Use of *NN'*-Isopropylidene Dipeptides in Peptide Synthesis

By **Paul M. Hardy** * and **David J. Samworth**, Department of Chemistry, The University, Exeter EX4 4QD

The direct condensation of dipeptides with acetone has been found generally useful for the preparation of 2-(2,2,4-trialkyl-5-oxoimidazolidin-1-yl)alkanoic acids. Peptide synthesis using these *NN'*-isopropylidene dipeptides may be conveniently carried out with dicyclohexylcarbodi-imide; such couplings are racemisation-free even in the absence of additives. Subsequent deprotection may be effected by hydrolysis under neutral conditions. If required, *NN'*-isopropylidene dipeptides may be stabilised temporarily towards hydrolysis by conversion into their *N*-benzyloxycarbonyl or *N*-nitroso-derivatives.

THE first *NN'*-alkylidene dipeptide † derivative to be fully characterised was *NN'*-isopropylidene-*S*-benzylcysteinyltyrosine ‡ (1a), prepared in 1965 for the synthesis of acetone-inactivated oxytocin.[1] Shortly afterwards the acetone-induced conversion of ampicillin into hetacillin (2) was shown by an *X*-ray study to involve the generation of an imidazolidinone ring.[2] The preparation of *NN'*-isopropylidene dipeptide salts [3] and a few esters [4] has been described subsequently, but the potential of compounds of this type in peptide synthesis has not been explored systematically. The linkage of an *NN'*-isopropylidene dipeptide (1a) to a heptapeptide by the mixed anhydride procedure is the sole coupling hitherto described, although the acylation of leucine t-butyl ester by *NN'*-cyclohexylideneglycylalanine has been examined and the alanine residue found susceptible to racemisation.[5]

In this paper we report on the preparation, stability, deprotection, and coupling of *NN'*-isopropylidene dipeptide acids and their resistance to racemisation. The low solubility of the sodium salts of *NN'*-isopropylidene dipeptides in aprotic solvents and the difficulty of preparing them from dipeptides whose *N*-terminal amino-acids have small side-chains,[3] together with our finding that all attempts to convert them into the free acids leads to concomitant hydrolysis of the imidazolidinone ring, led us initially to examine the generality of the reaction of free zwitterionic dipeptides with acetone. Of nineteen dipeptides studied, it proved possible to prepare solid *NN'*-isopropylidene derivatives directly from eleven either by stirring in the cold or under reflux with acetone. A pure product could not be obtained from valylisoleucine, and alanylalanine, alanylglycine, and valylasparagine required the presence of methanol for successful conversion. Four dipeptides, in which an *N*-terminal glycine residue was bound to alanine, phenylalanine, valine, or glycine, respectively, could not be derivatised even in the presence of polar solvents. *NN'*-Isopropylideneglycylglycine was obtained indirectly by acylating sodium *NN'*-isopropylideneglycylglycinate with benzyl chloroformate and subjecting the resulting *N*-benzyloxycarbonyl-*NN'*-isopropylidene dipeptide acid (1b) to catalytic hydrogenolysis to cleave the urethane. This route would no doubt be applicable to other compounds of this type not available directly. The ^{1}H n.m.r. spectra of the *NN'*-isopropylidene dipeptides prepared were all consistent with the imidizolidinone structure.

The *NN'*-isopropylidene dipeptides could be deprotected by heating aqueous 10% w/v solutions at 60 or 100 °C for a few hours. In some cases methanol was added to dissolve the compounds initially. With the exception of alanylglycine, of the derivatives studied those slowest to be hydrolysed all contained *N*-terminal valine. The relative resistance to cleavage of *NN'*-isopropylidenealanylglycine was unexpected in view of the rates observed with other dipeptides containing alanine and glycine. In the case of *NN'*-isopropylidene-*S*-benzylcysteinylglycine the hydrolysis was slow at 60 °C (*ca.* 60% in 24 h), but the 2,5-dioxopiperazine formation from *S*-benzylcysteinylglycine known to occur at higher temperature [6] could be circumvented by solvolysis in 20% acetic acid, complete hydrolysis at 60 °C then being achieved in 4 h. The retention of stereochemical integrity during the formation and decomposition of the imidazolidinone ring system was checked by converting phenylalanylalanine into its *NN'*-isopropylidene derivative and hydrolysing the product without recrystallisation of crude materials to preclude optical fractionation. Neither ^{1}H n.m.r. spectroscopy [7] nor paper chromatography [8] revealed the presence of any L,D-diastereoisomer in the regenerated dipeptide.

The coupling of imidazolidinone-protected dipeptides was explored with *NN'*-isopropylidenevalylglycine and phenylalanine methyl ester. As in the original experiment of du Vigneaud,[1] the mixed anhydride reaction

† For convenience 2-(2,2,4-trialkyl-5-oxoimidazolidin-1-yl)-alkanoic acids are referred to as *NN'*-alkylidene dipeptides.

‡ All amino-acids are of the L-configuration unless otherwise stated.

[1] D. Yamashiro, H. L. Aanning, and V. du Vigneaud, *Proc. Nat. Acad. Sci., U.S.A.*, 1965, **54**, 166; D. Yamashiro and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1968, **90**, 487; V. J. Hubry, D. Yamashiro, and V. du Vigneaud, *ibid.*, p. 7106.

[2] G. A. Hardcastle, jun., D. A. Johnson, C. A. Panetta, A. I. Scott, and A. S. Sutherland, *J. Org. Chem.*, 1966, **31**, 897.

[3] C. A. Panetta and M. Pesh-Iman, *J. Org. Chem.*, 1972, **37**, 302.

[4] Y. Ariyoshi and N. Sato, *Bull. Chem. Soc. Japan*, 1972, **45**, 2015.

