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HYDROXAMIC ACIDS AND THEIR DERIVATIVES—IV[1]

FURTHER STUDIES ON THE USE OF ESTERS OF PIVALOHYDROXAMIC ACID FOR PEPTIDE SYNTHESIS*

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Abstract– Lossen rearrangement is revealed as the major side-reaction in the use of pivalohydroxamic acid esters for the synthesis of larger peptides.

IN THE previous paper[1] we reported the addition of N-protected amino acids to pivalonitrile oxide to form esters of pivalohydroxamic acid which could subsequently be coupled with amino acid esters to form peptides. In order to define the scope and limitations of this method, we decided to synthesize the following two tetrapeptide sequences:

Pro-Phe-Leu-Val and Pro-Leu-Phe-Val.

I II

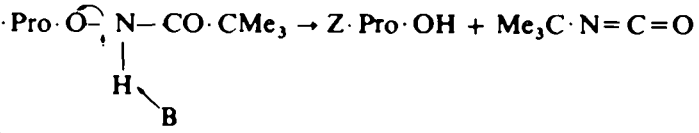
In a parallel study, these two tetrapeptides were also synthesized using DCCI; this enabled us to compare the yields in the two methods and evaluate the utility of pivalonitrile oxide for the synthesis of larger peptides.

Method. In both cases, the synthesis was carried out stepwise from the C-terminal end, one unit being added each time. The carbobenzoxy group was used for N-protection and was removed at each stage by hydrogenolysis. In the pivalonitrile oxide method, the active esters from carbobenzoxyphenylalanine and carbobenzoxyleucine were crystalline,[1] whereas carbobenzoxyproline gave only a gummy product which was used as such for the coupling reaction.

For the coupling stage, it was found preferable to have the amine component as the acetic acid salt. The next best alternative was to use the amine hydrochloride in conjunction with one equivalent of sodium acetate. The least efficient process was to use the free base liberated from its hydrochloride by means of triethylamine.

Results. The yields in the various coupling stages are listed in the following Table.

In the last reaction, no tetrapeptide could be isolated by our method. The only product obtained (36·7% yield) could be assigned structure III, arising obviously from a Lossen rearrangement of the active ester followed by reaction of the isocyanate with the amine component:



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[1] Part III. *Tetrahedron*

$$Me_3C{\cdot}N{=}C{=}O + H{\cdot}Leu{\cdot}Phe{\cdot}Val{\cdot}OMe \rightarrow Me_3C{\cdot}NH\,CO{\cdot}Leu{\cdot}Phe{\cdot}Val{\cdot}OMe$$

III

Analytical values are in agreement with this structure, and in the NMR spectrum, the hydrogens of the t-butyl group occurred as a singlet at 80 c/s in addition to the side-chain methyls of valine and leucine at 50–60 c/s.

TABLE 1

Peptide	Yields*	
	Pivalohydroxamic acid method	DCCI
Z·Leu·Val·OMe[a]	85%	90%
Z·Phe·Leu·Val·OMe	28·4%	46·3%
Z·Pro·Phe·Leu·Val·OMe	5·6%	20%
Z·Phe·Val·OMe[b]	70·4%	83·7%
Z·Leu·Phe·Val·OMe	37·4%	41·5%
Z·Pro·Leu·Phe·Val·OMe	-	17·5%

* Yields refer to the peptide bond produced at the point indicated by dotted line.

[a] Oil; known compound; E. L. Smith, D. H. Spackman and W. J. Polglase, *J. Biol. Chem.* **199**, 801 (1952).

[b] cf. *Chem. Abstr.*, **60**, 8128 (1964). Details not available.

A similar side reaction has been observed in the two tripeptide stages as well, leading to the following compounds, but in lower yields.

$$Me_3C{\cdot}NH{\cdot}CO{\cdot}Phe{\cdot}Val{\cdot}OMe \qquad IV$$

$$Me_3C{\cdot}NH{\cdot}CO{\cdot}Leu{\cdot}Val{\cdot}OMe \qquad V$$

Occurrence of the Lossen rearrangement is thus a serious drawback in this method, especially when used for the synthesis of larger peptides.

