Amino-acids and Peptides. Part XXVIII.[1] Determination of Racemization in Peptide Synthesis by Nuclear Magnetic Resonance Spectroscopy

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Diastereoisomeric aromatic alanyl peptides possess different n.m.r. spectra owing to a diamagnetic shielding phenomenon. Observation of the shifted methyl resonance is an excellent procedure for the study of racemization in peptide synthesis. Eight alanyl dipeptides are available as models for the evaluation and testing of any *N*-acyl protecting group or coupling agent. The method is general, convenient, rapid, and sensitive.

RACEMIZATION during the coupling of amino-acid components is an important problem in the synthesis of peptides. Procedures developed to detect the degree of racemization include countercurrent distribution,[2] fractional crystallization,[3-5] gas–liquid partition,[6,7] ion-exchange,[8,9] paper,[10,11] and thin-layer [12,13] chromatography, fluorine n.m.r. spectroscopy,[14] and isotopic dilution.[15] Unfortunately, many of these techniques are not in wide use, as they require specific *N*-protecting groups or are limited to a few amino-acids and peptides. Moreover, one must always be aware of the possibility of kinetic resolution in the synthetic scheme or optical fractionation in subsequent purification procedures. Since retention of configuration in peptide bond formation is dependent on the nature of the activating agent and on changes in acyl groups, amino-components, bases, salts, solvents, and temperature, alternative methods may offer some advantages in studying these many factors in detail.[16-20]

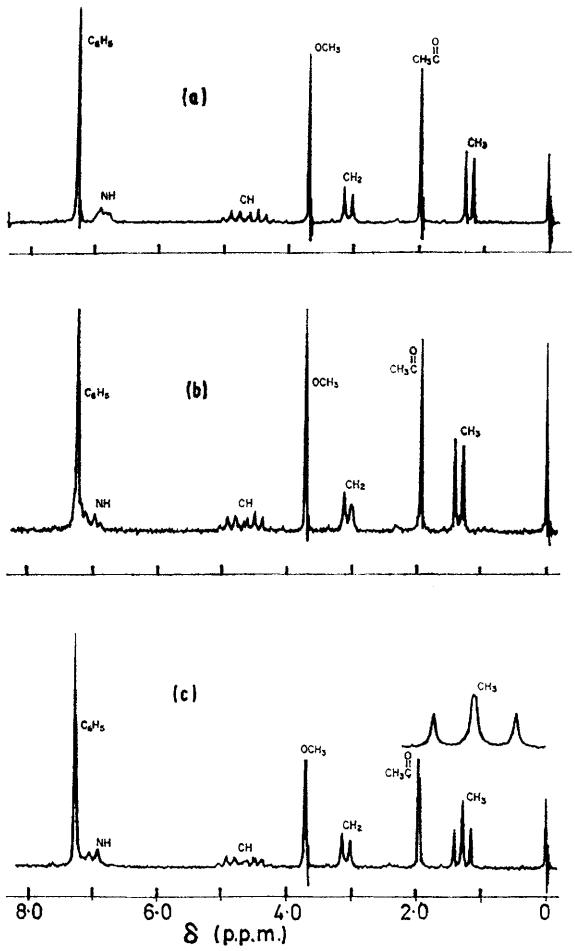
Although the introduction of n.m.r. spectroscopy as a tool for racemization studies is relatively new, observations based on the n.m.r. spectra of peptides have been common for the past decade. For example, a shielding phenomenon is known to exist in *free* dipeptides containing adjacent aromatic and aliphatic amino-acid residues.[21] In the chief explanation it is assumed that the aliphatic side-chain in the L,L state is more extended and is deshielded by the charged amino species as compared to the more compact D,L conformation.[22,23] Diamagnetic shielding by the aromatic ring is not implicated, because in these diastereomers the signals of the protons of the benzene ring coincide exactly. Magnetic non-equivalence has been noted in the absence of an aromatic group, such as in methyl L-α-chloroacyl-L-valinates,[24] glycyl dipeptides,[25] and various alanyl peptides,[26] but the effects seem due to special steric or conformational factors.

In contrast, *blocked* alanylphenylalanyl dipeptides are unable to ionize in solution, and diamagnetic effects are considerably enhanced in these compounds. The resulting shift in the alanyl methyl signal offers a new method for the determination of racemization in peptide synthesis.[27] By use of this technique it became possible to examine the influence of several coupling agents and *N*-acyl protecting groups on the extent of racemization during peptide synthesis.[28] This idea has been applied to the use of thiazolidine-2,5-dione intermediates,[29] to assign configurations in some alanylcycloserine derivatives,[30] to evaluate phosphonitrilic chloride as a peptide

