specific inter- and intramolecular interactions are known for crystals of this protein. Several other systems of crystallographic interest are being considered, in particular ribonuclease, lysozyme, and carboxypeptidase.

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The Synthesis of 2′,3′-Dideoxyadenosine from 2′-Deoxyadenosine

Sir:

We wish to report the first preparation of the unusual purine nucleoside 2′,3′-dideoxyadenosine (IV). The present synthesis utilizes the first recorded successful acidic removal of the 5′-triphenylmethyl (trityl) blocking group from a derivative of 2′-deoxyadenosine. The reaction scheme also employs the displacement of a secondary tosylate by alkyl mercaptide and emphasizes this reaction as a powerful new synthetic tool in the preparation of deoxynucleosides.

Interest in 2′,3′-dideoxyadenosine (IV) arises from the fact that such a compound (as a 5′-phosphate derivative) should inhibit biosynthesis of DNA by acting as a polynucleotide chain terminator due to the

TrOCH₂ — O — B
TsO
I
B = 9-adenyl
NH₂

HOCH₂ — O — B
TsO
II

HOCH₂ — O — B
SR
III
R = C₂H₅

HOCH₂ — O
IV
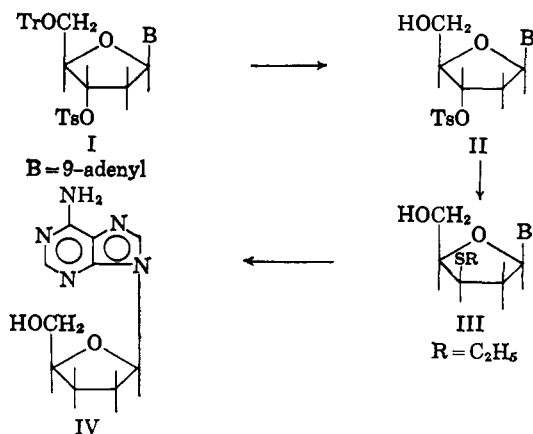
absence of the 3′-hydroxyl group. A related nucleoside antibiotic cordecypin[1–3] has recently been shown to be identical with 3′-deoxyadenosine.[4–6] The action of cordecypin on nucleic acid synthesis appears to be due to the accumulation of phosphorylated derivatives of the antibiotic[7,8] which are not able to substitute for structurally related adenosine phosphates due to the missing 3′-hydroxyl. There is also good evidence that such a pool of the purine 3′-deoxynucleotide acts as a

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(6) E. A. Kackzka, N. R. Trenner, B. Arison, R. W. Walker, and K. Folkers, *ibid.*, **14**, 456 (1964).

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specific inhibitor of purine nucleotide biosynthesis.[9,10] Although 3′-deoxyadenosine is an inhibitor of both DNA and RNA synthesis,[11,12] 2′,3′-dideoxyadenosine would be expected to exert selective inhibition of DNA biosynthesis.

The synthesis and biological activity of 9-(tetrahydro-2-furyl)adenine[13,14] suggested some time ago that 2′,3′-dideoxyadenosine would be a compound of considerable biochemical interest due to its closer structural relationship to 2′-deoxyadenosine. In the present study 5′-O-trityl-2′-deoxyadenosine[15] was treated with p-toluenesulfonyl chloride in pyridine to yield 5′-O-trityl-3′-O-tosyl-2′-deoxyadenosine (I) which was purified on alumina to give a chromatographically homogeneous foam in 65% yield. *Anal.* Calcd. for C₃₆H₃₃N₅O₅S: C, 66.8; H, 5.14; N, 10.8. Found: C, 66.8; H, 5.43; N, 10.7. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,500), $\lambda_{shoulder}^{MeOH}$ 226 mμ (ε 24,400); infrared band 705 (OTr) and 1170 cm.⁻¹ (OTs). Previous detritylations of purine 2′-deoxyribofuranoside derivatives have met with very limited success.[15–17]

