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-cyclopentanecarboxanamide (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]

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(16) Since dihydroxylation proceeds through bulky, cyclic manganese and osmate adducts,[14] addition to the less hindered *exo* side should be overwhelmingly favored.

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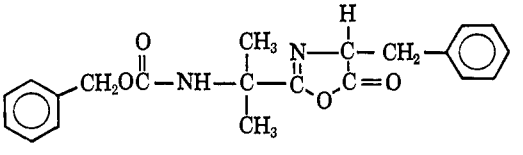
Retention and Racemization Reactions during Peptide Synthesis

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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Table I. Per Cent of Optically Active Tripeptide Derivatives Obtained after Reaction between Oxazolone and Various Amino Acid Esters

Amino acid ester	Toluene	Chloroform	Ethyl acetate	Dioxane
Ethyl glycinate	78	66	38	14
Methyl DL-alaninate	52	32	11	0
Methyl α-aminoisobutyrate	0	0	0	0

is allowed to react with equimolar solutions of various amino acids esters in a number of solvents. The per cent retention of optical activity in the tripeptide product in each instance was obtained by direct comparisons with the optical rotations of the same tripeptides synthesized by the nonracemizing azide-coupling route.

The figures show that for any given solvent the least amount of racemization occurs when ethyl glycinate is the attacking nucleophile.[6] The ratio of nucleophilicity to basicity of a particular amino acid ester governs the amount of racemization observed. Our data indicate that for ethyl glycinate the nucleophilicity-to-basicity ratio is most favorable for retention of optical activity. Schnabel[7] prepared a partially racemized oxazolone from carbobenzoxyglycyl-L-phenylalanine and allowed it to react with ethyl glycinate. The product he obtained exhibited some optical activity.

In the case of methyl DL-alaninate the nucleophilicity-to-basicity ratio is less favorable and more racemization is observed than with ethyl glycinate. For methyl α-aminoisobutyrate, the ratio favors basicity because of steric hindrance. As a result, when this amino acid ester is allowed to react with the pure oxazolone, only complete racemization is encountered. Hence, in a specific peptide coupling procedure, if an optically pure product results when methyl α-aminoisobutyrate is used as the attacking agent, one could state with certainty that the technique is free from oxazolone formation, the major route for racemization during peptide synthesis.

As can be seen from Table I, the solvent plays an important role in the racemization process. Toluene, the least polar of the four solvents, gives the best retention results. Chloroform is also a good solvent, perhaps because it contains an acidic proton. Dioxane, the most basic of the solvents, gives the highest extent of racemization probably because it solvates the proton leaving the oxazolone ring.

Our findings do not appear to agree with those of Vaughan.[8] He found that in using the mixed anhydride coupling method, dioxane as a solvent led to less racemization than chloroform as a solvent. This difference in results could arise from the fact that in dioxane less oxazolone forms, or that Vaughan used amino acid ester hydrochlorides and tertiary amines, a combination known to give rise to the "chloride ion" effect[4] in chloroform. We employed the free amino acid esters in all cases.

In addition to the above findings, we also wish to present direct chemical evidence to explain why optically pure hydrazides are obtained when esters are allowed to react with hydrazine hydrate. Nowak and Siemion[9] reported that the noncrystalline 2-methyl-L-4-isobutyloxazolone reacts with hydrazine hydrate to give optically active products.

When we allowed oxazolone I to react with a huge excess of hydrazine hydrate in methanol, we obtained a product identical with the hydrazide made by the traditional method of allowing dipeptide ester to react with hydrazine hydrate in refluxing methanol. The hydrazide Z-Aib-L-Phe-$NHNH_2$,[10] obtained from the oxazolone reaction, had mp 51–56°, $[\alpha]^{25}D$ −33.8° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{21}H_{26}N_4O_4$: C, 63.31; H, 6.53; N, 14.07. Found: C, 63.24; H, 6.61; N, 13.89.

We expected the dipeptide oxazolone to be much more susceptible to racemization than the corresponding methyl ester in the strongly basic medium. Since identical products were obtained by both methods, it is clear that even if the oxazolone formed from the ester, no racemization would be encountered. Thus this key displacement step in the azide method does not involve racemization.

In an analogous reaction, Czonka and Nicolet[11] observed that an amino acid in acetic anhydride solution reacts with ammonium thiocyanate to form an optically active thiohydantoin. They postulated that an oxazolone intermediate was involved. We have evidence that hydroxylamine reacts with oxazolone I to give an optically active hydroxamic acid.[12] These extraordinarily enhanced nucleophilicities are largely due to what Edwards[13] has called "the α effect" and are apparently characteristic of those nucleophiles containing unshared pairs of electrons on the atom attached to the nucleophilic center.

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Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from *Camptotheca acuminata*[1,2]

Sir:

Camptothecin (I), an alkaloid with a novel ring system exhibiting potent antileukemic and antitumor activities in animals, has been isolated from the tree *Camptotheca acuminata*,[3] *Nyssaceae*. The stem wood

(1) This investigation was conducted under Contract SA-43-ph-4322, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

(2) X-Ray investigations at the University of Illinois were supported under Grant GB2878, National Science Foundation.

(3) The only previous chemical examination of *C. acuminata* is