Nuclear Magnetic Resonance Spectra of Benzoyldipeptide Esters. A Convenient Test for Racemisation in Peptide Synthesis

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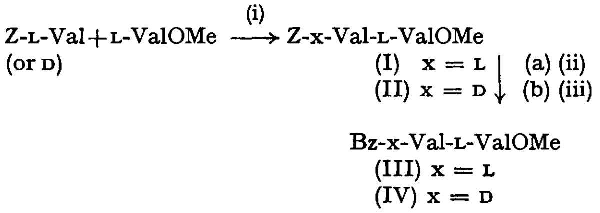
Summary The different chemical shifts of the ester methyl protons in diastereoisomeric benzoyldivalyl methyl esters provides a convenient method for assessing racemisation during peptide synthesis.

Methods for detecting racemisation during peptide coupling[1] rely on the accurate determination of the composition of a mixture of diastereoisomers. Diamagnetic shielding effects in the n.m.r. spectra of aromatic alanyl dipeptides have recently been used successfully[2] as a convenient racemisation test. We now report that an *N*-terminal benzoyl group creates sufficient differential in the chemical shift of *C*-terminal ester methyls to give a convenient analysis of the diastereoisomeric mixture.

The method originates from the observation that methyl *N*-benzoyl-L-valyl-L-valinate (III) and methyl *N*-benzoyl-D-valyl-L-valinate (IV), both produced stereochemically pure (summarised in the Scheme), show ester methyl signals at τ 6·35 and 6·42 respectively in deuteriochloroform solution. Experiments on standard mixtures of the diastereoisomers showed a quantitative relationship between integration of the signals and mixture composition.

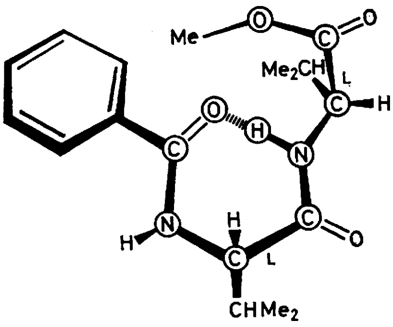


$$\begin{array}{lcl} \text{Z-L-Val} + \text{L-ValOMe} & \xrightarrow{\text{(i)}} & \text{Z-x-Val-L-ValOMe} \\ \text{(or D)} & & \text{(I) x = L} \quad \text{(a) (ii)} \\ & & \text{(II) x = D} \downarrow \text{(b) (iii)} \\ & & \text{Bz-x-Val-L-ValOMe} \\ & & \text{(III) x = L} \\ & & \text{(IV) x = D} \end{array}$$

SCHEME

Conditions:
(i) mixed anhydride coupling; (ii) HBr/AcOH; (iii) BzCl/NaOH. Z = benzyloxycarbonyl, Bz = benzoyl.

The ester signals are solvent-dependent; a mixture of (III) and (IV) analysed in $(CD_3)_2SO$ shows coincident ester signals. The phenomenon can also be associated with the position of the benzene ring since the benzyloxycarbonyl-protected compounds (I) and (II) show no differences in their signals. The nature of the side chain does not seem to influence the chemical shifts since the ^{1}H n.m.r. spectra of the diastereoisomers of *N*-benzoylvalylalanine methyl ester, *N*-benzoylalanylvaline methyl ester, and *N*-benzoylalanylalanine methyl ester all show ester methyl signals at τ *ca.* 6·35 (L-L) and 6·42 (D-L). A ^{13}C n.m.r. spectrum of a 50 : 50 mixture of L-L- and D-L-*N*-benzoylvalylvaline methyl ester showed all lines co-incident except for a slight separation (0·25 p.p.m.) of a secondary carbon signal associated with one of the isopropyl side chains.



The separate ester signals in the ^{1}H n.m.r. spectra can be explained by a hydrogen-bonded structure [Figure (L-L form)]. Changes in the stereochemistry of the *N*-terminal asymmetric centre could induce a conformational change at the *C*-terminal residue thus altering the spatial relationship between the benzene ring and the ester methyl group.

This phenomenon can be used to detect racemisation in the following way. *N*-Benzoyl-L-valine and L-valine methyl ester are coupled using a particular reagent, and then the excess of reagent is removed by mineral acid and HCO_3 extraction. The residual benzoyl dipeptide ester is dissolved in deuteriochloroform and the composition of the mixture analysed by integration of the ester signals at τ 6·35 (L-L) and 6·42 (D-L) in the 100 MHz ^{1}H n.m.r. spectrum of the solution. Results of a brief survey of coupling methods are summarised in the Table.

TABLE

Degree of racemisation during peptide bond formation

Coupling agent	% D-L in product†
Azide[a]	None observable
Dicyclohexylcarbodi-imide[b]	48
Dicyclohexylcarbodi-imide with *N*-hydroxy-succinimide[c]	None observable‡
Dicyclohexylcarbodi-imide with 1-hydroxy-benzotriazole[d]	13
Mixed anhydride[e] (Et_3N as base)	46
Mixed anhydride (*N*-methylmorpholine as base)	21
Ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ)[f]	22
N-Ethyl-5-(3-sulphonatophenyl)isoxazolium salt (Woodward's Reagent K)[g]	33

For reaction conditions see: [a] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1963, 881; [b] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067; [c] F. Weygand, D. Hofmann, and E. Wünsch, *Z. Naturforsch*, 1966, **21b**, 426; [d] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 789; [e] J. R. Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; [f] B. Belleau and G. Malek, *ibid.*, 1968, **90**, 1651; [g] R. B. Woodward, R. A. Olofson, and H. Mayer, *ibid.*, 1961, **83**, 1010.

Note: All couplings involving DCCI were carried out in dichloromethane.

† Average of duplicate determinations.

‡ *Added in proof:* During more recent experiments a small amount (10%) of racemisation has been detected.

This method is chemically similar to the Young racemisation test for which it has been stated[1] that the model reaction is ten times more susceptible to racemisation than most typical peptide coupling reactions. We have also confirmed[3] that the valyl residue is more susceptible to racemisation than the alanyl residue.[4] The combination of these factors provides a stringent test for a coupling method. A very low result for the degree of racemisation implies that a coupling agent should be satisfactory when used with well established and less racemisation-prone *N*-protecting groups.

We thank the S.R.C. and Roche Products Ltd. for financial support.

(*Received*, 27*th September* 1974; *Com.* 1218.)

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[1] J. H. Jones in 'Amino-acid, Peptides, and Proteins,' Vol. **3**, ed. G. T. Young, The Chemical Society, 1971, p. 239.

[2] B. Weinstein and A. E. Pritchard, *J.C.S., Perkin I*, 1972, 1015.

[3] I. Tomida, H. Kayahara, and R. Iriye, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2557.

[4] It has been brought to our attention by a referee that the higher value for valine may be due to asymmetric induction of an oxazolone intermediate: F. Weygand, W. Steglich, and X. Barocio de la Lama, *Tetrahedron*, 1966, Suppl. **8**, 9.