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PEPTIDES—XIX*

THE ISOMERIZATION OF SOME OXAZOLONES DERIVED FROM TRIPEPTIDES

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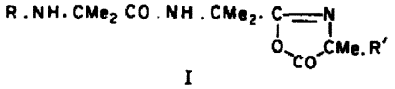
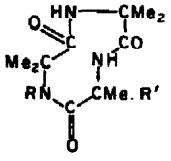
Abstract—The oxazolone (I) of α-methylalanyl-α-methylalanyl-α-methylalanine isomerizes in ethyl acetate solution at room temperature to an amidine (IV), which cyclizes with great ease to the imidazolone (V). Conclusive evidence for the amidine structure (IV), in preference to a previously suggested cyclol structure (III), is provided by ^{14}C-labelling.

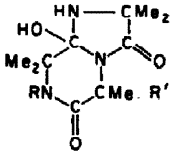
IN PART XVII of this series we described[1] the preparation of the tripeptide oxazolone (I) by hydrogenolysis of its benzyloxycarbonyl derivative (I, R = $C_6H_5 \cdot CH_2 \cdot O \cdot CO$). Like the analogous oxazolones derived from the di-, tetra-, penta-, and hexa-α-methylalanine peptides,[1] the tripeptide oxazolone proved to be a very reactive compound and polymerized rapidly in the solid state.[1] In contrast to the other members of this series, however, solutions of the tripeptide oxazolone deposited not polymer but a crystalline, monomeric condensation product (A) in high yield.[2] After removal of traces of polymer by recrystallization, A did not melt below 300°, but gradually yielded, by loss of one mole of water, a sublimate (B) m.p. 255°. Dehydration of A could also be effected under much milder conditions, e.g. by the action of acetic anhydride at room temperature, and even prolonged storage of A led to complete decomposition into B.



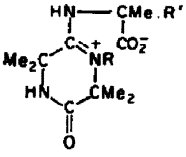
I



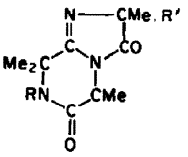
II



III



IV



V

(R = H and R′ = Me unless stated otherwise in the text)

A priori, four possible structures could be considered for the isomerization product A:

(i) *The cyclic tripeptide* (II). This would result from the normal intramolecular reaction between the terminal amino group and the oxazolone carbonyl, and molecular models indicate that the two reacting groups could easily come within bonding

* Part XVIII, *J. Chem. Soc.* in press.

[1] D. S. Jones, G. W. Kenner, J. Preston and R. C. Sheppard, *J. Chem. Soc.* in press.

[2] For preliminary accounts of part of this work see [a] D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Experientia* **19**, 126 (1963); [b] D. S. Jones, G. W. Kenner, J. Preston and R. C. Sheppard, *Proc. 6th European Peptide Symposium*, Athens, 1963, Pergamon Press, Oxford, in press.

distance without strain. Cyclic tripeptide structures for condensation products of activated tripeptide derivatives have been proposed many times in the past,[3] but in several cases, re-examination has led to the substitution of cyclic hexapeptide structures.[4] Apart from the question of the existence of the rather strained cyclic tripeptide system in general, there can be no doubt that such a structure composed exclusively of α-methylalanyl residues would be sterically impossible if the planarity of the amide groups were retained. Molecular models of cyclic tripeptides with planar *cis* amide groups show that each of the three α-carbon atoms has one substituent directed towards the centre of the ring, and serious non-bonding interactions occur if any one of these substituents is other than a hydrogen atom. One consequence of this is that a cyclic tripeptide structure is possible only if composed of residues of glycine or optically active α-amino-acids of one configuration.[5] For αα-dialkyl amino-acids, the cyclic tripeptide structure is extremely improbable since three alkyl groups would then be crowded in the centre of the macrocycle.

(ii) *The analogous cyclic hexapeptide.* As mentioned above, many examples are known of the dimerization of activated tripeptide derivatives leading to cyclic hexapeptides.[6] However, a dimeric structure for the isomerization product may be eliminated on several grounds, e.g., (a) the mol. wt of the dehydration product, B, was determined mass spectrometrically to be 237, establishing its monomeric nature and almost certainly that of its precursor, A. (b) Only polymer could be obtained when the oxazolone derived from the analogous hexapeptide was reacted under the conditions which led to a high yield of isomerization product from the tripeptide oxazolone (I).

