

## Synthesis of Phosphopeptides

### III \*. Derivatives of D-Seryl-L-leucine Obtained by Separation from a DL-L Diastereoisomeric Mixture

GEORG FÖLSCH

*Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden*

The two diastereoisomers of N-carbobenzoxy-DL-seryl-L-leucine benzyl ester, were separated by fractional crystallisation. The D-seryl derivative was converted to D-seryl-L-leucine, O-phosphoryl-D-seryl-L-leucine and O-monophenylphosphoryl-D-seryl-L-leucine.

The use of racemic amino acids in peptide synthesis yields mixtures of diastereoisomers when  $\alpha$ -amino acids other than glycine are used, as all other  $\alpha$ -amino acids contain at least one asymmetric center. The components of such diastereoisomeric mixtures are sometimes separated by fractional crystallisation<sup>2-3</sup>. For large scale preparation of dipeptides containing a difficultly accessible antipode of an amino acid, such procedures may be of value, as the amino acid in question can be used in the generally more easily accessible racemic form.

In this paper the resolution of the carbobenzoxy peptide benzyl ester (I) obtained from DL-serine and L-leucine is described. The difference in solubility of the two isomers made it comparatively easy to isolate the higher melting isomer, *i.e.* N-carbobenzoxy-D-seryl-L-leucine benzyl ester (II). After two recrystallizations, the m.p. and optical rotation had the values of an authentic sample, prepared from pure D-serine. The isolation of pure L-L derivative (III) was, however, somewhat more difficult since this isomer, in none of the solvent systems tried, was less soluble than compound (II), and five recrystallizations were necessary to obtain the m.p. and rotation values of a previously prepared<sup>1</sup> sample of (III).

Compound (II) was converted to D-seryl-L-leucine by hydrogenolysis, and to O-phosphoryl-D-seryl-L-leucine and O-monophenylphosphoryl-D-seryl-L-leucine by phosphorylation with diphenylphosphoryl chloride followed by hydrogenolysis. The phosphopeptides may be of considerable interest in

\* For part II, see Ref.<sup>1</sup>

current studies on the action of phosphatases<sup>4</sup> and proteolytic enzymes on phosphorylated peptides, as it has been shown, that the enzyme phosphoserine phosphatase acts on O-phosphoryl-D-serine as well as on the L-isomer<sup>5-7</sup>.

### EXPERIMENTAL

*N*-Carbobenzoxy-DL-seryl-L-leucine benzyl ester (I). To a solution of 12.0 g (50 mmoles) of N-carbobenzoxy-DL-serine<sup>8</sup>, 13.0 g (50 mmoles) of L-leucine benzyl ester hydrochloride<sup>9</sup> and 7.2 ml (50 mmoles) of triethylamine in 400 ml of tetrahydrofuran-acetonitrile (1:1) was added 10.5 g (50 mmoles) of N,N'-dicyclohexylcarbodiimide. The mixture was shaken for 2 h and then left over night. After filtration, the solvent was distilled off *in vacuo* and the residue dissolved in ethyl acetate. After washing with water, 1 N hydrochloric acid, water, saturated bicarbonate solution, and water, followed by drying over sodium sulphate, the ethyl acetate was distilled off *in vacuo* and the residue brought to solid state by addition of light petroleum. The product was collected and then dried. Yield 20.0 g (90 %) of crude (I).

*Separation of isomers.* The above mixture of diastereoisomers was dissolved in 200 ml of ethyl acetate, and light petroleum was added to slight turbidity. When left for 24 h at +4°, crystals were formed which, after filtration, washing with ethyl acetate-light petroleum (1:1) and drying weighed 8.1 g (73 % of theory). M. p. 110–111°. After one additional crystallization from the same solvent system, 7.1 g (64 %) of a compound (II) with m. p. 115°  $[\alpha]_D^{25} - 19.9^\circ$  (ethanol, *c* 6.7, *l* 1);  $[\alpha]_D^{25} - 4.0^\circ$  (chloroform, *c* 11, *l* 1) was obtained. For the L-L derivative, a m. p. of 83–84° has been reported<sup>1</sup>. Therefore, the actual compound had to be the D-L isomer, and this was proved by comparison with an authentic sample of N-carbobenzoxy-D-seryl-L-leucine (III). This was synthesized by the carbodiimide procedure from 0.6 g (2.5 mmoles) of N-carbobenzoxy-D-serine<sup>8</sup> as described above, whereby a yield of 0.95 g (86 %) was obtained after recrystallization from ethyl acetate-light petroleum. M. p. 114°; mixed with II, m. p. 114°.  $[\alpha]_D^{25} - 20.2^\circ$  (ethanol, *c* 3.4, *l* 1);  $[\alpha]_D^{25} - 4.2^\circ$  (chloroform, *c* 12, *l* 1).

The mother liquors and washings from compound (II) were evaporated to dryness and the residue repeatedly crystallized from methanol-water. After five crystallizations, 4.6 g (42 %) were collected (IV). M. p. 83–84°, mixed with pure N-carbobenzoxy-L-seryl-L-leucine benzyl ester, m. p. 83–84°;  $[\alpha]_D^{25} - 26.5^\circ$  (ethanol, *c* 6.1, *l* 1).

