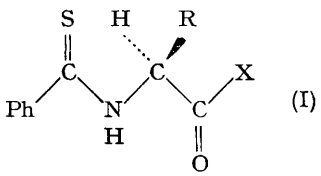
Solvent-dependent Circular Dichroism of Terminal *N*-Thiobenzoyl Peptides, in Relation to a Spectroscopic Method for the *N*-Terminal Analysis of Polypeptides

By G. C. BARRETT

(*Department of Chemistry, West Ham College of Technology, Romford Road, London, E.*15)

THE circular dichroism (c.d.) exhibited by *N*-thiobenzoyl-L-α-amino-acids (I; X = OH) is dependent on solvent[1–3] and temperature,[4] these factors influencing not only the magnitude, but also the sign and the wavelength location, of the $n\rightarrow\pi^*$ c.d. maximum of these compounds near 390 mμ. The effects of varying the solvent, *e.g.*,



(I)

from water through the C_1–C_4 aliphatic alcohols, on the c.d. behaviour of this series, are particularly striking;[4] moreover, the family of c.d. curves exhibited by each *N*-thiobenzoyl-L-α-amino-acid in a few specified solvents is quite distinctive, an observation which might be exploited for the *N*-terminal analysis of polypeptides.

Thus, terminal *N*-thiobenzoyl derivatives of peptides (I; X = peptide residue) should show solvent-dependent c.d. characteristic of the *N*-terminal amino-acid residue, if the factors responsible for the c.d. behaviour of such compounds were merely the positions of conformational equilibria (rotation of the thiobenzamide chromophore relative to the adjacent asymmetric centre) and/or of solvational equilibria involving only the *N*-terminus. Indications that the peptide residue might be passive in this respect are the reports (a) that *N*-thionoethoxycarbonyl derivatives of peptides yield Cotton effect curves (solvent not specified) whose sign depends upon the configuration of only the *N*-terminal amino-acid,[5] and (b) that the terminal *N*-dimedone derivative of glycyl-L-leucine ethyl ester exhibits no Cotton effect near 280 mμ.[6] On the other hand, additional Cotton effect contributions might be expected[7] in compounds such as (I; X = peptide residue) where the chromophore adjoins a helical peptide sequence.

Circular dichroism data for a number of terminal *N*-thiobenzoyl peptides (Table) reveal that, in fact, the peptide residue influences the c.d. behaviour of these compounds in two ways: (a) its steric requirements modify the factors which determine the solvent-dependence of the c.d. developed at the *N*-terminus in these compounds (shown well by the data for the series

TABLE

Circular dichroism of terminal N*-thiobenzoyl derivatives of representative amino-acids, amino-acid amides, and peptides*

N-Thiobenzoyl derivative of:	($\epsilon_L-\epsilon_R$) Values at c.d. maximum near 390 mμ:				
	H_2O	MeOH	Bu^{t}OH	Dioxan	Et_2O
L-Valine	+1·25	+0·42*	−0·39	−0·24	−1·44*
L-Valineamide	—	+0·97†	—	−0·06†	—
L-Valylglycine	+1·68	+0·84	−0·25	−0·23	−1·00
L-Valyl-L-leucine	+1·21	+1·51	+0·36	+0·36	−0·62
Glycyl-L-leucine	−0·13	+0·10	0·00	+0·16	+0·12
L-Leucine	+3·12	+1·26*	+0·44	+1·47	−0·83*
L-Leucinamide	insoluble	+1·92‡	+0·61	+0·70‡	−0·23
L-Leucylglycine	+3·30	+1·76	+0·39	+0·69	−0·63
L-Alanine	+0·94	−0·07*§ / +0·04	−0·65	−1·00	−1·32*
L-Alanylglycine	+1·35	+0·12	−1·05	−1·21	−1·42
Glycyl-L-alanine	−0·04	+0·13	+0·10	−0·06	+0·07
L-Serine	+0·46	−0·14*	−0·52	−1·64	−1·22*
L-Seryl-L-prolylglycine	+0·66	+0·36	−0·44	−0·96	insoluble
Glycyl-L-proline	−0·06	+0·13	+0·05	+0·06	−0·12

* from ref. 1 and 2. † from ref. 3. ‡ c.d. data published for these solutions (ref. 3) yield ($\epsilon_L-\epsilon_R$) values approximately 8% larger. § "Double-humped" c.d. curve (*cf.*, ref. 2).

N-thiobenzoyl-L-valyl-X, and *N*-thiobenzoyl-L-seryl-X); and (b) the c.d. data for *N*-thiobenzoylglycyl-L-α-amino-acids show that the penultimate *N*-terminal amino-acid residue can contribute significantly to the c.d. of terminal *N*-thiobenzoyl peptides.

The peptide unit in (I; R = H) adopts an extended conformation typical[8] of simple peptides in solution, since the c.d. of *N*-thiobenzoylglycyl-L-α-amino-acids is generally of opposite sign (in aqueous or in ethereal solution) to that of corresponding *N*-thiobenzoyl-L-α-amino-acids in the same solvents. Nevertheless, in spite of the relatively large separation of amino-acid side-chains in this conformation, the present results show that solvent-modified steric interactions between the chromophore and the first and second *N*-terminal amino-acid residues play a major role in obscuring the empirical c.d.–structure correlations upon which a spectroscopic method for *N*-terminal analysis of polypeptides might have been based. Even the more limited objective, assignment of absolute configuration to *N*-terminal amino-acid residues in polypeptides through the sign of the $n \rightarrow \pi^*$ c.d. displayed by their terminal *N*-thiobenzoyl derivatives, is shown to hold true only for specified solvents (water or ether, so far as solubility allows); even with this limitation, derivatives of peptides carrying *N*-terminal α-imino-acid residues are exceptional in aqueous solutions (*N*-thiobenzoyl-L-prolylglycine shows negative $n \rightarrow \pi^*$ c.d.), and derivatives with *N*-terminal phenylalanyl, tyrosyl, and related aromatic amino-acid residues, may prove to be exceptions to a correlation for ethereal solutions.[1,2] Therefore, the same empirical correlation,[5] for optical rotatory dispersion studies with analogous *N*-thionoethoxycarbonyl peptides, will probably be subject to similar limitations. Furthermore, the correlation should be limited to exclude derivatives with helical peptide sequences adjacent to an *N*-terminal chromophore, until c.d. contributions from this source have been assessed.

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