(iii) *The cyclol tripeptide* (III). Initially[2a] we considered this possibility very favourably, both because a plausible reaction scheme for its formation was evident, and also because it provided a ready explanation of the very facile dehydration. Molecular models of the tripeptide oxazolone (I) show clearly that intramolecular attack of the amino group on the oxazolone carbonyl results also in close juxtaposition of the peptide carbonyl group and the ring nitrogen atom. The concerted electronic shifts shown in Ia (p. 3212) therefore seemed feasible, and for reasons already given, this mechanism is perferable to one involving transannular reaction of a preformed cyclic tripeptide. No steric congestion is evident in models of III. On the basis of this structure for the tripeptide oxazolone isomerization product, its ready dehydration (to the imidazolone, V) is understandable, and close analogy is available for this latter reaction.[7]

(iv) *The dipolar amidine structure* (IV). Although the carbonyl group of an oxazolone ring is almost invariably the point of nucleophilic attack by amines, we have

[3] R. A. Boissonnas and I. Schumann, *Helv. Chim. Acta* **35**, 2229 (1952); M. Winitz and J. S. Fruton, *J. Amer. Chem. Soc.* **75**, 3041 (1953); H. Brockmann, H. Tummes and F.-A. von Metzsch, *Naturwiss.* **41**, 37 (1954); J. C. Sheehan and W. L. Richardson, *J. Amer. Chem. Soc.* **76**, 6329 (1954); P. W. G. Smith, *J. Chem. Soc.* 3985 (1957).

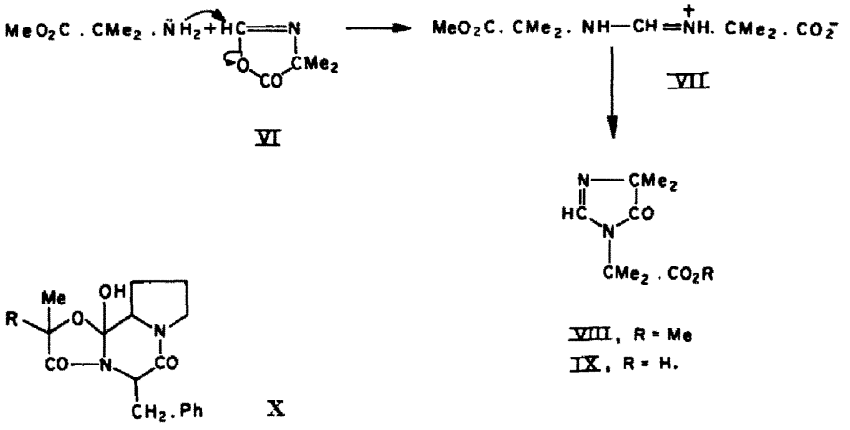
[4] C. H. Bamford and F. J. Weymouth, *J. Amer. Chem. Soc.* **77**, 6368 (1955); J. C. Sheehan, M. Goodman and W. L. Richardson, *Ibid.* **77**, 6391 (1955); H. Brockmann and M. Springorum, *Naturwiss.* **49**, 514 (1962).

[5] G. W. Kenner and J. M. Turner, *Chem. & Ind.* 602 (1955); G. W. Kenner, *J. Chem. Soc.* 3692 (1956).

[6] See also R. Schwyzer, J. P. Carrión, B. Gorup, H. Nolting and A. Tun-Kyi, *Helv. Chim. Acta* **47**, 441 (1964) and previous papers for further examples and discussion of this reaction.

[7] V. K. Antonov, Ts. E. Agadzhanyan, T. R. Telesmina and M. M. Shemyakin, *Tetrahedron Letters* 730 (1964); M. Rothe, *Angew. Chem.* **74**, 725 (1962).

recently encountered examples where steric factors direct the reaction to the methine carbon atom. A particularly pertinent example is the reaction between 4,4-dimethyloxazolone (VI) and α-methylalanine methyl ester, leading to the amidine derivative (VII)[8]:



A similar (intramolecular) reaction of the tripeptide oxazolone (Ib) leads to structure IV for its isomerization product, A. This structure also accounts very easily for the facile dehydration, visualized now as a cyclization reaction leading to the same bicyclic imidazolone (V). Excellent analogy is available for this cyclization,[8,9] e.g. ring closure of VII to a mixture of VIII and IX.[8]

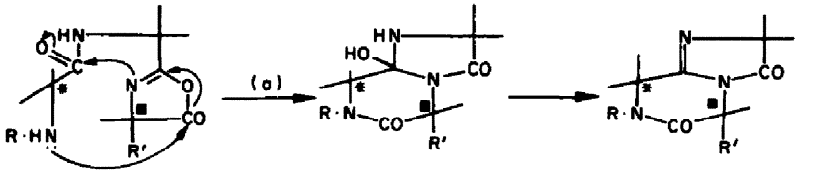
Thus, two structures (III and IV) deserve serious consideration, although previously we had assigned structure III to A.[2a] If substantiated, III would be the first example of a cyclol tripeptide, although the possible existence of cyclol forms of peptides has been discussed at length for many years.[10] Many examples are known of analogous compounds containing one hydroxy acid residue,[11] e.g. (X, R = H), and this system is present in the naturally occurring ergot alkaloids, e.g. ergotamine (X, R = lysergylamino).[12] It seemed possible that previous attempts to prepare cyclol peptides had been frustrated *inter alia* by an inherent instability, typified by dehydration of III to V, which would not be shown by oxygen analogues such as X. The possible formulation of the tripeptide oxazolone isomerization product as a cyclol derivative therefore assumed some importance, and we have examined the chemistry of its formation in some detail in order to distinguish decisively between the two possible structures III and IV.

