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L-SERYLGLYCYLGLYCINE

By Erich Baer, Jonas Maurukas, and Donald D. Clarke
L-SERYLGLYCYGLYCINE

BY ERICH BAER, JONAS MAURUKAS, AND DONALD D. CLARKE

ABSTRACT

A synthesis of the hitherto unreported L-serylglycylglycine by two different methods is described. N-carbobenzoxy L-serylglycylglycine benzyl ester, an intermediate in the preparation of the tripeptide, was found to be a suitable derivative in the synthesis of L-α-phosphatidyl L-serylglycylglycine.

In previous publications from this laboratory (1-9) methods for the synthesis of choline, ethanolamine, and serine-containing glycerolphosphatides have been described. A comparison of the synthetic and naturally occurring phosphatides led to an unambiguous elucidation of the structure and configuration of several naturally occurring lecithins, cephalins, and phosphatidyl serine. The recent discovery of naturally occurring phosphatides with peptides as nitrogenous moieties (10, 11, 18, 21, 24) challenged us to attempt the synthesis of a phosphatidyl peptide. Obviously, the difficulty of preparing peptides of even moderate chain length made it imperative that in the beginning we restrict ourselves to the synthesis of a phosphatidyl peptide with a relatively simple peptide moiety. L-Serylglycylglycine was chosen as it was expected that its preparation would not offer too many difficulties and that the phosphatidyl tripeptide would exhibit some of the properties of natural phosphatidyl peptides. Serylglycylglycine, however, is not a suitable compound for phosphorylation because of the presence of a free amino and carboxyl group. Hence, the N-carbobenzoxy L-serylglycylglycine benzyl ester, in which both groups are blocked by readily removable substituents and which is a precursor in the synthesis of the tripeptide, was used. This paper describes the synthesis of L-serylglycylglycine, a tripeptide not previously reported.

The specific rotation of L-serylglycylglycine \( [\alpha]^{20}\_D +32.5^\circ \) was found to be higher than that of L-serylglycine \( [\alpha]^{20}\_D +30.5^\circ \) reported by Fruton (19) although by analogy with peptides of a similar constitution (20) the addition of a glycine moiety to L-serylglycine should have resulted in a lower rotation. Hence L-serylglycylglycine was synthesized by a second procedure. The specific rotation of the tripeptide again was found to be +32.5°. Repeated checks bore out the correctness of this value. In addition the optical activity of L-serylglycylglycine was measured at various temperatures within the range of 11°-50°. The results are recorded in Table I.

1 Manuscript received May 31, 1956.

Contribution from the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario. Supported by a Research Grant of the National Research Council of Canada, Division of Medical Research, Ottawa, Canada. An account of the work described in this paper was presented at the Symposium on Phospholipids at the University of Western Ontario, London, Ontario, Canada, October 12-13, 1955.

2 A section of this paper forms part of a thesis submitted by Jonas Maurukas to the Department of Pathological Chemistry of the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
TABLE I
OPTICAL ROTATION OF L-SERYLGLYCYLGlyCINE
AT VARIOUS TEMPERATURES

<table>
<thead>
<tr>
<th>Temp. (°C.)</th>
<th>$[\alpha]_D^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>+35.4</td>
</tr>
<tr>
<td>17</td>
<td>+34.1</td>
</tr>
<tr>
<td>25</td>
<td>+32.5</td>
</tr>
<tr>
<td>30</td>
<td>+32.2</td>
</tr>
<tr>
<td>33</td>
<td>+31.6</td>
</tr>
<tr>
<td>45</td>
<td>+29.7</td>
</tr>
<tr>
<td>50</td>
<td>+28.6</td>
</tr>
</tbody>
</table>

*Concentration: 5.4% in 1 N HCl.

