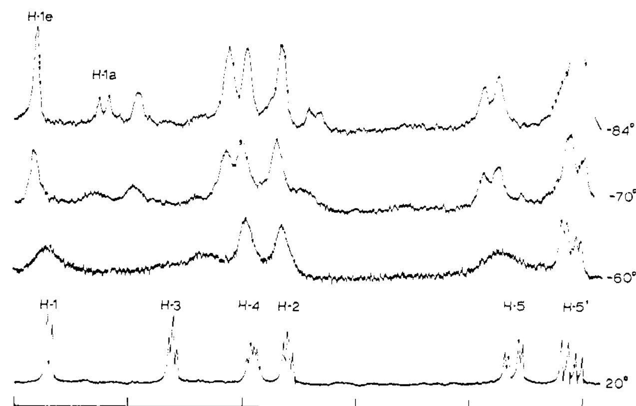

Figure 1. The low-field portion of the 220-MHz nmr spectrum of β-D-ribopyranose tetraacetate (**1**) in acetone-d_6, at 20, −60, −70, and −84°. Each scale division corresponds to 100 Hz.

The 220-MHz spectrum of **1** in acetone-d_6 at room temperature (Figure 1, lowest curve) was completely first order, and analysis[1b,11] gave the following first-order couplings: $J_{1,2}$ = 4.8 Hz, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 3.2 Hz, $J_{4,5'}$ = 5.6 Hz, $J_{3,5}$ = ∼0.5 Hz, and $J_{5,5'}$ = 12.0 Hz. These values are intermediate between those predicted[1b,5,11] for **1** in the *C1* conformation ($J_{1,2}$ = 8–10 Hz) and those predicted for **1** in the *1C* conformation ($J_{1,2}$ = 1–2 Hz). As the temperature was lowered the spectrum showed progressive changes; at −32° slight line-broadening was evident, and with decreasing temperature the H-1, H-3, and H-5 signals collapsed to broad humps, and the shapes of the other signals broadened, until at −60° the spectrum shown in Figure 1 (second curve) was observed. New signals began to appear as the temperature was lowered further, and the appearance of the spectrum at −70° is shown in Figure 1 (third curve). The signals became sharper at still lower temperatures, and the spectrum observed at −84° (Figure 1, top curve) was not substantially different from that observed at −78°. The signals for the acetyl groups also showed changes, from the simple four-line pattern observed at 20° to a more complex pattern at −84°.

The observed data indicate that substance **1** exists at room temperature as a mixture of conformers undergoing rapid interconversion, and that at −84° the interconversion is slow on the nmr time scale, so that spectra of the separate conformers are seen. Assuming that the H-1 proton of either conformer gives its signal at lower field than those of the other ring protons, the broadened singlet at lowest field and the wide doublet at next higher field can be assigned to H-1 of the two conformers. The former signal is assigned to H-1 of the *1C* conformation ($J_{1,2}$ = ∼1 Hz), and the latter is assigned to H-1 of the *C1* conformation ($J_{1,2}$ = 8.0 Hz), and the relative magnitudes of the signals indicate that the *1C* and *C1* conformers are present in 2:1 proportion, corresponding to a free-energy difference of $\Delta G°$ = 0.3 kcal/mole. Upfield from the H-1 signals can be observed three signals of intensity similar to the H-1e signal; these are presumably those of the methine protons of the *1C* conformer. Two additional signals, of intensity equal to that of the H-1a signal, are observed and are presumably those of two of the methine protons of the *C1* conformer, and the signal of the remaining methine proton of the *C1* conformer appears as a broad resonance partially overlapped by one of the stronger signals.

The separation of the H-1e and H-1a signals at −84° is 58.5 Hz. From this it may be calculated[12] that at −60°, the approximate temperature at which the H-1e and H-1a signals coalesce to give a broad singlet, the rate of interconversion of the *C1* and *1C* chair conformers is approximately 130 times per second.