*D*-Seryl-L-leucine (V). By catalytic hydrogenolysis of 2.2 g of compound (II) in ethanol-water, using palladium catalyst, followed by filtration, evaporation to dryness and recrystallization from water-ethanol-ether, a yield of 0.9 g (76 %) of the free peptide as the monohydrate was obtained.  $[\alpha]_D^{25} - 38^\circ$  (1 N HCl, *c* 6.7, *l* 1). (Found: C 45.9; H 8.3; N 11.8. Calc. for C<sub>9</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub> · H<sub>2</sub>O (236.3): C 45.7; H 8.5; N 11.8.)

*O*-Phosphoryl-D-seryl-L-leucine (VI). To 4.4 g (10 mmoles) of compound II in 10 ml of anhydrous pyridine was added 3.2 g (12 mmoles) of diphenylphosphoryl chloride, the temperature being kept below 40°. After 4 h, the excess of acid chloride was destroyed by addition of water, and the product taken up in 100 ml of water and 100 ml of ether. The ether layer was washed with 4 N sulphuric acid, water, saturated sodium bicarbonate and water and was then dried over sodium sulphate. After the ether had been distilled off *in vacuo*, 6.2 g (92 %) of oily O-diphenyl-phosphoryl-N-carbobenzoxy-D-seryl-L-leucine benzyl ester was obtained. This oil was dissolved in 100 ml of ethanol and the solution, after addition of water to slight turbidity was then shaken in an atmosphere of pure hydrogen, 1.0 g of 10 % palladium on charcoal being used as catalyst. In 4 h, 480 ml of hydrogen was used. The catalyst was filtered off and was well washed with a small quantity of hot acetic acid. The filtrate and washings were evaporated to dryness and

\* In a study of O →<sup>c</sup>N acyl shifts in serine derivatives, Josefsson and Edman<sup>10</sup> reported the preparation of compound (I) *via* the mixed chlorocarbonic anhydride of N-carbobenzoxy-DL-serine. The isolated compound was obviously practically pure D-seryl-L-leucine derivative and had a m. p. 107°.

the residue again hydrogenolysed, but this time in glacial acetic acid solution and with 1.0 g of Adams platinum oxide as catalyst. The hydrogen uptake was completed in 8 h. After filtration and evaporation *in vacuo*, 2.8 g of crystalline residue was obtained. This residue was recrystallized from boiling water. Insoluble material (VII) was filtered off, and in the cold room the aqueous solution gave nicely crystallizing O-phosphoryl-D-seryl-L-leucine as monohydrate, 2.1 g (66 %). M. p. 138–140° (decomp.);  $[\alpha]_D^{25}$  –28.1 (1 N HCl, *c* 3.6, *l* 1). (Found: C 33.8; H 6.9; N 8.6; P 9.6. Calc. for  $C_9H_{10}O_7N_2P, H_2O$  (316.2): C 34.2; H 6.7; N 8.9; P 9.8.) A sample dried to constant weight at 65° and 0.05 mm Hg lost 7.7 % of weight and the compound then analyzed correctly for anhydrous phosphopeptide. (Found: C 36.3; H 6.3; N 9.4; P 10.4. Calc. for  $C_9H_{10}O_7N_2P$  (298.2): C 36.2; H 6.4; N 9.4; P 10.4.)

*O*-Monophenylphosphoryl-D-seryl-L-leucine (VII). The water-insoluble material from the recrystallization of (VI) was monophenylphosphoryl-D-seryl-L-leucine and crystallized from acetic acid-water, as monohydrate at a yield of 0.35 g (9 %). M. p. 213–215° (decomp.). (Found: C 45.8; H 6.4; N 7.1; P 7.8. Calc. for  $C_{15}H_{23}O_7N_2P, H_2O$  (392.4): C 45.9; H 6.4; N 7.1; P 7.9.)

## REFERENCES

1. Fölsch, G. *Acta Chem. Scand.* **13** (1959) 1407.
2. Polglase, W. J. and Smith, E. L. *J. Am. Chem. Soc.* **71** (1949) 3081.
3. Schnabel, E. and Zahn, H. *Ann.* **614** (1948) 141.
4. Mellander, O., Bennich, H. and Fölsch, G. *Acta Soc. Med. Upsaliensis* **64** (1959) 303.
5. Borkenhagen, L. F. and Kennedy, E. P. *Biochim et Biophys. Acta* **28** (1958) 222.
6. Schramm, M. *J. Biol. Chem.* **233** (1958) 1169.
7. Neuhaus, F. C. and Byrne, W. L. *J. Biol. Chem.* **234** (1959) 113.
8. Baer, E. and Maurukas, J. *J. Biol. Chem.* **212** (1955) 25.
9. Miller, H. K. and Wäelsch, H. *J. Am. Chem. Soc.* **74** (1952) 1092.
10. Josefsson, L. and Edman, P. *Biochim. Biophys. Acta* **25** (1957) 614.

Received May 13, 1959.