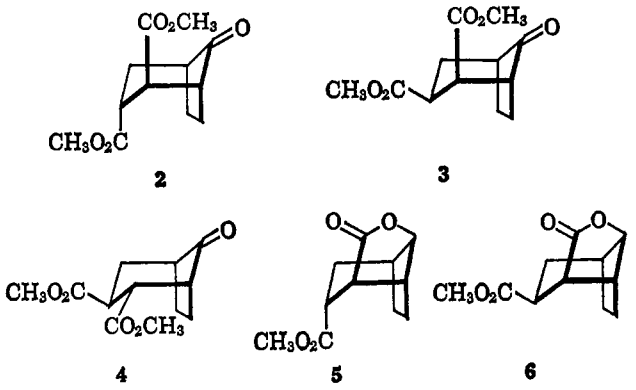
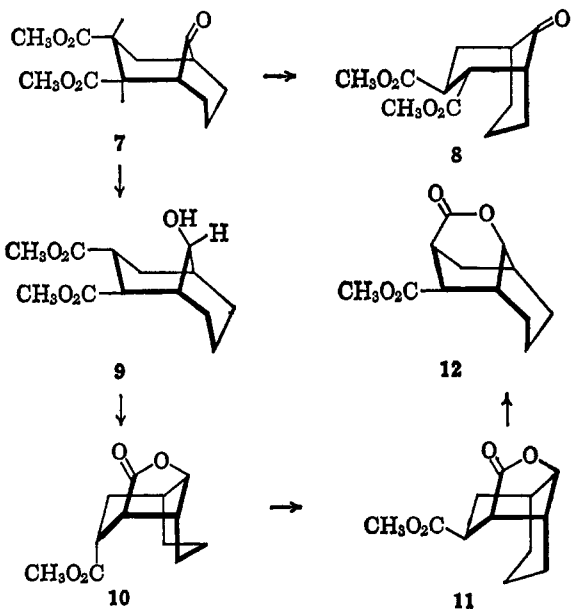


(quartet), 7.47 (multiplet), 7.77 (multiplet), and 8.10 (multiplet); dinitrophenylhydrazone, $C_{18}H_{20}N_4O_8$, mp 186.5–188°].

In a similar condensation of bromomesaconic ester **1** with the pyrrolidinenamine of cyclohexanone,[4] there was produced a 76% yield of a bicyclononanone diester [II; $C_{13}H_{18}O_5$; bp 175–179° (2.5 mm); $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1735, 1175, 1040; τ_{CDCl_3} (ppm) 6.29 (3 H, singlet), 6.30 (3 H, singlet), 7.40 (5 H, multiplet), and 8.08 (7 H, multiplet); dinitrophenylhydrazone, $C_{19}H_{22}O_8N_4$, mp 201.5–202.5°]. In contrast to the initially formed major bicyclooctanone diester **2**, the nonanone exhibited nearly identical ester OCH_3 absorptions in the nmr which suggested a different stereochemistry or conformation or both. Treatment of the keto diester with sodium methoxide–methanol afforded a crystalline isomer [$C_{13}H_{18}O_5$; mp 96–97.5°; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1735, 1280, and 1173; τ_{CDCl_3} (ppm) 6.27 (3 H, singlet), 6.31 (3 H, singlet), 6.62 (multiplet), 7.22 (multiplet), 7.50 (multiplet), and 7.96 (multiplet); dinitrophenylhydrazone, $C_{19}H_{22}N_4O_8$, mp 149–150.5°] assumed to have the most stable configuration (**8**). Borohydride reduction of the first formed keto diester (II) afforded a