The two tetrapeptide sequences (I and II) have been suggested as possible structures for a peptide isolated from Linseed.[2] We have not yet made a direct comparison of our synthetic samples with the original naturally occurring specimen. We are currently engaged in isolating the peptide from the same natural source and a future communication would deal with this aspect of the problem.

EXPERIMENTAL

All amino acids used had the *L*-configuration.

N-Benzyloxycarbonyl leucyl valine methyl ester

(a) O-(N-benzyloxycarbonyl leucyl) pivalohydroxamic acid[1] (3·64 g) in DMF (20 ml) was added to a

[2] H. P. Kaufmann and A. Tobschirbel, *Chem. Ber.* **92**, 2805 (1959).

stirred mixture of valine methyl ester hydrochloride (1·68 g) and AcONa.$3H_2O$ (1·4 g) in DMF (20 ml) Stirring was continued at room temp overnight and the product isolated as usual (cf. Ref. 1). The oil (3·2 g) after passage through silica gel had identical R_f value on TLC with that of the product obtained by the DCCI method.

(b) A mixture of N-benzyloxycarbonyl leucine (3·7 g) and valine methyl ester hydrochloride (2·35 g) in DMF (50 ml) was cooled in ice salt and treated with Et_3N (1·4 g), followed by DCCI (3·24 g). The mixture was stirred overnight, and the product isolated as usual. (~90% yield, oil).

Leucyl valine methyl ester. Hydrogenolysis of the above oil in MeOH–AcOH and evaporation gave the acetic acid salt of leucyl valine methyl ester.

Addition of methanolic HCl gave the hydrochloride (single spot on paper chromatogram), which was used for the next experiment.

N-Benzyloxycarbonyl phenylalanyl leucyl valine methyl ester

(a) About 2·75 g of the above acetate salt in 25 ml DMF was stirred for 20 hr with O-(N-benzyloxycarbonylphenylalanyl) pivalohydroxamic acid[1] (3·6 g) in 25 ml acetonitrile. The usual work-up gave 4·9 g of crude product which was exhaustively chromatographed over 50 g of silica gel. The following fractions were collected:

5 × 100 ml benzene; 4 × 150 ml chloroform; 3 × 150 ml chloroform: ethyl acetate (3:1).

Recrystallization of the product from fractions 10 and 11 from AcOEt–petrol gave 0·7 g of the tripeptide. Fractions 7, 8, 11 and 12 were combined and re-chromatographed to provide an additional 0·65 g of the tripepetide, m.p. 139–140°. (Found: C, 66·59; H, 7·11. $C_{29}H_{39}O_6N_3$ requires: C, 66·26; H, 7·48%.)

Reaction as usual of the hydrochloride salt (3·45 g) with the active ester (4·9 g) in presence of AcONa (1·7 g) and exhaustive chromatography of the product gave the tripeptide (1·35 g) and compound V (100 mg), m.p. 203–206°. (Found: C, 59·69; H, 9·95. $C_{17}H_{33}O_4N_3$ requires: C, 59·45; H, 9·68%.) NMR and IR spectra are in agreement with this structure.

(b) A mixture of benzyloxycarbonylphenylalanine (7·0 g), leucyl valine methyl ester hydrochloride (from 7·0 g of Z·Leu·Val·OMe), Et_3N (3·3 ml) and DCCI (6·4 g) in DMF (90 ml) and acetonitrile (30 ml) was stirred overnight and worked up as usual. Chromatography of the product as before gave 4·5 g of the tripeptide, identical with the previous product, m.p. 139–140°. (Found: C, 65·90; H, 7·22. $C_{29}H_{39}O_6N_3$ requires: C, 66·26; H, 7·48%.) $[\alpha]_D^{26}$ −34·9° (*c*, 2 in ethanol).

