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THE UNUSUAL CYCLIC PRODUCTS FROM TETRA- AND PENTA-PEPTIDES OF α-METHYLALANINE

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Abstract—Tetra- and pentapeptides of α-methylalanine give with PCl_5, $SOCl_2$ etc. intermediates which, with or without loss of amino acids, lead to bi- and tricyclic products containing imidazole and piperazine rings through different routes.

During attempts to prepare cyclic peptides of α-methylalanine (α-aminoisobutyric acid), a series of abnormal reactions were encountered.[1] From the tetra- and pentapeptides of α-methylalanine, under the conditions of acid chloride formation with PCl_5, $SOCl_2$, etc. and alkaline conditions of cyclization, corresponding cyclic peptides were not obtained; instead, bi-(IV) and tricyclic(V) products containing imidazole and piperazine ring systems were formed. Ali *et al.*[1] proposed a mechanism, on the basis of the deuteration experiments, to explain the formation of the bi-(IV) and tricyclic(V) products from the tetrapeptide.

Deuteration experiments with the pentapeptide gave evidence of the formation of bicyclic product (IV) similarly (Ia → II → IV) (Fig. 1), however, in case of the tricyclic product (V), a different route seemed to lead to this product. We present further evidence, on the basis of the deuteration experiments, that the tricyclic bis-imidazolone(V) has not been formed from the pentapeptide through the similar route (Ia → II → IV).

Two pentapeptides of α-methylalanine, containing amino acid residues fully deuterated at both α-methyl groups at 2- and 1,3-positions of the peptide chain have been synthesized systematically according to Ali *et al.*[1] The isotopic purity of the labelled residue in labelled pentapeptides was *ca* 98% as determined by NMR and mass spectrometry. The labelled pentapeptides were subjected to reaction with phosphorus pentachloride in acetyl chloride. The chlorinated intermediates were cyclized in pyridine under the conditions of high dilution and the cyclic products were examined by IR, NMR and mass spectrometry (Table 1).

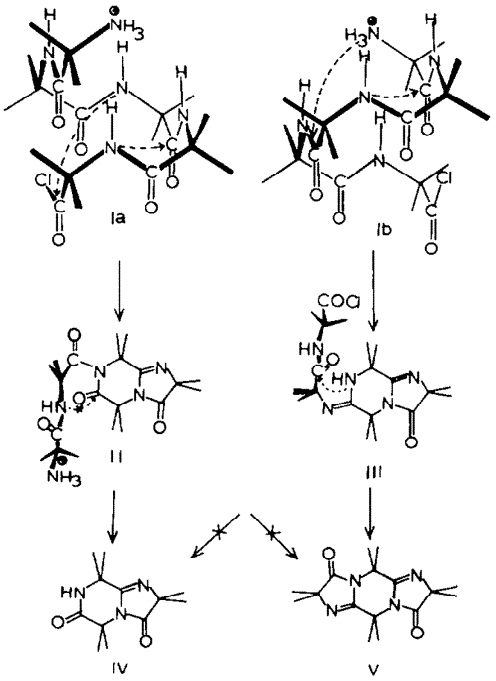

Fig. 1.

Earlier ^{14}C-labelling experiments by Kenner *et al.*[2] and deuteration experiments by Ali *et al.*[1] led to the assignment of the Me proton absorption peaks of the bicyclic imidazolone(IV) and the tricyclic bis-imidazolone(V). The line at 1.35 ppm was assigned to the Me protons of the imidazolone ring in the bicyclic imidazolone(IV). The lines at 1.33 ppm and 1.89 ppm of the tricyclic bis-imidazolone(V) were assigned by inference to the Me protons of the imidazolone and the piperazine rings respectively. NMR absorption characteristics of the bicyclic imidazolone(IV) and the tricyclic bis-imidazolone(V) obtained from the pentapeptides containing deuteromethyl groups in the 2- and 1,3-residues of the peptide chain are shown in the Fig. 2.

In agreement to the previous deuteration experiments[1] on the pentapeptides, the currently synthesized pentapeptides containing deutero-Me groups in the 2-residue gave bicyclic imidazolone(IV) with no duetero-Me groups; while that containing duetero-Me groups in the 1,3-residues gave bicyclic imidazolone(IV) with deutero-Me groups in the piperazine ring only, following the reaction route Ia → II → IV. This is in conformity to the mechanism proposed by Ali *et al.*[1] for the formation of the bicyclic imidazolone(IV) and the tricyclic bis-imidazolone(V) from the tetrapeptide.