There can be little doubt that the dehydration product, B, is correctly formulated as the bicyclic imidazolone derivative (V) and indeed it is difficult to visualize mechanistically acceptable alternatives corresponding to the molecular formula. The IR spectrum strongly supports this structure with ν_{max} 1730 (unsaturated γ-lactam of imidazolone ring, c.f. VIII with ν_{max} 1724 cm^{-1}), 1670 (δ lactam, cf. 2,2,5,5-tetramethyl-3,6-dioxopiperazine with ν_{max} 1670 cm^{-1}), and 1640 cm^{-1} (C=N of imidazolone).

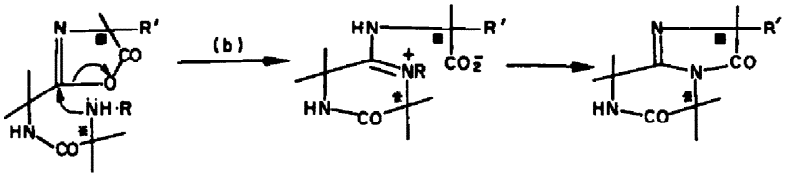
[8] M. T. Leplawy, D. S. Jones, G. W. Kenner and R. C. Sheppard, *Tetrahedron* **11**, 39 (1960).
[9] S. Petersen and E. Tietze, *Liebigs Ann.* **623**, 166 (1959).
[10] D. Wrinch, *Chemical Aspects of the Structure of Small Peptides.* Munksgaard, Copenhagen (1960) and Refs there cited.
[11] E.g. H. Ott, A. J. Frey and A. Hofmann, *Tetrahedron* **19**, 1675 (1963); M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, Y. N. Sheinker and L. B. Senyavina, *Tetrahedron Letters* 701 (1962).
[12] A. Hofmann, H. Ott, R. Griot, P. A. Stadler and A. J. Frey, *Helv. Chim. Acta* **46**, 2306 (1963).

The NMR spectrum shows that as expected, all six methyl groups are intact. B cannot be titrated, but it shows very weakly basic properties. It is soluble in strong hydrochloric acid (but not in water), and evaporation yields a rather unstable hydrochloride which readily reverts to the free base. This behaviour parallels that of other related imidazolones, e.g. VIII.

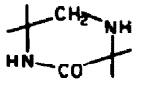
Investigation of A (i.e. III or IV) was hampered by its ready dehydration to V (see below). However, we realised that a conclusive distinction between structures III and IV might be possible by using the observation that the N- and C-terminal residues (* and ■ in the scheme below) of the tripeptide oxazolone (I) appear in relatively different positions in the imidazolone (Va or b) depending on whether the reaction passes through the intermediate cyclol III (route a), or amidine IV (route b). Thus a suitable labelling experiment followed by degradation of the final imidazolone (possibly by hydrolysis to α-methylalanine and tetramethyldioxopiperazine) should enable a decision to be reached. A corollary of the indicated labelling pattern is that a terminally N-methylated tripeptide oxazolone (I, R = Me) should yield an N-methylimidazolone (Va, R = Me) if the reaction followed path (a), but should yield a stable amidine derivative (IV, R = Me) incapable of conversion into a simple imidazolone if it followed path (b). In the event, this last experiment was unsuccessful, because although the oxazolone derived from benzyloxycarbonyl-α,N-dimethylalanyl-α-methylalanyl-α-methylalanine was prepared without difficulty, hydrogenolysis yielded a free methylamino oxazolone (I, R = Me) which proved to be quite inert to further reaction. However, experiments in which the C-terminal residue was labelled either with an additional methyl group (i.e. replacement of the terminal α-methylalanine by α-ethylalanine), or with radiocarbon were successful.



Ia III Va



Ib IV Vb



XI

(R = H and R′ = Me unless stated otherwise in the text)

The imidazolone (V) proved to be a remarkably stable compound and its hydrolysis could not be achieved, even by prolonged heating with mineral acids or alkalies. However it was found possible to effect a reductive cleavage of A itself under very mild conditions which did not effect its transformation into the imidazolone (V). Reduction of the isomerization product A with a large excess of sodium borohydride in aqueous solution yielded free α-methylalanine and the oxopiperazine (XI).[1] In itself, this reaction supported the amidine structure (IV) but reductive cleavage of the cyclol system was conceivable. It was clear that in any case the oxopiperazine (XI) resulted from the existing six membered ring in III or IV, and that the free α-methylalanine was therefore derived from the central or C-terminal α-methylalanyl residue of the original tripeptide oxazolone (I), depending on whether its isomerization product A had structure III or IV.

