assigned to the methyl protons of the C-ethyl group (see I).

The mass spectrum showed a molecular ion peak at *m/e* 475 while the base peak occurred at *m/e* 187. A series of characteristic peaks was present which can also be detected in the mass spectrum of emetine[4,5]: those occurring at *m/e* 288, 272–275, 246, and 244 are characteristic of the quinolizidine moiety, whereas other peaks (*m/e* 206, 205, 192, and 191), also found in the emetine spectrum,[4,5] are representative of isoquinoline ions. Two other significant peaks in the mass spectrum of tubulosine (I) occur at *m/e* 201 and 187. They represent the tetrahydro-β-carboline portion of the molecule as indicated in I. These assignments were confirmed in two ways.

First, the mass spectrum of totally synthetic III[6] gave all the peaks derived from the quinolizidine moiety, but the peaks at *m/e* 201 and 187 were absent. Instead, new peaks at *m/e* 185 and 171 appeared, the 16 mass unit difference being due to the additional hydroxyl group of tubulosine (I). This is in accordance with the mass spectrometric shift technique first formulated by Biemann,[7] which implies that two alkaloids differing only in the substitution of an aromatic nucleus should exhibit identical mass spectra except for displacement of those fragments which contain the additional sub-

(1) Presented in part at the I.U.P.A.C. Congress, London, July, 1963.

(2) Paper LIV in the Stanford Series "Mass Spectrometry in Structural and Stereochemical Problems." For preceding paper see R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, in press.

(3) G. Dalma and B. Mateu Amengual, *Arch. Farm. Bioquim. Tucumán*, **4**, 59 (1948).

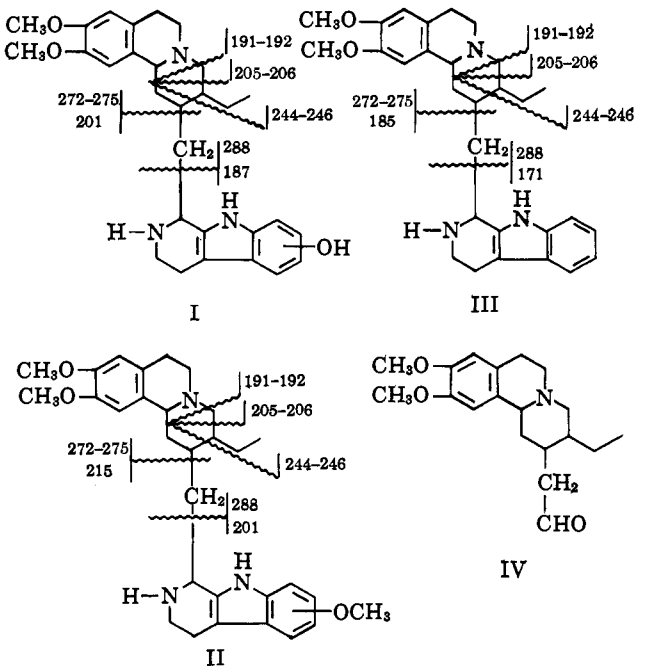
(4) G. Spiteller and M. Spiteller-Friedman, *Tetrahedron Letters*, 153 (1963).

(5) H. Budzikiewicz, S. C. Pakrashi, and H. Vorbrüggen, *Tetrahedron*, **20**, 399 (1964).

(6) A. R. Battersby, J. C. Davidson, and J. C. Turner, *J. Chem. Soc.*, 3899 (1961).

(7) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., New York, N. Y., 1962, Chapter 8.

stituent. This approach has been used successfully for structure proposals of many indole alkaloids.[8]



Second, the noncrystalline product (II) from the methylation of tubulosine with diazomethane gave a mass spectrum in which all the peaks originating from the quinolizidine moiety were also present, but the peaks at *m/e* 201 and 187 were shifted to *m/e* 215 and 201 (base peak). The observed peak movements (see II) are in full accord with the proposed structure of tubulosine. Mechanistic proposals for the formation of the fragments indicated schematically in structures I–III may be found in the original literature[4,5] dealing with emetine.

In the biogenesis of Ipecacuanha alkaloids it is assumed that the isoquinoline nucleus is formed from a phenylethylamine precursor, which (formally speaking) condenses with another intermediate which can be represented by protoemetine (IV).[9] By substituting a tryptamine precursor for the phenylethylamine component, the biogenetically plausible, but hitherto unencountered, structure of tubulosine (I) is created.

Acknowledgment.—We thank Dr. W. Simon (E. T. H., Zürich) for the pK_{MCS} determinations, Prof. A. R. Battersby (University of Liverpool) for the generous gift of a sample of the hydrochloride of III, Dr. M. J. Vernengo (University of Buenos Aires) for assistance in part of this work, and Dr. D. P. Hollis (Varian Associates for the n.m.r. spectrum. The work at Stanford University was supported by grant No. AM-04257 from the National Institutes of Health of the U. S. Public Health Service.