[1] Part XXVII, A. Ali, J. H. R. Faesel, D. Sarantakis, D. Stevenson, and B. Weinstein, *Experientia*, 1971, **27**, 1138.
[2] D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, 1957, 1398.
[3] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1958, **80**, 2902.
[4] N. A. Smart, G. T. Young, and M. W. Williams, *J. Chem. Soc.*, 1960, 3902; M. W. Williams and G. T. Young, *ibid.*, 1963, 882.
[5] S. Goldschmidt and K. K. Gupta, *Chem. Ber.*, 1965, **98**, 2831.
[6] F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem.*, 1963, **75**, 282; F. Weygand, A. Prox, and W. König, *Chem. Ber.*, 1966, **99**, 1451.
[7] B. Halpern and J. W. Westley, *Biochem. Biophys., Res. Comm.*, 1965, **19**, 361; B. Halpern, L. F. Chew, and J. W. Westley, *Analyt. Chem.*, 1967, **39**, 399.
[8] N. Izumiya and M. Muraoka, *J. Amer. Chem. Soc.*, 1969, **91**, 2391.
[9] F. H. C. Stewart, *Austral. J. Chem.*, 1970, **23**, 1073.
[10] E. Taschner, T. Sokolowska, J. F. Biernat, A. Chimiak, C. Wasielewski, and B. Rzeszotarska, *Annalen*, 1963, **663**, 197; T. Sokolowska and J. F. Biernat, *J. Chromatog.*, 1964, **13**, 269.
[11] G. Losse, H. Rave, and K. Koehler, *Z. Chem.*, 1967, **7**, 105.
[12] Z. Pravda, K. Poduska, and K. Blaha, *Coll. Czech. Chem. Comm.*, 1964, **29**, 2626.
[13] E. Taschner, L. Lubiewska, M. Smulkowski, and H. Wojciechowska, *Experientia*, 1968, **24**, 521.
[14] R. E. Sievers, E. Bayer, and P. Hunziker, *Nature*, 1969, **223**, 5202.
[15] D. S. Kemp, S. W. Wang, G. Busby, tert., and G. Hugel, *J. Amer. Chem. Soc.*, 1970, **92**, 1043.
[16] S. Sakakibara and M. Itoh, *Bull. Chem. Soc. Japan*, 1967, **40**, 656.
[17] M. Bodanszky and A. Bodanszky, *Chem. Comm.*, 1967, 591.
[18] G. T. Young, in 'Peptides, Proceedings of the Eighth European Peptide Symposium,' eds. H. C. Beyerman, A. van de Linde, and W. Maassen van den Brink, North-Holland, Amsterdam, 1967, p. 55.
[19] N. Nakamizo, *Tampakushitsu Kakusan Koso*, 1968, **13**, 586.
[20] M. Goodman and C. Glaser in 'Peptides: Chemistry and Biochemistry,' ed. B. Weinstein, Marcel Dekker, New York, 1970, p. 267.
[21] F. A. Bovey and G. V. D. Tiers, *J. Amer. Chem. Soc.*, 1959, **81**, 2870.
[22] T. Wieland and H. Bende, *Chem. Ber.*, 1965, **98**, 504.
[23] R. U. Lemieux and M. A. Barton, *Canad. J. Chem.*, 1971, **49**, 767.
[24] B. Halpern, J. W. Westley, and B. Weinstein, *Chem. Comm.*, 1967, 160.
[25] J. W. Westley and B. Weinstein, *Chem. Comm.*, 1967, 1232.
[26] B. Halpern, D. E. Nitecki, and B. Weinstein, *Tetrahedron Letters*, 1967, 3075.
[27] B. Halpern, L. F. Chew, and B. Weinstein, *J. Amer. Chem. Soc.*, 1967, **89**, 5051.
[28] B. Weinstein in 'Peptides: Chemistry and Biochemistry,' ed. B. Weinstein, Marcel Dekker, New York, 1970, p. 371.
[29] R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Palevada, jun., H. Schwam, H. Barkenmeyer, B. H. Arison, D. F. Veber, R. G. Denkelwalter, and R. Hirschmann, *J. Amer. Chem. Soc.*, 1968, **90**, 3254.
[30] R. A. Payne and C. H. Stammer, *J. Org. Chem.*, 1968, **33**, 2421.

coupling agent,[31] to study the photochemical alkylation of methyl *N*-acetylglycylalaninate by phenylalanine,[32] to monitor the coupling of L-histidine thiocarbamate with L-alanylglycine,[33] and to try acyloxysilanes as acylating agents.[34]

We present evidence that methyl *N*-acetylphenylalanylalaninate can be employed as a model compound for racemization studies. The advantages are as follows: the acetyl group is known to be poor from a protection viewpoint—thus, an index of racemization can be constructed for a large array of coupling agents; next, the phenylalanylalanine unit affords a satisfactory shielding value, which allows integration of the methyl doublet areas without difficulty; and thirdly the acetyl and methyl ester n.m.r. signals provide convenient internal standardization values. These points are illustrated in the Figure.



N.m.r. spectra of diastereoisomers of methyl *N*-acetylphenylalanylalaninate: L,D (top); L,L (middle); L,D plus L,L (bottom) (Varian A-60 spectrometer; deuteriochloroform as solvent and tetramethylsilane as reference)

This compound has been utilized to test a variety of coupling agents for the extent of racemization during a typical peptide synthesis. Table 1 summarizes the results. Generally, the data confirm earlier reports on the usefulness of such intermediates. We did not seek optimum conditions, so these values may represent upper limits.

TABLE 1

Degree of racemization during peptide bond formation

Coupling agent	% D,L in product
Azide	<3
NN'-Carbonyldi-imidazole	<3
Dicyclohexylcarbodi-imide with *N*-hydroxysuccinimide	<3
Ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate	<3
1-(3-Dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride with *N*-hydroxysuccinimide	<3
Piperidyl ester	<3
Ethylene phosphorochlorodite	13
N-Ethyl-5-(3-sulphonatophenyl)isoxazolium	15
Diethyl chlorophosphite	27
Dicyclohexylcarbodi-imide	30
1-(3-Dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride	32
Diethyl chlorophosphate	37
1-Cyclohexyl-3-(4-ethylmorpholin-2-yl)carbodi-imide methotoluene-*p*-sulphonate	45
Ethyl chloroformate	50

TABLE 2

Methyl resonances (in Hz from Me_4Si) of *N*-acyl dipeptide derivatives

Compound	Methyl resonance [a]	Shift difference L,L − L,D (or D,L)
Z-L(or D)-Ala-L(or D)-Ala-OMe	83·5	0
Z-L-His-L-Ala-OMe	76·5	2·5
Z-L-His-D-Ala-OMe	74·0	
N^{α}-Z-N^{im}-Bzl-L-His-L-Ala-OMe	73·0	−4·5
N^{α}-Z-N^{im}-Bzl-L-His-D-Ala-OMe	77·5	
Z-L-Phe-L-Ala-OMe	79·5	6·0
Z-L-Phe-D-Ala-OMe	73·5	
Z-L-Ala-L-Phe-OMe	78·0	2·0
Z-D-Ala-L-Phe-OMe	76·0	
Z-L-Trp-L-Ala-OMe	74·0	8·0
Z-L-Trp-D-Ala-OMe	66·0	
Z-L-Ala-L-Trp-OMe	77·5	2·0
Z-D-Ala-L-Trp-OMe	75·5	
Z-L-Tyr-L-Ala-OMe	79·5	4·0
Z-D-Tyr-L-Ala-OMe	75·5	
Z-L-Ala-L-Tyr-OMe	79·5	0·0
Z-D-Ala-L-Tyr-OMe	79·5	
NO-di-Z-L-Tyr-L-Ala-OMe	79·0	5·5
NO-di-Z-L-Tyr-L-Ala-OMe	73·5	

[a] Determined on a Varian A-60 spectrometer; the centre of gravity of the signal is quoted (J 7·2 ± 0·3 Hz); 7·5% (w/v) solutions in $CDCl_3$ [$(CD_3)_2SO$ for the last two].

