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THE STERIC PURITY OF MODEL PEPTIDES BY N.M.R. SPECTROSCOPY

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Magnetic nonequivalence in the diastereoisomeric peptide pair alanyl-tyrosine was noted recently,[1] but a second study using strict pH control concluded that diastereoisomeric alanyl peptides have identical nuclear magnetic resonance (n.m.r.) spectra.[2] A reexamination of a more complete series of alanyl peptides now reveals a small change in chemical shift of the methyl resonances between L-L and L-D peptides (Table I).

TABLE I

Methyl Resonances of Alanyl Peptides

Peptide[a]	N-Terminal[b]	Central	C-Terminal
L-alanyl-L-alanine	92.8	-	80.9
D-alanyl-D-alanine	93.0	-	80.8
L-alanyl-D-alanine	91.1	-	81.1
L-alanyl-L-alanyl-L-alanine	93.1	84.1	79.6
L-alanyl-D-alanyl-L-alanine	91.2	83.0	78.9
D-alanyl-L-alanyl-L-alanine	91.5	83.9	79.6
D-alanyl-D-alanyl-L-alanine	92.9	83.4	79.0

[a] All spectra were determined on a Varian A-60 spectrometer with the center of gravity of the chemical shift given in c.p.s. downfield from sodium dimethylsilapentylsulfonic acid (SDSS). The compounds were dissolved in deuterium oxide and the pH values were adjusted to 5-6 with addition of deuteroacetic acid or sodium deuteroxide. The error of measurement was ±0.5 c.p.s.

[b] N-terminal and C-terminal methyl resonances were assigned by observing the shift in spectral lines with varying pH.[3]

Although the assignment of configuration in optically pure di- and trialanyl peptides is difficult, the observable chemical shift is sufficient to detect the contamination of one diastereoisomer with the other in an artificial mixture (Fig. Ia). The presence of an aromatic amino acid in the peptide greatly enhances the methyl shift; in this case, the optical purity, the configuration, and the location of the alanyl unit in either the N-terminal or C-terminal position can be determined simultaneously by inspection of the n.m.r. spectrum (Fig. Ib, Ic, and Id). Similar information can be obtained from the presence of an alanyl residue in a tripeptide (Fig. Ie and If). The increased chemical shift seen for the alanyl methyl group in phenylalanine or tyrosine containing diastereoisomers must be attributed to a deshielding effect caused by the ring current in the adjacent aromatic system.

The observed chemical shifts and integrated intensities constitute useful parameters for racemization studies in the area of peptide synthesis. Present techniques rely on the separation of "model" diastereoisomers by countercurrent distribution,[4] fractional crystallization,[5,6] vapor phase chromatography,[7,8] paper chromatography,[9,10] and optical rotation.[11] An examination of the methyl resonances in various N-acylated peptide derivatives shows that these n.m.r. measurements are a convenient tool for the quantitative analysis of N-protected diastereoisomeric peptide reaction mixtures (Table II).

TABLE II

Methyl Resonances of Various N-Acyl Peptide Derivatives

Peptide[a]	L-L(or D-D)[b]	D-L(or L-D)
N-acetyl-L or D-alanyl-O-benzyl-L-tyrosine methyl ester	78.0	74.0
N-acetyl-L or D-alanyl-L-phenylalanine methyl ester	78.4	70.4
N-benzoyl-L or D-phenylalanyl-L-alanine methyl ester	79.5	74.5
N-boc-L-phenylalanyl-L or D-alanine methyl ester	80.9	75.4
N-formyl-L-alanyl-L or D-phenylalanine methyl ester	80.5	74.8
N-cbz-glycyl-L or D-alanyl-L-phenylalanine	75.5	74.5
N-cbz-glycyl-L or D-phenylalanyl-D-alanine benzyl ester	77.5	69.5

[a] Compounds listed here were prepared by standard procedures and had physical constants in agreement with literature values.

[b] All spectra were determined on a Varian A-60 spectrometer with the center of gravity of the chemical shift given in c.p.s. downfield from tetramethylsilane. The compounds were dissolved in deuterochloroform.