L-Serine methyl ester hydrochloride
  ↓
L-Serine methyl ester
  ↓
N-Cbz. L-serine methyl ester
  ↓
N-Cbz. L-serinhydrazide
  ↓
N-Cbz. L-serinazide

Glycylglycine benzyl ester
  ↓
Glycine methyl ester
  ↓
N-Cbz. L-serglycine methyl ester
  ↓
N-Cbz. L-serglycinhydrazide
  ↓
N-Cbz. L-serglycinazide
  ↓
Glycine benzyl ester
  ↓
N-Cbz. L-serglyclylglycine benzyl ester
  ↓
L-Serylglyclylglycine

Cbz. = carbobenzoxy

REACTION SCHEME
EXPERIMENTAL

N-CARBOBENZOXY L-SERYLGLYCYLGLYCINE BENZYL ESTER

Procedure I

N-Carbobenzoxy L-Serinhidrazide

A synthesis of N-carbobenzoxy L-serinhydrazide via the N-carbobenzoxy L-serine methyl ester hydrochloride has been reported by Fruton (19). Since his description is rather brief, the procedure is reported in greater detail here.

N-carbobenzoxy L-serine methyl ester: In a 500 ml. two-necked round-bottomed flask equipped with a motor-driven stirrer were placed 100 ml. of water, 48 gm. of sodium bicarbonate, 75 ml. of ether, and 13.2 gm. of L-serine methyl ester hydrochloride (17). The flask was immersed in an ice-cold water bath and 10 min. later 21.0 ml. of freshly prepared carbobenzoxy chloride was added in the course of 20 min., while stirring vigorously. After two hours the bath was removed and the stirring was continued for an additional 30 min. The ether and water layers were separated, and the water layer was washed with two 35 ml. portions of ether. The combined ether solutions were freed from carbobenzoxy chloride by the addition of pyridine in just sufficient amounts to destroy the unreacted portion of the chloride, and then were washed successively with water, 2.5 N hydrochloric acid, and water. Drying the ether solution with anhydrous sodium sulphate and subsequent evaporation of the solvent under diminished pressure yielded N-carbobenzoxy L-serine methyl ester. (This compound was also obtained in a good yield by methylating N-carbobenzoxy L-serine (7) with diazomethane.)

Hydrazide: The N-carbobenzoxy L-serine methyl ester, without further purification, was dissolved in 100 ml. of 99% ethanol, and to its solution was added 4.7 ml. of hydrazine hydrate. The N-carbobenzoxy L-serinhydrazide began to crystallize almost immediately. After it had been left at room temperature for 10 hr., the mixture was filtered with suction, the hydrazide was washed with cold 99% alcohol followed by ether and dried in vacuo. Obtained were 13 gm. of N-carbobenzoxy L-serinhydrazide (54% of the theory based on serine), m.p. 181°. Reported by Fruton (19), m.p. 181°.

N-Carbobenzoxy L-Serinazide

To a cold (-5°) and vigorously stirred solution of 13 gm. of N-carbobenzoxy L-serinhydrazide in a mixture of 60 ml. of glacial acetic acid and 223 ml. of 0.5 N hydrochloric acid was added a concentrated aqueous solution of 3.9 gm. of sodium nitrite. The azide formed immediately and precipitated as an oil. Crystallization of the azide was induced by mechanical means and the crystals were washed with ice-cold water. The dry and crystalline N-carbobenzoxy L-serinazide, which weighed 10.4 gm., and which appeared to be quite stable at room temperature, melted from 61° to 63° with the evolution of gas. On further heating the melt solidified to melt again at 169°-171°.

The higher melting substance evidently is 4-carbobenzoxyaminooxazolidone-2 (m.p. 171°), which was obtained by Fruton (19) by rearrangement of N-carbobenzoxy L-serinazide in ethyl acetate solution at 40°. When Fruton's
procedure was repeated, 4-carbobenzoxyaminooxazolidone-2, m.p. 169°–171°, with a specific rotation of \([\alpha]_D^{25} = -50°\) in ethyl acetate \((c, 0.6)\), was obtained. The retention of optical activity on rearrangement is in agreement with the mechanism suggested by Kenyon and Young (22) for the Curtius rearrangement.

**Glycylglycine Benzyl Ester Hydrochloride**

Glycylglycine (14.4 gm.) was converted to its benzyl ester benzenesulphonate as described by Miller and Waelsch for glycine (23); 39 gm. (95%) of crude product was obtained. This material was added to 325 ml. of 99% ethanol, and dry hydrogen chloride gas was passed into the mixture with swirling until a clear solution resulted. After a few minutes crystallization set in. The mixture was cooled in an ice bath, filtered with suction, and the precipitate was washed with cold 99% ethanol followed by dry ether. On recrystallization of the crude product (15.2 gm.) from 200 ml. of hot 99% ethanol, 13.0 gm. of glycylglycine benzyl ester hydrochloride (50%) was obtained, m.p. 159°–161°C.