Formation of the tricyclic bis-imidazolone(V) from the pentapeptides, currently synthesized, apparently took place by a different route, i.e. Ib → III → V. The tricyclic bis-imidazolone(V) obtained from the pentapeptide containing deutero-Me groups in the 2-residue showed absorption peaks at 1.33 and 1.89 ppm, the 1.33 ppm peak being reduced to half the intensity, indicating the deutero-Me groups in the imidazolone ring (Fig. 2). The pentapeptide containing the deuteromethyl groups in the 1- and 3-residues gave the tricyclic bis-imidazolone(V) showing only one absorption peak at 1.33 ppm, and the 1.89 ppm peak being missing, indicating deutero-Me groups in the piperazine ring only (Fig. 2). Previous deuteration experiments[1] on pentapeptides containing the deuterated amino acid residues in the 1- and 4-residues of the peptide chain

Table 1. Properties of cyclic compounds from pentapeptides

	Pentapeptide d_6 in 2-residue		Pentapeptide d_6 in 1- and 3-residues	
	IV	V	IV	V
Mol. ion (mass spectrometry)	237	310	243	316
Double bond abs. in IR	1730, 1670, 1640 cm^{-1}	1720, 1630 cm^{-1}	1730, 1670, 1640 cm^{-1}	1720, 1630 cm^{-1}
δ_{TMS} $CDCl_3$	1.78, 1.68, 1.35	1.89, 1.33*	1.78, (i), 1.35	(ii), 1.33

* Intensity reduced to half.
(i) 1.68 ppm peak missing.
(ii) 1.89 ppm peak missing.

gave tricyclic bis-imidazolones(V) containing deutero-Me groups in the piperazine and imidazolone rings respectively; while that containing deutero-Me groups in the 5-residue gave the tricyclic bis-imidazolone(V) with no deutero-Me groups.

From study of the NMR spectra of the tricyclic bis-imidazolone(V). obtained from the pentapeptides containing the deuterated amino acid residues at different positions of the peptide chain, and assuming that any intermediate chloroimine double bond, as well as the parent amide group, is exclusively in trans, and that chain folding can only occur at the α-carbon to give part of a 4-helix (Ia & Ib), a reaction mechanism (Ib → III → V) can be proposed for the case of the pentapeptide (Fig. 1). Initial imidazolone formation† at the 2-residue, followed by piperazine formation in the alkaline medium as the amino group of 1-residue is liberated and drawn closer to the 3-residue, and finally, elimination of 5-residue of the side chain as 8-membered ring formation is unlikely, might lead to the formation of the tricyclic bis-imidazolone(V). Probably more reactive —NH— of 1-residue of the amidine III (cf. —NH— of amide II) easily couples with the 4-residue with elimination of 5-residue to form the tricyclic bis-imidazolone according to the route Ib → III → V. Such amidines (III) are known to form cyclopeptides and cyclols under the influence of bases.[6]

EXPERIMENTAL

Derivatives and oligomers of α-methylalanine were prepared according to Kenner *et al.*[4] and Ali *et al.*[1] Reactions were followed by TLC on silica gel G using $CHCl_3$–EtOAc (2 : 1) solvent system. The chromatograms were developed with I_2 vapour. Blocked peptides gave brown spots, free carboxyl peptides showed white peaks from the point of application, while free amino peptides showed wide deep-brown spots. Evaporations were carried out under reduced pressure. Neutral products were isolated by washing in EtOAc successively with 0.1 N HCl, water, 0.5 M $NaHCO_3$, water and finally dried over Na_2SO_4 and evaporated. M.ps are uncorrected.

α-Methylalanine-d_6 methyl ester. Freshly distilled $SOCl_2$ (5 ml) was slowly added to MeOH (45 ml) at −5°, in which α-methylalanine-d_6 (5.45 g = 50 mmol), prepared according to Ali *et al.*[1] from acetone-d_6, slowly added. After stirring for 1 hr at −5° and 2 hr at 50° the soln was evaporated and α-methylalanine-d_6 methyl ester hydrochloride was recrystallized from MeOH–ether (6.4 g = 80%), m.p. 183°.[1] The hydrochloride was dissolved in $CHCl_3$, washed with dilute NH_4OH and water and the $CHCl_3$ layer was dried and evaporated (4 g), b.p. 181–3°.[1] The integrated NMR and mass spectra showed that *ca* 98% of the α-Me protons were deuterated.