Phenylalanyl leucyl valine methyl ester

Hydrogenolysis of the above tripeptide in MeOH–AcOH and addition of methanolic HCl gave the *hydrochloride*, m.p. 195–200°. Recrystallization of a sample from MeOH–Et_2O raised the m.p. to 203–204°. (Found: C, 57·05; H, 7·84. $C_{21}H_{34}O_4N_3Cl \cdot H_2O$ requires: C, 56·54; H, 8·14%.) The compound showed a single spot on paper chromatogram.

N-Benzyloxycarbonyl prolyl phenylalanyl leucyl valine methyl ester

(a) Carbobenzoxyproline was reacted in the usual manner with pivalonitrile oxide to yield the gummy active ester.

A mixture of the above active ester (0·5 g), the tripeptide hydrochloride (0·61 g) and AcONa (0·2 g) in DMF (30 ml) was stirred at room temp for 2 days. The usual work-up gave 0·3 g of crude material. This was chromatographed over 5 g of silica gel and the following fractions were collected: 2 × 50 ml chloroform; 2 × 50 ml chloroform: ethyl acetate (3:1). The residue from fraction 3 gave 50 mg of the required *tetrapeptide*, m.p. 117–122°.

In one experiment, a product, m.p. 197–200° was obtained, which could be $Me_3C \cdot NH \cdot CO \cdot Phe \cdot Leu \cdot Val \cdot OMe$. But in most other experiments, a compound m.p. 140–154° was obtained, which appeared to be a mixture of the above two pure materials.

(b) A mixture of carbobenzoxyproline (2·8 g) and the tripeptide hydrochloride (4·8 g) in DMF (80 ml) and acetonitrile (60 ml) was cooled and treated with Et_3N (1·6 ml) followed by DCCI (2·8 g). After stirring overnight, the product was isolated as usual. Digestion with ether, followed by recrystallization from AcOEt–petrol gave the *tetrapeptide*, (1·5 g), m.p. 122–125°. (Found: C, 65·62; H, 7·23. $C_{34}H_{46}O_7N_4$ requires: C, 65·57; H, 7·45%.) $[\alpha]_D^{26}$ −64·15° (*c*, 2 in EtOH).

Prolyl phenylalanyl leucyl valine methyl ester hydrochloride

The above tetrapeptide was hydrogenolysed in MeOH–AcOH and then converted to the *hydrochloride*, m.p. 128–135°. (Found: C, 58·31; H, 8·10. $C_{26}H_{41}O_5N_4Cl·0·5\ H_2O$ requires: C, 58·48; H, 7·93%.) The compound showed a single spot (ninhydrin and isatin) on paper chromatogram.

N-*Benzyloxycarbonyl phenyl alanyl valine methyl ester*

(a) To a stirred mixture of valine methyl ester hydrochloride (1·68 g) and AcONa (1·4 g) in DMF (20 ml) was added O-(N-benzyloxycarbonyl phenylalanyl) pivalohydroxamic acid[1] (4 g) in dry acetonitrile (15 ml). Stirring was continued overnight and the product worked up as usual to give the *dipeptide* (2·7 g) m.p. 108–109°. Recrystallization from AcOEt petrol raised the m.p. to 110–111°. (Found: C, 66·84; H, 7·09. $C_{23}H_{28}O_5N_2$ requires: C, 66·97; H, 6·84%.) $[\alpha]_D^{25}$ −14·8° (*c*, 2 in EtOH).

(b) 2·99 g of carbobenzoxyphenylalanine and 1·67 g of valine methyl ester hydrochloride gave by the DCCI procedure 3·45 g of the dipeptide, m.p. 109–111°. (Found: C, 66·70; H, 6·81. $C_{23}H_{28}O_5N_2$ requires: C, 66·97; H, 6·84%.)

Phenylalanyl valine methyl ester hydrochloride

Hydrogenolysis of the above dipeptide in MeOH AcOH, followed by treatment with methanolic HCl and evaporation gave the hydrochloride which was used as such for the next experiment.