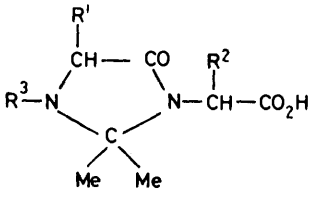
[5] F. Cardinaux and M. Brenner, 'Peptides 1971,' North-Holland Publishing Co., Amsterdam, 1973, p. 65.

[6] L. C. King and F. H. Suydam, *J. Amer. Chem. Soc.*, 1952, **74**, 5499.

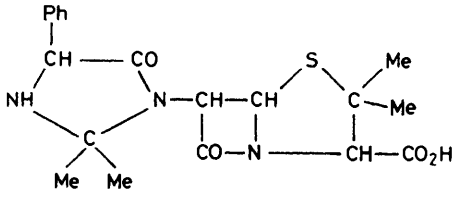
[7] B. Weinstein and A. E. Pritchard, *J.C.S. Perkin I*, 1972, 1015.

[8] T. Sokolovska and J. F. Biernat, *J. Chromatog.*, 1964, **13**, 269.

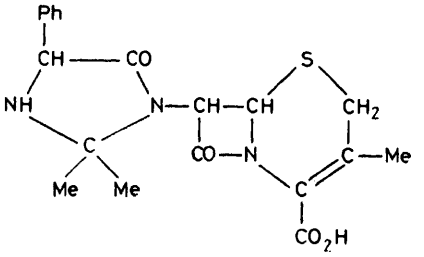
using isobutyl chloroformate gave only *ca.* 40% yield of the tripeptide; it seems that the imidazolidinyl imino-function is sufficiently nucleophilic for side reactions to occur during the coupling. Although *N*-benzyloxycarbonylalanine 2,4,5-trichlorophenyl ester could not be



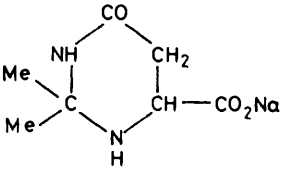
(1)a; R^1 = $CH_2{\cdot}S{\cdot}CH_2Ph$, R^2 = $CH_2{\cdot}C_6H_4{\cdot}OH$-*p*, R^3 = H
b; R^1 = R^2 = H, R^3 = $PhCH_2{\cdot}O{\cdot}CO$
c; R^1 = R^2 = H, R^3 = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO$
d; R^1 = CH_2Ph, R^2 = $CH_2{\cdot}CHMe_2$, R^3 = NO



(2)



(3)



(4)

induced to acylate *NN'*-isopropylidenevalylglycine, the more highly activated *N*-benzyloxycarbonylglycyl chloride gave a 13% yield of *N*-benzyloxycarbonylglycyl-*NN'*-isopropylideneglycylglycine (1c) on treatment with *NN'*-isopropylideneglycylglycine. As might be expected, the imidazolidinone ring of this product was resistant to hydrolysis; no decomposition occurred in 80% acetic acid at 60 °C for 24 h. The failure of an active ester to acylate the imino-nitrogen suggested the use of such derivatives of *NN'*-isopropylidene dipeptides for coupling purposes. *NN'*-Isopropylidenevalylglycine, however, gave only impure oily *p*-nitrophenyl and 2,4,5-trichlorophenyl esters, and the crystalline pentachlorophenyl ester could not be freed from contaminating pentachlorophenol, although it coupled with phenylalanine methyl ester in moderate yield. The most successful coupling procedure found was that with *NN'*-dicyclohexylcarbodi-imide; by this means *NN'*-isopropylidenevalylglycylphenylalanine was prepared in 77% yield. To demonstrate the application of *NN'*-isopropylidene dipeptides to peptide synthesis this tripeptide was converted into the heptapeptide phenylalanylalanylvalylalanylvalylglycylphenylalanine by two successive stages of deprotection and coupling with *NN'*-isopropylidene dipeptides. The intermediate imidazolidinones were not isolated, but hydrolysed directly to the peptide esters. The pentapeptide ester was characterised as the oxalate (42% yield) and the heptapeptide ester, which was only readily soluble in dimethyl sulphoxide or hexamethylphosphoramide, was obtained in 39% yield after purification by extraction with boiling methanol. 1-Hydroxysuccinimide had been added during the couplings, but since under these conditions *NN'*-isopropylidenephenylalanylglycine coupled with proline t-butyl ester in 61% yield, the rather low yields probably reflect steric hindrance in coupling to valine residues.

The possibility that partial racemisation might be occurring during coupling remained to be examined. Although oxazolone formation is precluded by the involvement of the amide nitrogen in the imidazolidinone ring, other mechanisms of racemisation can operate. The coupling of *NN'*-isopropylidenephenylalanylalanine to the relatively hindered valine methyl ester was selected as a test reaction to enable any L,D,L-diastereoisomer generated during peptide bond formation to be determined by the ^{1}H n.m.r. method of Weinstein and Pritchard.[7] Crude *N*-benzyloxycarbonylphenylalanylalanylvaline methyl ester, prepared from *N*-benzyloxycarbonylphenylalanylalanine by a *NN'*-dicyclohexylcarbodi-imide condensation, had in its ^{1}H n.m.r. spectrum two doublets for the alanine methyl protons (τ 8.72 and 8.84); integration showed the presence of 30% of the L,D,L-isomer. Two signals were also observed for the ester methyl protons. On crystallisation of this crude protected tripeptide mixture the first crop comprised virtually pure L,L,L-isomer. Experiments with mixtures of crude and purified material established that the n.m.r. technique could be used to detect down to 1% of L,D,L-diastereoisomer. Catalytic hydrogenolysis of crude protected tripeptide gave oily phenylalanylalanylvaline methyl ester, and crystallisation of the oxalate of this compound did not result in optical fractionation. *NN'*-Isopropylidenephenylalanylalanine was therefore coupled to valine methyl ester using *NN'*-dicyclohexylcarbodi-imide, the product hydrolysed without isolation, and the resulting oil crystallised as its oxalate. The ^{1}H n.m.r. spectrum showed only one doublet attributable to the alanine methyl group (τ 8.63). The product was thus

indistinguishable from optically pure L,L,L-phenylalanylalanylvaline methyl ester oxalate, establishing that less than 1% of racemisation had occurred.