hydroxy diester, **9**, which did not undergo lactonization until heated to 170° for 2 hr. Since the γ-lactone ester **10**[6] [$C_{12}H_{16}O_4$; mp 106–107°; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1770, 1725, 1160, and 995; τ_{CDCl_3} (ppm) 6.20 (3 H, singlet), 5.55 (1 H, triplet), 6.85 (multiplet), 7.27 (multiplet), 7.45 (multiplet), 7.75 (multiplet), 8.05–8.60 (multiplet)] produced by this treatment was converted to an epimeric γ-lactone ester [**11**; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1775, 1730, 1155, and 1005; τ_{CDCl_3} 6.28 (3 H, singlet), 5.60 (1 H, multiplet), 7.10 (multiplet), 7.50 (multiplet), 7.20 (multiplet), 8.43 (multiplet)] with *t*-butoxide–*t*-butyl alcohol, we conclude that II has a *trans* orientation of the ester functions as did I, but has at least the substituted ring in a boat conformation (**7**).[7] Additional justification for the above assignments was obtained from the observed facile transformation of the γ-lactone ester **10** to a δ-lactone ester (**12**) [$C_{12}H_{16}O_4$; liquid; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1755, 1735, 1265, 1135, and 1050 (three peaks); τ_{CDCl_3} (ppm) 6.27 (3 H, singlet), 5.75 (1 H, triplet), 7.10 (1 H, multiplet), 7.33 (2 H, multiplet), 7.69 (1 H, multiplet), and 8.45 (8 H, multiplet)] with sodium methoxide–methanol at room temperature. Undoubtedly the resistance to γ-lactone formation is a consequence of the interaction of the 3-carbomethoxy group with the methylenes of the unsubstituted cyclohexane ring which develops as a result of the conformational inversion prior to lactone formation (**9** → **10**). δ-Lactone formation must occur by epimerization at position 3, opening of the γ-lactone, conformational inversion to a boat form, and formation of δ-lactone (**12**).

Since both the enamine and α-(1-haloalkyl)-unsaturated ester would seem able to include many diverse features, this synthesis provides a general method for the construction of a wide variety of structures. The scope, limitations, and logical extensions of this reaction are under active investigation as are the further uses of the products in synthetic and conformational studies.[8]

(6) The unsubstituted ring must reside in a boat conformation so as to relieve the 3-carbomethoxy–7-H interaction.

(7) The substituted ring might assume a very flat chair conformation; however, this would bring the 3-carbomethoxy group much closer to the methylenes of the unsubstituted ring. Thus, these compounds appear to be the first examples of the bicyclo[3.3.1]nonane system having a boat form. *Cf.* G. Eglinton, J. Martin, and W. Parker, *J. Chem. Soc.*, 1243 (1965); W. A. C. Brown, G. Eglinton, J. Martin, W. Parker, and G. A. Sim, *Proc. Chem. Soc.*, 57 (1964).

(8) Preliminary studies on the mechanism indicate that of the available pathways, the C-alkylation followed by proton transfer and Michael addition process is that preferred over the Michael–elimination–Michael or N-alkylation–rearrangement–Michael routes.

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9-[β-DL-2α,3α-Dihydroxy-4β-(hydroxymethyl)-cyclopentyl]adenine, the Carbocyclic Analog of Adenosine[1,2]

Sir:

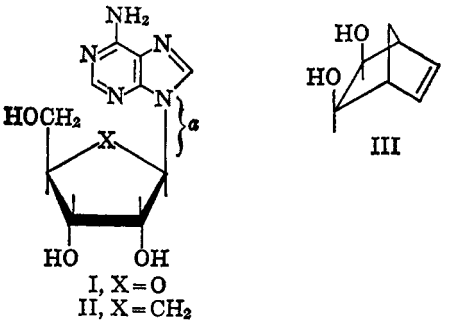
During recent years much interest has been manifested in analogs of the ribofuranosyl- and deoxyribofuranosylpurines and -pyrimidines that occur in nucleic acids. Investigations of such analogs are exemplified

(1) This investigation was supported by the C. F. Kettering Foundation and by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

(2) Analytical data for compounds II, III, III-diacetate, IV, IV-anhydride, V, VI, VII (R_1 = $COCH_3$, R_2 = H or $COCH_3$), VIII, IX, XI, II-isopropylidene derivative, and XII were satisfactory.