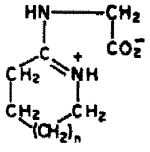
Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-DL-α-*ethyl*alanine t-butyl ester was prepared by the oxazolone method,[1,8] and converted into the free acid by cleavage with trifluoroacetic acid. Dehydration with acetic anhydride yielded the oxazolone (I, R = $C_6H_5CH_2$·O·CO, R′ = Et) which was hydrogenolysed and the free amine (I, R′ = Et) allowed to stand in ethyl acetate solution for several days. A crystalline isomerization product separated with IR spectrum almost identical with that of the tri-α-methylalanine analogue, A. Like the latter, it sublimed with loss of water, yielding an imidazolone (Va or Vb, R′ = Et), again with IR spectrum almost identical with that of B. Reduction of the isomerization product (III or IV, R′ = Et) with sodium borohydride yielded the tetramethyloxopiperazine (XI) and free α-ethylalanine. No trace of α-methylalanine could be detected by paper chromatography. Thus, in this series, there is no doubt that the isomerization product has the amidine structure (IV, R′ = Et), and strong presumptive evidence is provided that the analogue A likewise has the amidine structure (IV). This point was finally established by a ^{14}C labelling experiment.

The C-terminally labelled tripeptide oxazolone (I) was prepared by way of the benzyloxycarbonyl-dipeptide and ^{14}C-enriched α-methylalanine t-butyl ester. This labelled oxazolone was allowed to isomerize in ethyl acetate solution, and the product reduced with sodium borohydride. The isolated oxopiperazine (XI) had 4% of the mean radioactivity of the tripeptide derivative and this dropped to 2% after purification by one sublimation. The α-methylalanine was isolated as its benzyloxycarbonyl derivative, and had 98% of the activity of the benzyloxycarbonyl-α-methylalanine from which the original t-butyl ester was prepared. Clearly it is the C-terminal residue of I which is liberated by reduction of A with borohydride, and A therefore has structure IV. The cyclol structure (III), previously advanced,[2a] must be unequivocally withdrawn.[2b]

Before these decisive results were obtained, attempts were made to solve the structural problem by more conventional means, but a method of converting A into a more stable derivative was not found. For example, treatment of A with acetic anhydride, benzyl chloroformate, *p*-bromophenacyl chloride, or even acetic acid, led smoothly to V. A reaction characteristic of cyclol derivatives such as X is the facile methylation of the acidic hydroxyl group.[11] Treatment of A with a large excess of methyl iodide and silver oxide yielded an unstable monomethyl derivative, but this could not be obtained completely pure as it was transformed into V at room temperature. However the IR and NMR spectra of the crude methylated product suggested its formulation as a methyl *ester*, favouring the amidine structure (IV) for A. Treatment

of A with diazomethane led directly to V. This behaviour parallels that of the amidine (VII), which under similar conditions yielded VIII.[8]

An attempt to distinguish between the dipolar amidine (IV) and cyclol (III) structures by titration experiments was inconclusive, as the dissociation constants of A ($pK_a < 3$ and > 10) fell outside the easily accessible range. However the model amidines (XII and XIII)[9] behaved similarly, and the cyclol (X) has been reported to have pK_a 9·07. Aqueous solutions of A showed a small positive dielectric increment effect[13] (dielectric increment 10·5, based on an assumed value for glycine of 22·6) again favouring the amidine structure.



XII, n = 1
XIII, n = 2

EXPERIMENTAL

All evaporations were under reduced pressure.

Preparation and isomerization of 2(1′-α-*methylalanylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyloxazolone* (I)

The amidine (IV). Hydrogen was passed through a solution of 2-(1′-benzyloxycarbonyl-α-methyl-alanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (0·389 g, 1 mmole) in dry ethyl acetate (200 ml) containing 5% Pd–C catalyst (0·2 g) until evolution of CO_2 ceased (1½ hr). After filtration of the catalyst, the ethyl acetate solution of I was concentrated† to 10 ml and set aside at room temp. After 3 days the crystalline precipitate (0·201 g, 79%) was collected. For analysis the amidine was recrystallized twice from EtOH–ether yielding short prisms, no m.p. below 300°, ν_{max} 1165, 1200, 1247, 1337, 1378, 1470, 1585, 1632 and 1685 cm^{-1}. (Found: C, 56·65; H, 8·4; N, 16·2. $C_{12}H_{21}O_3N_3$ requires: C, 56·45; H, 8·3; N, 16·5%.) Recrystallization from aqueous MeOH yielded a solvate (needles), ν_{max} 1160, 1180, 1200, 1230, 1240, 1345, 1360, 1385, 1450, 1600 and 1650 cm^{-1}. (Found: C, 51·2; H, 8·5; N, 13·75. $C_{12}H_{21}O_3N_3 \cdot CH_3OH \cdot H_2O$ requires: C, 51·1; H, 8·8, N, 13·8%) which reverted to the anhydrous form after prolonged drying *in vacuo*.