Attempts to couple *NN'*-isopropylidenephenylalanylleucine, the most easily hydrolysed imidazolidinone we had prepared, with proline t-butyl ester or glycine benzyl ester gave mixtures of products. The imidazolidinone ring of hetacillin (2) has been stabilised as its *N*-nitroso-derivative during the conversion of this compound into cephalexin (3),[9] and this prompted us to examine *N*-nitrosation of our *NN'*-isopropylidene derivatives. Nitrous acid was found to convert our compounds smoothly into the pale yellow, highly crystalline *N*-nitrosoimidazolidinones in *ca.* 70% yield. *N*-Nitroso-*NN'*-isopropylidenephenylalanyl-leucine (1d) could be coupled with proline t-butyl ester in 92% yield, demonstrating the effectiveness of the modification. The protecting *N*-nitroso-group resists aqueous 80% acetic acid at 60 °C and 90% formic acid and 70% trifluoroacetic acid at 20 °C, requiring the use of 4M-hydrogen chloride in dioxan at 0 °C for its removal Disruption of the imidazolidinone ring does not occur under these conditions, the *NN'*-isopropylidene peptide hydrochloride being isolated.

The only peptides which cannot by the nature of their structure form imidazolidinone derivatives are those in which proline is adjacent to the *N*-terminal residue (an *NN'*-isopropylidene derivative has been prepared from L-prolyl-L-leucylglycinamide [1] and there is thus no problem if proline is *N*-terminal). Although in principle glutamine and asparagine could react with acetone to form cyclic structures, no reaction could be induced in the case of the former although a tetrahydropyrimidine derivative (4) was isolated from the latter. However, use of the sodium salt was necessary for reaction to occur, and it seems unlikely that this type of condensation would compete with imidazolidinone formation from asparaginyl-peptides or -amino-acids under our normal conditions for derivatisation (*i.e.* in the absence of base).

[9] W. J. Gottstein, P. F. Misco, and L. C. Cheney, *J. Org. Chem.*, 1972, **37**, 2765.

The foregoing experiments demonstrate the availability of *NN'*-isopropylidene dipeptides and their use in peptide synthesis. In contrast to other *N*-α-protected dipeptides containing chiral *C*-terminal amino-acids, coupling with dicyclohexylcarbodi-imide in the absence of other additives proceeds without racemisation. However, the most useful feature is their deprotection by hydrolysis under mild neutral conditions. Since they are relatively water-soluble, this suggests their use in semisynthesis,[10] *i.e.* partial synthesis of peptides from smaller peptides of biological origin. Protection against hydrolysis may be obtained if required by *N*-nitrosation or *N*-benzyloxycarbonylation; these groups may be removed under conditions which leave the imidazolidinyl ring intact.

EXPERIMENTAL

Optical rotations were measured with a Bendix-NPL 143 polarimeter. N.m.r. spectra were recorded at 33.5 °C with a Perkin-Elmer R10 60 MHz spectrometer or at 30 °C with a JEOL JMH 100 MHz instrument. Organic solutions were dried over magnesium sulphate and evaporated at 10—20 mmHg with a rotary evaporator. Microanalyses were carried out with a Carlo Erba 1102 elemental analyser.

TABLE 1

Free dipeptides

Compound	Cryst. solvent	M.p. (°C)	$[\alpha]_D$ (°)	Analysis
Z-Leu-Ser(OBu^t)-OMe	Ether–petroleum	85.5—86.5	+13.0 (22 °C) (*c* 2 in AcOH)	Found: C, 62.5; H, 8.2; N, 6.3. $C_{22}H_{34}N_2O_6$ requires C, 62.5; H, 8.1; N, 6.6%
Z-Leu-Ser(OBu^t)-OH	Ethyl acetate–petroleum	106—108	+5.6 (22 °C) (*c* 2 in MeOH)	Found: C, 61.7; H, 8.0; N, 6.9. $C_{21}H_{32}N_2O_6$ requires C, 61.7; H, 7.9; N, 6.9%
H-Leu-Ser(OBu^t)-OH	Methanol–ether		+30.0 (23 °C) (*c* 2 in H_2O)	Found: C, 55.1; H, 9.3; N, 9.9. $C_{13}H_{26}N_2O_4$, $0.5H_2O$ requires C, 55.1; H, 9.3; N, 9.9%
Z-Val-Ile-OH	Ethyl acetate–petroleum	158—159	−12.7 (26 °C) (*c* 2 in MeOH)	Found: C, 62.8; H, 7.6; N, 7.4. $C_{19}H_{28}N_2O_5$ requires C, 62.6; H, 7.7; N, 7.7%
H-Val-Ile-OH	Methanol–ether		+34.0 (26 °C) (*c* 1 in M-HCl)	Found: C, 56.3; H, 9.5; N, 11.9. $C_{11}H_{22}N_2O_3$,$0.25H_2O$ requires C, 56.3; H, 9.7; N, 12.0%
Z-Val-Asn-OH	Methanol–ether	176—178	+9.0 (23 °C) (*c* 2 in AcOH)	Found: C, 55.7; H, 6.4; N, 11.3. $C_{17}H_{23}N_3O_6$ requires C, 55.9; H, 6.35; N, 11.5%
H-Val-Asn-OH	Aqueous ethanol		+19.0 (21 °C) (*c* 2 in H_2O)	Found: C, 46.6; H, 7.5; N, 17.8. $C_9H_{17}N_3O_4$ requires C, 46.7; H, 7.4; N, 18.2%

Free Dipeptides.—These were prepared from *N*-benzyloxycarbonyl derivatives by hydrogenolysis in methanol over 5% palladised charcoal. Table 1 lists the properties of dipeptides and their precursors not hitherto described. The dipeptides used in synthesis were chromatographically homogeneous and had elemental analyses and optical rotations in accord with reported values.