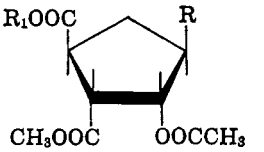
by both chemical and biological studies of cordycepin[3] (3′-deoxyadenosine) and other deoxyadenosines,[4] psicofuranine and angustmycin[5] A, 9-β-D-arabinofuranosyladenine and the other 9-β-D-pentofuranosyladenines,[6] 3-isoadenosine,[7] and 1-β-D-arabinofuranosylcytosine.[8] Studies of mutant cell lines that have lost adenosine kinase or a purine nucleotide pyrophosphorylase indicate that adenosine kinase is essential for the activation of many adenosine (I) analogs.[9] These and other studies suggest that replacement of the oxygen atom in the furanose ring with a methylene group—that is, replacement of the tetrahydrofuran ring with a cyclopentane ring—might produce analogs with interesting biochemical and therapeutic properties. These carbocyclic analogs ought to be conformationally similar to the naturally occurring ribofuranosyl derivatives;[10,11] at the same time, the carbon–nitrogen bond (a) at the purine, or pyrimidine, ring should have the same order of stability as a simple 9-alkylpurine or 3-alkylpyrimidine. It is anticipated that the carbocyclic analogs will be less susceptible to enzymatic fission of this bond than are the furanosyl heterocycles. Murdock and Angier[12] have synthesized the cyclopentane analog of thymidine and have discussed the probable steric similarity of the cyclopentane and the tetrahydrofuran derivatives of thymine. We now report the synthesis of the carbocyclic analog (II) of adenosine (I).

Since methods[13] are available for preparing 9-alkylpurines from alkylamines, the principal problem was the synthesis of the requisite cyclopentylamine (X) with the correct *cis–trans* relationships among the four groups. The strategy of the synthesis was to begin with a cyclopentane with four groups already in the proper geometrical configuration and to convert this derivative into X. The starting cyclopentane was 2α,3α-diacetoxy-1β,4β-cyclopentanedicarboxylic acid (IV), mp 170–171° after recrystallization from ethyl acetate–hexane, obtained as follows: *exo,cis*-5-norbornene-2,3-diol (III) was prepared from norbornadiene and either potassium permanganate or osmium tetroxide, the *cis*-diol was acetylated with acetic anhydride in pyridine, and the diacetoxy derivative was oxidized to the acid (IV) with sodium permanganate. Dihydroxylation of norbornadiene with *cis*-hydroxylating agents ensures the *cis* relationship of the hydroxyl groups,[14] and the rule of *exo* addition[15] determines their *exo* configuration.[16] In addition, the melting point (117–118°, after

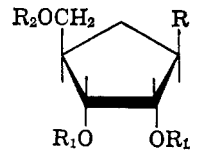


I, X = O
II, X = CH_2

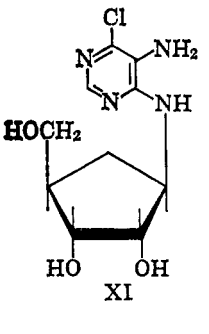
III



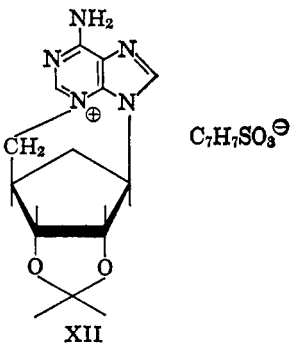
IV, R = COOH, R_1 = H
V, R = $CONH_2$, R_1 = H
VI, R = $NHCOCH_3$, R_1 = CH_3



VII, R = $CONH_2$
VIII, R = $NHCOCH_3$, R_1 = R_2 = $COCH_3$
IX, R = $NHCOCH_3$, R_1 = R_2 = H
X, R = NH_2, R_1 = R_2 = H



XI



XII

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sublimation) of *exo,cis*-5-norbornene-2,3-diol (III) differed from that of the *endo,cis* isomer[17] (176–179° dec), the melting point of the hydrogenation product of III differed from that reported for *endo,cis*-norbornane-2,3-diol,[17,18] and the melting point and infrared spectrum of the hydrogenation product were in agreement with these properties of *exo,cis*-norbornane-2,3-diol.[14a,18] Oxidation of the alkene group of the diacetate of III then generates *cis*-carboxyl groups on the side of the ring opposite to the acetoxy groups.

