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PEPTIDES—XI*

SYNTHESIS OF PEPTIDES DERIVED FROM ALPHA-METHYLALANINE

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Abstract—Union of α-methylalanyl residues in a peptide chain is severely sterically hindered. This hindrance can be overcome by employing either the mixed anhydride (I) of pivalic acid and benzyloxycarbonyl-α-methylalanine or, more generally, oxazolones (II), including 4,4-dimethyl-2-trifluoromethyloxazolone (II; R = CF_3). 4,4-Dimethyloxazolone (II; R = H) has been obtained in solution and characterized by its infra-red spectrum; it is attacked by α-methylalanine methyl ester at the methine group, in contrast to the normal reaction with cyclohexylamine at the carbonyl group.

INCORPORATION of α-methylalanine (α-aminoisobutyric acid) in synthetic peptides has received little attention because this amino acid has not been generally regarded as a constituent of proteins, although this possibility was mooted when 5,5-dimethylhydantoin was reported as a pyrolytic fragment of common proteins.[1] Our discovery of α-methylalanine as a component of an antibiotic[2] prompted us to examine the problem with the eventual aim of synthesizing both cyclic oligopeptides and open-chain peptides, built entirely or partly from α-amino acids lacking a hydrogen atom at the α-position. Not unexpectedly, we encountered difficulties arising from steric hindrance,‡ but on the other hand the problem of preserving optical activity was absent and practical methods for the synthesis of open-chain oligopeptides of this class can now be described.

Abderhalden *et al.*[4,5] prepared peptides of α-methylalanine by Fischer's method, but comparison with the material prepared by us from the benzyloxycarbonyl derivative shows that even the simple dipeptide, α-methylalanyl-α-methyl-alanine, was impure. Bergmann *et al.*[6] synthesized dipeptides of glycine or alanine and α-methylalanine by the carbobenzoxy(benzyloxycarbonyl) method. Although they prepared the benzyloxycarbonyl derivative of α-methylalanine via the ethyl ester, it is available directly, in excellent yield, from the amino acid (see Experimental)§ and it crystallizes well. On the other hand the acid chloride, which they used, without

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‡ The extraordinary slowness[3] of the "polymerization" of N-carboxy-α-methylalanine anhydride is a noteworthy example of this effect.

§ Yields from some other acylations of α-methylalanine have been reported[7] to be low or negligible, presumably as a result of steric hindrance.

[1] T. Yabuta, Z. Saito, K. Takeda and K. Tamari, *Chem. Zentr.* **1**, 221 (1940).
[2] G. W. Kenner and R. C. Sheppard, *Nature, Lond.* **181**, 48 (1958).
[3] H. Weingarten, *J. Amer. Chem. Soc.* **80**, 352 (1958).
[4] [a] E. Abderhalden and F. Gebelein, *Z. physiol. Chem.* **152**, 125 (1926); [b] E. Abderhalden and E. Rossner, *Ibid.* **163**, 149 (1927).
[5] E. Abderhalden and W. Zeisset, *Fermentforschung* **13**, 310 (1932).
[6] M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider and H. Schleich, *J. Biol. Chem.* **109**, 325 (1935).
[7] e.g. W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.* 3009 (1950); A. Kjaer, *Acta Chem. Scand.* **6**, 327 (1952).

isolation, for reaction with glycine benzyl ester to obtain small amounts of the dipeptide, has eluded us; even under very mild conditions the N-carboxyanhydride is formed by elimination of benzyl chloride. The similar cyclization of benzyloxycarbonylalanine occurs more easily than with the glycine derivative,[8] and evidently a second α-methyl group facilitates it still more.

A serviceable route to dipeptide derivatives in this series is through the chlorides of the tosylamino acids. Thus a 71 per cent yield was obtained from the coupling, in aqueous acetone buffered with magnesium oxide, of glycine with tosyl-α-methylalanyl chloride, and this was superior to the coupling of the acid with glycine ester by the carbodi-imide or mixed anhydride procedures. In contrast, the aqueous coupling of tosylglycyl chloride with α-methylalanine failed, but the methyl ester of the tosyldipeptide was prepared in 71 per cent yield from the chloride and the amino acid ester under anhydrous conditions. The difference between these two aqueous couplings recalls the kinetic data on the formation of amides from esters; the reaction of methyl trimethylacetate with ammonia is approximately 100 times slower than that of methyl propionate,[9] while the reaction between methyl acetate and t-butylamine is immeasurably slow.[10] As a general rule steric hindrance of the usual peptide-forming reactions at the amino group can be expected to be more severe than hindrance of reactions at the carbonyl group in derivatives of α-methylalanine. The aqueous coupling of tosyl-α-methylalanyl chloride with α-methylalanine also failed; in this instance toluene-*p*-sulphonamide was a major product. Evidently the coupling was so slow that it was supplanted by alkaline degradation (Reaction 1) of the acid chloride.[11]

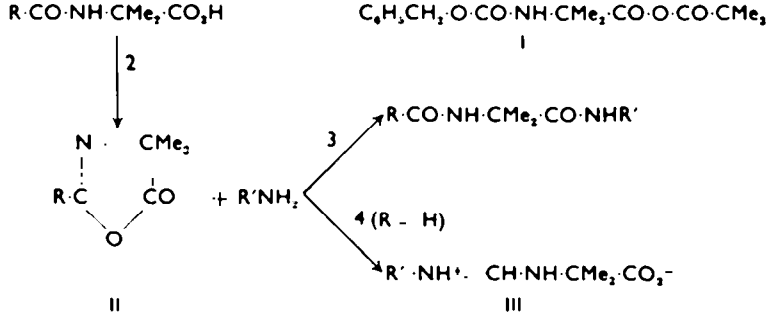
$$Tos{\cdot}NH{\cdot}CMe_2{\cdot}CO.Cl \cdot\cdot OH \xrightarrow{I} Tos{\cdot}NH_2 \cdot\cdot Me_2CO \vdots CO \cdot Cl^-$$

Even when this reaction was prevented by N-methylation, the yield of N-methylated dipeptide derivative was very poor and the main reaction was hydrolysis. However, the methyl ester of tosyl-α-methylalanyl-α-methylalanine was obtained satisfactorily by coupling the acid chloride with the amino acid ester in dry acetone.