Benzyloxycarbonyl - α - methylalanyl - α - methylalanine - d_6. Benzyloxycarbonyl-α-methylalanine (11.85 g = 50 mmol) and α-methylalanine-d_6 methyl ester (6.2 g = 5 mmol) in acetonitrile (300 ml) was cooled to 0° and dicyclohexylcarbodiimide (10.7 g = 52 mmol) added. The mixture was stirred at room temp (24 hr) and evaporated. The neutral product was isolated in the usual manner and recrystallized from EtOAc–light petroleum (10.7 g = 32 mmol), m.p. 110°.[1] TLC showed a single spot (R_f = 0.74). Hydrolysis[1] gave benzyloxycarbonyl-α-methylalanyl-α-methylalanine-d_6, recrystallized from aqueous MeOH (8.9 g = 85%), m.p. 161°.[1] (cf. non-deuterated compound, m.p. 161°.1)

Benzyloxycarbonyl - α - methylalanyl - α - methylalanyl - d_6 - (α - methylalanyl)$_2$ - α - methylalanine. Benzyloxycarbonyl - α - methylalanyl - α - methylalanine - d_6 (1.6 g = 5 mmol) was converted into 2-(1′-benzyloxycarbonylamino-1′-methyl) ethyl-4,4-dideuteromethyloxazolone[1] and allowed to react with (α-methylalanyl)$_2$-α-methylalanine t-butyl ester[1] (1.6 g = 5 mmol) in acetonitrile (50 ml) under reflux for 7 days. The neutral pentapeptide-d_6 derivative was isolated in the usual way and recrystallized from acetonitrile (1.9 g = 60%), m.p. 236° (dec).[1] TLC showed a single spot (R_f = 0.45). Transesterification[1] in TFA for 15 min at 0° afforded the desired product, recrystallized from aqueous MeOH (1.7 g), m.p. 256° (dec?) (cf. non-deuterated compound decomposes at 256°).[1]

Benzyloxycarbonyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanine. Benzyloxycarbonyl - α - methylalanyl - d_6 - α - methylalanine[1] (4.92 g = 15 mmol) was dehydrated to the corresponding oxazolone[1] (4.3 g = 92%), m.p. 126°. Part of it (2.5 g = 8 mmol) was reacted with α - methylalanine t-butyl ester[1] (1.3 g) to yield benzyloxycarbonyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanine t-butyl ester (3.2 g = 86%), m.p. 167°.[1] Hydrogenolysis[1] of the t-butyl ester in MeOH (100 ml) using Pd–C (5%) catalyst (0.8 g) afforded α - methylalanyl - d_6 - α - methylalanyl - α - methylalanine t-butyl ester (2.2 g) which was reacted with other part of the dipeptide oxazolone-d_6 (1.8 g = 6 mmol) in acetonitrile (40 ml) at reflux temp for 7 days to yield the pentapeptide-d_{12} derivative, recrystallized from acetonitrile (2.5 g = 66%), m.p. 236° (dec).[1] TLC showed a single spot (R_f = 0.45). Transesterification[1] in TFA in the usual way afforded the desired product (2.1 g), m.p. 256° (dec?).

† Formation of the 5-membered ring is facilitated in case of the peptides of α-methylalanine, probably due to gem-dimethyl effect. Benzyloxycarbonyl-α-methylalanyl chloride[2,3,5] and acid chlorides of di-[4] and tripeptides[1] of α-methylalanine easily forms the oxazolones. Benzoyl-α-methylalanine amide[5] forms imidazolone simply by heating.