Glycylglycine benzyl ester hydrochloride was also prepared according to the procedure of Cook et al. (13, 14) for the synthesis of glycy peptides, by coupling 2-thio-5-thiazolidone with glycine benzyl ester. The chloroform which was used by these authors was replaced by dichloromethane as this solvent facilitated the crystallization of the benzyl ester hydrochloride, m.p. 159°–160°C.

Neither of these preparations gave melting point depressions with glycylglycine benzyl ester hydrochloride prepared by the method of Zervas and Theodoropoulos (26).

**Glycylglycine Benzyl Ester**

Glycylglycine benzyl ester hydrochloride (13.0 gm.) suspended in 130 ml. of dry chloroform was treated with dry ammonia gas in excess and the fine precipitate of ammonium chloride was removed by filtration. The solvent was distilled off under reduced pressure to remove excess ammonia, and the glycylglycine benzyl ester was redissolved in 100 ml. of chloroform.

**N-Carbobenzoxy L-Serylglycylglycine Benzyl Ester**

The solution of glycylglycine benzyl ester in chloroform, obtained in the previous step, was added to a solution of 10.4 gm. of N-carbobenzoxy L-serinhydrazide in 200 ml. of dry ether. A precipitate was formed immediately. The mixture, after having been kept overnight at room temperature, was cooled to −10°, filtered with suction, and the solid was washed first with dry chloroform and then with dry ether. The crude material, weighing 18 gm., on recrystallization from 100 ml. of 99% ethanol yielded 13 gm. of N-carbobenzoxy L-serylglycylglycine benzyl ester (58% based on N-carbobenzoxy L-serinhydrazide), m.p. 150°–152°. A further recrystallization from ethanol yielded a material melting from 151° to 153°. Anal. Calc. for C_{22}H_{28}O_{7}N_{3} (443.45): C, 59.58; H, 5.68; N, 9.48. Found: C, 59.11; H, 5.62; N, 9.50
**Procedure II**

**Glycine Methyl Ester**

The ester was prepared from glycine methyl ester hydrochloride by the procedure of Curtius and Goebel (15), except that a small and controlled amount of water was added to ensure a rapid reaction. The modified procedure is as follows.

A mixture of 14 gm. (111.5 mM.) of finely powdered glycine methyl ester hydrochloride, 12.9 gm. (55.7 mM.) of finely powdered silver oxide, and 85 ml. of anhydrous ether, to which 15 drops of distilled water had been added, was vigorously shaken for three hours. At the end of this period the ether was decanted and the residue was washed with three 20 ml. portions of anhydrous ether. The combined ether solutions, which contained approximately 70% of the theoretical amount of the glycine methyl ester, were dried with barium oxide. The glycine methyl ester is sufficiently stable in ether solution that, if necessary, it can be stored in this form for several days without suffering decomposition.

**N-Carbobenzoxy L-Serylglycine Methyl Ester**

This substance was prepared from N-carbobenzoxy L-serinazide and glycine methyl ester following Erlanger and Brand’s procedure for the preparation of substituted glycine and alanine dipeptides (16). A dry and ice-cold solution of N-carbobenzoxy L-serinazide prepared as described above from 13.0 gm. of N-carbobenzoxy L-serinazide was added to an ether solution of glycine methyl ester obtained from 14 gm. of glycine methyl ester hydrochloride. The formation of the N-carbobenzoxy L-serylglycine methyl ester took place rapidly; most of it precipitated during the first 10 min. After it had been standing for 20 hr. at room temperature and one hour at $-10^\circ$, the mixture was filtered by suction, the solid was washed with cold ether and dried in vacuo over sodium hydroxide. The crude material, weighing 14 gm., on re-precipitation from 75 ml. of warm ethyl acetate by the addition of low-boiling petroleum ether, yielded 12.5 gm. (78.5%) of crystalline N-carbobenzoxy L-serylglycine methyl ester, m.p. 100–101°. Anal. Calc. for C$_{14}$H$_{18}$O$_{6}$N$_{2}$ (310.3): N, 9.03. Found: N, 9.20.