Most of the other conventional methods of peptide synthesis are of negligible value for the joining of α-methylalanyl residues. For example, dicyclohexyl carbodi-imide failed to condense formyl-α-methylalanine with α-methylalanine methyl ester. Likewise, there was negligible diminution in the infra-red absorption of the isocyanate derived from this ester when it was heated with benzyloxycarbonyl-α-methylalanine, although it has been reported to combine normally, albeit comparatively slowly, with benzyloxycarbonylglycine.[12] Again, the cyanomethyl ester of benzyloxycarbonyl-α-methylalanine was recovered completely after a prolonged period of attempted reaction with α-methylalanine methyl ester, and the mixed carbonic anhydride method also failed; the yield of dipeptide derivative from the sulphuric anhydride method was only 8 per cent. Our assumption that steric hindrance was responsible for these failures prompted the trial of the mixed pivalic anhydride (I), in which the steric effect would be counter-balanced. Pivaloyl chloride was included in an early survey[13] of

[8] M. Hunt and V. Du Vigneaud, *J. Biol. Chem.* **124**, 699 (1938).
[9] M. Gordon, J. G. Miller and A. R. Day, *J. Amer. Chem. Soc* **70**, 1946 (1948).
[10] E. M. Arnett, J. G. Miller and A. R. Day, *J. Amer. Chem. Soc.*, **72**, 5635 (1950).
[11] [a] R. H. Wiley and R. P. Davis, *J. Amer. Chem. Soc.* **76**, 3496 (1954); [b] A. F. Beecham, *Ibid.* **79**, 3257 (1957).
[12] S. Goldschmidt and M. Wick, *Liebigs Ann.* **575**, 217 (1952).
[13] J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.* **73**, 5553 (1951).

reagents for the mixed carboxylic anhydride method of peptide synthesis, but it appeared slightly inferior to isovaleryl chloride. Recently, however, pivaloyl chloride has proved valuable for the condensation of tosyl-α-amino acids and in the asparagine series.[14] With the triethylammonium salt of benzyloxycarbonyl-α-methylalanine it yielded the crystalline and relatively stable mixed anhydride (I) quantitatively. In turn, reaction between the anhydride (I) and α-methylalanine methyl ester in toluene solution afforded the benzyloxycarbonyldipeptide ester in excellent yield.* With the triethylammonium salt of benzyloxycarbonyl-α-methylalanyl-α-methylalanine, however, pivaloyl chloride yielded (Reaction 2) the oxazolone (II; $R = C_6H_5CH_2{\cdot}O{\cdot}CO{\cdot}NHCMe_2$) rather than a mixed anhydride, and ethyl chloroformate behaved similarly. This oxazolone was more conveniently obtained by dehydration of the benzyloxycarbonyldipeptide with acetic anhydride (see below).



While the absence of a hydrogen atom from the alpha position in α-methylalanine diminishes the value of the customary methods of peptide synthesis, it favours the oxazolone method because there can be neither racemization nor acylation at the alpha carbon atom. Consequently the oxazolones (II) derived from α-methylalanine are, in general, easily manipulated, and they give exceptionally high yields in addition reactions (3) with amines. Indeed the early literature contains examples of the preparation of acetyl and benzoyl peptides containing a residue of α-methylalanine by the oxazolone method.[15] 4,4-Dimethyl-2-trifluoromethyloxazolone (II; R CF_3), prepared (Reaction 2) by a modification of the general method of Weygand and Glöckler[16], is an excellent reagent for the addition of a single α-methylalanyl residue to an amine. Together, this coupling and removal of the trifluoroacetyl group by methanolic hydrogen chloride provide a repetitive method for lengthening the peptide chain of methyl esters. However, alkaline hydrolysis of the trifluoroacetylamino group, and simultaneously the ester group, is more efficient. Thus trifluoroacetyl-α-methylalanyl-α-methylalanine methyl ester was converted, by direct carbobenzoxylation of the alkaline hydrolysate, almost quantitatively into the benzyloxycarbonyl dipeptide. In turn this was dehydrated, as mentioned above, to the crystalline oxazolone, which also reacted cleanly with α-methylalanine methyl ester. Similarly

* With the more polar dimethylformamide as solvent, the yield was lower and some pivaloyl-α-methylalanine methyl ester was also formed.

[14] M. Zaoral, *Angew. Chem.* **71**, 743 (1959).
[15] E. Mohr, *J. Prakt. Chem.* **81**, 49, 473 (1910); C. Gränacher and M. Mahler, *Helv. Chim. Acta* **10**, 246 (1927); R. E. Steiger, *Ibid.* **17**, 563 (1934).
[16] F. Weygand and U. Glöckler, *Chem. Ber.* **89**, 653 (1956).

the tosyl derivatives of the di-, tri-, and tetra-peptides of α-methylalanine all gave crystalline, well behaved oxazolones; the tosyl dipeptide was lengthened at the carboxyl end up to the tosyl pentapeptide in 50 per cent overall yield. The oxazolone from tosyl-α-methylalanyl-glycine was obtained in only 60 per cent yield, probably owing to side reactions of the methylene group, but it reacted satisfactorily with α-methylalanine methyl ester in boiling acetone in one hour, conditions too mild for the derivative of α-methylalanyl-α-methylalanine. For this and its homologues acetonitrile was the preferred solvent, and it was better to employ, as the other reagent, the free ester, than its hydrochloride and triethlyamine.

As the formyl protecting group can be removed easily with methanolic hydrogen chloride, we were attracted by 4,4-dimethyl-oxazolone (II; R = H). We have not yet isolated this rather unstable compound, but it is well characterized by the infra-red spectrum of the carbon tetrachloride solution after reaction between formyl-α-methyl-alanine and thionyl chloride in the presence of triethylamine.* (Reaction between this acid and acetic anhydride gave an N-acetylated mixed anhydride (IV) instead of the oxazolone). The solution of 4,4-dimethyloxazolone gave the expected reaction 3 with cyclohexylamine and with glycine ester, although the yield was only 47 per cent in the latter instance. With α-methylalanine methyl ester almost the entire reaction took Path 4, which has an analogy in part of the penillic acid change.[18] Just as aldehydes are more reactive than ketones, so 4,4-dimethyloxazolone should be the oxazolone (II) most prone to reaction 4 and evidently the competition between 3 and 4 in this case is mainly determined by steric hindrance of the normal Reaction 3. Presumably some of the reaction with glycine ester goes along Path 4.

The structure (III; R' = $CMe_2 \cdot CO_2Me$) of the abnormal product from 4,4-dimethyloxazolone and α-methylalanine methyl ester is secured by the following evidence. It formed a hydrochloride which was a relatively strong acid (p*Ka* 2·84), comparable to α-amino acid hydrochlorides. On being heated, the amidine (III; R' = $CMe_2 \cdot CO_2Me$) cyclized with loss of methanol yielding the imidazolone carboxylic acid (V; R" = H); a minor product was the corresponding methyl ester (V; R" = Me) formed by the alternative cyclization with loss of water. This imidazolone methyl ester was obtained directly by reaction between α-methylalanine methyl ester and ethyl orthoformate. In addition to confirming the structure assigned to the imidazolones (V), this reaction shows that the abnormal product (III) results from attack by the amine at the methine group (Position 2) in 4,4-dimethyloxazolone.

Attempts to obtain the free amidine dimethyl ester corresponding to the

* Unsuccessful attempts to prepare unsubstituted oxazolone hydrochloride have been mentioned.[17] Infra-red spectroscopy has not detected free oxazolone as being produced from formylglycine under our conditions, i.e. in the presence of triethylamine.

[17] F. E. King, J. W. Clark-Lewis, D. A. A. Kidd and G. R. Smith, *J. Chem. Soc.* 1039 (1954).
[18] R. B. Woodward, *Chemistry of Penicillin* (Edited by H. T. Clarke, J. R. Johnson and Sir Robert Robinson) p. 445. University Press, Princeton (1949).

zwitterion (III; R = $CMe_2 \cdot CO_2Me$) were unsuccessful. Thus α-methylalanine methyl ester was converted by formimidoisopropyl ether to the imido ether (VI),[19] but this with α-methylalanine methyl ester again yielded the imidazolone (V; R″ = Me). The imidazolone was also formed by reaction between α-methylalanine methyl ester and dichloromethyl methyl ether, and also when the hydrochloride of (III; R = $CMe_2 \cdot CO_2Me$) was treated with 2 equivalents of diazomethane. Evidently the amidine dimethyl ester cyclizes too rapidly to be isolated. With cyclohexylamine, the imido ether (VI) yielded the N-cyclohexylimidazolone, also formed by thermal dehydration of formyl-α-methylalanine cyclohexylamide in the presence of acid.