Khorana and co-workers[18] have recently made use of the more acid labile tris(p-anisyl)methyl group in order to circumvent simultaneous cleavage of the purine base during deblocking. The study of this problem in our laboratory revealed that the 3′-tosyl function (I) contributed significantly to the stability of the glycosidic linkage.[19] Thus 5′-O-trityl-3′-O-tosyl-2′-deoxyadenosine (I) was heated for 12 min. at 100° in 80% acetic acid to give an essentially quantitative yield of 3′-O-tosyl-2′-deoxyadenosine (II) which was recrystallized from an ethanol–ether mixture to give fine needles, m.p. 184–184.5°. *Anal.* Calcd. for C₁₇H₁₉N₅O₅S: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.6; H, 4.59; N, 17.3. Spectral data showed: λ_{max}^{MeOH} 259 and 228 mμ (ε 15,900 and 13,500); strong infrared band at 1170 cm.⁻¹, band at 705 cm.⁻¹ absent. This would appear to provide a general synthetic route to a variety of previously inaccessible 3′-substituted derivatives of purine 2′-deoxynucleosides by replacement of the tosylate group. Nucleophilic displacement of the tosylate of 3′-O-tosyl-2′-deoxyadenosine (II, 6.5 g.) with ethyl mercaptide in a sodium ethoxide–ethanol solution at 80° yielded 1.2 g. (25%) of 6-amino-9-(3′-S-ethyl-3′-thio-2′,3′-dideoxy-β-D-threo-pentofuranosyl)purine (III) by an assumed Walden inversion. III crystallized from ethanol in colorless needles, m.p. 210–212°. *Anal.* Calcd. for C₁₂H₁₇N₅O₂S: C, 48.8; H, 5.76; N, 23.7. Found: C, 48.7; H, 5.72; N, 23.6. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,600). Sponge nickel[20] de-

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(18) See H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3821 (1963), and references cited therein.

(19) D. M. Brown, G. D. Fasman, D. I. Magrath, and A. R. Todd, *J. Chem. Soc.*, 1448 (1954).

(20) Davison Sponge Nickel Catalyst, W. R. Grace & Co., Davison Chemical Division, Cincinnati, Ohio.

sulfurization of III (1.5 g.) proceeded at 100° in a refluxing ethanol–Methyl Cellosolve solution in the presence of a 15-fold weight of catalyst to give 0.8 g. (67%) of crude 2',3'-dideoxyadenosine. After three recrystallizations from ethanol, colorless crystals of IV (0.25 g.) were obtained. chromatographically homogeneous. Pure 2',3'-dideoxyadenosine (IV) melted at 184–186°, $[\alpha]^{25}D$ −25.2° (c 1.01, H_2O); λ_{max}^{MeOH} 259.5 mμ (ϵ 14,800). Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.54; N, 29.8. Found: C, 50.9; N, 5.32; N, 29.6; R_f 0.45, $R_{Adenine}$ 1.80 (NH_4OH:DMF:i-PrOH, 10:25:65); R_f 0.36, $R_{Adenine}$ 1.19 (n-BuOH saturated with H_2O). The proton magnetic resonance spectrum of IV in D_2O showed a complex multiplet corresponding to four protons at δ 2.0 to 2.8 (C-2' and C-3' protons) and no absorption at δ 4.63 in the region of the C-3' proton in 2'-deoxyadenosine in the same solvent.

These procedures are presently being applied to the preparation of other novel purine deoxy- and polydeoxynucleosides utilizing the commercially available deoxynucleosides obtained from DNA.

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Synthesis of Deoxyribonucleoside-3',5' Cyclic Phosphates by Base-Catalysed Transesterification

Sir:

Hydrolysis of p-nitrophenyl thymidine-3' phosphate in aqueous sodium hydroxide produces both thymidine-3' and thymidine-5' phosphates, thymidine-3',5' cyclic phosphate being an intermediate in the reaction.[1] This communication describes the reaction of p-nitrophenyl esters of deoxyribonucleotides with base in anhydrous solvents where deoxyribonucleoside-3',5' cyclic phosphates are produced in excellent yields.