In order to extend the work, a related diastereoisomeric series has been prepared with the remaining aromatic amino-acids histidine, tryptophan, and tyrosine. A similar alanyl shift apparently exists in most of these systems, too; the n.m.r. data are presented in Table 2.

Methyl *N*-benzyloxycarbonyl-L-alanyl-L-tyrosinate

[31] C. S. Levine and C. H. Stammer, *J. Org. Chem.*, 1972, **37**, 148; K. C. Das, Y.-Y. Lin, and B. Weinstein, *Experientia*, 1969, **25**, 1238.

[32] D. Elad and J. Sperling, *J. Chem. Soc.* (*C*), 1969, 1579.

[33] R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Palevada, jun., H. Schwan, H. Barkemeyer, B. H. Arison, D. F. Veber, R. G. Strachan, J. Milkowski, R. G. Denkelwalter, and R. Hirschmann, *J. Org. Chem.*, 1971, **36**, 49.

[34] T. H. Chan and L. T. L. Wong, *J. Org. Chem.*, 1971, **36**, 850.

and methyl *N*-benzyloxycarbonyl-D-alanyl-L-tyrosinate exhibit identical spectra. This unexpected result is possibly due to steric requirements that prevent the juxtaposition of the side-chains in each isomer. Additionally, the aliphatic shift for the L,D-isomer relative to the L,L-isomer in the methyl $N^{\alpha}N^{im}$-bisbenzyloxycarbonylhistidylalaninates is downfield instead of upfield, in contrast to the other dipeptide pairs. Models indicate that the aliphatic side-chain is diamagnetically shielded by the N^{im}-benzyl substituent in the L,L-isomer, but the reverse is true for the L,D-isomer.

If there are difficulties involved in the use of aromatic residues in this type of analysis, then the dipeptides benzyl *N*-benzyloxycarbonyl-L-alanyl-L-alaninate and benzyl *N*-benzyloxycarbonyl-L-alanyl-D-alaninate are of interest, since a related shift is found for the methyl group in the second alanyl residue due to the proximity of the benzyl ester ring.[35]

In summary, the n.m.r. procedure for the analysis of racemization in peptide synthesis has several practical and theoretical advantages as compared to other schemes reported. Any *N*-protecting group or coupling agent is easily evaluated. There is no need to synthesize special diastereoisomeric peptides or to isolate individual isomers. Apart from the time needed for the reaction and work-up procedures, an n.m.r. scan takes only a few minutes, including integration. A typical value is useful to within $\pm 3\%$; however, CAT or ^{13}C side-band measurements increases the accuracy at least ten-fold. Finally, at least eight alanyl dipeptides and sixteen glycylalanyl or alanylglycyl tripeptides can furnish methyl doublet data. Thus, by choosing any one or a combination of a number of these twenty-four compounds, a host of secondary factors involved in racemization, such as changes in solvent or base concentration, can be studied.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus and are corrected. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. I.r. spectra, for potassium bromide pellets, were obtained on a Perkin-Elmer 257 spectrometer, n.m.r. spectra were recorded on a Varian A-60 spectrometer, and u.v. spectra on a Cary 14 spectrophotometer. Evaporations were performed under reduced pressure (water pump) in a rotatory evaporator at minimum temperature. Sodium and magnesium sulphates were used for drying purposes. All solvents were reagent grade; light petroleum had b.p. 30—60°.

N-*Acetyl*-L-*phenylalanine*.—L-Phenylalanine (4·5 g, 27 mmol) was dissolved in sodium hydroxide solution (2N; 10·9 ml) at 0° and eight additions of sodium hydroxide (2N; 9·0 ml) and acetic anhydride (0·9 ml) were made at intervals of 2 min with constant stirring. Sulphuric acid (6N; 28 ml) was added (to Congo Red end-point) after 45 min and the solution was evaporated until the acetyl derivative began to crystallize. Recrystallization from water gave *N*-acetyl-L-phenylalanine (4·63 g, 83%), m.p. 168—169°, $[\alpha]_D^{20\cdot0}$ +46·5° (*c* 2·00 in EtOH) {lit.,[36] m.p. 172°, $[\alpha]_D^{26\cdot0}$ +51·4° (*c* 1·00 in EtOH); lit.,[37] m.p. 170—171°, $[\alpha]_D^{22\cdot0}$ +47·6° (*c* 1·00 in EtOH)}.

Methyl N-*Acetyl*-L-*phenylalaninate*.—To methyl L-phenylalaninate hydrochloride (2·16 g, 10 mmol) and triethylamine (1·38 ml, 10 mmol) in chloroform (50 ml) at −40°, acetyl chloride (0·705 ml, 10 mmol) was added during 20 min. The mixture was stirred for 40 min at room temperature, the chloroform was evaporated off, and the residue was triturated with ethyl acetate. The resulting solution was filtered, washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution, and water, dried, and evaporated. Recrystallization from ethyl acetate–light petroleum gave methyl *N*-acetyl-L-phenylalaninate (1·94 g, 88%), m.p. 90—91°, $[\alpha]_D^{20\cdot0}$ +16·5° (*c* 2·00 in MeOH) {lit.,[38] m.p. 89—90°, $[\alpha]_D^{25\cdot0}$ +19·5° (*c* 2·00 in MeOH)}.

