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A NEW SYNTHESIS OF NEBULARINE AND 7-D-RIBOFURANOSYLPURINE

(SYNTHESIS IN NUCLEOSIDE ANTIBIOTICS, PART I)

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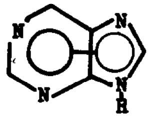
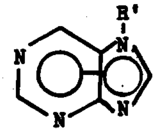
Ehrenberg et. al[1] have isolated nebularine from Agaricus (Clitocybe) nebularis Batsch. Later, Isono[2] et. al. have detected it as a metabolic product of a streptomycete.
The compound shows a strong activity against mouse Sarcoma 180 and mycobacteria. The structure has been determined as 9-β-D-ribofuranosylpurine[3] and confirmed by synthesis[4].



I. R=H, purine
II. R=2',3',5'-tri-O-acetyl-β-D-ribofuranosyl
III. R=β-D-ribofuranosyl, nebularine



I. R'=H, purine
IV. R'=2',3',5'-tri-O-acetyl-D-ribofuranosyl
V. R'=D-ribofuranosyl, 7-isonebularine

The synthesis by Brown et. al.[4] principally rests on Davoll-Lowy method and the yield is reported to be 34 % starting from the chloromercuri complex of purine and 2', 3', 5'-tri-O-acetyl-D-ribofuranosyl chloride. In another synthesis described by Fox et. al.[5], the over-all yield is calculated to be 32% starting from inosine.

Recently, we have shown that the catalytic effect of bis-(p-nitrophenyl) hydrogen phosphate is superior than known catalysts for fusion procedure which has been exploited for nucleoside synthesis[6]. Utilizing this catalyst for the fusion of purine (I) with tetra-O-acetyl-D-ribofuranose, we have synthesized 2', 3', 5'-tri-O-acetylnebularine (II) and 7-(2', 3', 5'-tri-O-acetyl-D-ribofuranosyl)-purine (IV) in quantitative yield. Removal of the acetyl groups from (II) and (IV) has furnished nebularine (III) and 7-D-ribofuranosylpurine(7-isonebularine, V), respectively. This seems to be the first example of simultaneous production of both 7- and 9-β-D-nucleosides by the fusion method. We wish to report this facile synthesis of nebularine and 7-isonebularine.

Purine and tetra-O-acetyl-D-ribofuranose were made by the methods described by earlier workers[7,8].

Finely pulverized mixture of 2g. of purine[7] and 4.1g. of tetra-O-acetyl-β-D-ribofuranose[8] was fused at 170°-180°. To the mixture was added 200mg. of anhydrous bis-(p-nitrophenyl) hydrogen phosphate and mixed well. The mixture was kept at 170°-180° for 15 min. under reduced pressure by water pump. The reaction product was extracted with chloroform. The chloroform layer was washed with water, dried, and then evaporated to dryness yielding 5.2g. of oil. The product was dissolved in ethylacetate, poured into the top of the silicic acid column, 3 cm. i. d., and 49 cm. in length, packed with silicic acid (100 mesh, Mallinckrodt), and then eluted with ethylacetate. The eluates were collected by fraction collector. Measuring optical densities of the eluates at 260 mμ, the chromatogram involving three peaks was obtained. From the fractions corresponding to the first peak, 0.6g. of unreacted sugar was recovered. From the fractions corresponding to the second peak, 2.09g (yield 70%, calculated from the reacted sugar) of II was obtained as oil. From the third fractions, 0.60g. (yield 20%, based on reacted sugar) of IV was obtained as oil.

Removal of the acetyl groups from 1.7g of II in 70 ml. of methanolic ammonia and recrystallization from ethanol afforded 886 mg. of III in 79% yield, as colorless needle, m.p. 182-183° (Reported: 181-182°[3,4], 180-181°[2]), $[\alpha]_D^{35}$-46.8° (C.,2, water), Reported[3]: $[\alpha]_D^{25}$-47.5° (C., 2, water))

$\lambda_{max}^{H_2O}$ 262.5 mμ (ε, 7, 020); $\lambda_{max}^{N\text{-}HCl}$ 262.8 mμ (5, 900);

$\lambda_{max}^{pH\ 9.18}$* 263.0 mμ (7, 110) (Reported: $\lambda_{max}^{H_2O}$ 263 mμ[3];

$\lambda_{max}^{0.1N\text{-}HCl}$ 262 mμ[2]: $\lambda_{max}^{0.1N\text{-}NaOH}$ 263 mμ[2]),

Anal. Calcd. for $C_{10}H_{12}O_4N_4$:C, 47.62; H, 4.80; N, 22.22

Found. C, 47.67; H, 4.87; N, 22.37

Upon admixture with natural nebularine**, the compound showed no depression of melting point. The o.r.d. curve was

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* Borate buffer, pH 9.18±0.02(at 25°), was used.