Dehydration of the amidine (IV)

The bicyclic imidazolone (V). (a) The amidine IV (0·05 g, 0·196 mmole) was heated at 170–240° and 0·2 mm press. The pure *imidazolone* (0·042 g, 95%) sublimed, m.p. 255° (sealed tube), ν_{max} 1135, 1175, 1208, 1238, 1305, 1360, 1380, 1395, 1640, 1670 and 1730 cm^{-1}. (Found: C, 60·8; H, 7·85; N, 17·5. $C_{12}H_{19}O_3N_3$ requires: C, 60·7; H, 8·1; N, 17·7%.)

(b) A solution of IV (0·103 g) in anhydrous acetic acid (10 ml) was set aside at room temp for 3 days. Evaporation of the solvent at room temp yielded a crystalline residue of the imidazolone (0·092 g, 96%) identified by IR spectrum. In a separate experiment, replacement of the acetic acid by acetic anhydride yielded the pure imidazolone after 16 hr at room temp.

Attempted methylation of the amidine (IV)

(a) An ethereal solution (20 ml) of excess diazomethane was added to the amidine (0·101 g) dissolved in dioxan (10 ml). After 3 days at room temp the solution was evaporated yielding V (0·09 g) m.p. 250° (sealed tube).

(b) Silver oxide (0·075 g) was added to a suspension of the amidine (0·167 g) in redistilled MeI

† The crystalline oxazolone (I) m.p. 82–85°, can be obtained by evaporation,[1] but rapidly polymerizes on storage.

[13] P. M. Hardy, G. W. Kenner and R. C. Sheppard, *Tetrahedron* **19**, 95 (1963).

(20 ml). The mixture was shaken for 3 hr and then set aside overnight. Next morning the filtered solution was evaporated and the residue (0·075 g) recrystallized from ethyl acetate–light petroleum (ν_{max} 1160, 1195, 1230, 1280, 1460, 1525, 1660, 1720, 3220 and 3400 cm^{-1}). (Found: C, 58·5; H, 8·7; N, 15·8. $C_{12}H_{22}O_3N_2$ requires: C, 58·0; H, 8·55; N, 15·6%.) The NMR spectrum (in $CDCl_3$ solution) slowly changed on standing, and after 2 weeks was identical with that of V. Evaporation of this solution yielded the pure V (IR spectrum).

Reduction of the amidine (IV) *with sodium borohydride*

Sodium borohydride (0·102 g) was added in several portions to a solution of IV (0·105 g) in water (5 ml), and the mixture then set aside overnight at room temp. Next morning the solution was acidified to pH 2 and washed with ethyl acetate. The aqueous solution was adjusted to pH 9 and re-extracted with ethyl acetate. Evaporation of the dried ($MgSO_4$) extract yielded a crystalline residue (0·0137 g) of 2,2,5,5-*tetramethyl*-3-*oxopiperazine*. For analysis a sample was sublimed at 100° and 0·1 mm. (Found: C, 61·4; H, 10·5. $C_8H_{16}ON_2$ requires: C, 61·5; H, 10·3%.) The IR spectrum was identical with that of material prepared[1] by hydrogenolysis of 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone. The aqueous solution was shown to contain α-methylalanine as the only ninhydrin-positive product by paper chromatography in the solvent system butan-1-ol (4), acetic acid (1), water (5) (R_f = 0·36).

Tosyl-α-N-*dimethylalanine*

This compound was prepared by the method of Leplawy *et al.*[8] except that the heating at 100° was extended from 5 min to 1 hr. Recrystallization from aqueous MeOH gave the N-methyl derivative (78%, m.p. 143–145°) (lit.[8] 50%, m.p. 142·5–145°). (Found: C, 53·2; H, 6·3; N, 5·2. Calc. for $C_{12}H_{17}O_4NS$: C, 53·1; H, 6·3; N, 5·2%.)