In an alternative synthesis, methyl L-phenylalaninate hydrochloride (4·31 g, 20 mmol) and triethylamine (2·76 ml, 20 mmol) were added to tetrahydrofuran (20 ml) at 0°. After 15 min, *p*-nitrophenyl acetate (3·98 g, 27 mmol) was added and the mixture was stirred at room temperature for 4 days. The solution was diluted with ethyl acetate (100 ml), then washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution, and water, dried, and evaporated. Crystallization from hot ethyl acetate gave methyl *N*-acetyl-L-phenylalaninate (3·06 g, 69%), m.p. 90·5—91·5°, $[\alpha]_D^{20\cdot0}$ +16·5° (*c* 2·00 in MeOH).

N-*Acetyl*-L-*phenylalanine Hydrazide*.—Methyl *N*-acetyl-L-phenylalaninate (5·52 g, 25 mmol) was dissolved in anhydrous ethanol (25 ml) and hydrazine hydrate (99—100%; 6·06 ml, 125 mmol) was added. Next day the product was filtered off and washed with cold, anhydrous ethanol. Recrystallization from ethanol gave *N*-acetyl-L-phenylalanine hydrazide (2·3 g, 83%), m.p. 182—184°, $[\alpha]_D^{20\cdot0}$ +17·0° (*c* 2·00 in EtOH) {lit.,[39] m.p. 164—166°, $[\alpha]_D^{20\cdot0}$ +20·0° (*c* 1·2 in EtOH)}, $\nu_{max.}$ 3280 (NH), 2920 (CH), 1630 (C=O), 1530 (amide II), 750 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 264, 258, 252, and 246 nm (ε 112, 180, 230, 207, and 200).

Methyl N-*Acetyl*-L-*phenylalanyl*-L-*alaninate*.—*N*-Acetyl-L-phenylalanine hydrazide (0·884 g, 4 mmol) was dissolved in dimethylformamide (20 ml) and the solution was cooled to −10°. Hydrochloric acid in tetrahydrofuran (3·6N; 3·3 ml) and n-butyl nitrite (0·625 ml, 5 mmol) were added and the solution was stirred for 20 min. Then, triethylamine (1·80 ml, 13 mmol) in dimethylformamide (20 ml) was added, followed by methyl L-alaninate (0·554 g, 4 mmol). The mixture was stirred for 20 min and adjusted to pH 8 with a few drops of triethylamine; it was then stored for 1 h at −10°, and placed in a refrigerator for 3 days. After work-up in the usual manner, crystallization from ethyl acetate–light petroleum gave *methyl* N-*acetyl*-L-*phenylalanyl*-L-*alaninate* (0·91 g, 78%), m.p. 193—194°, $[\alpha]_D^{20\cdot0}$ −9·5° (*c* 1·00 in MeOH), $\nu_{max.}$ 3260 (NH), 2950 (CH), 2930 (CH), 1765 (C=O), 1680 (C=O), 1640br (C=O), 1560br (amide), 700 (Ph), and 670 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 258, 252, and 247 nm (ε 100, 163, 205, 166, and 126), δ ($CDCl_3$) 7·23 (s, aromatic), 6·84—7·17 (complex, NH), 4·3—5·0 [complex, C(α)H], 3·70 (s, CO_2Me), 3·22 [d, *J* 7 Hz, C(β)H_2 in phenylalanine], 1·93 (s, Ac), and 1·35 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 62·05; H, 7·05; N, 9·6. $C_{15}H_{20}N_2O_4$ requires C, 61·65; H, 6·9; N, 9·6%).

[35] B. Weinstein and H.-H. Chang, unpublished observations.
[36] V. du Vigneaud, *J. Biol. Chem.*, 1932, **98**, 295.
[37] R. S. Coffey, M. Green, and G. W. Kenner, *J. Chem. Soc.*, 1959, 4100.
[38] A. T. Huang, R. J. Foster, and C. Niemann, *J. Amer. Chem. Soc.*, 1952, **74**, 105.
[39] I. Z. Siemion and J. Morawiec, *Bull. Acad. Polon. Sci., Ser. Sci. Chim.*, 1964, **12**, 295.

Methyl N-*Acetyl*-L-*phenylalanyl*-D-*alaninate*.—The procedure described for the L-alaninate isomer was used. Crystallization from ethyl acetate–light petroleum gave the D-*alaninate* (0·82 g, 70%), $[\alpha]_D^{20\cdot0}$ +31·5° (*c* 1·00 in MeOH), $\nu_{max.}$ 3290 (NH), 2950 (NH), 2930 (CH), 1745 (C=O), 1640br (C=O), 1545 (amide), 705 (Ph), and 685 (Ph) cm^{-1}; $\lambda_{max.}$ (MeOH) 258, 255, 249, 243, and 238 nm (ε 110, 174, 217, 180, and 145), δ ($CDCl_3$) 7·25 (s, aromatic), 6·75—7·08 (complex, NH), 4·2—5·0 [complex, C(α)H], 3·70 (s, CO_2Me), 3·09 [d, *J* 7 Hz, C(β)H_2 in phenylalanine], 1·97 (s, Ac), and 1·22 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 61·65; H, 7·0; N, 9·5%).

Methyl N$^{\alpha}$-*Benzyloxycarbonyl*-L-*histidyl*-L-*alaninate*.—To a solution of methyl L-alaninate (0·70 g, 5 mmol) in *NN*-dimethylformamide (10 ml) at 0° was added triethylamine (0·69 ml, 5 mmol), and the mixture was stirred for 15 min. N$^{\alpha}$-Benzyloxycarbonyl-L-histidine (1·45 g, 5·02 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride (0·97 g, 5·50 mmol) were then added and the solution was stirred overnight. Ethyl acetate (50 ml) was added, the solution was washed with water and dilute sodium hydrogen carbonate solution, dried, and evaporated. Crystallization from hot water gave *methyl* N$^{\alpha}$-*benzyloxycarbonyl*-L-*histidyl*-L-*alaninate* (1·12 g, 60%), m.p. 133—136°, $\nu_{max.}$ 3310 (NH), 2950 (CH), 1745 (C=O), 1690 (urethane), 1660 (C=O), 1535 (amide II), 1225 (CO), 740 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 260, 257, 251, and 246 nm (ε 115, 175, 162, 212, 166, and 127), δ (Me_2SO) 7·37 (s, aromatic), 7·60 and 6·85—7·1 (s and complex, imidazole), 5·03 (s, $PhCH_2{\cdot}O$), 4·0—4·5 [C(α)H], 3·63 (s, CO_2Me), 2·7—3·2 [C(β)H_2 in histidine], and 1·28 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 57·5; H, 6·05; N, 15·15. $C_{18}H_{22}N_4O_5$ requires C, 57·75; H, 5·9; N, 14·95%).

