Conformation of Small Peptides. I. Secondary Structure in a Tetrapeptide

Sir:

Previous work in this laboratory has shown that a branched hexapeptide is hydrogen-bonded intramolecularly between the two tripeptide moieties.[1] Goodman had earlier reported that the onset of secondary structure occurred at the pentapeptide level in small peptides.[2] It appears from our data that the tetrapeptide *t*-butyloxycarbonyl-L-valyl-L-valyl-L-alanylglycine ethyl ester (I) has a secondary structure in chloroform, in methanol, and even in hexafluoroacetone sesquihydrate (HFA). Only in 0.5 *M* KF in HFA does I seem to show no secondary structure.

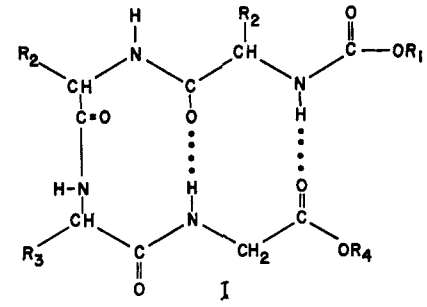
The amino acid structure of I corresponds to the sequence 133–136 of the β chain of human hemoglobin A. This occurs at approximately the center of helix H of the β chain, a long helical segment which extends to the carboxyl terminus of the native polypeptide.[3]

Tetrapeptide I was synthesized in a stepwise manner by the dicyclohexylcarbodiimide[4] method. Carbobenzoxy-L-alanine and glycine ethyl ester hydrochloride gave carbobenzoxy-L-alanylglycine ethyl ester,[5] mp 100–103°, which was treated with hydrogen bromide in glacial acetic acid and coupled with carbobenzoxy-L-valine to give carbobenzoxy-L-valyl-L-alanylglycine ethyl ester, mp 192–192.5°, $[\alpha]^{22}D$ +5° (*c* 2.0, dimethylformamide). This tripeptide was similarly treated to give the crystalline tripeptide L-valyl-L-alanylglycine ethyl ester hydrobromide, mp 181.5–182°, $[\alpha]^{22}D$ +33° (*c* 2.0, water), which was coupled with *t*-butyloxycarbonyl-L-valine to give I, mp 212–213°, $[\alpha]^{22}D$ +77° (*c* 2.0, methanol). *Anal.* Calcd for $C_{22}H_{40}N_4O_7$: C, 55.91; H, 8.53; N, 11.86. Found: C, 55.94; H, 8.51; N, 11.75.

Solutions of I in deuteriochloroform were examined in the infrared at concentrations from 1.45×10^{-2} down to 1.45×10^{-5} *M*. The absorption band at 3330 cm^{-1} due to hydrogen-bonded N–H showed an initial decrease on the first tenfold dilution, so that its intensity at 1.45×10^{-3} *M* was roughly half that at 1.45×10^{-2} *M*. However, the two subsequent dilutions produced no significant changes in the relative intensities of the 3330- and 3430-cm^{-1} bands compared to the 1.45×10^{-3} *M* sample. This concentration-independent absorption indicates the presence of intramolecularly hydrogen bonded N–H.

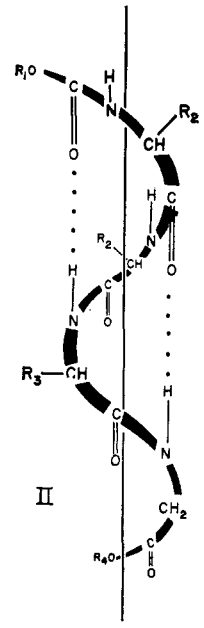
The optical rotatory dispersion of I in the far-ultraviolet was found to show two troughs and a positive peak in both methanol and HFA (see Table I). In methanol, the troughs occurred at 235 and 205 mμ with the peak lying below 200 mμ. A similar curve was obtained in HFA: troughs at 227 and 205 mμ and the peak again below 200 mμ. On examination of the peptide in 0.5 *M* KF in HFA, the trough at *ca.* 230 mμ was no longer present. The trough at 205 mμ and a peak at 189 mμ were the only distinguishable features.

With molecular models, one can build three structures



I

Figure 1. The folded β form.



II

Figure 2. The 3_{10} helix.[7]

Table I. Mean Residue Rotations of ORD Troughs

Solvent	$[m]_{205}$, deg	$[m]_{235}$, deg
Methanol	−2800	−4100
HFA	−4200	−4800[a]
0.5 *M* KF in HFA	−7000	...[b]

[a] Minimum shifted to 227 mμ. [b] Minimum absent.

for I with internal hydrogen bonds. One of these represents the beginning of an α helix and allows formation of a single intramolecular bond The differences between the other two appear to be slight, and both allow formation of two hydrogen bonds. Structure I (Figure 1) corresponds to the folded, antiparallel β-sheet conformation proposed by Schwyzer[6] for cyclization of hexapeptides. The other, structure II (Figure 2), is the 3_{10} helix of Bragg, Kendrew, and Perutz[7] after Huggins.[8] Our models appear to allow either a left-

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handed or a right-handed screw sense in the 3_{10} structure.