It was suggested in early investigations[13] that the conformers of α-D-lyxopyranose tetraacetate (**2**) have approximately the same energy. However, the 220-MHz spectrum of **2** in acetone-d_6 showed $J_{1,2}$ = 3.0 Hz, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 8.8 Hz, $J_{4,5e}$ = 4.8 Hz, and $J_{4,5a}$ = 9.2 Hz, in a spectrum that was fully first order, these data support the *C1* form as the major or sole chair conformer. As the temperature was lowered to −76°, the H-3 signal moved upfield and the positions of the acetyl-group signals changed, but no new signals were detected in the anomeric-proton region,[14] indicating that any of the *1C* form present could not have comprised more than 2% of the total.

The greater stability of the *1C* form of **1**, having three of the four substituents axial, over the *C1* form, having only one axial substituent, and the failure to observe any detectable proportion of the *1C* form of **2** in equilibrium with the *C1* form, even though both forms have two axial and two equatorial substituents, further illustrate[1c,11] the strong influence of the "anomeric effect" in determining the favored conformation of tetrahydropyran derivatives.

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Received September 7, 1967

Removal of Protected Peptides by Hydrazinolysis after Solid-Phase Synthesis

Sir:

The effectiveness of the solid-phase synthesis of polypeptides[1] has been documented in the syntheses of the A and B chains of insulin,[2] bradykinin,[3] and angiotensin II.[4] The synthesis of longer peptides or proteins might be facilitated by the availability of methods for the preparation of fully protected α-N-blocked peptides suitable for successive coupling to a growing COOH-terminal fragment. We have recently described[5] a modified solid-phase procedure which has proved useful in the preparation of a number of protected peptide

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fragments of the polypeptide chain of staphylococcal nuclease.[6] This communication reports an alternative procedure, the hydrazine cleavage of a blocked peptide from the Merrifield polymer.[7] This method of cleavage yields the protected peptide hydrazide, which may be directly coupled to another peptide after conversion to the azide. Kessler and Iselin have shown that hydrazine cleavage of a peptide from the Merrifield polymer is accompanied by side reactions causing low yields.[8] However, this procedure seems to be applicable for the preparation of water-insoluble, blocked peptide hydrazides which do not contain ω-protected aspartic or glutamic acid residues or other groups labile to hydrazine. In preliminary experiments, both *t*-butyloxycarbonylglycyl polymer and *t*-butyloxycarbonyl-L-leucyl-L-tyrosyl-L-alanyl polymer were treated with 30 equiv of hydrazine in dimethylformamide. Complete cleavage of the blocked amino acid or peptide from the polymer support was observed. Amino acid analyses of the cleaved products in the case of the protected tripeptide indicated equimolar amounts of each amino acid. The solid-phase polymers, after cleavage, were hydrolyzed with hydrochloric acid–dioxane. No traces of amino acid residues were found upon analysis.