Methyl N$^{\alpha}$-*Benzyloxycarbonyl*-L-*histidyl*-D-*alaninate*.—The procedure described for the L-alaninate isomer was used. Crystallization from hot water gave the D-*alaninate* (1·04 g, 55%), m.p. 175—178°, $\nu_{max.}$ 3290 (NH), 2950 (CH), 1740 (C=O), 1690 (urethane), 1650 (C=O), 1550 (amide II), 1220 (CO), 750 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263 260, 258, 252, and 247 nm (ε 151, 210, 190, 243, 190, and 146), δ (Me_2SO) 7·30 (s, aromatic), 7·6 and 6·75—7·0 (s and complex, imidazole), 5·03 (s, $PhCH_2{\cdot}O$), 4·0—4·7 [complex, C(α)H], 3·63 (s, CO_2Me), 2·86 [d, *J* 7 Hz, C(β)H_2 in histidine], and 1·23 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 57·7; H, 6·15; N, 14·8%).

Methyl N$^{\alpha}$-*Benzyloxycarbonyl*-N^{im}-*benzyl*-L-*histidyl*-L-*alaninate*.—To a solution of methyl L-alaninate hydrochloride (1·40 g, 10 mmol) in chloroform (15 ml) at 0° was added triethylamine (1·38 ml, 10 mmol). The mixture was stirred for 15 min, then N$^{\alpha}$-benzyloxycarbonyl-N^{im}-benzyl-L-histidine (3·79 g, 10·5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride (2·11 g, 11 mmol) were added. The solution was stirred at room temperature for 24 h, then evaporated, and the residue was triturated with ethyl acetate (50 ml). The organic phase was washed with water (3 × 40 ml), dilute sodium hydrogen carbonate solution, and water again, dried, and evaporated. Crystallization from ethyl acetate–light petroleum gave *methyl* N$^{\alpha}$-*benzyloxycarbonyl*-N^{im}-*benzyl*-L-*histidyl*-L-*alaninate* (2·7 g, 58%), m.p. 113—115°, $\nu_{max.}$ 3320 (NH), 2960 (CH), 1755 (C=O), 1715 (urethane), 1660 (C=O), 1530 (amide II), 1215 (CO), 730 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 272, 263, 260, 257, 251, and 246 nm (ε 233, 346, 334, 424, 346, and 275), δ ($CDCl_3$) 7·37 (s, aromatic), 6·73 and 7·0—7·3 (s and broad band, imidazole), 5·15 (s, $PhCH_2$), 5·03 (s, $PhCH_2{\cdot}O$), 4·3—4·7 [complex, C(α)H], 3·70 (s, CO_2Me), 2·98—302), [C(β)H_2 in histidine], and 1·22 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 64·15; H, 6·0; N, 12·1. $C_{25}H_{28}N_4O_5$ requires C, 64·65; H, 6·1; N, 12·05%).

Methyl N$^{\alpha}$-*Benzyloxycarbonyl*-N^{im}-*benzyl*-L-*histidyl*-D-*alaninate*.—The procedure described for the L-alaninate isomer was used. Crystallization from ethyl acetate–light petroleum gave the D-*alaninate* (2·4 g, 52%), m.p. 144—146°, $\nu_{max.}$ 3310 (NH), 2960 (CH), 1740 (C=O), 1680 (urethane), 1645 (C=O), 1540 (amide II), 1230 (CO), 755 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 260, 257, 252, and 247 nm (ε 243, 367, 353, 452, 359, and 281), δ ($CDCl_3$) 7·37 (s, aromatic), 6·70 and 7·1—7·3 (s and complex, imidazole), 5·15 (s, $PhCH_2$), 5·03 (s, $PhCH_2{\cdot}O$), 4·3—4·7 [complex, C(α)H], 3·70 (s, methyl ester), 2·8—3·2 [complex, C(β)H_2 in histidine], and 1·28 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 64·35; H, 5·65; N, 12·0%).

The following dipeptides were made by the standard *NN'*-dicyclohexylcarbodi-imide procedure, which is described in detail for the first compound only. The products were crystallized from ethyl acetate–light petroleum, unless otherwise noted.

Methyl N$^{\alpha}$-*Benzyloxycarbonyl*-L-*tyrosyl*-L-*alaninate*.—Methyl L-alaninate hydrochloride (1·40 g, 10 mmol) and triethylamine (1·38 ml, 10 mmol) were added to dimethylformamide (15 ml) at 0°. The solution was stirred for 15 min and N$^{\alpha}$-benzyloxycarbonyl-L-tyrosine (3·32 g, 10·5 mmol) was added followed by *NN'*-dicyclohexylcarbodi-imide (2·27 g, 11 mmol). The solution was stirred overnight at room temperature, filtered, and diluted with ethyl acetate (100 ml). The ethyl acetate solution was washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution, and water, dried, and evaporated. Crystallization from ethyl acetate–light petroleum gave *methyl* N$^{\alpha}$-*benzyloxycarbonyl*-L-*tyrosyl*-L-*alaninate* (2·8 g, 70%), m.p. 157—159°, $\nu_{max.}$ 3300br (NH), 2950 (CH), 1690br (C=O and urethane), 1660 (C=O), 1615 (Ph), 1595 (Ph), 1230 (CO), 1515 (amide II), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 278 nm (ε 1650), δ ($CDCl_3$) 7·30 (s, $PhCH_2{\cdot}O$), 6·87 (q, $J = 9$ Hz, aromatic in tyrosine), 5·39 (d, *J* 7 Hz, NH), 6·44 (d, *J* 7 Hz, NH), 5·10 (s, $PhCH_2{\cdot}O$), 4·2—4·7 [C(α)H], 3·70 (s, CO_2Me), 2·98 [d, *J* 7Hz, C(β)H_2 in tyrosine], and 1·33 p.p.m. (*J* 7 Hz, CH_3 in alanine) (Found: C, 62·9; H, 6·1; N, 6·9. $C_{21}H_{24}N_2O_6$ requires C, 63·0; H, 6·05; N, 7·0%).