5'-O-Di-p-methoxytritylthymidine[2,3] was reacted with p-nitrophenyl phosphate and dicyclohexylcarbodiimide in dimethylformamide–pyridine[4] to yield, after acetic acid treatment, p-nitrophenyl thymidine-3' phosphate. The nucleotide (20 μmoles) as its ammonium salt in dimethyl sulfoxide (2.0 ml.)[5] was treated with molar potassium t-butoxide in t-butyl alcohol (1.0 ml.)[6] at 20°. Immediately an intense yellow color developed and chromatography in isopropyl alcohol–concentrated ammonia–water (7:1:2) indicated that formation of thymidine-3',5' cyclic phosphate was quantitative and complete in less than 5 min. The nucleotide was isolated by ion-exchange chromatography on diethylaminoethyl cellulose[7] and characterized by its spectral properties, paper chromatography in

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(4) This solvent system was first described by R. K. Ralph, W. J. Connors, H. Scballer, and H. G. Khorana, ibid., 85, 1983 (1963), and was used here because p-nitrophenyl phosphate is insoluble in anhydrous pyridine.

(5) Dimethyl sulfoxide is a useful solvent in nucleotide chemistry; see J. G. Moffatt, Can. J. Chem., 42, 599 (1964).

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three systems, electrophoresis at pH 7.5, and hydrolysis to thymine in molar hydrochloric acid at 50°.[7-9]

Although p-nitrophenyl uridine-5' phosphate is not hydrolysed by aqueous alkali via the nucleoside-3',5' cyclic phosphate,[1] the reaction of p-nitrophenyl thymidine-5' phosphate (sodium salt) was next examined. Under the conditions described above, conversion to thymidine-3',5' cyclic phosphate was complete in 60 min.[10] Similarly, p-nitrophenyl deoxyadenosine-5' phosphate[11] was completely converted to deoxyadenosine-3',5' cyclic phosphate, although the reaction proceeded at about 80% of the rate of the thymidine-5' nucleotide. Deoxyadenosine-3',5' cyclic phosphate was characterized by its ion-exchange, spectral, chromatographic, and electrophoretic properties, by its resistance to molar hydrochloric acid at 50°, and by its hydrolysis by the adenosine-3',5' cyclic phosphate diesterase of brain.[7,12]

When formamide was substituted for dimethyl sulfoxide as solvent,[13] there was no detectable reaction of p-nitrophenyl thymidine-5' phosphate after 60 min. In dimethylformamide, thymidine-3',5' cyclic phosphate was produced at about 75% of the rate in dimethyl sulfoxide.

Experiments to determine the utility of this reaction in the synthesis of other deoxyribonucleoside-3',5' cyclic phosphates,[7] ribonucleoside-3',5' cyclic phosphates,[14] and internucleotide linkages are in progress.

(7) G. I. Drummond, M. W. Gilgan, E. J. Reiner, and M. Smith, J. Am. Chem. Soc., 86, 1626 (1964).

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(9) These criteria do not exclude the possibility of anomerization (at the glycosidic linkage). However, other experiments involving t-butoxide catalysis indicate that this is improbable. See R. Letters and A. M. Michelson, J. Chem. Soc., 1410 (1961); A. M. Michelson and W. E. Cohn, Biochemistry, 1, 490 (1962).

(10) Thymidylyl-(5'→3')-thymidine is unaffected under the same conditions (unpublished results).

(11) Kindly donated by Dr. W. E. Razzell.

(12) G. I. Drummond and S. Perrot-Yee, J. Biol. Chem., 236, 1126 (1961).

(13) Formamide was used as solvent in the potassium t-butoxide catalysed transesterification of ribonucleic acid to ribonucleoside-2',3' cyclic phosphates; see D. Lipkin and P. T. Talbert, Chem. Ind. (London), 143 (1955).

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Heat of Hydrogenation of Bicyclo[2.2.2]octa-2,5,7-triene

Sir:

In view of recent commentary on the question of delocalization energy in bicyclo[2.2.2]octa-2,5,7-triene ("barrelene"),[1] the author wishes to report the value obtained in this laboratory for the heat of hydrogenation of this substance. A purified sample, kindly provided by Dr. H. E. Zimmerman, was reduced in acetic acid solution at 25° with the uptake of 2.99 molar equivalents of hydrogen. The heat of hydrogenation was −93.78 ± 0.31 kcal./mole.

Since the heat of hydrogenation of bicyclo[2.2.2]octa-2,5-diene is −56.21 ± 0.10 kcal./mole,[2] the heat evolved in reduction of the first double bond of barrelene

(1) H. E. Zimmerman and G. L. Grunewald, J. Am. Chem. Soc., 86, 1434 (1964), footnote 2.

(2) R. B. Turner, W. R. Meador, and R. E. Winkler, ibid., 79, 4116 (1957).