Treatment of the cyclic anhydride (mp 162°) of the dicarboxylic acid (IV) with ammonia gave 2α,3α-diacetoxy-4β-carbamoyl-1β-cyclopentanecarboxylic acid (V), mp 183°. Two routes were employed in the conversion of V to the amine X. In the first route, the carboxamide group was transformed by the Hofmann hypobromite reaction, known to occur with retention of configuration,[19] to an amino group, the carboxyl group was esterified with methanol and hydrogen chloride, and the crude methyl ester was acetylated. The resulting methyl 4β-acetamido-2α,3α-diacetoxy-1β-cyclopentanecarboxylate (VI) was purified by chromatography on silica gel and characterized; yield, 52% from V; mp, 115°. Reduction of VI with lithium borohydride, acetylation of the crude reduction product, and chromatography on silica gel gave the tetraacetyl derivative VIII as a colorless, analytically pure syrup. The second route for the preparation of VIII consisted of the following steps: conversion of the carboxyl group of V to the acid chloride, reduction of the acid chloride to the hydroxymethyl group with sodium borohydride, conversion of the carboxamide group to an amino group by the Hofmann reaction, and reacetylation. A specimen of VIII from the second route was prepared for analysis by vapor phase chromatography. The intermediate 2α,3α-diacetoxy-4β-hydroxymethyl-1β-cyclopentanecarboxamide (VII, R_1 = $COCH_3$, R_2 = H; mp 94°) and the triacetate (VII, R_1 = R_2 = $COCH_3$; mp 86–89°) were isolated and characterized. Specimens of the tetraacetyl derivative (VIII) obtained from the two routes were shown to be identical by their infrared spectra and by thin layer chromatography.

Deacetylation of VIII with base gave crystalline N-(2α,3α-dihydroxy-4β-hydroxymethyl-1β-cyclopentyl)-acetamide (IX), mp 114–116°. Complete deacetylation of VIII with hydrochloric acid and treatment of the crude aminotriol (X) with 5-amino-4,6-dichloropyrimidine gave the trihydroxycyclopentylaminopyrimidine (XI); mp 182–184°; λ_{max}^{EtOH} in mμ ($\epsilon \times 10^{-3}$): 297 (10.0), 267 (8.9), and 207 (18.4). The pyrimidine (XI) was converted to the 6-chloropurine derivative with triethyl orthoformate, and from a reaction of the crude 6-chloropurine and ammonia the desired adenosine analog was isolated by chromatography on Amberlite CG-120 (H^+) ion exchange resin and was recrystallized from water; mp 238–242° dec; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 261 (14.8) in phosphate buffer (pH 7), 258 (14.5) and 212 (20.6) in 0.1 *N* HCl.

The 2′,3′-isopropylidene derivative of II was obtained in good yield and was converted *via* its 5′-*p*-toluenesulfonate to the cyclonucleoside derivative XII. These reactions confirm that the 2′- and 3′-hydroxyl groups are *cis* and that the 5′-hydroxymethyl group is *cis* to the purine ring.[20]

Compound II may function as an antagonist of adenosine, or, because of its stereochemical resemblance to adenosine, it conceivably could mimic some of the biochemical functions of adenosine or, after phosphorylation, of adenine nucleotides. Initial studies indicate that II is highly cytotoxic and suggest that it may be phosphorylated.[21]

Acknowledgment. The authors express their appreciation to Drs. W. J. Barrett, W. C. Coburn, Jr., P. D. Sternglanz, and associates of this Institute for microanalytical and spectral data; to Mr. W. E. Fitzgibbon and associates of the Organic Preparations Section for large quantities of intermediates; and to Dr. J. A. Montgomery for cooperation and advice.

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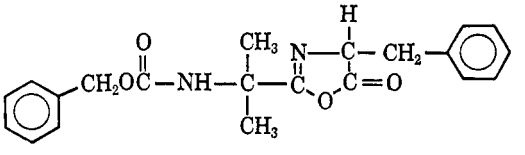
Retention and Racemization Reactions during Peptide Synthesis

Sir:

Among the most important chemical methods to measure the extent of racemization during peptide synthesis are the techniques developed by Anderson[1] and Young.[2–4] In their approaches, an activated amino acid or peptide derivative is allowed to react with ethyl glycinate as the attacking amino acid ester. In this communication we will show that the nature of the amino acid ester and the solvent play fundamental roles in the extent of racemization.

We recently[5] reported the isolation of the crystalline, optically active peptide oxazolone, carbobenzoxyaminoisobutyryl-L-phenylalanine oxazolone (I). We



I

used this activated compound to study the effects of different amino acids esters and solvents on the extent of racemization. Since all the activated peptide derivative is present as the oxazolone, we have in this material a most stringent system to study racemization during peptide coupling. In Table I are listed the amounts of optically active tripeptides obtained when oxazolone I

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