Methyl N$^{\alpha}$-benzyloxycarbonyl-L-alanyl-L-tyrosinate (2·5 g, 64%) had m.p. 124° (lit.,[40] 121—122°), $\nu_{max.}$ 3400 (OH), 3320 (NH), 2940 (CH), 1740 (C=O), 1710 (urethane), 1690 (C=O), 1660 (C=O), 1615 (Ph), 1595 (Ph), 1520 (amide II), 1180 (CO), 750 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 278 nm (ε 1580), δ ($CDCl_3$) 7·37 (s, $PhCH_2{\cdot}O$), 6·82 (q, *J* 9 Hz, aromatic in tyrosine), 6·56 (d, *J* 7 Hz, NH), 5·39 (d, *J* 7 Hz, NH), 5·13 (s, $PhCH_2{\cdot}O$), 4·1—4·4 and 4·7—4·9 [complex, C(α)H], 3·73 (s, CO_2Me), 3·06 [d, *J* 6 Hz(β)CH_2 in tyrosine], and 1·33 p.p.m. (d, *J* 7 Hz, CH_3 in alanine).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-D-*alanyl*-L-*tyrosine* (2·7 g, 68%) had m.p. 149—151°, $\nu_{max.}$ 3360 (OH), 3290 (NH), 2940 (CH), 1730 (C=O), 1705 (urethane), 1660 (C=O), 1615 (Ph), 1595 (Ph), 1520 (amide II), 1250br (CO), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 277 nm (ε 1720), δ ($CDCl_3$) 7·37 (s, $PhCH_2{\cdot}O$), 6·82 (q, *J* 9 Hz, aromatic in tyrosine), 5·13 (s, $PhCH_2{\cdot}O$), 3·73 (s, CO_2Me), 3·05 [d, *J* 6 Hz, C(β)H_2 in

[40] K. Jost, V. Devalov, H. Nesvadba, and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1964, **29**, 419.

tyrosine], and 1·33 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 63·0; H, 6·1; N, 7·0%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-L-*tyrosyl*-D-*alaninate* (2·3 g, 58%) had m.p. 162—164°, $\nu_{max.}$ 3330 (OH), 3300 (NH), 2940 (CH), 1690br (C=O and urethane), 1660 (C=O), 1615 (Ph), 1595 (Ph), 1515 (amide II), 1235 (CO), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 277 nm (ε 1610), δ ($CDCl_3$) 7·35 (s, *Ph*CH_2·O), 6·90 (q, J 9 Hz, aromatic in tyrosine), 5·13 (s, PhC*H*$_2$·O), 4·2—4·7 [C(α)*H*], 3·71 (s, CO_2Me), 3·01 [d, J 7 Hz, C(β)H_2 in tyrosine], and 1·24 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 62·6; H, 6·05; N, 6·85%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-L-*tryptophanyl*-L-*alaninate* (1·1 g, 26%) had m.p. 131—133°; $\nu_{max.}$ 3300br (NH), 2940 (CH), 1700br (C=O and urethane), 1650 (C=O), 1530 (amide II), 1220 (C=O), 740 (Ph), and 690 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 290, 282, and 274 nm (ε 6100, 6950, and 6530), δ ($CDCl_3$) 7·36 (s, Ph), 7·05—7·3 (complex, indole), 6·34 (d, J 7 Hz, NH), 5·56 (d, J 7 Hz, NH), 5·14 (s, PhC*H*$_2$·O), 4·3—4·8 [complex, C(α)H], 3·64 (s, CO_2Me), 3·25 [d, J 6 Hz, C(β)H_2 in tryptophan], and 1·22 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 65·15; H, 6·0; N, 9·7. $C_{23}H_{26}N_3O_5$ requires C, 65·05; H, 6·2; N, 9·9%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-L-*tryptophanyl*-D-*alaninate* (1·8 g, 42%) had m.p. 164—166°, $\nu_{max.}$ 3300 (NH), 2940 (CH), 1715 (C=O and urethane), 1655 (C=O), 1530 (amide II), 1230 (CO), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 290, 281, and 273 (ε 6080, 6970, and 6480), δ ($CDCl_3$) 7·35 (s, Ph), 7·0—7·3 (complex, indole), 6·26 (d, J 7 Hz, NH), 5·54 (d, J 7 Hz, NH), 5·12 (s, PhC*H*$_2$·O), 4·2—4·8 [complex, C(α)H], 3·60 (s, CO_2Me), 3·26 [d, J 7 Hz, C(β)H_2 in tryptophan], and 1·09 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 65·3; H, 6·05; N, 9·95%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-D-*alanyl*-L-*tryptophanate* (2·15 g, 51%) had m.p. 124—126°, $\nu_{max.}$ 3350br (NH), 3940 (CH), 1720br (C=O and urethane), 1660 (C=O), 1520 (amide II), 1230br (CO), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 290, 281, and 273 nm (ε 6460, 7400, and 6950), δ ($CDCl_3$) 7·35 (s, Ph), 6·9—7·3 (complex, indole), 6·73 (d, J 7, NH), 5·44 (d, J 7 Hz, NH), 3·9—4·4 [complex, C(α)H], 3·65 (s, CO_2Me), 3·30 [d, J 6 Hz, C(β)H_2 in tryptophan], and 1·26 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 65·1; H, 6·05; N, 10·0%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-L-*alanyl*-L-*tryptophanate* (2·2 g, 52%) had m.p. 126—128°, $\nu_{max.}$ 3350br (NH), 2930 (CH), 1720 (C=O), 1690 (urethane), 1650 (C=O), 1510 (amide II), 735 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 289, 282, and 273 nm (ε 4950, 5590, and 5300), δ ($CDCl_3$) 7·35 (s, Ph), 6·9—7·3 (complex, indole), 6·67 (d, J 7 Hz, NH), 5·39 (d, J 7 Hz, NH), 5·07 (s, PhC*H*$_2$·O), 3·9—4·4 [complex, C(α)H], 3·65 (s, CO_2Me), 3·30 [d, J 6 Hz, C(β)H_2 in tryptophan], and 1·29 p.p.m. (d, J 6 Hz, CH_3 in alanine) (Found: C, 65·25; H, 5·95; N, 9·85%).