The trough at 235 mμ observed for I in methanol suggests the 233-mμ minimum found for α-helical polypeptides,[9] while the 227-mμ trough in HFA suggests the minimum found for certain proteins thought to have the β-sheet structure.[10] The 205-mμ trough found in all our systems is probably the same as that found for the random-coil[9] structure of polypeptides.

The mean residue rotations observed for small peptides at the extrema of the optical rotatory dispersion curves have been found to be much smaller than in the cases for high molecular weight polyamino acids. This is to be expected, since the amide groups involved in intramolecular hydrogen bonding in these peptides are so engaged on only one of their sides,[2] leaving the other free to interact with solvent. Our data on dipeptides and tripeptides are similar in nature but do not indicate the presence of such secondary structure as has been found for tetra- and higher peptides.[2]

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Metalated Carboxylic Acids. I. Alkylation

Sir:

Aliphatic carboxylic acids and their salts have not been reported to metalate by a simple, generalized process on treatment with Grignard reagents,[1] organolithium reagents,[2,3] or alkali metal amides.[4] Except for salts of acetic acid[4a] and methacrylic acid,[4b] homologous metalated carboxylic acid salts apparently decompose under conditions used for their formation.[4a] Arylacetic acids, as well as their salts and simple carboxyl derivatives, are exceptional in that they produce "Ivanov" reagents on treatment with Grignard reagents or "Ivanov-like" reagents on treatment with other organometallic agents.[5] Phenylacetic acid has been metalated also with sodium (potassium) amide in ammonia.[6] This communication reports the metalation of aliphatic carboxylic acids to be a general phenomenon which is illustrated by the metalation of isobutyric acid[7] (**1**, R = CH_3) on treatment with lithium diisopropylamide.

$$\underset{\mathbf{1}}{\mathrm{RCH(CH_3)CO_2H}} + 2\mathrm{LiN}(i\text{-Pr})_2 \longrightarrow \underset{\mathbf{2}}{\mathrm{RLiC(CH_3)CO_2Li}} \xrightarrow{\mathrm{R'X}} \underset{\mathbf{3}}{\mathrm{RR'C(CH_3)CO_2H}}$$

The addition of isobutyric acid, **1** (R = CH_3), to lithium diisopropylamide, prepared from *n*-butyllithium and diisopropylamine, in tetrahydrofuran–heptane (hexane) at 0° produced **2** (R = CH_3) in homogeneous solution. Metalated isobutyric acid, **2** (R = CH_3), is apparently quite stable in the indicated solvent system at least up to 40°. The success of the metalation can be attributed in large measure to the use of lithium as metal, which, among other factors, confers favorable solubility on the metalated species.[8] Homogeneous solutions of **2** (R = CH_3) of at least 1 *M* in tetrahydrofuran–heptane (hexane) (1:5, v/v) have been prepared. The existence of **2** (R = CH_3) could be demonstrated by alkylation with *n*-butyl bromide (iodide) to produce 2,2-dimethylhexanoic acid in preparatively useful yields (Table I).

The alkylation of **2** (R = CH_3) has synthetic utility for the preparation of highly hindered trialkylacetic acids. Several examples are illustrated in Table I. Useful yields were obtained for those alkyl halides that were not especially susceptible to elimination or related side reactions. The alkylation of dilithium derivative **2** (R = CH_3) appears to be superior, even for such a sterically hindered example, to the alkylation of sodium sodioacetate[9] and more direct than most syntheses of trialkylacetic acids.[10] The yields of product are comparable to those obtained for the alkylation of lithioisobutyronitrile.[11] When **2** (R = CH_3) was similarly treated with aliphatic dihalides, the tetramethyldicarboxylic acids listed in Table II were obtained.[12]

Table I. Synthesis of Alkyldimethylacetic Acids from Metalated Isobutyric Acid

$$\mathrm{LiC(CH_3)_2CO_2Li} + \mathrm{RX} \longrightarrow \mathrm{RC(CH_3)_2CO_2H}$$

Alkylating agent	Alkyldimethylacetic acid,[a] %
n-Butyl bromide	80
n-Butyl iodide	89
Allyl chloride	61
Benzyl chloride	46
β-Bromophenetole	41
2-Bromoethyl ethyl ether	66
Cyclohexyl bromide	6

[a] Satisfactory combustion analyses and consistent spectral data have been obtained for all products, as well as satisfactory comparison of physical properties with reported values. Yields are based on purified product.

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(2) The reaction of carboxylic acids with 2 equiv of an organolithium has preparative value for the synthesis of ketones, particularly methyl ketones.[3]
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(5) The terms "Ivanov" and "Ivanov-like" reagents apparently originated with F. F. Blicke and H. Raffelson, *J. Am. Chem. Soc.*, **74**, 1730 (1952). For a recent review see P. E. Wright, Ph.D. Dissertation, University of Michigan, 1959; *Dissertation Abstr.*, **21**, 3642 (1960).
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(7) The metalation of other aliphatic, olefinic, araliphatic, and toluic acids will be subjects of future reports.
(8) Contrast with the poor solubility of sodium sodioacetate.[4a,9]
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