Tosyl-α-N-*dimethylalanyl*-α-*methylalanine methyl ester*

Tosyl-α-N-dimethylalanyl chloride was prepared by the method of Leplawy *et al.*[8] except that the heating at 45° was extended from 10 to 40 min. The crude acid chloride (100%, m.p. 75–80°) (lit.[8] 63%, m.p. 79–80°) (3·01 g, 10 mmoles) was added in 3 portions to an ice-cold solution of α-methylalanine methyl ester (2·53 g, 21 mmoles) in dry acetone (20 ml). After 2 days at room temp the solution was filtered and evaporated and the neutral fraction isolated in the usual manner. Recrystallization from benzene–light petroleum (charcoal) yielded the *dipeptide derivative* (3·2 g, 87%) m.p. 102–103°. (Found: C, 55·3; H, 7·0; N, 7·6. $C_{17}H_{26}O_5N_2S$ requires: C, 55·1; H, 7·1; N, 7·6%.)

Tosyl-α,N-*dimethylalanyl*-α-*methylalanine*

A mixture of the foregoing methyl ester (0·556 g, 1·5 mmoles) and 2 N NaOH (1·5 ml) was shaken at room temp until a clear solution was obtained (5 hr). The solution was diluted with water (3 ml) and acidified with HCl aq. The precipitated dipeptide derivative (0·528 g, 99%) m.p. 210–214° was collected and recrystallized from aqueous MeOH, m.p. 210·5–215°. (Found: C, 53·9; H, 6·7; N, 7·7. Calc. for $C_{16}H_{24}O_5N_2S$: C, 53·9; H, 6·8; N, 7·9%.) Leplawy *et al.*[8] gave m.p. 210·5–212° for material prepared by reaction between tosyl-α,N-dimethylalanyl chloride and α-methylalanine (yield 8%).

2-(1′-N-*Tosyl-methylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyloxazolone*

The general method[8] for the preparation of oxazolones applied to tosyl-α,N-dimethylalanyl-α-methylalanine (0·339 g) yielded the *oxazolone* (0·320 g, 100%) m.p. 127–128° (sealed tube). (Found: C, 57·1; H, 6·6; N, 8·2. $C_{16}H_{22}O_4N_2S$ requires: C, 56·8; H, 6·55; N, 8·3%.)

Tosyl-α,N-*dimethylalanyl*-α-*methylalanyl*-α-*methylalanine methyl ester*

A solution of the foregoing oxazolone (0·311 g) and α-methylalanine methyl ester (0·416 g) in anhydrous acetonitrile (10 ml) was heated under reflux for 21 hr. Evaporation, finally at 0·1 mm yielded the *tripeptide derivative* (0·415 g, 99%) m.p. 132–133°, unchanged on recrystallization from benzene–light petroleum. (Found: C, 55·65; H, 7·3; N, 9·0. $C_{21}H_{33}O_6N_3S$ requires: C, 55·4; H, 7·3; N, 9·2%.)

Tosyl-α,N-dimethylalanyl-α-methylalanyl-α-methylalanine

A mixture of tosyl-α,N-dimethylalanyl-α-methylalanyl-α-methylalanine methyl ester (0·267 g) and 2 N NaOH (1 ml) was shaken at room temp until all the solid had dissolved (7 hr). The solution was diluted with water and acidified with HCl aq, and the precipitated *tripeptide derivative* (0·259 g, 100%) m.p. 181–185° recrystallized from aqueous MeOH, m.p. 183·5–185°. (Found: C, 54·4; H, 6·8; N, 9·3. $C_{20}H_{31}O_6N_3S$ requires: C, 54·4; H, 7·1; N, 9·5%.)

Benzyloxycarbonyl-α-N-dimethylalanyl-α-methylalanyl-α-methylalanine

Sodium was added to a solution of the foregoing tosyl derivative (3·31 g) in liquid ammonia (250 ml) until a blue colour permanent for 3 min was obtained. The blue colour was discharged by the addition of ammonium acetate, and the solution allowed to evaporate. The residue was dissolved in a mixture of water (20 ml) and acetone (15 ml) and the solution adjusted to pH 10·9. Benzyl chloroformate (10 g) dissolved in acetone (25 ml) was added during 45 min to this stirred solution, the pH being maintained at 10·9–11 by concurrent addition of 1 N NaOH. After stirring for a further 3 hr the solution was concentrated and the acidic fraction isolated in the usual manner. The *benzyloxycarbonyl derivative* (2·85 g, 90%) had m.p. 178–179° after recrystallization from aqueous MeOH. (Found: C, 59·8; H, 7·6; N, 10·0. $C_{21}H_{31}O_6N_3$ requires: C, 59·8; H, 7·4; N, 10·0%.)

2-(1′-*Benzyloxycarbonyl-α,N-dimethylalanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone*

The general method for the preparation of oxazolones[8] applied to the foregoing tripeptide derivative yielded the *oxazolone* (100%) m.p. 122–124°. (Found: C, 62·7; H, 7·3; N, 10·5. $C_{21}H_{29}O_5N_3$ requires: C, 62·5; H, 7·25; N, 10·4%.)