NN'-*Isopropylidene Dipeptides* (*Table* 2).—*Method A.* The free dipeptide was heated under reflux in acetone (100 ml per g). *Method B.* The dipeptide was stirred in acetone (100 ml per g) at 20 °C. *Method C.* The dipeptide was heated under reflux in methanol–acetone (9 : 1 v/v; 100 ml per g). *Method D.* The dipeptide was heated under reflux in methanol–acetone (15 : 1 v/v; 300 ml per g). *Method E.* The dipeptide was stirred at 20 °C in methanol–acetone (9 : 1 v/v; 200 ml per g). In each case any un-

[10] See, for example, the review by R. E. Offord in 'Peptides 1972, Proceedings of the Twelfth European Peptide Symposium,' ed. H. Hanson and H-D. Jakubke, North-Holland Publishing Co., Amsterdam and London, 1973, p. 52.

dissolved solid was filtered off, the filtrate evaporated, and the residue recrystallised.

NN'-*Isopropylideneglycylglycine*.— *NN'*-Isopropylideneglycylglycine sodium salt [3] (874 mg, 4.5 mmol) in water (10 ml) was treated with benzyl chloroformate at pH 9.5. Work-up gave N-*benzyloxycarbonyl*-NN'-*isopropylideneglycylglycine* as a gum (1.23 g, 4.0 mmol, 89%); τ (60 MHz; $CDCl_3$) 0.97 (1 H, s, D_2O-exchangeable, CO_2H), 2.66 (5 H, s, Ph), 4.87 (2 H, s, ZCH_2), 5.95 (2 H, s, Gly-CH_2), 6.06 (2 H, s, Gly-CH_2), and 8.38 (6 H, s, CH_3). Dicyclohexylamine (0.33 g, 2 mmol) was added to this material (0.50 g, 1.63 mmol) in diethyl ether (10 ml) and the mixture then saturated with light petroleum. The precipitate was filtered off and recrystallised from light petroleum to give N-*benzyloxycarbonyl*-NN'-*isopropylideneglycylglycine dicyclohexylammonium salt* (470 mg, 0.96 mmol, 59%), m.p. 155.5—156.5 °C (Found: C, 66.6; H, 8.5; N, 8.2. $C_{27}H_{41}N_3O_5$ requires C, 66.5; H, 8.5; N, 8.6%). The bulk of the *N*-benzyloxycarbonyl-*NN'*-isopropylideneglycylglycine (1.2 g, 3.9 mmol) was hydrogenolysed over palladised charcoal (5%; 0.3 g). After 2 h filtration, evaporation, and recrystallisation of the residue from methanol–diethyl ether afforded NN'-*isopropylideneglycylglycine* (540 mg, 3.14 mmol, 90%), m.p. 140 °C (decomp.) (Found: C, 48.7; H, 7.1; N, 16.4. $C_7H_{12}N_2O_3$ requires C, 48.8; H, 7.0; N, 16.3%).

Hydrolysis of NN'-*Isopropylidene Dipeptides*.—The hydrolysis of *NN'*-isopropylidene dipeptides [10% solutions (w/v) in water] was monitored by t.l.c. in ethyl acetate–pyridine–acetic acid–water (100 : 44 : 12 : 12 v/v). When hydrolysis was complete (Table 3), the solution was evaporated and the n.m.r. spectrum and optical rotation were recorded. In every case the results were indistinguishable from those obtained for the original free dipeptides.

A suspension of *NN'*-isopropylidenephenylalanylalanine (500 mg) in water (50 ml) was heated under reflux for 12 h and then kept at 4 °C for 24 h. The crystalline precipitate was filtered off and dried *in vacuo* to give *cyclo*-alanylphenylalanine (38 mg), m.p. 270—271 °C (lit.,[11] 290—291 °C) (Found: C, 66.0; H, 6.5; N, 12.8. Calc. for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.5; N, 12.8%). The filtrate was evaporated and dried *in vacuo* to give phenylalanylalanine, $[\alpha]_D$[19]