The infra-red spectra[20] of the amidine (III; R = $CMe_2 \cdot CO_2Me$), N,N′-diphenyl and N,N′-dicyclohexylformamidines and their hydrochlorides are recorded in the Experimental section, since these show anomalies doubtless due to strong molecular association.

EXPERIMENTAL

Melting points are uncorrected. Infra-red spectra are of Nujol mulls determined with a Perkin–Elmer Model 21 Spectrophotometer (NaCl optics) unless otherwise stated. Evaporations are under reduced pressure. Neutral products were isolated by washing in ethyl acetate solution successively with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water.

General method for the preparation of oxazolones. A solution of the acylamino acid or peptide (5 mmoles) in acetic anhydride (20 cc) was heated at 110–120° (oil bath) for 15 min and then evaporated. Last traces of acetic anhydride were removed by addition of toluene (20 cc) and evaporation, and the residual oxazolone recrystallized.

α-Methylalanine methyl ester. The methyl ester hydrochloride was prepared from α-methylalanine[21] by the usual Fischer method. The methyl ester (70% yield from the amino acid), b.p. 130–138°, was liberated by ammonia in chloroform solution.[22]

α-Methylalanyl-α-methylalanine. The *hydrobromide*, m.p. 213–215° (Found: C, 35·5; H, 6·6; N, 10·2. $C_8H_{17}O_3N_2Br$ requires: C, 35·7; H, 6·4; N, 10·4%) was obtained from the benzyloxycarbonyl derivative (see below) by the action of hydrogen bromide in acetic acid. Passage of an aqueous solution of the hydrobromide through a column of Dowex-1 × 4 anion-exchange resin (acetate form) afforded the free *dipeptide*, no m.p. below 300°, ν_{max} 3378, 3077, 2564, 2151, 1684, 1653, 1634, 1610, 1575, 1493, 1351, 1300, 1290, 1241, 1209, 1168, 1094, 1003, 955, 939, 918, 880, 798, 794, 769 cm^{-1} (Found: C, 51·2; H, 8·75; N, 14·7. $C_8H_{16}O_3N_2$ requires: C, 51·05; H, 8·6; N, 14·9%). Abderhalden and Gebelein[16] give m.p. 244–246°.

Trifluoroacetyl Derivatives

Trifluoroacetyl-α-methylalanine

Trifluoroacetic anhydride (4·41 g, slight excess) was added gradually to an ice-cooled solution of α-methylalanine (2·06 g, dried at 100°) in trifluoroacetic acid (8·5 g, distilled from a little phosphoric oxide). After standing overnight at room temp. the solution was evaporated and the crystalline residue extracted with ether. Dilution of the ether with light petroleum and partial evaporation afforded needles of *trifluoroacetyl-α-methylalanine* (3·61 g, 90%), m.p. 167–171·5°. The analytical sample, m.p. 170·5–172°, was sublimed at 140°/14 mm (Found: C, 36·2; H, 4·2; N, 7·3. $C_6H_8O_3NF_3$ requires: C, 36·2; H, 4·1; N, 7·0%).

4,4-*Dimethyl-2-trifluoromethyloxazolone* (II; R = CF_3)

Throughout the preparation, atmospheric moisture was excluded by drying tubes containing phosphoric oxide. Trifluoroacetyl-α-methylalanine (5·20 g, dried at 80°) was dissolved in thionyl chloride (6 cc) at 50–70°, with vigorous evolution of hydrogen chloride. The excess thionyl chloride

[19] cf. J. W. Cornforth and R. H. Cornforth, *J. Chem. Soc.* 96 (1947).
[20] cf. J. Fabian, M. Legrand, and P. Poirier, *Bull. Soc. Chim. Fr.* 1499 (1956).
[21] H. T. Clarke and H. J. Bean, *Org. Syntheses* Coll. Vol. II, 29 (1943).
[22] G. Hillman, *Z. Naturf.* 1, 682 (1946).

was evaporated at room temp and quinoline (3·87 g, slight excess) added to the residue. The mixture was heated for a few min at 80–120° (oil bath) and then distilled, yielding *4,4-dimethyl-2-trifluoromethyloxazolone* (3·76–4·27 g, 80–91%), b.p. 112–116°, ν_{max} (film) 3003, 2950, 1835, 1689, 1462, 1439, 1385, 1366 cm^{-1} (Found: C, 40·2; H, 3·3; N, 7·5. $C_6H_6O_2NF_3$ requires: C, 39·8; H, 3·3; N 7·7%). The compound is rapidly hydrolysed by atmospheric moisture but otherwise stable. In the absence of quinoline the acid chloride, unlike those of other trifluoroacetylamino acids,[16] sublimes unchanged.

Trifluoroacetyl-α-methylalanyl-α-methylalanine methyl ester

α-Methylalanine methyl ester (1·17 g, slight excess) was added gradually to an ice-cold solution of 4,4-dimethyl-2-trifluoromethyloxazolone (1·27 g) in dry acetonitrile (5 cc). The mixture was kept for 10 min before being heated (steam bath) for 10 min and evaporated. Recrystallization of the residue from benzene afforded the *dipeptide derivative* (2·06 g, 98%), m.p. 111·5–115° raised to 113·5–115·5° by a second recrystallization (Found: C, 44·4; H, 5·6; N, 9·3. $C_{11}H_{17}O_4N_2F_3$ requires: C, 44·3; H, 5·7; N, 9·4%).

Trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester

Hydrogen chloride was bubbled through a boiling solution of the foregoing dipeptide derivative (1·5 g, 5 mmoles) in dry methanol (100 cc) during 2½ hr. After evaporation, the hygroscopic residue (1·15 g), was dissolved in dry acetonitrile (20 cc), triethylamine (0·51 g, 5 mmoles) added and the solution filtered. 4,4-Dimethyl-2-trifluoromethyloxazolone (0·906 g, 5 mmoles) was added to the ice-cooled filtrate, which was kept for 10 min before being heated (steam bath) for 10 min. The acetonitrile was evaporated and the neutral fraction isolated. When the dried (Na_2SO_4) ethyl acetate solution was concentrated and cooled it furnished the *tripeptide derivative* (1·14 g, 60%), m.p. 197–199° (Found in material sublimed at 150–175°/0·1 mm: C, 47·1; H, 6·2; N, 11·0. $C_{14}H_{24}O_5N_3F_3$ requires: C, 47·0; H, 6·3; N, 11·0%).