(8) See H. Budzikiewicz, C. Djerassi, and D. H. Williams," Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, Inc., San Francisco, Calif., 1964.

(9) A. R. Battersby and B. J. T. Harper, *J. Chem. Soc.*, 1748 (1959); C. Szántay and L. Töke, *Tetrahedron Letters*, 1323 (1963).

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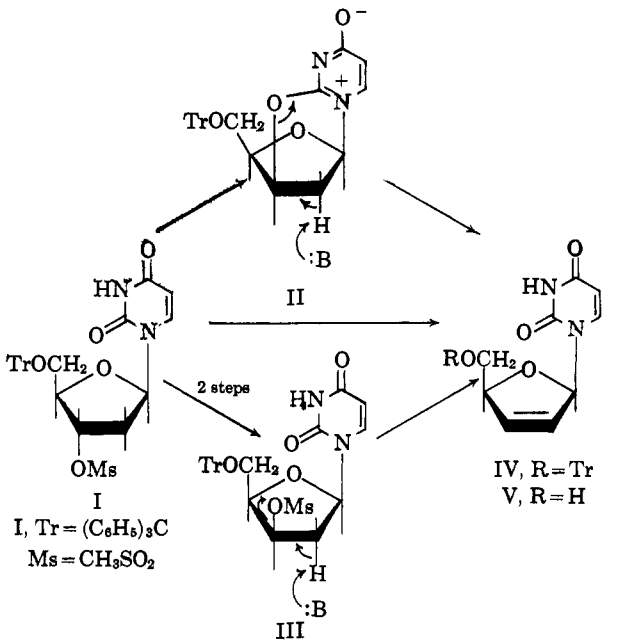
RECEIVED MARCH 6, 1964

Nucleosides. VI. The Introduction of Unsaturation into the Carbohydrate of a Pyrimidine Nucleoside *via* a 2,3′-Anhydro Bond

Sir:

We wish to report the introduction of a 2′,3′-double bond into the carbohydrate moiety of a pyrimidine nucleoside *via* a novel, base-catalyzed elimination reaction in which a uracilyloxy group behaves as a leaving group.[1] The unsaturated derivatives are viewed as potentially useful intermediates for the synthesis of a number of unusual pyrimidine nucleosides.

3′-*O*-Mesyl-5′-*O*-trityl-2′-deoxyuridine[2] (I) was readily converted to 2,3′-anhydro-1-(2′-deoxy-5′-*O*-trityl-β-D-lyxosyl)uracil (II)[3] in high yield on treatment with 1 equiv. of sodium hydroxide in ethanol.[4] The reaction of II with potassium *t*-butoxide in dimethyl sulfoxide at room temperature for 0.5 hr. afforded a solid (70% yield) with properties consistent with 1-(5′-*O*-trityl-2′,3′-dideoxy-2′-ene-β-D-*glycero*-pentofuranosyl)uracil[5] (IV), m.p. 194–196°, $[\alpha]^{25}D$ −56°



(*c* 0.4, ethanol), $\lambda^{EtOH}_{max,\,min}$ (mμ) 261, 242 (ε 9770, 6000). The same base–solvent system (2 equiv. of base) applied to either 1-(3′-*O*-mesyl-5′-*O*-trityl-2′-deoxy-β-D-lyxosyl)uracil (III)[6] or I gave a product identical in every respect with IV. The corresponding detritylated product V, m.p. 155–156° dec., $[\alpha]^{25}D$ −88° (*c* 0.3, water), $\lambda^{H_2O}_{max,\,min}$ (mμ) 261, 231 (ε 10,380, 2300), was obtained in 81% yield on treatment with 1 equiv. of hydrogen chloride in chloroform.

(1) The concept of an anhydro bond comprising a leaving group was first advanced by K. C. Murdock and R. B. Angiers [*J. Am. Chem. Soc.*, **84**, 3748 (1962)] in connection with the formation of a halogenated 1-cyclopentane derivative of thymine (a thymidine isostere) from an anhydro intermediate. It was proposed that a positive charge (acid solution) on the anhydro oxygen atom enables a thyminyloxy group to behave as a leaving group.

(2) J. P. Horwitz, J. Chua, M. Noel, and M. A. DaRooge, *J. Med. Chem.*, **7**, 385 (1964).

(3) Analytical values for all compounds described in this work were consistent with the indicated structures.

(4) The procedure is essentially the same as that described previously [J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel, *J. Org. Chem.*, **28**, 942 (1963)] for the synthesis of 5′-*O*-trityl-2,3′-anhydrothymidine.

(5) This nomenclature is in accord with that employed by R. K. Ness and H. G. Fletcher, Jr.; *ibid.*, **28**, 435 (1963).

(6) The mesylation of 1-(5′-*O*-trityl-2′-deoxy-β-D-lyxofuranosyl)uracil (see ref. 2) gives III in good yield.