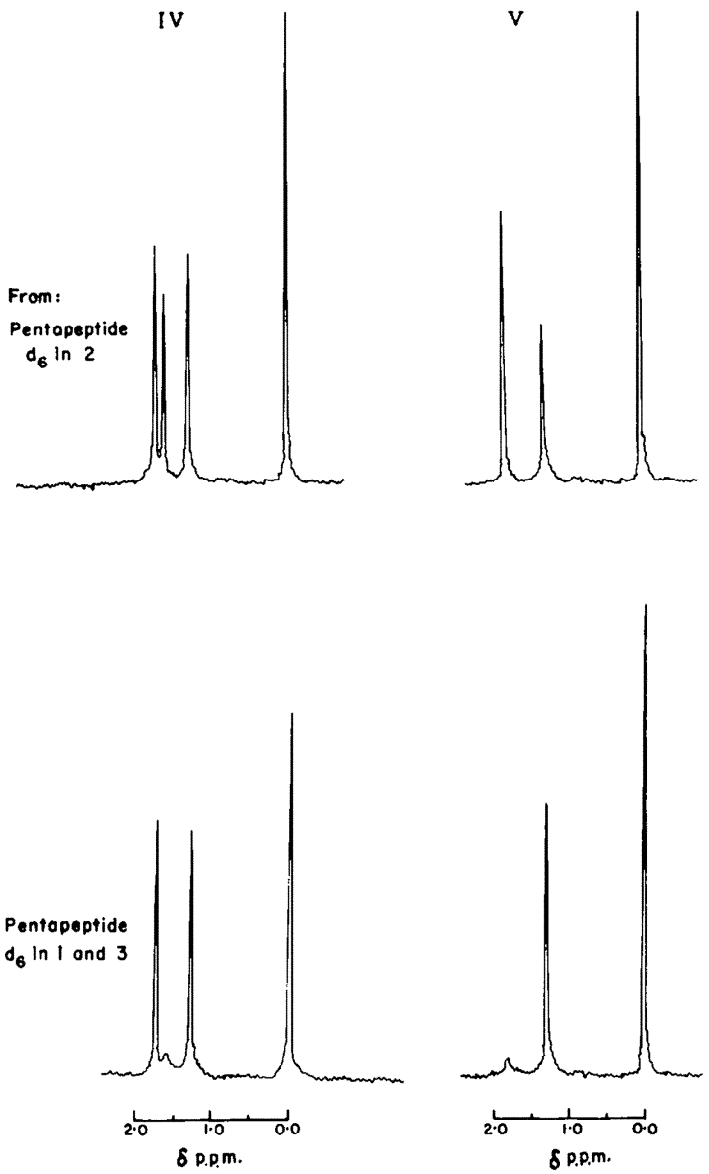

Fig. 2. NMR-spectra at 60 MHz in $CDCl_3$-solution of bicyclic imidazolone(IV) and tricyclic bis-imidazolone(V) formed from deuterated peptides.

Imidazolone(IV) and bis-imidazolone(V) from α - methylalanyl - α - methylalanyl - d_6 - (α - methylalanyl)$_2$ - α - methylalanine Hydrogenolysis[1] of the benzyloxycarbonyl - α - *methylalanyl* - α - methylalanyl - d_6 - (α-methylalanyl)$_2$ - α - methylalanine (1.5 g = 2 mmol) in methanol (50 ml) using Pd–C catalyst (5%, 0.4 g) yielded α - methylalanyl - α - methylalanyl - d_6 - (α - methylalanyl)$_2$ - α - methylalanine (0.8 g = 90%), precipitated from acetone ether.

The pentapeptide-d_6 (0.23 g = 0.5 mmol) was suspended in freshly distilled acetyl chloride (40 ml), cooled to 0° and PCl_5 (0.5 g) added. The clear soln which appeared after few min was stirred at room temp for 20 hr, when a white substance precipitated. The mixture after evaporation showed absorption at $\bar{\nu}_{max}$ 1790, 1730 and 1690 cm^{-1}. The residue was dissolved in dry dimethyl-formamide (25 ml) and added during 1 hr to dry pyridine (700 ml) at 70° with stirring, the mixture was kept at this temp for 4 hr. After evaporation, the residue was dissolved in $CHCl_3$ and washed with water. The water layer contained polymers, unreacted peptide and α-methylalanine. The $CHCl_3$ soln was dried over Na_2SO_4 and evaporated, and the imidazolone(IV) and the bis-imidazolone(V) were fractionally crystallized from MeOH and further purified by sublimation. IV, sublimes at 90–110°/0.02 mmHg, 30 mg = 25%, m.p. 255° (sealed tube), δ_{TMS} $CDCl_3$ 1.78, 1.68, 1.35 (1 : 1 : 1), *m/e* 237; V, sublimes at 100–120°/0.02 mmHg, 16 mg = 10%, m.p. 253° (sealed tube), δ_{TMS} $CDCl_3$ 1.89, 1.33 (2 : 1), *m/e* 310. (cf. non-deuterated IV, m.p. 255° (sealed tube), δ_{TMS} $CDCl_3$ 1.78, 1.68, 1.35 (1 : 1 : 1), *m/e* 237; V, m.p. 253° (sealed tube), δ_{TMS} $CDCl_3$ 1.89, 1.33 (1 : 1), *m/e* 304).[1]

Imidazolone(IV) and bis - imidazolone(V) from α - methylalanyl - d_6 - α - methylalanyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanine.

Benzyloxycarbonyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanyl - d_6 - α - methylalanyl - α - methylalanine (1.18 g = 2 mmol) was hydrogenolysed in methanol (50 ml) as in the preceding experiment to yield the pentapeptide-d_{12}—precipitated from acetone ether, 0.8 g (95%).

The pentapeptide-d_{12} (0.3 g = 0.66 mmol) was reacted with PCl_5 (0.55 g) in acetyl chloride (15 ml), and after evaporation of the solvent, the residue was cyclized in pyridine and the cyclic products were isolated as in the preceding experiment; IV, 45 mg = 28%, δ_{TMS} $CDCl_3$ 1.78, 1.35, *m/e* 243, and V, 22 mg = 10%, δ_{TMS} $CDCl_3$ 1.33, *m/e* 316.

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