Hydrogenolysis of 2-(1′-benzyloxycarbonyl-α,N-dimethylalanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone

Hydrogen was passed over the surface of a stirred solution of the foregoing benzyloxycarbonyl derivative (0·79 g) in anhydrous ethyl acetate (200 ml) containing 5% Pd–C catalyst (0·9 g) until CO_2 evolution ceased (3 hr). The solution was concentrated to 25 ml after filtration, and set aside for 3 days. No precipitation occurred. A sample (5 ml) was evaporated. The residual oil had ν_{max} 907, 972, 1050, 1210, 1365, 1380, 1470, 1510, 1690, 1830 and 3400 cm^{-1}, almost identical to the IR spectrum of 2-(1′-α-methylalanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone.

No change in the IR spectrum was observed when a solution of the oxazolone in either ethyl acetate or toluene was heated under reflux during 21 hr.

Benzyloxycarbonyl-DL-α-ethylalanine t-butyl ester

A solution of benzyloxycarbonyl-DL-α-ethylalanine (14·5 g) and conc. H_2SO_4 (0·6 ml) in CH_2Cl_2 (150 ml) was saturated with isobutene and then set aside for 3 days at room temp. The solution was washed with 5% Na_2CO_3 aq (50 ml) and water, dried ($MgSO_4$), and evaporated. Crystallization of the residual oil from light petroleum yielded the *t-butyl ester* (14·2 g, 80%) m.p. 56–58°. (Found: C, 66·6; H, 8·2; N, 4·5. $C_{17}H_{25}O_4N$ requires: C, 66·4; H, 8·2; N, 4·6%.)

DL-α-*Ethylalanine t-butyl ester*

Hydrogen was passed over the surface of a stirred solution of the foregoing benzyloxycarbonyl derivative (14·1 g) in MeOH (500 ml) containing 5% Pd–C catalyst (0·8 g) until CO_2 evolution ceased (12 hr). The solution was filtered and distilled yielding DL-α-*ethylalanine t-butyl ester* (6·11 g, 77%) b.p. 60–70° at 15 mm. The *picrate* had m.p. 142–143°. (Found: C, 45·1; H, 5·5; N, 14·0. $C_{16}H_{22}O_9N_4$ requires: C, 44·8; H, 5·5; N, 13·9%.)

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-DL-α-ethylalanine t-butyl ester

A solution of 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (1·864 g, 6 mmoles) and DL-α-ethylalanine t-butyl ester (1·407 g, 8 mmoles) in acetonitrile (60 ml) was heated under reflux for 10 hr. The acetonitrile was replaced by dioxan (25 ml) and the solution heated under reflux for a further 20 hr. Isolation of the neutral fraction in the usual manner and recrystallization from ethyl acetate–light petroleum yielded the *tripeptide derivative* (2·199 g, 75%) m.p. 106–108°. (Found: C, 62·95; H, 8·2; N, 8·8. $C_{25}H_{39}O_6N_3$ requires: C, 62·9; H, 8·2; N, 8·8%.)

*Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-*DL*-α-ethylalanine*

A solution of the foregoing ester (2·134 g) in trifluoroacetic acid (40 ml) was kept at room temp for 1½ hr and then evaporated. Isolation of the acidic fraction in the usual manner and recrystallization from aqueous MeOH yielded the *benzyloxycarbonyl-tripeptide* (1·396 g, 74%) m.p. 180–181°. (Found: C, 59·8; H, 7·3; N, 9·8. $C_{21}H_{31}O_6N_3$ requires: C, 59·8; H, 7·4; N, 10·0%.)

2-(1′-*Benzyloxycarbonyl-α-methylalanylamino-*1′-*methyl)ethyl-4-ethyl-4-methyloxazolone*

The general method[8] for the preparation of oxazolones applied to the foregoing tripeptide derivative yielded the oxazolone (99%) m.p. 91–92°. (Found: C, 62·7; H, 7·4; N, 10·2. $C_{21}H_{29}O_5N_3$ requires: C, 62·5; H, 7·25; N, 10·4%.)

Hydrogenolysis of 2-(1′-*benzyloxycarbonyl-α-methylalanylamino-*1′-*methyl)ethyl-4-ethyl-4-methyloxazolone*

The amidine (IV, R = H, R′ = Et). Hydrogen was passed over the surface of a stirred solution of the foregoing oxazolone (0·38 g) in ethyl acetate (100 ml) containing 5% Pd–C catalyst (0·3 g) until evolution of CO_2 ceased (3 hr). The solution was filtered, concentrated to 10 ml and set aside at room temp. After 3 days the precipitated *amidine* (0·102 g, 40%) was collected and recrystallized from MeOH–ether, ν_{max} 825, 910, 1010, 1040, 1160, 1180, 1200, 1230, 1310, 1370, 1450, 1600, 1650, 3100, 3200 and 3450 cm^{-1}. (Found: C, 55·6; H, 8·8; N, 13·8. $C_{18}H_{33}O_5N_3 \cdot CH_3OH$ requires: C, 55·8; H, 9·0; N, 13·95%.)