N-*Benzyloxycarbonyl leucyl phenylalanyl valine methyl ester*

(a) The above hydrochloride (1·75 g) in DMF (20 ml) was stirred overnight with O-(N-benzyloxycarbonyl-leucyl) pivalohydroxamic acid[1] (2·0 g) and AcONa (0·78 g) in acetonitrile (20 ml). The crude product (2·65 g) was chromatographed over 25 g silica gel. 4 × 150 ml fractions of $CHCl_3$-eluate were collected. The residue from the first 2 fractions gave 1·0 g solid (m.p. 145–152°) on trituration with ether. The mother liquor from this was combined with fraction 3 and rechromatographed to provide another 75 mg of the same solid. Recrystallization from AcOEt petrol gave the *tripeptide*, m.p. 152–154°. (Found: C, 66·58; H, 7·40. $C_{29}H_{39}O_6N_3$ requires: C, 66·26; H, 7·48%.)

Fraction 4 from the above chromatography gave 50 mg solid, which was recrystallized from AcOEt–petrol, to provide *compound* IV, m.p. 200–204°. (Found: C, 63·76; H, 8·24. $C_{20}H_{31}O_4N_3$ requires: C, 63·63; H, 8·28%.) The NMR spectrum is in agreement with this structure.

(b) 2·86 g of carbobenzoxyleucine and 3·32 g of the dipeptide hydrochloride gave by the DCCI method 2·35 g of the *tripeptide* (after chromatography and recrystallization), m.p. 152–154°. (Found: C, 66·38; H, 7·38. $C_{29}H_{39}O_6N_3$ requires: C, 66·26; H, 7·48%.) $[\alpha]_D^{25}$ −42·85° (*c*, 2 in EtOH).

Leucyl phenylalanyl valine methyl ester hydrochloride

The above tripeptide was hydrogenolysed as usual, converted to the *hydrochloride* and recrystallized from MeOH–ether, m.p. 246–248°. (Found: C, 58·93; H, 8·01. $C_{21}H_{34}O_4N_3Cl$ requires: C, 58·94; H, 8·01%.)

N-*Benzyloxycarbonyl prolyl leucyl phenyl alanyl valine methyl ester*

(a) 0·78 g of the carbobenzoxy proline active ester in 20 ml acetonitrile was added to a stirred mixture of the tripeptide hydrochloride (0·95 g) and AcONa (0·31 g) in 20 ml DMF. After 4 days, the soln was filtered. The filtrate showed no colour with $FeCl_3$ aq. The usual work-up gave 0·4 g of a solid, m.p. 188–190°. Recrystallization from AcOEt petrol gave *N-(t-butyl carbamoyl) leucyl-phenylanalyl-valine methyl ester* (III), m.p. 194–196°. (Found: C, 63·47; H, 8·76. $C_{26}H_{42}O_5N_4$ requires: C, 63·64; H, 8·63%.) No tetrapeptide could be obtained in this reaction.

(b) A mixture of the tripeptide hydrochloride (2·7 g), carbobenzoxyproline (1·43 g) and Et_3N (0·9 ml) in DMF was cooled and treated with DCCI (1·45 g). Stirring was continued for 2 days; after the usual work-up, the crude product (3·2 g) was chromatographed on 30 g of silica gel. The following fractions were collected: 6 × 50 ml chloroform; 100 ml chloroform: ethyl acetate (4:1). The last fraction gave a solid which was rechromatographed and recrystallized from AcOEt petrol to provide the pure *tetrapeptide*, m.p. 176–178°. (Found: C, 65·55; H, 7·43. $C_{34}H_{46}O_7N_4$ requires: C, 65·57; H, 7·45%.) $[\alpha]_D^{24}$ −75·0° (*c*, 2 in EtOH).

Prolyl leucyl phenyl alanyl valine methyl ester hydrochloride

The above protected tetrapeptide was hydrogenolysed, converted to the hydrochloride and recrystallized from MeOH–ether to give the product, m.p. 125–145°. (Found: C, 57·01; H, 8·04. $C_{26}H_{41}O_5N_4Cl·H_2O$ requires: C, 57·50; H, 7·98%.)

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