Benzyloxycarbonyl Derivatives

Benzyloxycarbonyl-α-methylalanine

To an ice-cold solution of α-methylalanine (5·02 g) in 1·67 N NaOH (30 cc) and acetone (25 cc), adjusted to pH 10·9, was gradually added benzyl chloroformate (16 g of 80% pure) in dry acetone (25 cc) during 70 min with stirring. The pH of the solution was maintained at 10·8–10·9 by simultaneous addition of 2 N NaOH (37 cc). The mixture was stirred during 2 hr at room temp before being concentrated and acidified. The precipitated oil was extracted into ethyl acetate and the acidic fraction isolated with sodium carbonate solution in the usual manner. The oily product crystallized slowly, and was recrystallized from ether–light petroleum (10·9 g, 92%), m.p. 67–70° raised to 72·5–74·5° by one further recrystallization from benzene–light petroleum (Found: C, 60·7; H, 6·6; N, 5·95. Calc. for $C_{12}H_{15}O_4N$: C, 60·75; H, 6·4; N, 5·9%). Bergmann *et al.*[6] report m.p. 78°.

Benzyloxycarbonyl-α-methylalanine cyanomethyl ester

A mixture of benzyloxycarbonyl-α-methylalanine (5·93 g), triethylamine (5·28 cc), and chloroacetonitrile (7·55 g) was kept at 70–75° during 40 min. After the excess chloroacetonitrile had been evaporated the neutral product (6·33 g, 90%), m.p. 42–47°, was isolated with ethyl acetate in the usual manner. Recrystallization from ether at −10° furnished the pure *cyanomethyl ester*, m.p. 45–48·5° (Found: C, 61·1; H, 5·5; N, 10·05. $C_{14}H_{16}O_4N_2$ requires: C, 60·9; H, 5·8; N, 10·1%). This ester (2·76 g) was recovered in 97% yield after being heated during 4 hr with a boiling mixture of ethyl acetate (3 cc), α-methylalanine methyl ester (2·34 g), and acetic acid (0·030 g) either with or without triethylamine (5 cc).

Benzyloxycarbonyl-α-methylalanine cyclohexylamide

The general route[23] via the lithium salt of benzyloxycarbonyl-α-methylalanine and the sulphuric anhydride gave an 83% yield of the crude neutral product. Recrystallization from benzene–light petroleum furnished the pure *cyclohexylamide* (76%), m.p. 124–127° (Found: C, 68·1; H, 7·95; N, 8·8. $C_{18}H_{26}O_3N_2$ requires: C, 67·9; H, 8·2; N, 8·8%).

[23] D. W. Clayton, J. A. Farrington, G. W. Kenner and J. M. Turner, *J. Chem. Soc.* 1398 (1957).

Benzyloxycarbonyl-α-methylalanine pivalic acid mixed anhydride (I)

Pivaloyl chloride (3·6 g, 30 mmole) was added to a stirred and cooled (−5°) solution of benzyloxycarbonyl-α-methylalanine (7·11 g, 30 mmoles) and triethylamine (3·03 g, 30 mmoles) in dry toluene (15 cc). After 2 hr at 5° and 1 hr at room temp, the solution was filtered and the filtrate evaporated to yield the *mixed anhydride* (9·52 g, 99%), m.p. 82–86°. The analytical sample had m.p. 81–83° after recrystallization from toluene–light petroleum, ν_{max} 3290, 1805, 1736, 1678 and 1531 cm^{-1} (Found: C, 63·6; H, 7·3; N, 4·6. $C_{17}H_{23}NO_5$ requires: C, 63·5; H, 7·2; N, 4·3%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester

The foregoing mixed anhydride (6·42 g, 20 mmoles) was added to dry toluene (100 cc) containing α-methylalanine methyl ester (2·67 g, 22 mmoles) and the solution heated at 60° for 3 hr. After standing overnight at room temp, the reaction mixture was diluted with ethyl acetate and the neutral product isolated in the usual manner. Recrystallization from ether–light petroleum afforded the *dipeptide derivative* (5·9 g, 89%), m.p. 107–109°. A sample recrystallized once further had m.p. 109–111° (Found: C, 60·7; H, 7·2; N, 7·9. $C_{17}H_{24}O_5N_2$ requires: C, 60·7; H, 7·2; N, 8·3%). When the toluene was replaced by dimethylformamide as solvent, the crude neutral product contained pivaloyl-α-methylalanine methyl ester (20–30%, I.R. spectrum), and the isolated yield of pure dipeptide derivative was lower (57%).

Pivaloyl-α-methylalanine methyl ester

This *compound*, prepared from α-methylalanine methyl ester hydrochloride (1·53 g, 10 mmoles), triethylamine (2·02 g, 20 mmoles), and pivaloyl chloride (1·20 g, 10 mmoles) in toluene (20 cc) kept overnight at room temp, had m.p. 92–93° after two crystallizations from ether (Found: C, 60·1; H, 9·7; N, 6·7. $C_{10}H_{19}NO_3$ requires: C, 59·7; H, 9·5; N, 7·0%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanine

(a) A mixture of trifluoroacetyl-α-methylalanyl-α-methylalanine methyl ester (2·98 g) and N NaOH (30 cc) was kept overnight at room temp. Trifluoroacetic acid was added to pH 6 and the crystalline residue from evaporation dissolved in water (20 cc) and acetone (15 cc) and brought to pH 10·9 by addition of N NaOH. Benzyl chloroformate (3·20 g of 80%) in acetone (20 cc) was added during 35 min to the cooled and stirred solution of dipeptide, the pH being maintained at 10·7–11 by further addition of alkali. Stirring was continued at room temp for 1 hr, and then the usual extraction procedure furnished the *benzyloxycarbonyl dipeptide* (3·05 g, 94%), m.p. 157–159° raised by recrystallization from aqueous methanol to 161–162·5° (Found: C, 59·7; H, 6·6; N, 8·9. $C_{16}H_{22}O_5N_2$ requires: C, 59·6; H, 6·9; N, 8·7%). Diazomethane in ethereal solution converted this acid into the foregoing methyl ester, m.p. and mixed m.p. 111°.

(b) A solution of benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester (0·336 g, 1 mmole) in dioxan (5 ml) containing 2 N HCl (1 cc) was heated under reflux for 6 hr and then left overnight at room temp. After evaporation of the dioxan, the acidic product was isolated with ethyl acetate and sodium carbonate solution in the usual way. Evaporation yielded the benzyloxycarbonyl dipeptide (0·233 g, 72·5%), m.p. 140–154°, raised by one recrystallization from aqueous methanol to 157–160°.

2-(1′-*Benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone*

(II; R = $C_6H_5CH_2$·O·CO·NH·CMe_2)

(a) This *oxazolone* was prepared from benzyloxycarbonyl-α-methylalanyl-α-methylalanine (1·23 g) by the general procedure already described. Yield 1·19 g (98%), m.p. 125–126·5° (Found: C, 63·3; H, 6·7; N, 9·2. $C_{16}H_{20}O_4N_2$ requires: C, 63·1; H, 6·6; N, 9·2%).

(b) Ethyl chloroformate (0·11 cc, 1 mmole) was added to a solution at −5° of benzyloxycarbonyl-α-methylalanyl-α-methylalanine (0·322 g, 1 mmole) and triethylamine (0·14 cc, 1 mmole) in dry toluene (10 cc). The mixture was kept at 5° for 2 hr and at room temp for 1 hr before being filtered and evaporated at low temp. There was obtained 0·320 g (103%) of crude oxazolone, m.p. 120–123°, identical in I.R. spectrum with the above product.