Dehydration of the amidine (IV, R = H, R′ = Et)

The imidazolone (V, R = H, R′ = Et). The foregoing amidine (0·034 g) was heated in a sublimation tube at 160–180° and 0·1 mm press. After 3 hr the sublimed *imidazolone* (0·032 g, 94%) was collected, ν_{max} 905, 920, 945, 1000, 1135, 1180, 1210, 1240, 1315, 1365, 1410, 1450, 1645, 1675, 1730, 3050 and 3150 cm^{-1}. (Found: C, 61·8; H, 8·2; N, 16·6. $C_{18}H_{31}O_3N_3$ requires: C, 62·1; H, 8·4; N, 16·7%.)

Reduction of the amidine (IV, R = H, R′ = Et) *with sodium borohydride*

Sodium borohydride (0·1675 g) was added in portions to a solution of IV (R = H, R′ = Et; 0·173 g) in water (8 ml). After 14 hr at room temp the solution was acidified and washed with ethyl acetate. The aqueous solution was brought to pH 10, extracted with ethyl acetate, and the extract dried ($MgSO_4$) and evaporated. The residual 2,2,5,5-tetramethyl-3-oxopiperazine (0·0216 g, 22%) was identified by its IR spectrum. The aqueous solution was shown to contain α-ethylalanine and no α-methylalanine by paper chromatography.

^{14}C-*Derivatives of α-methylalanine*

Carboxyl-labelled α-methylalanine (6 μc) was diluted to 2·06 g (20 mmoles) and reacted with benzyl chloroformate by the method of Leplawy *et al.*[8] The resulting benzyloxycarbonyl-α-methylalanine (4·52 g, 95%) 0·327 μc/mmole, m.p. 78–79° (lit. m.p. 72·5–74°) was converted to the t-butyl ester[1] (5·16 g, 88%) 0·320 μc/mmole, m.p. 60–61° (lit. m.p. 60–61°). Hydrogenolysis of the benzyloxycarbonyl derivative (5·1 g) yielded crude α-methylalanine t-butyl ester[1] (1·50 g, 54%) which without purification was reacted[1] with 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone[8] (2·432 g). The resulting benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-1-^{14}C-α-methylalanine t-butyl ester (3·70 g, 100%) had 0·312 μc/mmole, m.p. 166–167° after recrystallization from ethyl acetate–light petroleum (lit.[1] 166·5–167·5°). Cleavage of the t-butyl ester (2·42 g) with trifluoracetic acid yielded benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-1-^{14}C-α-methylalanine (2·05 g, 95%) 0·329 μc/mmole, m.p. 203–206° (lit.[1] 205–206°). Dehydration of the benzyloxycarbonyl-tripeptide (1·22 g) with acetic anhydride yielded 2-(1′-benzyloxycarbonyl-α-methylalanylamino-1′-methyl)ethyl-4,4-dimethyl-5-^{14}C-oxazolone (1·08 g, 93%) 0·333 μc/mmole, m.p. 124–125° (lit,[1] 124–125°). Hydrogenolysis of the benzyloxycarbonyl derivative (1·08 g) and reaction in ethyl acetate solution for 2 days yielded the labelled amidine (IV; 0·285 g, 40%) 0·345 μc/mmole, with IR spectrum identical with that of the inactive compound previously described.

Sodium borohydride reduction of the ^{14}C*-labelled amidine* (IV)

The radioactive amidine (0·210 g) dissolved in water (10 ml) was reduced with $NaBH_4$ (0·204 g) as previously described for the inactive compound. The aqueous solution at pH 10 was extracted with ethyl acetate and the extract dried ($MgSO_4$) and evaporated. The residual 2,2,5,5-tetramethyl-3-oxopiperazine (0·0216 g, 17%), identified by its IR spectrum had 0·013 μc/mmole. This material (0·0149 g) was sublimed at 100° and 0·1 mm, yielding 0·0132 g, 0·007 μc/mmole. (Found: M, 156 (mass spectrometry). $C_8H_{16}ON_2$ requires: M, 156). The aqueous solution contained α-methylalanine (paper chromatography), which was isolated as its benzyloxycarbonyl derivative.[8] After recrystallization from ethyl acetate–light petroleum, there was obtained 0·1225 g (63%) 0·321 μc/mmole, m.p. 74·5–77° (lit.[8] 72–74·5°).