**N-Carbobenzoxy L-Serylglycinhydrizide**

The hydrazide was prepared as follows (12, 16): A solution of 11.2 gm. of N-carbobenzoxy L-serylglycine methyl ester and 4.5 ml. of hydrazine hydrate in 100 ml. of 99% ethanol was refluxed for one hour. During this time most of the hydrazide precipitated as a crystalline mass. After it had been left for 12 hr. at room temperature, the mixture was filtered by suction, the hydrazide was washed with cold 99% ethanol, followed by ether, and was dried in vacuo over sodium hydroxide. Yield 9.4 gm. (83.9% of theory); m.p. 186°–187°, reported m.p. 181°–182° (20). This substance is relatively stable and can be stored, if necessary, for future use. Anal. Calc. for C$_{13}$H$_{18}$O$_{6}$N$_{4}$ (310.31): N, 18.05. Found: N, 17.74.
N-Carbobenzoxy L-Serylglycylglycine Benzyl Ester

Azide: To a clear solution of 9.5 gm. (30.6 mM.) of N-carbobenzoxy L-seryl-glycinhydrazide in 65 ml. of glacial acetic acid and 325 ml. of 0.5 N hydrochloric acid was added with shaking a concentrated aqueous solution of 2.1 gm. (31.0 mM.) of sodium nitrite, and the mixture was cooled to -5°. The N-carbobenzoxy L-serylglycinazide was extracted with two 250 ml. portions of an ice-cold mixture of ether-ethyl acetate (1:1, v/v). The combined extracts were washed with a cold and concentrated aqueous solution of bicarbonate until the washings remained alkaline to red litmus paper. The cold solution of the azide, after being dried briefly with anhydrous sodium sulphate, was added to the dry ethereal solution of glycine benzyl ester that had been prepared from 9.2 gm. of glycine benzyl ester hydrochloride by the procedure of Miller and Waelsch (23). After the solution had been left for six hours at room temperature and one hour at 0°, the crystalline material was filtered off, washed with ether, and dried over sodium hydroxide in vacuo. The crude product (9.5 gm.) on reprecipitation from a solution in 10 ml. of 99% ethanol by the gradual addition of ether yielded 8.5 gm. of N-carbobenzoxy L-serylglycylglycine benzyl ester (62.7% based on N-carbobenzoxy L-serylglycinhydrazide), m.p. 151°-153°.

Tripeptide

L-Serylglycylglycine

Two grams of N-carbobenzoxy L-serylglycylglycine benzyl ester, prepared by either one of the above procedures, was dissolved in 60 ml. of 80% acetic acid (12), and to the solution was added 0.2 gm. of palladium black (25). The mixture was shaken vigorously at room temperature in an atmosphere of pure hydrogen at an initial pressure of approximately 50 cm. of water until the consumption of hydrogen had ceased completely. The hydrogen was replaced by nitrogen, the catalyst was filtered off, and both hydrogenation vessel and catalyst were washed with distilled water. The combined filtrate and washings were evaporated to dryness under reduced pressure (bath 35°-40°). The residue was dissolved in 10 ml. of water and to the solution was added with stirring 20 ml. of 99% ethanol. After cooling to -10°, the mixture was filtered with suction and the precipitate was washed with dry ethanol. The L-serylglycylglycine, which frequently was obtained in the form of a monohydrate, after being dried in vacuo at 120°, weighed 0.9 gm. (91% of theory). The tripeptide, obtained from N-carbobenzoxy L-serylglycylglycine benzyl ester prepared either by procedure I or II, melted with decomposition at 216° (the capillary was placed in an oil bath at 200° and the temperature of the bath was raised 10° per min.) and had a specific rotation [α]D 25° +32.5° in 1 N hydrochloric acid (c, 5.4). Anal. Calc. for C7H13O5N3 (219.2): C, 38.35; H, 5.98; N, 19.17. Found: C, 38.24; H, 5.74; N, 18.83 (Dumas).

REFERENCES