(c) Dehydration of the dipeptide derivative (1 mmole) with pivaloyl chloride (1·5 mmole) and triethylamine (1·5 mmoles) under the same conditions as in the preceding experiment likewise

afforded the crude oxazolone (98%), m.p. 122–123° after recrystallization from ethyl acetate–light petroleum (yield 80%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester

A solution of the foregoing oxazolone (0·364 g) and α-methylalanine methyl ester (0·35 g) in acetonitrile (8 cc) was kept at 100° during 6 hr and then evaporated. Recrystallization of the residue from aqueous methanol afforded the *tripeptide derivative* (0·461 g, 91%), m.p. 145–146° (Found: C, 59·8; H, 7·2; N, 10·25. $C_{21}H_{31}O_6N_3$ requires: C, 59·8; H, 7·4; N, 10·0%).

Tosyl Derivatives

Tosylglycyl-α-methylalanine methyl ester

A solution of tosylglycyl chloride (2·48 g, 10 mmoles) in dry acetone (20 cc) was added in 4 portions to a cooled, shaken solution of α-methylalanine methyl ester (2·46 g, 21 mmoles) in acetone (25 cc). The mixture was cooled for 30 min further and then left for 1 hr at room temp. The precipitated α-methylalanine methyl ester hydrochloride (1·02 g) was collected before the acetone was evaporated. Concentration of the solution of the neutral product in ethyl acetate yielded the *dipeptide derivative* (2·33 g, 71%), m.p. 113–116°. The analytical sample was recrystallized from aqueous methanol, m.p. 113·5–115 (Found: C, 51·4; H, 6·4; N, 8·5. $C_{14}H_{20}O_5N_2S$ requires: C, 51·2; H, 6·1; N, 8·5%). A similar preparation (threefold scale), in which a mixture of α-methylalanine methyl ester hydrochloride (5·06 g, 33 mmole), triethylamine (6·6 g, 66 mmoles) and dimethylformamide (30 cc) was substituted for the solution of ester (63 mmoles) in acetone, yielded only 47% of the dipeptide derivative.

Tosylglycyl-α-methylalanine

A solution of the foregoing methyl ester (6·23 g, 19 mmoles) in 2 N NaOH (20 cc) was shaken at room temp during 4 hr and then acidified. Recrystallization from aqueous methanol afforded *tosylglycyl-α-methylalanine* (5·80 g, 97%), m.p. 177–178·5° (Found: C, 49·6; H, 5·7; N, 8·9. $C_{13}H_{18}O_6N_2S$ requires: C, 49·7; H, 5·8; N, 8·9%).

Tosyl-α-methylalanine

The following procedure is preferable to published methods. A solution of tosyl chloride (41·8 g, 0·22 mole) in dry acetone (100 cc) and simultaneously 2 N NaOH (110 cc) were added dropwise during 1 hr to a vigorously stirred and cooled solution of α-methylalanine (20 g, 0·2 mole) in 2·5 N NaOH (80 cc) and acetone (80 cc). Stirring was continued for 20 min and then the solution was allowed to reach room temp and left for 1 hr. Acidification after partial evaporation yielded material (35·5 g, 69%) sufficiently pure for further work, m.p. 142–145° raised by recrystallization from aqueous methanol to 151–152° (Found: C, 51·3; H, 6·1; N, 5·2. Calc. for $C_{11}H_{14}O_4NS$: C, 51·4; H, 5·9; N, 5·45%). Previously reported m.p.s are 147°,[24] 143°,[25] 149–150°.[11b]

Tosyl-α-methylalanyl chloride

To avoid darkening of the solution, the reaction between tosyl-α-methylalanine and thionyl chloride was carried out at 10–18° during 3 hr and it yielded 73% of material sufficiently pure for further work, m.p. 116–117° (dec) raised by recrystallization from benzene to 120–121° (Found: C, 48·2; H, 5·4; N, 5·0; Cl, 12·9. Calc. for $C_{11}H_{14}O_3NSCl$: C, 47·9; H, 5·1; N, 5·1; Cl, 12·9%). Previously m.p. has been reported[11b] as 115–116°.

Tosyl-α-methylalanylglycine

A solution of tosyl-α-methylalanyl chloride (2·10 g, 75 mmoles) in dry acetone (8 cc) was added gradually during 1 hr to a well shaken and cooled mixture of glycine (1·12 g, 15 mmoles), magnesium oxide (0·80 g), acetone (5 cc), and water (15 cc). The thick mixture was shaken at room temp for 1½ hr before being diluted with water (20 cc) and acidified with strong hydrochloric acid. Recrystallization, from water containing a few drops of methanol, of the crude product (2·05 g) obtained by

[24] F. Fichter and M. Schmid, *Helv. Chim. Acta* **3**, 704 (1920).
[25] R. H. Wiley, N. R. Smith and J. P. Johansen, *J. Amer. Chem. Soc.* **74**, 6298 (1952).

concentration and cooling, furnished *tosyl-α-methylalanylglycine* (1·70 g, 71%), m.p. 150–151° (Found: C, 49·65; H, 5·8; N, 8·7. $C_{13}H_{18}O_6N_2S$ requires: C, 49·7; H, 5·8; N, 8·9%).

Tosyl-α-methylalanylglycine ethyl ester

(a) Ethyl chloroformate (2·05 g, 20 mmoles) was added gradually to a solution, stirred at −5°, of tosyl-α-methylalanine (5·15 g, 20 mmoles) and triethylamine (2·05 g, 20 mmoles) in dry toluene (80 cc); the mixture set to a gel. After 30 min a solution of glycine ethyl ester hydrochloride (2·80 g 20 mmoles) and triethylamine (2·05 g) in dry dimethylformamide (50 cc) was added dropwise, and stirring at −2° was continued for 1½ hr. Next day the *dipeptide derivative* (1·65 g, 24%) was isolated in the usual way, m.p. 141–144° raised, by recrystallization from aqueous methanol, to 149–151° (Found: C, 52·4; H, 6·2; O, 23·7; N, 8·0. $C_{16}H_{22}O_6N_2S$ requires: C, 52·6; H, 6·5; O, 23·4; N, 8·2%).

(b) Glycine ethyl ester (2·48 g, 24 mmoles) was added to a solution of tosyl-α-methylalanine (3·08 g, 12 mmoles) in acetonitrile (100 cc). After a few min crystals separated. Dicyclohexyl carbodi-imide (2·48 g, 12 mmoles) was added and the mixture was shaken for 10 hr. The yield of dipeptide derivative, m.p. 142–146°, was 2·20 g (53%).

2-(1′-*Methyl*-1′-*toluene*-p-*sulphonamido*)*ethyloxazolone*

Application of the general method to tosyl-α-methylalanylglycine and recrystallization of the product from toluene furnished the *oxazolone* (61%), m.p. 172–173° (Found: C, 52·6; H, 5·4; N, 9·0. $C_{13}H_{14}O_4N_2S$ requires: C, 52·7; H, 5·4; N, 9·5%).