TABLE 2

NN'-Isopropylidene dipeptides

NN'-Isopropylidene dipeptide	Method and reaction time (h)	% Yield	Cryst. solvent	M.p. (°C)	$[\alpha]_D$ (°) in MeOH	Analysis
Ala-Ala	C, 4	67	Methanol–ether	140	−13.5 (26 °C; *c* 2.0)	Found: C, 54.1; H, 8.2; N, 13.9. $C_9H_{16}N_2O_3$ requires C, 54.0; H, 8.1; N, 14.0%
Ala-Gly	D, 24	67	Methanol–ether	140	+11.0 (26 °C; *c* 2.0)	Found: C, 51.2; H, 7.7; N, 14.8. $C_8H_{14}N_2O_3$ requires C, 51.6; H, 7.6; N, 15.0%
Ala-Phe	A, 24	80	Acetone	130	−150.0 (26 °C; *c* 2.0)	Found: C, 65.1; H, 7.4; N, 10.3. $C_{15}H_{20}N_2O_3$ requires C, 65.2; H, 7.3; N, 10.1%
Cys(Bzl)-Gly	A, 4	51	Acetone–petroleum	55—57	+14.5 (25 °C; *c* 1.3)	Found: C, 57.2; H, 6.7; N, 8.8. $C_{15}H_{20}N_2O_3S,0.5H_2O$ requires C, 56.8; H, 6.7; N, 8.8%
Leu-Ser(OBu^t)	B, 24	70	Acetone	220	−76.2 (25 °C; *c* 2.0)	Found: C, 61.0; H, 9.8; N, 8.8. $C_{16}H_{30}N_2O_4$ requires C, 61.1; H, 9.6; N, 8.9%
Phe-Ala	A, 23	78	Acetone–petroleum	150	−104.0 (21 °C; *c* 2.0)	Found: C, 65.4; H, 7.4; N, 10.1. $C_{15}H_{20}N_2O_3$ requires C, 65.2; H, 7.3; N, 10.1%
Phe-Gly	A, 1½	85	Acetone–petroleum	118—120	−57.5 (27 °C; *c* 2.0)	Found: C, 64.5; H, 7.7; N, 9.5. $C_{14}H_{18}N_2O_3,0.5Me_2CO$ requires C, 63.9; H, 7.3; N, 9.6%
Phe-Leu	B, 48	83	Acetone	200	−51.6 (21 °C; *c* 3.1)	Found: C, 67.8; H, 8.5; N, 8.4. $C_{18}H_{26}N_2O_3$ requires C, 67.9; H, 8.2; N, 8.8%
Trp-Gly	A, 2	78	Acetone–petroleum	90	−98.0 (22 °C; *c* 2.0)	Subject to atmospheric oxidation
Val-Ala	A, 48	81	Acetone–petroleum	140	−41.2 (25 °C; *c* 2.0)	Found: C, 57.7; H, 9.0; N, 12.4. $C_{11}H_{20}N_2O_3$ requires C, 57.9; H, 8.8; N, 12.3%
Val-Asn	E, 18	50	Methanol–petroleum	60	−95.0 (25 °C; *c* 1.0)	Found: C, 50.0; H, 7.7; N, 14.6. $C_{13}H_{21}N_3O_4,H_2O$ requires C, 49.8; H, 8.0; N, 14.5%
Val-Gly	A, 24	87	Acetone–petroleum	140	−13.5 (24 °C; *c* 2.0)	Found: C, 52.2; H, 8.6; N, 13.0. $C_{10}H_{18}N_2O_3$ requires C, 56.1; H, 8.5; N, 13.1%
Val-Tyr	A, 2	82	Acetone–petroleum	140	−167.5 (22 °C; *c* 2.1)	Found: C, 60.3; H, 81.; N, 8.0. $C_{17}H_{24}N_2O_4,H_2O$ requires C, 60.3; H, 7.7; N, 8.3%
Leu-Gly	A, 2	62	Acetone–petroleum	130	−17.2 (26 °C; *c* 2.0)	Found: C, 57.7; H, 9.2; N, 12.3. $C_{11}H_{20}N_2O_3$ requires C, 57.9; H, 8.8; N, 12.3%

[11] D. E. Nitecki, B. Halpern, and J. W. Westley, *J. Org. Chem.*, 1968, **33**, 864.

+13.2° (*c* 2.0 in H_2O), τ (100 MHz; D_2O) 8.62 (3 H, d, *J* 7.5 Hz, CH_3). No second Ala CH_3 doublet due to the L,D-diastereoisomer was visible.

NN′-*Isopropylidenevalylglycylphenylalanine Methyl Ester.*—A solution of *NN′*-isopropylidenevalylglycine (529 mg, 2.47 mmol) and phenylalanine methyl ester hydrochloride (523 mg, 2.47 mmol) in dichloromethane (10 ml) containing triethylamine (0.34 ml, 2.47 mmol) was cooled to 0 °C and a solution of *NN′*-dicyclohexylcarbodi-imide (509 mg, 2.47 mmol) in dichloromethane (10 ml) added. The mixture was kept at 0 °C for 30 min and then overnight at 20 °C. The precipitated *NN′*-dicyclohexylurea was filtered off and the filtrate washed in succession with water (10 ml), M-sodium hydrogen carbonate (10 ml), water (10 ml), and brine (10 ml), dried, and evaporated. The residue was taken up in a little acetone and the solution kept at −10 °C for 12 h; it was then filtered and evaporated. Crystallisation of the residual oil from acetone–light petroleum gave NN′-*isopropylidenevalylglycylphenylalanine methyl ester* (719 mg, 77%), m.p. 97—98 °C, $[\alpha]_D^{21}$ +2.4° (*c* 2.1 in acetone). The same compound was obtained by using isobutyl chloroformate in tetrahydrofuran to effect a mixed anhydride coupling (44%); m.p. 97—98 °C, $[\alpha]_D^{20}$ +2.0° (*c* 2.1 in acetone) (Found: C, 64.3; H, 8.2; N, 11.2. $C_{20}H_{29}N_3O_4$ requires C, 64.0; H, 7.8; N, 11.2%).

TABLE 3

NN′-Isopropylidene dipeptide	Time (h) for complete hydrolysis 60 °C	100 °C
Phe-Leu	1.5	
Leu-Ser	1.5	
Ala-Ala	6.0	
Ala-Phe	6.0	
Phe-Ala	16	1.5
Gly-Gly	18	1.5
Phe-Gly		1.5
Leu-Gly		3.0
Val-Ala		3.0
Val-Gly		3.5
Val-Tyr		4.0
Val-Asn		4.0
Ala-Gly		4.0

Valylglycylphenylalanine Methyl Ester Hydrochloride.—*Method* (i). A solution of *NN′*-isopropylidenevalylglycylphenylalanine methyl ester (508 mg, 1.35 mmol) in 50% aqueous methanol (50 ml) was heated at 60 °C for 24 h, cooled, and evaporated. The residue was dissolved in methanol (5 ml), ethereal 3M-hydrogen chloride (5 ml) was added, and the solution was kept at 4 °C for 24 h. Filtration and recrystallisation from methanol–ether gave the pure *tripeptide ester hydrochloride* (424 mg, 84%), m.p. 194—195 °C, $[\alpha]_D^{22}$ +45.4° (*c* 1.94 in Me_2NCHO).