Methyl N^{α}-benzyloxycarbonyl-L-phenylalanyl-L-alaninate (1·8 g, 47%) had m.p. 131—133° (lit.,[41] 130—131°), $\nu_{max.}$ 3300 (NH), 2930 (CH), 1750 (C=O), 1695 (urethane), 1655 (C=O), 1540 (amide II), 1215 (CO), 750 (Ph), and 700 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 258, 252, and 246 nm (ε 199, 311, 395, 311, and 216), δ ($CDCl_3$) 7·35 (s, *Ph*CH_2·O), 7·27 (s, Ph in phenylalanine), 5·44 (d, J 7 Hz, NH), 6·48 (d, J 7 Hz, NH), 5·12 (s, PhC*H*$_2$·O), 4·25—4·65 [complex, C(α)*H*], 3·72 (s, CO_2Me), 3·09 [d, J 7 Hz, C(β)H_2 in phenylalanine], and 1·33 p.p.m. (d, J 7 Hz, CH_3 in alanine).

Methyl N^{α}-benzyloxycarbonyl-L-phenylalanyl-D-alaninate (2·0 g, 52%) had m.p. 135—136° (lit.,[10] 145—147°), $\nu_{max.}$ 3300 (NH), 2950 (CH), 2930 (CH), 1735 (C=O), 1690 (urethane), 1655 (C=O), 1535 (amide II), 1240 (CO), 745 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 268, 264, 258, 252, and 247 nm (ε 196, 305, 388, 302, and 221), δ ($CDCl_3$) 7·35 (s, *Ph*CH_2·O), 7·28 (s, Ph in phenylalanine), 6·39 (d, J 7 Hz, NH), 5·53 (d, J 7 Hz, NH), 5·12 (s, PhC*H*$_2$·O), 4·25—4·65 [complex, C(α)H], 3·20 (s, CO_2Me), 3·09 [d, J 7 Hz, C(β)H_2 in phenylalanine], and 1·23 p.p.m. (d, J 7 Hz, CH_3 in alanine).

Methyl N^{α}-benzyloxycarbonyl-L-alanyl-L-phenylalaninate (2·0 g, 52%) had m.p. 103—104° (lit.,[10] 96—98°), $\nu_{max.}$ 3300 (NH), 2940 (CH), 1760 (C=O), 1745 (C=O), 1690 (urethane), 1660 (C=O), 1540 (amide II), 1225 (CO), 735 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 256, 251, and 247 nm (ε 202, 319, 404, 319, and 232), δ ($CDCl_3$) 7·33 (s, *Ph*CH_2·O), 7·20 (s, Ph in phenylalanine), 6·88 (d, J 7 Hz, NH), 5·68 (d, J 7 Hz, NH), 5·08 (s, PhC*H*$_2$·O), 4·2—4·4 and 4·7—5·0 [complex, C(α)H], 3·66 (s, CO_2Me), 3·24 [d, J 6 Hz, C(β)H_2 in phenylalanine], and 1·30 p.p.m. (d, J 7 Hz, CH_3 in alanine).

Methyl N^{α}-benzyloxycarbonyl-D-alanyl-L-phenylalaninate (1·6 g, 42%) had m.p. 113—114° (lit.,[10] 112°), $\nu_{max.}$ 3300 (NH), 2960 (CH), 2930 (CH), 1730 (C=O), 1690 (urethane), 1650 (C=O), 1545 (amide II), 1240 (CO), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 258, 252, and 247 nm (ε 201, 312, 387, 308, and 231), δ ($CDCl_3$) 7·33 (s, *Ph*·CH_2·O), 7·22 (s, Ph in phenylalanine), 6·81 (d, J 7 Hz, NH), 5·61 (d, J 7 Hz, NH), 5·08 (s, PhC*H*$_2$·O), 4·0—4·4 and 4·7—5·0 [complex, C(α)H], 3·68 (s, CO_2Me), 3·08 [d, J 7 Hz, C(β)H_2 in phenylalanine], and 1·27 p.p.m. (d, J 7 Hz, CH_3 in alanine).

Methyl N^{α}-benzyloxycarbonyl-L-alanyl-L-alaninate (1·3 g, 42%) had m.p. 117—118° (lit.,[42] 105·5°), $\nu_{max.}$ 3280 (NH), 2940 (CH), 1755 (C=O), 1705 (urethane), 1660 (C=O), 1550 (amide II), 1210 (CO), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 268, 264, 261, 258, 252, and 247 nm (ε 137, 209, 194, 257, 197, and 149), δ ($CDCl_3$) 7·35 (s, Ph), 6·76 (d, J 7 Hz, NH), 5·56 (d, J 7 Hz, NH), 5·12 (s, PhC*H*$_2$·O), 4·2—4·7 [complex, C(α)H], 3·73 (s, CO_2Me), and 1·38 p.p.m. (d, J 7 Hz, CH_3 in alanine).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-L-*alanyl*-D-*alaninate* (1·3 g, 42%) had m.p. 138—140°, $\nu_{max.}$ 3290 NH), 2960 (CH), 1740 (C=O), 1690 (urethane), 1645 (C=O), 1540 (amide II), 1230 (CO), and 690 (Ph) cm^{-1}, δ ($CDCl_3$) 7·34 (s, Ph), 6·7—7·0 (NH), 5·59 (d, J 7 Hz, NH), 5·13 (s, PhC*H*$_2$·O), 4·2—4·8 [complex, C(α)H] 3·72 (s, CO_2Me), and 1·21 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 58·5; H, 6·55; N, 8·9. $C_{15}H_{20}N_2O_5$ requires C, 58·45; H, 6·55; N, 9·1%).