Tosyl-α-methylalanylglycyl-α-methylalanine methyl ester

A mixture of the foregoing oxazolone (0·266 g, 0·9 mmole), α-methylalanine methyl ester (0·212 g 1·8 mmoles), and dry acetone (10 cc) was kept for 30 min at room temp and then boiled for 30 min. Next day the solution was evaporated. Recrystallization, from ethyl acetate containing a trace of acetone, of the residue gave the *tripeptide derivative* (0·26 g, 70%), m.p. 193–194·5° (Found: C, 52·1; H, 6·8; N, 10·5. $C_{18}H_{27}O_6N_3S$ requires: C, 52·3; H, 6·6; N, 10·2%).

Tosyl-α,N-dimethylalanine

Dimethyl sulphate (7·5 g) and 4 N NaOH (12·5 cc) were added gradually during 1 hr to a shaken, cooled solution of tosyl-α-methylalanine (5·15 g) and sodium hydroxide (1·2 g) in water (30 cc). The mixture was shaken at room temp for 2½ hr further and then heated to 100° for 5 min. After removal of the precipitated solid (1·6 g), the product was obtained by acidification as an oil which slowly crystallized. Fractional crystallization from benzene and aqueous methanol afforded *tosyl-α,N-dimethylalanine* (2·68 g, 50%), m.p. 142·5–145° (Found: C, 52·8; H, 6·2; N, 5·1. $C_{12}H_{17}O_4NS$ requires: C, 53·1; H, 6·3; N, 5·2%).

Tosyl-α,N-dimethylalanyl chloride

Reaction, initially at room temp and after 2 hr at 45° for 10 min, between the foregoing acid and thionyl chloride, and recrystallization of the product from light petroleum (b.p. 60–80°) furnished *tosyl-α,N-dimethylalanyl chloride* (63%), m.p. 79–80° (Found: C, 50.0; H, 5.5;; N, 4·6. $C_{12}H_{16}O_3NSCl$ requires: C, 49·7; H, 5·6; N, 4·8%). Tosyl-α,N-dimethylalanine (88%) was recovered from hydrolysis of this acid chloride by sodium hydroxide in aqueous acetone.

Tosyl-α,N-dimethylalanyl-α-methylalanine

The foregoing acid chloride was reacted with α-methylalanine as in the preparation of tosyl-α-methylalanylglycine already described. The product, m.p. 112–160°, was a mixture of tosyl-α,N-dimethylalanine and the required *tosyl dipeptide*, which was separated through its insolubility in benzene and purified by recrystallization from aqueous methanol (yield about 8%), m.p. 210·5–212° (Found: C, 53·6; H, 6·8; N, 7·45. $C_{16}H_{24}O_6N_2S$ requires: C, 53·9; H, 6·8; N, 7·9%).

Tosyl-α-methylalanyl-α-methylalanine methyl ester

A solution of tosyl-α-methylalanyl chloride (2·76 g, 10 mmoles) in dry acetone (15 cc) was added in 3 portions to a cooled solution of α-methylalanine methyl ester (2·46 g, 21 mmoles) in acetone (20 cc).

Shaking was continued at room temp for 30 min before α-methylalanine methyl ester hydrochloride (1·33 g) was filtered off and the acetone evaporated. The *dipeptide derivative* (2·97 g, 83%), m.p. 146 147·5° (Found: C, 54·1; H, 6·9; N, 7·8. $C_{16}H_{24}O_6N_2S$ requires: C, 53·9; H, 6·8; N, 7·9%), crystallized from the concentrated ethyl acetate solution of the neutral product. When a mixture of α-methylalanine methyl ester hydrochloride (1·2 equivalents), triethylamine (2·4 equivalents), and dimethylformamide was brought into reaction with the acid chloride, the yield was only 71%.

Tosyl-α-methylalanyl-α-methylalanine

A solution of the foregoing ester (3·56 g, 10 mmoles) in 2 N NaOH (10 cc) was shaken at room temp during 4 hr. Acidification with dil hydrochloric acid furnished the *tosyl dipeptide* (3·41 g, 99%), m.p. 214–218° raised, by recrystallization from methanol, to 216–218° (Found: C, 52·6; H, 6·5; N, 8·4. $C_{15}H_{22}O_6N_2S$ requires: C, 52·6; H, 6·5; N, 8·2%).

4,4-*Dimethyl*-2-(1′-*methyl*-1′-*toluene*-p-*sulphonamido*)*ethyloxazolone* (II; R = $C_7H_7SO_2NHCMe_2$)

Recrystallization from light petroleum (b.p. 80–100°) of the product from application of the general method to the foregoing tosyl dipeptide furnished the *oxazolone* (94%), m.p. 140–141·5° (Found: C, 55·2; H, 6·3; N, 8·4. $C_{15}H_{20}O_4N_2S$ requires: C, 55·55; H, 6·2; N, 8·6%).

Tosyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester

A solution of the foregoing oxazolone (0·649 g, 2 mmoles) and α-methylalanine methyl ester (0·351 g, 3 mmoles) in acetonitrile (10 cc) was kept at 100° during 4 hr and then evaporated. Recrystallization of the residue from benzene light petroleum furnished the *tripeptide derivative* (0·844 g, 95%), m.p. 156–158° (Found: C, 54·45; H, 7·1; N, 9·5. $C_{20}H_{31}O_8N_3S$ requires: C, 54·4; H, 7·1; N, 9·5%). An attempt to carry out the reaction under the conditions already described for tosyl-α methylalanylglycyl-α-methylalanine methyl ester was unsuccessful, and 80% of the oxazolone was recovered.

Tosyl-α-methylalanyl-α-methylalanyl-α-methylalanine

A solution of the foregoing ester (0·662 g, 1·5 mmoles) in 2 N NaOH (2 cc) was shaken at room temp during 5 hr and then acidified. The oil slowly crystallized, and recrystallization from aqueous methanol yielded the *tosyl tripeptide* (0·609 g, 89%), which was dried at 100°/0·1 mm to remove water of crystallization, m.p. 205–207° (Found: C, 53·1; H, 6·8; N, 9·4. $C_{19}H_{29}O_8N_3S$ requires C, 53·4; H, 6·8; N, 9·8%).

4,4-*Dimethyl*-2-(1′-*methyl*-1′-*tosyl*-α-*methylalanylamino*)*ethyloxazolone*
(II; R = $C_7H_7SO_2NHCMe_2CONHCMe_2$)

The *oxazolone* (99%), m.p. 148 149° (Found: C, 56·1; H, 6·5; N, 10·0. $C_{19}H_{27}O_5N_3S$ requires: C, 55·7; H, 6·65; N, 10·3%), was obtained by recrystallization from benzene light petroleum (b.p. 80–100°) of the product from the preceding tosyl-tripeptide.

Tosyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester

A solution of the foregoing oxazolone (3·27 g, 8 mmoles) and α-methylalanine methyl ester (1·40 g, 12 mmoles) in acetonitrile (15 cc) was kept at 100° during 3 hr. When it was cooled most (3·05 g) of the product, m.p. 217–218°, crystallized and a second crop was obtained from the liquor by extraction of the neutral material. Recrystallization of the total from methanol afforded the *tetrapeptide derivative* (3·36 g, 80%), m.p. 218–219° (Found: C, 55·2; H, 7·3; N, 10·9. $C_{24}H_{38}O_7N_4S$ requires: C, 54·7; H, 7·3; N, 10·6%).

Tosyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine

When hydrolysis was carried out as already described for the tripeptide derivative, it was incomplete and 23% of the ester was recovered through its insolubility in sodium hydrogen carbonate solution. When this fraction had been treated again with sodium hydroxide, the combined yield of directly crystallized *tosyl tetrapeptide* was 87%, m.p. 260 262° (Found: C, 53·6; H, 6·9; N, 10·7. $C_{23}H_{36}O_7N_4S$ requires: C, 53·9; H, 7·1; N, 10·9%).

4,4-*Dimethyl*-2-(1′-*methyl*-1′-*tosyl*-α-*methylalanyl*-α-*methylalanylamino*)*ethyloxazolone* (II; R $C_7H_7SO_2{\cdot}NH{\cdot}CMe_2{\cdot}CO{\cdot}NH{\cdot}CMe_2{\cdot}CO{\cdot}NH{\cdot}CMe_2$)

The *oxazolone* (95%), m.p. 224 225° (Found: C, 56·1; H, 6·9; N, 11·4. $C_{22}H_{34}O_6N_4S$ requires: C, 55·9; H, 6·9; N, 11·3%), was obtained by recrystallization from benzene of the product from the tosyl-tetrapeptide.

Tosyl-α-*methylalanyl*-α-*methylalanyl*-α-*methylalanyl*-α-*methylalanyl*-α-*methylalanine methyl ester*

(a) Trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester (1·532 g, 4 mmoles) was treated with methanolic hydrogen chloride and then triethylamine as in its own preparation, and then 4,4-dimethyl-2-(1′-methyl-1′-toluene-*p*-sulphonamido)ethyloxazolone (1·296 g, 4 mmoles) was added to the acetonitrile solution. The mixture was boiled for 2 hr and then the neutral product was isolated. Recrystallization from aqueous methanol of the material which had crystallized from the concentrated ethyl acetate extract furnished the *pentapeptide derivative* (0·451 g, 19%), m.p. 251-252·5 (Found: C, 54·8; H, 7·3; N, 11·5. $C_{25}H_{45}O_8N_5S$ requires: C, 55·0; H, 7·4; N, 11·45%).

(b) α-Methylalanine methyl ester was combined with the foregoing oxazolone, derived from the tosyl tetrapeptide, just as with the oxazolone from the tosyl tripeptide, and the product (91%), m.p. 251°, was isolated in the same way. By this route the overall yield from tosyl dipeptide was 50%.

Derivatives of Formyl-α-*methylalanine*

Formyl-α-*methylalanine*

Acetic anhydride (160 cc) was added during 15 min to a stirred solution kept at 7–12° of α-methylalanine (20·6 g) in 98% formic acid (400 cc). The mixture was stirred at 15° for 30 min and at room temp for 2 hr. Finally it was diluted with ice-cold water (160 cc) and concentrated. Cooling to −10° and then recrystallization (at −10°) from water (25 cc) afforded the water-soluble *formyl*-α-*methylalanine* (20·0 g, 76%), m.p. 145–147° (Found: C, 46·0; H, 6·9; N, 10·75. $C_4H_9O_3N$ requires: C, 45·8; H, 6·9; N, 10·7%).

N-*Acetyl*-N-*formyl*-α-*methylalanine acetic anhydride* (IV)

Application of the general method for preparing oxazolones to formyl-α-methylalanine (2·62 g) and recrystallization of the product from benzene–light petroleum (b.p. 60–80°) furnished the *mixed anhydride* (IV) (2·58 g, 60%), m.p. 64–66°, ν_{max} 1815, 1764, 1686 cm^{-1} (Found: C, 50·4; H, 6·2; N, 6·7. $C_8H_{13}O_5N$ requires: C, 50·2; H, 6·1; N, 6·5%). The same compound (3·72 g 86%) was obtained from a mixture of formyl-α-methylalanine (2·62 g, 20 mmoles), triethylamine (2·02 g, 20 mmoles) and acetic anhydride (35 cc) which had been kept during 32 hr at room temp and then evaporated at low temp. Its alkaline hydrolysis at room temp afforded acetyl-α-methylalanine, recrystallized from water, m.p. 198 200·5° (reported[26] m.p. 196°) (Found: C, 49·4; H, 7·6; N, 9·9. Calc. for $C_6H_{11}O_3N$: C, 49·6; H, 7·6; N, 9·65%).

N-(*Isopropoxymethylene*)-α-*methylalanine methyl ester* (VI)

α-Methylalanine methyl ester (1·89 g, 12·3 mmole) and formimidoisopropyl ether hydrochloride[19] (1·52 g, 12·3 mmoles) were added at 0° to aqueous potassium hydroxide (2·7 ml of 25%) under ether (20 ml). After shaking at 0° for 10 min and at room temp for 30 min, the ether layer was decanted, the salt sludge washed twice with fresh ether, and the combined ether extracts washed with a little water, dried (Na_2SO_4) and evaporated. Distillation of the oily residue (1·1 g, 48%) yielded the *ester*, b.p. 57 /4 mm (Found: C, 57·9; H, 9·3; N, 7·5. $C_9H_{17}O_3N$ requires C, 57·7; H, 9·15, N, 7·5%).

4,4-*Dimethyloxazolone* (II, R = H)

Triethylamine (4·04 g, 40 mmoles) was added to a suspension of formyl-α-methylalanine (2·62 g, 20 mmoles) in carbon tetrachloride (40 cc), and the mixture was warmed until a clear solution was obtained. This solution was cooled in ice and shaken while thionyl chloride (2·38 g, 20 mmoles) in

[26] P. A. Levene and R. E. Steiger, *J. Biol. Chem.* **93**, 581 (1931); N. C. Hancox, *Aust. J. Sci. Res.* **3A**, 450 (1950).

carbon tetrachloride (10 cc) was added in 3 portions during 3 min. There was immediate precipitation of triethylamine hydrochloride and evolution of sulphur dioxide. After the mixture had been shaken without cooling during 5 min, the triethylamine hydrochloride (5·64 g) was removed by filtration. The solution of 4,4-dimethyloxazolone had major peaks in the infra-red spectrum (after compensation for solvent absorption) at 1850, 1638, 1202, 1104, 1008, 963, 917, 875 cm^{-1} and it could be fractionally distilled at 76° and atmospheric pressure without qualitative change of the spectrum, but there was a non-volatile residue. The solution decomposed gradually during storage.

For preparative purposes benzene is slightly preferable to carbon tetrachloride as the solvent, but neither ether nor petroleum ether could be substituted for carbon tetrachloride on account of the insolubility of formyl-α-methylalanine; xylene was tolerably satisfactory, but 4,4-dimethyloxazolone was not separated from it by fractional distillation at atmospheric pressure, during which decomposition occurred.

Formyl-α-methylalanine cyclohexylamide

Cyclohexylamine (4 g) was added to a cooled solution of 4,4-dimethyloxazolone in benzene (prepared from 2·62 g of formyl-α-methylalanine). After a few min the crystalline *cyclohexylamide* (3·77 g, 89%) began to separate and was recrystallized from aqueous methanol, m.p. 142–145° (softening at 136°) (Found: C, 62·4; H, 9·5; N, 13·4. $C_{11}H_{20}O_2N_2$ requires: C, 62·2; H, 9·5; N, 13·2%). The yield from a similar run in carbon tetrachloride was 87%.

