SCHEME I

hydroxylactone form[3] and whose pseudo-ester is more stable, forms mainly normal ester under kinetic control.

In a comparable 15-min. experiment, 6-methyl-2-benzoylbenzoic acid forms *in 44% yield* a mixture of 40% normal–60% pseudo-ester whereas at equilibrium the ester is 63% normal–37% pseudo. These results may be rationalized by using a combination of esterifications *via* routes comparable to A, B, and D, route C being less involved because of conventional steric hindrance. We believe that the accelerative effect of the 6-methyl group on route A is largely responsible for the increase in rate of esterification, as in the case of alkaline hydrolysis.[2]

How general the above effects are in the cases of other keto acids is under investigation.

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Synthesis of 2,3-Didehydro-2,3-dideoxy and 2,3-Dideoxy Sugar Nucleosides of Known Configuration

Sir:

The antibiotic amicetin has been shown to have the structure I.[1] Initially reported in 1953,[2] the antibiotic

represents the first reported nucleoside containing a 2,3-dideoxy sugar. Two of the most important problems associated with the chemistry of this new class of compounds are the determination of the stereochemistry of the nucleoside bond and the synthesis of a 2,3-dideoxy sugar nucleoside of known configuration. This paper presents the first synthesis of a such a nucleoside *via* a 2,3-didehydro-2,3-dideoxy sugar nucleoside. Further, a 2,3-dideoxy sugar was converted directly into a nucleoside and the stereochemistry of nucleoside linkage established.

The starting nucleoside II, of known β-configuration, was prepared from 1(tetra-*O*-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1*H*)pyrimidone which in turn was made by the classic procedure of Hilbert and Jansen.[3] The four acetate groups were removed with catalytic amounts of ethoxide ion and the resulting product

(1) C. L. Stevens, P. Blumbergs, and F. A. Daniher, *J. Am. Chem. Soc.*, **85**, 1552 (1963); C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2991 (1962).

(2) J. W. Hinman, E. L. Caron, and C. DeBoer, *J. Am. Chem. Soc.*, **75**, 5864 (1953); E. H. Flynn, J. W. Hinman, E. L. Caron, and D. O. Woolf, Jr., *ibid.*, **75**, 5867 (1953).

(3) G. E. Hilbert and E. F. Jansen, *ibid.*, **58**, 60 (1936).

was treated with benzaldehyde and zinc chloride to give 63% of II, 1(4,6-*O*-benzylidene-β-D-glucosyl)4-ethoxy-2(1*H*)pyrimidone, m.p. 228–230°, [α]^{23}D +33.5° (*c* 1.3, CHCl₃).

Compound II was converted to a 2,3-epoxide by a procedure recently described by Goodman and Christensen.[4] Treatment with 1.2 equiv. of *p*-toluenesulfonyl chloride followed by excess acetic anhydride gave a monotosyl monoacetate in 47% yield, m.p. 143–144°, [α]$^{24.5}$D +7.2° (*c* 2.09, CHCl₃). The monotosyl monoacetate gave a crystalline oxide in 73% yield when allowed to react with 5 equiv. of ethoxide ion. The oxide, m.p. 184–185°, [α]^{21}D +70.6° (*c* 1.47, CHCl₃). is assigned the *manno* configuration on the basis of previous data[5] which indicate the initial tosylation takes place on the 2-carbon.

The oxide was opened with sodium iodide, acetic acid, and small amounts of sodium acetate in acetone to give 92% of an iodohydrin, m.p. 220–221°, [α]^{25}D +92.2° (*c* 1.14, CHCl₃). On the basis of diaxial opening, the iodohydrin is assigned the *altro* configuration. Mesyl-

ation at room temperature gave 67% of III, 1(4,6-*O*-benzylidene - 3-deoxy-3-iodo-2-*O*-methylsulfonyl-β-D-altrosyl)-4-ethoxy-2(1*H*)pyrimidone, m.p. 182–183°, [α]^{25}D +78.8° (*c* 1.84, CHCl₃).

The 2,3-unsaturated nucleoside IV, which to our knowledge represents the first such nucleoside reported in the chemical literature, was prepared in 99% yield from III with excess sodium iodide in acetone. The product, 1(4,6-*O*-benzylidene-2,3-didehydro-2,3-dideoxy-β-D-*erythro*-aldohexosyl)4-ethoxy-21(*H*)pyrimidone, IV, had m.p. 174–176°, [α]$^{25.5}$D +72.0° (*c* 0.7, CHCl₃).

Reduction of IV with platinum oxide in methanol was stopped after 1-mole uptake. Thin layer chromatography indicated a small amount of benzylidene reduction, but 60% of pure, recrystallized 2,3-dideoxy sugar nucleoside (V) was isolated.

This product V, 1(4,6-*O*-benzylidene-2,3-dideoxy-β-D-*erythro*-aldohexosyl)-4-ethoxy-2(1*H*)pyrimidone, had m.p. 181–182°; [α]^{25}D +69.5° (*c* 1.2, CHCl₃).

(4) L. Goodman and J. E. Christensen, *J. Org. Chem.*, **28**, 158 (1963).

(5) G. J. Robertson and C. F. Griffith, *J. Chem. Soc.*, 1193 (1935); H. R. Bollinger and D. A. Prins, *Helv. Chim. Acta*, **28**, 465 (1945); also *cf.* ref. 4.

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.
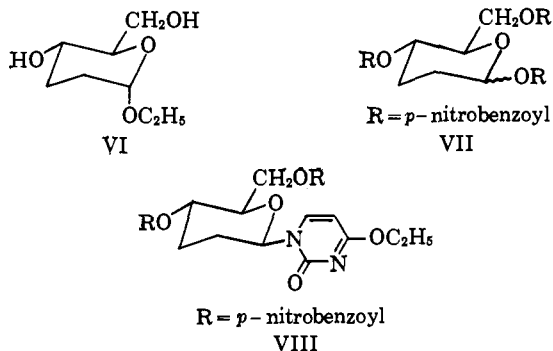
Compound VII was converted to the glycosyl chloride according to the procedure of Zorbach and Payne[7] using methylene chloride and dry hydrogen chloride, and the resulting glycosyl chloride was converted directly to the nucleoside using diethoxypyrimidine at 100°. Although the yield of VIII, m.p. 206–207°, $[\alpha]^{25.4}$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.

(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

(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. Fárm. Bioquim. Tucumán*, **4**, 59 (1948).

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 vaniline–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-

(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.