Method (ii). *N*-Benzyloxycarbonylvalylglycylphenylalanine methyl ester {2.0 g, 42.5 mmol; m.p. 153—154 °C; $[\alpha]_D^{24}$ −6.5° (*c* 2.0 in MeOH); prepared from *N*-benzyloxycarbonylvalylglycine} in methanol (100 ml) was hydrogenolysed for 1 h over palladised charcoal (5%; 0.5 g). The solution was filtered and evaporated. The residual oily tripeptide ester was converted as above into the *tripeptide ester hydrochloride* (557 mg, 75%), m.p. 194—195 °C, $[\alpha]_D^{21}$ +44.9° (*c* 1.9 in Me_2NCHO) (Found: C, 54.9; H, 7.4; N, 11.1. $C_{17}H_{26}ClN_3O_4$ requires C, 54.9; H, 7.1; N, 11.3%).

Phenylalanylalanylvalylalanylvalylglycylphenylalanine Methyl Ester.—*NN′*-Isopropylidenevalylalanine (1.126 g, 5.51 mmol) was added to a solution of valylglycylphenylalanine methyl ester (1.845 g, 5.50 mmol) and 1-hydroxysuccinimide (685 mg, 5.51 mmol) in tetrahydrofuran (40 ml), then the mixture was cooled to −10 °C before addition of *NN′*-dicyclohexylcarbodi-imide (1.136 g, 5.50 mmol) in tetrahydrofuran (10 ml). The mixture was kept at −10 °C for 2 h and then at 20 °C overnight. Dicyclohexylurea was filtered off, the filtrate evaporated, and the residue in chloroform (50 ml) washed successively with M-sodium hydrogen carbonate (50 ml), water (50 ml), and brine (50 ml) before being dried and evaporated. A solution of the residual pentapeptide derivative in 50% aqueous methanol (50 ml) was kept at 60 °C for 4 h and then evaporated. The crude product was distributed between chloroform (70 ml) and 2M-hydrochloric acid (50 ml). The organic phase was washed again with 2M-hydrochloric acid (50 ml). The combined aqueous extracts were basified to pH with aqueous 10% ammonia and then extracted with chloroform (4 × 50 ml). The combined chloroform solutions were washed with water (50 ml) and brine (50 ml), and evaporated to give oily *valylalanylvalylglycylphenylalanine methyl ester* (1.36 g, 2.69 mmol). A solution of anhydrous oxalic acid (250 mg, 2.70 mmol) in methanol (5 ml) was added to a solution of the pentapeptide ester in methanol (25 ml) and the mixture saturated with diethyl ether. The precipitated product, recrystallised from methanol–diethyl ether, gave pure *valylalanylvalylglycylphenylalanine methyl ester oxalate* (1.39 g, 42%), m.p. 190 °C (decomp.), $[\alpha]_D^{20}$ +17° (*c* 1.1 in Me_2NCHO); amino-acid ratios Ala, 1.00; Gly, 1.00; Phe, 0.96; Val, 2.12 (Found: C, 53.3; H, 7.2; N, 11.6. $C_{27}H_{41}N_5O_{10},0.5H_2O$ requires C, 53.6; H, 7.0; N, 11.6%). To a solution of this methyl ester oxalate (367 mg, 0.59 mmol) and 1-hydroxysuccinimide (70.3 mg, 0.60 mmol) in dimethylformamide (5 ml) containing triethylamine (0.083 ml, 0.59 mmol) was added *NN′*-isopropylidenephenylalanylalanine (169 mg, 0.61 mmol). The mixture was cooled to −5 °C and a solution of *NN′*-dicyclohexylcarbodi-imide (125 mg, 0.60 mmol) in dimethylformamide (6 ml) added; the mixture was kept at −5 °C for 1 h and then at 20 °C overnight. The precipitated solid was filtered off, water (15 ml) was added to the filtrate, and the solution was heated at 60 °C for 3 h. Evaporation gave a gelatinous mass which was stirred under boiling methanol (20 ml) for 2 h. Filtration gave, after crystallisation from aqueous dimethyl sulphoxide, *phenylalanylalanylvalylalanylvalylglycylphenylalanine methyl ester* (170 mg, 39%), m.p. 240 °C (decomp.), $[\alpha]_D^{22}$ −15.2° (*c* 0.92 in Me_2SO); amino-acid ratios: Ala, 2.12; Gly, 0.95; Phe, 1.93; Val, 2.00 (Found: C, 58.2; H, 7.3; N, 12.2. $C_{37}H_{53}N_7O_8,C_2H_6SO$ requires C, 58.5; H, 7.3; N, 12.2%).

Racemisation Test.—*Phenylalanylalanylvaline methyl ester oxalate.* A solution of *N*-benzyloxycarbonylphenylalanylalanine (1.85 g, 5.0 mmol), valine methyl ester hydrochloride (838 mg, 5.0 mmol), and triethylamine (0.70 ml, 5.0 mmol) in dichloromethane (30 ml) was cooled to −10 °C and a solution of *NN′*-dicyclohexylcarbodi-imide (1.03 g, 5.0 mmol) in dichloromethane (10 ml) added; the mixture was kept at −10 °C for 1 h and then at 20 °C for 6 h before filtration. Work-up of the filtrate and evaporation gave N-*benzyloxycarbonylphenylalanylalanylvaline methyl ester* (1.77 g, 73%), τ (60 MHz; $CDCl_3$) 6.33 (s, CO_2Me-L,L,L), 6.40 (s, CO_2Me-L,D,L), 8.72 (d, Ala CH_3-L,L,L), 8.84 (d, Ala CH_3-L,D,L). Integration of doublets at τ 8.72 and 8.84 indicated 30% of L,D,L-diastereoisomer. Crystallisation from ethyl acetate–light petroleum gave the almost