Methyl N$^{\alpha}$-*benzyloxycarbonyl*-D-*alanyl*-L-*alaninate* (1·2 g, 39%) had m.p. 138—138·5°, $\nu_{max.}$ 3290 (NH), 2960 (CH), 1740 (C=O), 1690 (urethane), 1645 (C=O), 1540 (amide II), 1230 (CO), and 690 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 268, 264, 261, 258, 252, and 247 nm (ε 100, 155, 145, 192, 146, and 104), δ ($CDCl_3$) 7·35 (s, Ph), 413·5 (d, J 7 Hz, NH), 5·63 (d, J 7 Hz, NH), 5·13 (s, PhC*H*$_2$·O), 4·1—4·8 [complex, C(α)H], 3·71 (s, CO_2Me), and 1·21 p.p.m. (d, J 7 Hz, CH_3 in alanine) (Found: C, 58·5; H, 6·5; N, 9·1%).

Methyl N$^{\alpha}$O-*bisbenzyloxycarbonyl*-L-*tyrosyl*-L-*alaninate* (3·1 g, 57%) had m.p. 195—198°, $\nu_{max.}$ 3290 (NH), 2940 (CH), 1740 (C=O), 1690 (urethane), 1650 (C=O), 1530 (amide II), 1250br (CO), 740 (Ph), and 690 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 261, 258, and 252 nm (ε 540, 735, 721, 743, and

[41] W. Grassman, E. Wünsch, and A. Riedel, *Chem. Ber.*, 1958, **91**, 455.

[42] J. Tonida, T. Ohashi, T. Tokuda, and M. Nakajima, *Nippon Nogeikagaku Kaishi*, 1965, **39**, 378.

578), δ ($CDCl_3$) 7·42 (Ph in *O*-benzyloxycarbonyl), 7·32 (Ph in *N*-benzyloxycarbonyl), 7·0—7·3 (complex, aromatic in tyrosine), 6·44 (d, *J* 7 Hz, NH), 4·57 (d, *J* 7 Hz, NH), 5·08 and 5·27 (s, 2 × $PhCH_2$·O), 4·2—4·7 [complex, C(α)H], 3·70 (s, CO_2Me), 3·06 [d, *J* 7 Hz, C(β)H_2 in tyrosine], and 1·32 p.p.m. (d, *J* 7 Hz, CH_3 in alanine) (Found: C, 64·75; H, 5·5; N, 5·25. $C_{29}H_{30}N_2O_8$ requires C, 65·15; H, 5·65; N, 5·25%).

Methyl N$^{\alpha}$O-*bisbenzyloxycarbonyl*-L-*tyrosyl*-D-*alaninate* (3·6 g, 67%) had m.p. 161—164°, $\nu_{max.}$ 3300 (NH), 2950 (CH), 1740 (C=O), 1685 (urethane), 1655 (C=O), 1530 (amide II), 1250br (CO), 740 (Ph), and 695 (Ph) cm^{-1}, $\lambda_{max.}$ (MeOH) 267, 263, 261, 258, and 252 nm (ε 500, 690, 678, 702, and 527), δ ($CDCl_3$) 7·40 (s, Ph in *O*-benzyloxycarbonyl), 7·32 (s, Ph in *N*-benzyloxycarbonyl), 7·0—7·3 (complex, aromatic in tyrosine), 7·98 (d, *J* 7 Hz, NH), 3·68 (s, CO_2Me), 5·08 and 5·25 (s, 2 × $PhCH_2$·O), 4·3—4·8 [complex, C(α)H], 3·06 [d, *J* 7 Hz, C(β)H_2 in tyrosine], and 1·23 p.p.m. (d, *J* 6 Hz, CH_3 in alanine) (Found: C, 65·1; H, 5·6; N, 5·3%).

Determination of Racemization.—General procedure. A solution of *N*-acetyl-L-phenylalanine and methyl L-alaninate or methyl L-alaninate hydrochloride is coupled with the aid of some suitable reagent. The organic phase is then washed one or more times with dilute citric acid and dilute sodium carbonate solutions and water, and dried, so as to remove any material giving extraneous n.m.r. signals in the aliphatic region. After evaporation of the solvent, the dipeptide is dissolved in deuteriochloroform for measurement purposes. To prevent a preferential concentration or fractionation of one of the optical isomers, the oily or solid product is not crystallized.

With a racemic product, three peaks are seen in the aliphatic region of the n.m.r. spectrum, as a result of an overlap of the L,L and D,L doublets. The signals are integrated to obtain the areas of the first two (downfield) peaks (due to the L,L doublet plus one half of the D,L doublet) and the area of the third (upfield) peak (due to one-half of the D,L doublet). Twice the area of the third (upfield) peak (the total area of the D,L doublet) divided by the total area of all three peaks (the total L,L plus D,L) gives the fraction of D,L isomer in the racemate. The summation can be done several times and the results averaged for a statistical treatment.

Racemization standardization [a]

% D,L Prepared	% D,L Found	Difference
6·00	5·19	−0·81
7·02	6·25	−0·77
9·32	9·51	+0·19
10·00	9·30	−0·70
16·80	16·50	−0·30
48·40	48·30	−0·10

[a] For mixtures with less than 10% D,L-isomer, the Varian T-60 spectrometer was used, instead of the A-60, since it gave better signal-to-noise ratios.

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