We report here the use of hydrazine cleavage of the synthesis of *t*-butyloxycarbonyl-L-leucyl-L-alanyl-L-tyrosyl-L-isoleucyl-L-tyrosyl-L-alanine hydrazide, which comprises residues 100–105 in the sequence of staphylococcal nuclease. The blocked hexapeptide polymer was synthesized from 5.0 g of *t*-butyloxycarbonyl-L-alanyl polymer (0.32 mmole/g) in the usual manner using N,N′-dicyclohexylcarbodiimide as the condensing agent and a threefold excess of each *t*-butyloxycarbonyl amino acid. Tyrosine was added as *t*-Boc-tyrosine, with an unprotected phenolic hydroxyl group. The blocked hexapeptide polymer was suspended in 25 ml of dimethylformamide, and 1.54 ml (48 mmoles) of anhydrous hydrazine was added. The mixture was allowed to stir for 2 days at room temperature. The supporting resin was removed by filtration and was washed with dimethylformamide. The filtrate and washings were combined and evaporated nearly to dryness *in vacuo*. The residue was then treated with water, whereupon 1.88 g of insoluble product was obtained. The product was recrystallized from methanol–ether (1.25 g, 95%, mp 224–225° dec). An aliquot (0.43 g) of the product was further purified by countercurrent distribution (upper phase 10 ml, lower phase 10 ml, 250 transfers), using the system chloroform–methanol–cyclohexane–dimethylformamide–water–acetic acid (5:5:2:2:2:0.2 by volume). The distribution pattern is shown in Figure 1. Fractions 117–160 were combined, neutralized with ammonia, and evaporated to dryness. Treatment of the residue with water yielded 0.37 g of the product. It was recrystallized from dimethylformamide–ether, 0.34 g, 79%, mp 225–226° dec, $[\alpha]^{20}D$ −24° (*c* 1.10, dimethylformamide). Single spots were obtained upon thin layer chromatography: $R_f{}^9$ 0.90, $R_f{}^{10}$ 0.62. *Anal.* Calcd for $C_{41}H_{62}O_{10}N_8 \cdot H_2O$: C, 58.27; H, 7.51; N, 13.26. Found: C, 58.06; H, 7.21; N, 13.85. Amino acid analysis of an acid hydrolysate (6 *N* HCl, sealed evacuated tube, 110°) gave the molar ratios: leucine 1.1, tyrosine 2.0, isoleucine 1.0, and alanine 2.0. The hexapeptide hydrazide ditrifluoroacetate ($R_f{}^9$ 0.79, $R_f{}^{11}$ 0.66, single spots) obtained from treatment of the *t*-butyloxycarbonyl hexapeptide hydrazide with anhydrous trifluoroacetic acid was digested with leucine aminopeptidase.[12] Amino acid analysis gave the ratios: leucine 1.1, tyrosine 2.0, isoleucine 1.0, and alanine 2.0.

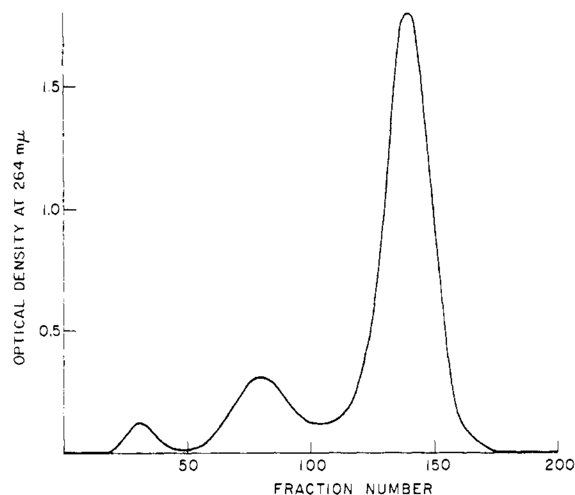

Figure 1. Countercurrent distribution pattern of the hydrazine cleavage product. The curve is based on measurements of 264-mμ absorption.

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(7) See ref 15 in M. Bodanszky and J. T. Sheehan, *Chem. Ind.* (London), 1423 (1964).
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(11) 1-Butanol–acetic acid–water (4:1:5).
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Additions and Corrections

Proximity Effects. XLIV. Stereospecific Synthesis and Solvolysis of *cis*- and *trans*-5-Phenylcyclooctyl and *cis*- and *trans*-5-Phenylcyclooctyl-1,2,2,8,8-d_5 Tosylates [*J. Am. Chem. Soc.*, **88**, 752 (1966)]. By ARTHUR C. COPE and ROBIN B. KINNEL. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

On page 760, the following material should be added immediately following column 1.

Elution with ether yielded 183 mg of crystalline alcohol. Fractional crystallization of the combined alcohol fractions gave 483 mg (32%) of **10**, mp 82.0–84.0°.

Anal. Calcd for $C_{14}H_{15}D_5O$: C, 80.32; H, 9.63. Found: C, 80.18; H, 9.83. Average number of D/molecule, 4.79 (falling drop).