pure L,L,L-diastereoisomer, m.p. 162—164 °C (Found: C, 64.7; H, 7.1; N, 8.8. $C_{26}H_{33}N_3O_6$ requires C, 64.6; H, 6.9; N, 8.7%); τ (100 MHz; $CDCl_3$) 8.69 (d, Ala CH_3-L,L,L), and 8.85 (d, Ala CH_3-L,D,L; only just visible, *i.e.* <1% impurity). *N*-Benzyloxycarbonylphenylalanylalanylvaline methyl ester (1.10 g, 2.27 mmol; crude material) was dissolved in methanol (50 ml) and hydrogenolysed over palladised charcoal (5%; 0.5 g) for 3 h. Filtration and evaporation yielded *phenylalanylalanylvaline methyl ester* (0.80 g, 100%), τ (100 MHz; $CDCl_3$) 6.25 (s, CO_2Me-L,L,L), 6.30 (s, CO_2Me-L,D,L), 8.62 (d, *J* 7.0 Hz, Ala CH_3-L,L,L), and 8.81 (d, *J* 7.0 Hz, Ala CH_3-L,D,L), integration indicating the presence of *ca.* 30% L,D,L-diastereoisomer. To this material (623 mg, 1.78 mmol) in diethyl ether (20 ml) was added a solution of oxalic acid (161 mg, 1.79 mmol) in diethyl ether (20 ml). The mixture was stirred for 10 min and evaporated. Crystallisation of the gelatinous residue from methanol–diethyl ether gave *phenylalanylalanylvaline methyl ester oxalate* (520 mg, 66%), m.p. 156—158 °C (Found: C, 53.7; H, 7.0; N, 9.3. $C_{20}H_{29}N_3O_8,0.5H_2O$ requires C, 53.6; H, 6.7; N, 9.4%). Integration of the n.m.r. doublets at τ 8.73 and 8.86 [100 MHz; $(CD_3)_2SO$] indicated the presence of 30% L,D,L-diastereoisomer; optical fractionation had not taken place on recrystallisation. A solution of *NN'*-isopropylidenephenylalanylalanine (546 mg, 1.96 mmol) and valine methyl ester hydrochloride (331 mg, 1.97 mmol) in dichloromethane (20 ml) was cooled to −10 °C before addition of a solution of *NN'*-dicyclohexylcarbodi-imide (404 mg, 1.96 mmol) in dichloromethane (10 ml). The mixture was kept at −10 °C for 1 h and then overnight at 20 °C. The precipitate was filtered off, the filtrate evaporated, and the residue in chloroform (10 ml) washed in succession with water (10 ml), M-sodium hydrogen carbonate (10 ml), and water (10 ml), then dried and evaporated. The residual oil in methanol (20 ml) was diluted with water (10 ml) and the mixture kept at 60 °C for 3 h and then evaporated. The residue was shaken with chloroform (10 ml) and 2M-hydrochloric acid (20 ml), the phases were separated, and the organic layer was extracted further with 2M-hydrochloric acid (20 ml). The combined aqueous extracts were basified to pH 10 with aqueous 12% ammonia and immediately extracted with chloroform (2 × 20 ml). The combined chloroform extracts were washed with water (10 ml), dried, and evaporated to give oily *phenylalanylalanylvaline methyl ester* (444 mg, 67%), τ (100 MHz; $CDCl_3$) 8.63 (3 H, d, Ala CH_3-L,L,L). There was no signal at τ 8.8, indicating the absence of the L,D,L-diastereoisomer. To a portion of this material (310 mg, 0.89 mmol) in diethyl ether (10 ml) was added oxalic acid (82 mg, 0.89 mmol) in diethyl ether (10 ml) and after stirring for 10 min the mixture was evaporated. Crystallisation of the gelatinous residue from methanol–diethyl ether gave *phenylalanylalanylvaline methyl ester oxalate* (356 mg, 91%), m.p. 170—172 °C, $[\alpha]_D^{20}$ −26.0° (*c* 1.0 in MeOH) (Found: C, 52.5; H, 6.6; N, 9.0. $C_{20}H_{29}N_3O_8,H_2O$ requires C, 52.5; H, 6.8; N, 9.2%), τ [100 MHz; $(CD_3)_2SO$] 6.36 (3 H, s, CO_2Me), 8.76 (3 H, d, *J* 7.0 Hz, Ala CH_3-L,L,L), and 9.11 (6 H, d, *J* 6.0 Hz, Val CH_3). No signal corresponding to Ala CH_3-L,D,L was observable.

N-*Benzyloxycarbonylglycyl*-NN'-*isopropylideneglycylglycine*.—A solution of *N*-benzyloxycarbonylglycyl chloride [12] (2.27 g, 10 mmol) in dioxan (15 ml) was added dropwise over 15 min to a solution of *NN'*-isopropylideneglycylglycine sodium salt (1.94 g, 10 mmol) in water (35 ml) at 5 °C. The pH of the solution was held at 9 by addition of M-sodium hydroxide; when the addition of alkali became slow the excess of acid chloride was removed by extraction with diethyl ether (60 ml) and the pH of the aqueous solution adjusted to 2 with 2M-hydrochloric acid. This acidfied aqueous solution was extracted with ethyl acetate (1 × 100 ml and 1 × 50 ml), and the organic extracts were combined and washed with water (50 ml) and brine (50 ml), dried, concentrated to *ca.* 50 ml, and kept at 20 °C for 24 h. The crystalline precipitate was filtered off and dried *in vacuo* to give N-*benzyloxycarbonylglycyl*-NN'-*isopropylideneglycylglycine* (490 mg, 13.5%), m.p. 200—220 °C (Found: C, 55.6; H, 5.8; N, 11.4. $C_{17}H_{21}N_3O_6,0.25H_2O$ requires C, 55.5; H, 5.9; N, 11.4%). A solution of this compound (100 mg) in 80% acetic acid (10 ml) was kept at 60 °C for 24 h. T.l.c. indicated that no decomposition had occurred.

