clinical evaluation indicates that V and VI have activities similar to I and II.

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SYNTHESIS OF 6-(1,2-DICARBOXYETHYLAMINO)-9-β-D-RIBOFURANOSYLPURINE AND THE STRUCTURE OF ADENYLOSUCCINIC ACID[1]

Sir:

The recent isolation[2] from mouse and rabbit livers of adenylosuccinic acid (AMPS) supports evidence from enzymatic studies[3,4] that AMPS is an intermediate in the biosynthesis of adenylic acid (AMP) from inosinic acid. Carter and Cohen[5a] assigned to AMPS the structure 6-(1,2-dicarboxyethylamino) - 9 - ribofuranosylpurine - 5′-phosphate on the basis of its physical properties, enzymatic reactions, and its acid degradation to authentic 6-(1,2-dicarboxyethylamino)-purine[5b].

6-(1,2-Dicarboxyethylamino)-9-β-D-ribofuranosylpurine (I) has been synthesized by an unambiguous route. 6-Methylmercapto-9-β-D-ribofuranosylpurine[6] (1.68×10^{-3} mole), *dl*-aspartic acid (1.68×10^{-2} mole), NaOH (3.02×10^{-2} mole), and water (7 cc.) were refluxed for 20 hours; methyl mercaptan was evolved; HCl (3.02×10^{-2} mole) was added. Paper chromatograms of the solution run in solvent B[7] were sprayed to detect acidic components[8] and *cis*-glycol systems[9]; I was identified as an ultraviolet light-absorbing spot which reacted positively in both tests. The solution was chromatographed on Dowex-1 (formate) (100 cc.). Elution with water (2 l.) followed by 0.2 *N* formic acid (1.6 l.) removed aspartic acid and ultraviolet light-absorbing by-products. Evaporation at 0.5 mm. of a 1 *N* formic acid eluate gave crude I as a gum (153 mg., 24%). Rechromatography at 3° on Dowex-1 (formate) (60 cc., height 17 cm.), employing gradient elution (2 *N* formic acid in reservoir; mixer volume, 1 liter), effected elution of a non-glycosidic substance[10] just prior to I. The product (75 mg.) was chromatogramed on paper in solvent A[7] to remove traces of an unidentified nucleoside (R_f 0.27). Elution with methanol followed by crystallization from methyl cyanide gave a white powder which in 2 cc. of ethanol deposited microplates of I (32 mg., m.p. 235–245° dec.) after 4 days at 25°. Calcd. for $C_{14}H_{17}N_5O_8$: C, 43.86; H, 4.47; N, 18.27. Found[11]. C, 43.81; H, 4.54; N, 18.20. Paper chromatography[7] and paper electrophoresis[12] showed no other components. Potentiometric titration revealed ionizing groups of pK_a 2.2 (±0.1), 5.1 (±0.1), and a third of intermediate pK_a; λ_{max} (*p*H 0.1) 268 mμ (A_M 15,500 ± 1000), (*p*H 8.2) 269 mμ (A_M 17,600 ± 1000). AMPS possesses very similar spectroscopic and ionization constants.[5c]

Treatment of AMPS[13] with phosphatases[14] yielded a nucleoside[15] (λ_{max} 269 mμ at *p*H 8.2) indistinguishable from I by paper chromatography[7] or electrophoresis.[12]

AMPS was heated in 0.1 *N* H_2SO_4 for 4 hours at 100°. The solution was neutralized with ammonia and chromatogramed on paper in four solvent systems,[16] together with a similar hydrolysate from AMP and samples of D-ribose-5′-phosphate, D-ribose, and Na_2HPO_4. Duplicates from each hydrolysate yielded evidence for much D-ribose-5′-phosphate,[17,18] traces of D-ribose[17] and of inorganic phosphate.[18] AMPS reacted as an unsubstituted *cis*-glycol in the periodate test.[9]

These findings afford strong evidence that AMPS is the 5′-phosphate of I, in agreement with the structure previously proposed.[5a]

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(2) W. K. Joklik, *Biochem. Biophys. Acta*, **22**, 211 (1956).

(3) R. Abrams and M. Bentley, THIS JOURNAL, **77**, 4179 (1955).

(4) I. Lieberman, *ibid.*, **78**, 251 (1956).

(5) (a) C. E. Carter and L. H. Cohen, *ibid.*, **77**, 499 (1955); (b) C. E. Carter, *Fed. Proc.*, **15**, 230 (1956); (c) C. E. Carter and L. H. Cohen, *J. Biol. Chem.*, **222**, 17 (1956).

(6) A. Hampton, J. J. Biesele, A. E. Moore, and G. B. Brown, THIS JOURNAL, **78**, 5695 (1956).

(7) R_f values (ascending solvents, Schleicher and Schuell No. 597 paper): (A) *n*-butanol 50, acetic acid 20, water 30, *n*-butyl acetate 30 (0.40); (B) *n*-butanol 50, acetic acid 25, water 25 (0.59); (C) 5% Na_2HPO_4, *iso*amyl alcohol (C. E. Carter, THIS JOURNAL, **72**, 1466 (1950)) (0.90).

(8) The reagent was a 0.1% solution of brom thymol blue in 0.02 *N* NaOH.

(9) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(10) This material, λ_{max} 276 in 0.1 *N* HCl, was probably the aglycone[5b] of I and could be detected in the presence of I on chromatograms run in pyridine–water (65:35).

(11) Analysis by J. F. Alicino, Metuchen, N. J.

(12) Migration distances towards the anode (Whatman 3MM paper, 800 volts, 0.04 *M* buffer) were: *p*H 4.7 (acetate–HCl buffer), 9.5 cm. in 1 hr.; *p*H 7.3 (phosphate), 10.5 cm. in 50 min.; *p*H 10.1 (glycine–NaOH), 8.5 cm. in 35 min.

(13) Kindly provided by Dr. C. E. Carter.

(14) Crudesnake venom phosphatases at *p*H 8.1, or human prostatic phosphatase at *p*H 5.4 caused complete conversion of AMPS in 24 hours at 37°.

(15) Purified by paper chromatography in solvent B.[7]

(16) *n*-Butanol–acetic acid–water (50:20:35); 1% aqueous $(NH_4)_2SO_4$–isopropyl alcohol (1:2) (N. Anand, V. M. Clark, R. H. Hall, and A. R. Todd, *J. Chem. Soc.*, 3665 (1952)); acetone–30% acetic acid (1:1) (S. Burrows, F. S. M. Grylls and J. S. Harrison, *Nature*, **170**, 800 (1952)); pyridine–ethyl acetate–water (1:2:2) (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(17) S. M. Partridge, *Nature*, **164**, 443 (1949).

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A CLEAVAGE REACTION INVOLVING α-METHYLSTYRENE OXIDE[1]

Sir:

It has been found that α-methylstyrene oxide (1,2-epoxy-1-methylethylbenzene) when allowed to

(1) This work was supported by the Air Research and Development Command under contract No. (AF 18(600)787) with the Ohio State University Research Foundation.