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negative plane in the range of 300-700 mμ. The n.m.r. spectrum is shown in Fig. I. The infrared spectrum was superimposable with that of natural nebularine.

Similarly, deacetylation of 298 mg. of IV and recrystallization from ethanol gave 167 mg. (85%) of V, as colorless needles. m.p. 184.5-185°, $[\alpha]_D^{38}$-37.8°(C., water), $\lambda_{max}^{H_2O}$ 264.0 mμ (7,480); λ_{max}^{N-HCl} 258.0 mμ(6,900); $\lambda_{max}^{pH\ 9.18}$ 265.0 mμ(7,300),

The ultraviolet spectra were similar to that of 7-methylpurine[9] among the spectra of 3-, 7-, and 9-methylpurines (e.g. 3-methylpurine[10]: $\lambda_{max}^{pH\ 1}$ 268 mμ(6,600); $\lambda_{max}^{pH\ 11}$ 275 mμ (6,050) 7-methylpurine[9]: $\lambda_{max}^{pH\ 0.23}$ 257.5 mμ(6,710); $\lambda_{max}^{pH\ 9.15}$ 266.5 mμ(8,140) 9-methylpurine[9]: $\lambda_{max}^{pH\ 0.62}$ 262.5 mμ(5,870); $\lambda_{max}^{pH\ 8.50}$ 264 mμ(7,900)), though spectral data of 1-methylpurine did not available. This seems to be indicative of 7-substitution, and the structure of 7-D-ribofuranosylpurine is tentatively assigned to this compound.

Anal. Calcd. For $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22
C, 47.59; H, 4.83; N, 22.21

On admixture with III, the compound showed depression of melting point. The o.r.d. curve was negative plane in the range described above. The n.m.r. spectra of this compound will be discussed fully together with that of nebularine in separate paper.

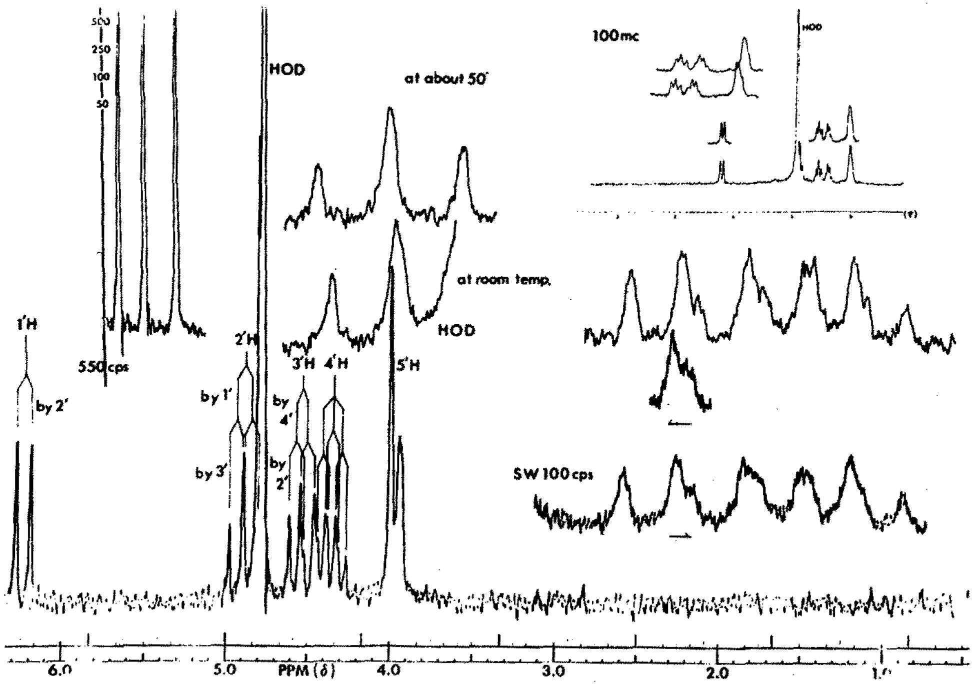

FIG.I. N.m.r. spectra of nebularine measured in D_2O.

Spectra were measured on a Varian A-60 and Japan Electron Optics Lab JNM-4H-100 (100 Mc) spectrometers.

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