1-*Cyclohexyl*-4,4-*dimethylimidazolone*

(a) A dry mixture of formyl-α-methylalanine cyclohexylamide (1·06 g, 5 mmoles) and tosyl-α-methylalanine (1·29 g, 5 mmoles) was heated gradually in a test-tube. At 170° evolution of bubbles began and it became rapid above 200°. Heating was continued to 230° when the *imidazolone* (0·88 g, 91%) sublimed; after resublimation at 120–140°/12 mm it had m.p. 110–113°, (Found: C, 67·85; H, 9·25; N, 14·2. $C_{11}H_{18}ON_2$ requires: C, 68·0; H, 9·3; N, 14·4%). Tosyl-α-methylalanine (0·97 g, 75%) was recovered by solution of the residue from the first sublimation in sodium hydrogen carbonate solution and subsequent acidification.

(b) A mixture of N-isopropoxymethylene-α-methylalanine methyl ester (0·375 g, 2 mmoles) and cyclohexylamine (0·20 g, 2 mmoles) was heated on the steam bath for 5 hr. The solid product obtained on cooling was recrystallized from ether to yield the imidazolone (0·282 g, 78%), m.p. 111–114°.

Formyl-α-methylalanylglycine ethyl ester

When a solution of glycine ethyl ester (2·83 g, 25 mmoles) in benzene (5 cc) was added to a solution of 4,4-dimethyloxazolone in benzene (prepared from 2·62 g, 20 mmoles, of formyl-α-methylalanine), there was slight evolution of heat and, after a few min, deposition of an oil. Next day, the supernatant solution was evaporated and the residue was reunited with the oil which had partially crystallized. Several extractions with hot benzene (20 cc portions) afforded *formyl-α-methylalanylglycine ethyl ester* (2·08 g, 47%), m.p. 102–103·5° (Found: C, 50·1; H, 7·3; N, 12·8. $C_9H_{16}O_4N_2$ requires: C, 50·0; H, 7·5; N, 13·0%). The insoluble residue was a gum, probably containing some amidine (III; $R' = CH_2{\cdot}CO_2Et$).

N,N′-*bis*(1-*Carboxy*-1-*methylethyl*)-*formamidine monomethyl ester* (III; $R' = CMe_2{\cdot}CO_2Me$)

A solution of α-methylalanine methyl ester (2·92 g, 25 mmoles) in benzene (5 cc) was added to a solution of 4,4-dimethyloxazolone in benzene (prepared from 2·62 g, 20 mmoles, of formyl-α-methylalanine). The oil, which soon began to separate, gradually crystallized, and next day the crystals (4·42 g), m.p. 132–137° (dec), were collected. Extraction with hot chloroform and dilution of the chloroform with benzene afforded the pure *amidine* (III; $R' = CMe_2{\cdot}CO_2Me$) (3·92 g, 85%), m.p. 145–146·5° (dec), ν_{max} 1742, 1709, 1570 or, in chloroform, 3175, 1736, 1709, 1634, 1558 (broad), 1513 (broad) cm^{-1} (broad absorption in region 2800–2600 cm^{-1} in both cases) (Found: C, 52·0; H, 7·9; N, 12·3. $C_{10}H_{18}O_4N_2$ requires: C, 52·2; H, 7·9; N, 12·2%). When hydrogen chloride was passed into a solution of the amidine in chloroform, heat was evolved and partial evaporation yielded the *hydro*-chloride, m.p. 155·5–156·5° (dec), p*Ka* 2·84, ν_{max} 3175, 1736, 1689, 1536 cm^{-1} (Found: C, 45·1; H, 7·2; N, 10·8; Cl, 13·3. $C_{10}H_{19}O_4N_2Cl$ requires: C, 45·1; H, 7·2; N, 10·5; Cl, 13·3%).

1-(1'-Carboxy-1'-methyl)ethyl-4,4-dimethylimidazol-5-one (V; R″ = H), *and its methyl ester* (V; R″ = Me)

(a) α-Methylalanine methyl ester (1·17 g, 10 mmoles) and ethyl orthoformate (0·50 g, 3·4 mmoles) were heated to reflux for 8 hr and allowed to stand overnight. Evaporation and crystallization of the residue from ether-light petroleum yielded the *imidazolone ester* (0·584 g, 81%), m.p. 87·92°, raised by recrystallization to 91·5–92·5° (Found: C, 56·6; H, 7·7; N, 12·9. $C_{10}H_{14}O_3N_4$ requires: C, 56·6; H, 7·6; N, 13·2%).

(b) The amidine (III; R′ = CMe_2CO_2Me, 2·30 g) was heated in a sublimation tube at 140° *in vacuo* for several hours. The loss in weight was 0·31 g; in a separate experiment conducted at atm press, the evolved methanol was collected and identified (infra-red spectrum). The subliming methyl ester (0·051 g, 2·5%) was collected, and after resublimation *in vacuo* had m.p. 88–91°. The involatile residue (1·93 g, 97%), m.p. 209 212°, crystallized from water as the *carboxylic acid monohydrate* (Found: C, 50·1; H, 7·5; N, 13·1. $C_9H_{14}O_3N_2 \cdot H_2O$ requires: C, 50·0; H, 7·5; N, 13·0%). Drying at 100° and 0·5 mm press afforded the anhydrous *acid*, m.p. 211–212° (Found: C, 54·9; H, 7·1; N, 13·8. $C_9H_{14}O_3N_2$ requires: C, 54·5; H, 7·1; N, 14·1%). Diazomethane converted the acid to the above methyl ester (m.p. and mixed m.p. 88·91°).

Infra-red spectra of formamidines

Diphenylformamidine, (m.p. 139°), ν_{max} 1681, 1661, 1587, 1488, 1462. In chloroform, ν_{max} 3390 (weak), 1653, 1592, 1488. Diphenylformamidine hydrochloride (m.p. 248–250°), ν_{max} 3086, 1704, 1623, 1605, 1572, 1502, 1462 and 1456 cm^{-1}.

Dicyclohexylformamidine, dimorphic, form A (needles, m.p. 99–101°, Found: C, 75·0; H, 11·65; N, 13·1. Calc. for $C_{13}H_{24}N_2$: C, 74·9; H, 11·6; N, 13·45%). ν_{max} 3226, 3040, 1639 and 1548 cm^{-1}. In chloroform, ν_{max} 2907, 2857, 1658 and 1447 cm^{-1}. Form B (prisms, m.p. 100 102°) ν_{max} 3205, 3125 and 1689 cm^{-1}, (identical to form A in chloroform solution). Grundmann and Kreutzberger[27] give m.p. 106°. *Dicyclohexylformamidine hydrochloride*, m.p. 229–230° (dec) (Found: C, 63·8; H, 9·85; N, 11·8. $C_{13}H_{25}N_2Cl$ requires: C, 63·8; H, 10·3; N, 11·45%). ν_{max} 3165, 1695 and 1563 cm^{-1}.

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[27] C. Grundmann and A. Kreutzberger, *J. Amer. Chem. Soc.* 77, 6559 (1955).