N-*Nitroso*-NN'-*isopropylidene dipeptides*.—*General procedure*. 6M-Hydrochloric acid (1.0 ml) was added dropwise over 5 min to a stirred mixture of the *NN'*-isopropylidene dipeptide (1 mmol), sodium nitrite (210 mg, 3 mmol), water (5 ml), and diethyl ether (5 ml) at 20 °C; stirring was continued for a further 5 min and the two phases were then

TABLE 4

N-Nitroso-derivatives

N-Nitroso-*NN'*-isopropylidene dipeptide	Cryst. solvent	% Yield	M.p. (°C)	$[\alpha]_D$ (°)	Analysis
Ala-Phe	Ethyl acetate–petroleum	67	177—178	−147 (26 °C) (*c* 1.8 in MeOH)	Found: C, 59.0; H, 6.2; N, 13.6. $C_{15}H_{19}N_3O_4$ requires C, 59.0; H, 6.3; N, 13.8%
Leu-Ser(OBu^t)	Ethyl acetate–petroleum	73	142—143	+7.2 (21 °C) (*c* 1.95 in MeOH)	Found: C, 56.0; H, 8.7; N, 12.4. $C_{16}H_{29}N_3O_5$ requires C, 56.0; H, 8.5; N, 12.2%
Phe-Gly	Ethyl acetate–petroleum	70	154—155	+18.6 (26 °C) (*c* 1.4 in MeOH)	Found: C, 57.7; H, 5.9; N, 14.5. $C_{14}H_{17}N_3O_4$ requires C, 57.7; H, 5.9; N, 14.4%
Phe-Leu	Ethyl acetate–petroleum	72	162—163	+13.2 (19.5 °C) (*c* 1.6 in MeOH)	Found: C, 62.2; H, 7.2; N, 12.2. $C_{18}H_{25}N_3O_4$ requires C, 62.2; H, 7.25; N, 12.1%
Val-Ala	Ether–petroleum	63	144—145	+48.5 (26 °C) (*c* 1.3 in MeOH)	Found: C, 51.0; H, 7.4; N, 16.2. $C_{11}H_{19}N_3O_4$ requires C, 51.35; H, 7.4; N, 16.3%

[12] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.

separated. The aqueous phase was extracted with diethyl ether (5 ml) and the organic phases were combined, washed with water (2 × 5 ml), and brine (5 ml), dried, and evaporated to leave the pale yellow *N*-nitroso-derivative (Table 4).

Removal of the N-*Nitroso Group*.—*N*-Nitroso-*NN'*-isopropylidenealanylphenylalanine (250 mg, 0.82 mmol) was added to ice-cold 4M-hydrogen chloride in dioxan. After 10 min the mixture was diluted with light petroleum, and the precipitated solid filtered off and dried *in vacuo* (NaOH). Recrystallisation from methanol–light petroleum gave NN'-*isopropylidenealanylphenylalanine hydrochloride* (230 mg, 90%), m.p. 163—164 °C, $[\alpha]_D^{28}$ −155° (*c* 1.08 in MeOH) (Found: C, 57.3; H, 6.6; N, 8.7. $C_{15}H_{21}ClN_2O_3$ requires C, 57.6; H, 6.8; N, 9.0%).

N-*Nitroso*-NN'-*isopropylidenephenylalanyl-leucylproline t-Butyl Ester*.—L-Proline t-butyl ester (0.70 ml, 4.0 mmol) was added to a solution of *N*-nitroso-*NN'*-isopropylidenephenylalanyl-leucine (1.39 g, 4.0 mmol) and 1-hydroxysuccinimide (461 mg, 4.0 mmol) in tetrahydrofuran (20 ml) and the mixture cooled to −10 °C before addition of *NN'*-dicyclohexylcarbodi-imide (825 mg, 4.0 mmol) in tetrahydrofuran (10 ml). The mixture was kept at −10 °C for 1 h and then at 20 °C overnight before filtration. The filtrate was evaporated, and the residual foam taken up in ethyl acetate (50 ml). This solution was washed successively with 2M-hydrochloric acid (2 × 30 ml), water (30 ml), M-sodium hydrogen carbonate (2 × 30 ml), water (30 ml), and brine (30 ml), dried, and evaporated. The residual oil in a little acetone was kept at 4 °C for 12 h; the solution was filtered and evaporated. Crystallisation of the residue from diethyl ether–light petroleum at −60 °C gave N-*nitroso*-NN'-*isopropylidenephenylalanyl-leucylproline t-butyl ester* (1.85 g, 92%), which turned to a foam at 55 °C without melting; $[\alpha]_D^{20.5}$ +20.6° (*c* 1.2 in MeOH) (Found: N, 11.2%; *m/e*, 500. $C_{27}H_{40}N_4O_5$ requires N, 11.2%; *M*, 500).

Reaction of L-*Asparagine with Acetone*.—L-Asparagine monohydrate (3.0 g, 20 mmol) was dissolved in M-sodium hydroxide (20 ml) and the solution evaporated to dryness under reduced pressure. The residue was refluxed for 4 h in methanol (45 ml) and acetone (5.0 ml); the solution was filtered and the filtrate evaporated. The residual glass was crystallised from methanol–diethyl ether to yield, after drying *in vacuo*, *sodium* 6-*hydroxy*-2,2-*dimethyltetrahydropyrimidine*-4-*carboxylate* (3.5 g, 18 mmol, 90%), $[\alpha]_D^{21}$ −100° (*c* 1.0 in MeOH) (Found: C, 38.7; H, 5.8; N, 12.8. $C_7H_{11}O_3N_2Na,1.25H_2O$ requires C, 38.8; H, 6.3; N, 12.9%); τ (60 MHz; D_2O) 6.30 (1 H, m, CH), 6.60 (2 H, m, CH_2), 8.55 (3 H, s, CH_2), and 8.62 (3 H, s, CH_3); $\nu_{max.}$ (KBr) 1 640, 1 590, 1 390, and 1 170 cm^{-1}.

We thank the S.R.C. for a research studentship (to D. J. S.) and Professor H. N. Rydon for his interest and discussions.

[7/489 *Received*, 21*st March*, 1977]