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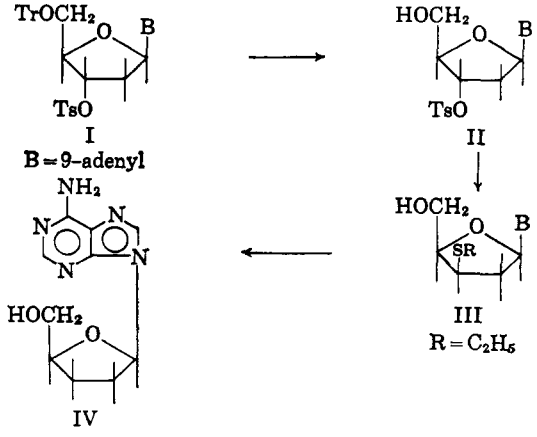
The Synthesis of 2',3'-Dideoxyadenosine from 2'-Deoxyadenosine

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



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 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 $C_{36}H_{33}N_5O_5S$: 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 $C_{17}H_{19}N_5O_5S$: 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 $C_{12}H_{17}N_5O_2S$: 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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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 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.

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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

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