As an example, the coupling of N-acetyl-L-alanine with L-phenylalanine methyl ester hydrochloride at 0° in the presence of a carbodiimide gave a mixture of N-acetyl-L-alanyl-L-

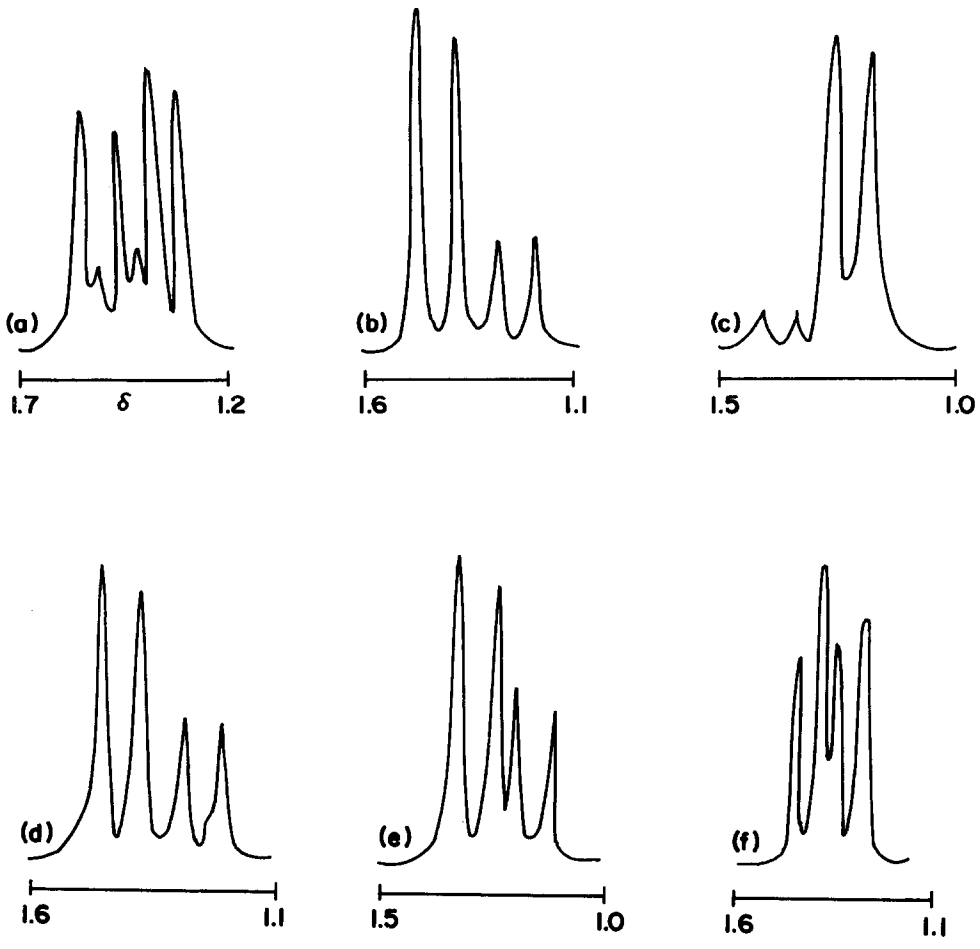

FIG. 1

N.m.r. spectra in the region of the methyl resonance for alanyl peptides. (a) L-alanyl-D-alanine and D-alanyl-D-alanine (1:9; D_2O; pH 5; SDSS); (b) L-alanyl-L-phenylalanine and L-alanyl-D-phenylalanine (3:1; D_2O; pH 3; SDSS); (c) L-phenylalanyl-L-alanine and L-phenylalanine-D-alanine (1:9; D_2O; pH <1; SDSS); (d) DL-alanyl-DL-phenylalanine (commercial sample; D_2O; pH 5; SDSS); (e) glycyl-DL-phenylalanyl-D-alanine (D_2O; pH 5; SDSS); (f) glycyl-DL-alanyl-L-phenyl-alanine (D_2O; pH >10; SDSS).

phenylalanine methyl ester and N-acetyl-D-alanyl-L-phenylalanine methyl ester in the ratio 75:25. This result is in good agreement with the value 70:30 obtained for the same reaction conditions, but using fractional crystallization to separate N-acetyl-L-leucyl-glycine ethyl ester from a condensation reaction.[12] This method is greatly superior in convenience, rapidity, and generality to previously used techniques.[4-11] The use of a time average device (C.A.T.) in conjunction with a 100 MHz spectrometer should permit a further reduction in sample size, and most importantly, increase the sensitivity of the technique. We will report later on the influence of coupling agents, solvents and N-acyl protecting groups on the degree of racemization during peptide synthesis.

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