[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Dipeptides of the Unnaturally Occurring *d*-Amino Acids

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In the course of work being carried out in connection with certain studies in this Laboratory, dipeptides of the unnaturally occurring amino acids were needed. We have prepared dipeptides containing two unnatural amino acids by means of the Bergmann[1] carbobenzoxy method. In previous syntheses of dipeptides of unnatural amino acids, Fischer's methods involving Walden inversions[2] or the coupling of optically pure alpha halogen acids[3] were used. Both of these methods are of limited applicability and the optical purity of the compounds was always questionable, due to the possibility of racemization.[4] The Bergmann method is free from this criticism and we obtained optically pure *d,d*-dipeptides in good yield. As far as we know, these are the first reported syntheses of optically pure *d,d*-dipeptides prepared by this method.

Two possible uses of *d,d*-dipeptides are immediately apparent. First, they may be used to define more rigorously the specificity of dipeptidase as reported in a fine summary by Bergmann,[5] second, they may be used as substrates for enzymes found in cancer tissue, in order to determine whether *d*-amino acid linkages[6] are actually present.

We are reporting in the experimental part a specific rotation of $+35.7°$ for glycyl-*d*-leucine. Fischer[7] reported $-35.1°$ ($\pm 0.5°$) for the synthetic antipode, glycyl-*l*-leucine. This is an indication of the optical purity of our compound. Due to the fact that we have insufficient material for precise determinations of the specific rotations of *d*-phenylalanyl-*d*-leucine and *d*-alanyl-*d*-leucine, we are unable to report rotations for these compounds at this time.

Experimental Part

Since the methods of synthesis of all these new dipeptides are quite similar, the preparation of one will be discussed in detail, and the other new compounds will be listed as prepared.

(1) Bergmann and Zervas, *Ber.*, **65**, 1192 (1932).
(2) Fischer and Warburg, *Ann.*, **340**, 165 (1905).
(3) Fischer and Ott, *Ber.*, **36**, 2106, 2982 (1903).
(4) C. L. A. Schmidt, "Chemistry of the Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1938, p. 259.
(5) Bergmann, Zervas, Fruton, Schneider and Schleich, *J. Biol. Chem.*, **109**, 325 (1935).
(6) Woodward, Reinhart and Dohan, *ibid.*, **138**, 677 (1941).
(7) Fischer and Steingrover, *Ann.*, **365**, 169 (1909).

d-Phenylalanyl-*d*-leucine

***d*-Leucine Methyl Ester Hydrochloride.**—This compound was prepared by the general method of E. Fischer.[8] Nineteen grams of *d*-leucine was covered with 100 cc. of absolute methanol and treated with dry hydrogen chloride for one hour. After removing the alcohol, the substance was recrystallized from alcohol–ether as long needles melting at 149–150°. The yield was quantitative.

Anal. Calcd. for $C_7H_{16}O_2NCl$: N, 6.28. Found: N, 6.09.

N-Carbobenzoxy-*d*-phenylalanine.—This was made from *d*-phenylalanine which in turn was prepared from *dl*-phenylalanine by Fischer's[9] method of resolution. Six and six-tenths grams of *d*-phenylalanine was dissolved in 20 cc. of 2 *N* sodium hydroxide, and the solution was treated in an ice-bath first with 6.8 g. of carbobenzoxy chloride, and then with an additional 20 cc. of *N* sodium hydroxide in two portions. After twenty minutes the mixture was acidified to congo red. The precipitate was filtered and washed and, on recrystallization from water–methanol, long matted needles formed; yield 84%, m. p. 126–128°; $[\alpha]^{24}D$ $-4.6°$ (*c* 4, glacial acetic acid).

Anal. Calcd. for $C_{17}H_7O_4N$: N, 4.68. Found: N, 4.51.

N-Carbobenzoxy-*d*-phenylalanyl-*d*-leucine Methyl Ester.—Four and one-half grams of carbobenzoxy-*d*-phenylalanine was shaken with 4.2 g. of phosphorus pentachloride n 50 cc. of ether in an ice-bath. When most of the material had dissolved, the mixture was filtered, and the ether was evaporated in the absence of water. The chloride was precipitated with cold petroleum ether and, on recrystallization, long needles formed, which were filtered and used immediately for coupling.

To prepare free *d*-leucine methyl ester from the hydrochloride described above, 5 g. of the hydrochloride was dissolved in 10 cc. of water and covered with 35 cc. of ether. The material was cooled in an ice-bath, and treated with 5 cc. of 33% sodium hydroxide. The aqueous solution was extracted with three 10-cc. portions of ether, and the ethereal solution was dried over sodium sulfate.

The acid chloride prepared above was added to the cold ethereal solution of the *d*-leucine methyl ester. After one hour, the *d*-leucine methyl ester hydrochloride, which had crystallized out, was removed and the filtrate was washed with dilute hydrochloric acid, dilute bicarbonate and finally with water. The ethereal solution was then dried over sodium sulfate. After filtering off the sulfate and removing the ether, crystals of carbobenzoxy-*d*-phenylalanyl-*d*-leucine methyl ester were obtained. On recrystallization from ethyl acetate–petroleum ether, 1.4 g. of the product came out as long prisms which melted sharply at 109°.

(8) E. Fischer, *Ber.*, **34**, 433 (1901).
(9) Fischer and Schoeller, *Ann.*, **357**, 4 (1907). The *d*-phenylalanine prepared had the rotation $[\alpha]^{20}D$ $+35.3°$. Fischer reported $[\alpha]^{20}D$ $+35$ ($\pm 0.5°$).

Anal. Calcd. for $C_{24}H_{30}O_5N_2$: N, 6.57. Found: N, 6.75.

d-**Phenylalanyl-*d*-leucine.**—To 1.25 g. of the ester in 100 cc. of methanol, 6 cc. of *N* sodium hydroxide was added. After one-half hour the solution was acidified to congo red. On evaporation of the methanol, a sirup resulted which was dissolved in ethanol and treated with water. Again a sirup resulted which was extracted with ethyl acetate and treated with dilute sodium bicarbonate solution. The aqueous solution was acidified, and the sirup which resulted (1 g.) was used for the next step.

The sirup of carbobenzoxy-*d*-phenylalanyl-*d*-leucine was dissolved in 10 cc. of methanol, a few drops of water and 1 cc. of glacial acetic acid. The mixture was hydrogenated over palladium black until complete evolution of carbon dioxide (one-half hour). The solution was filtered and evaporated under reduced pressure. Small needles crystallized out, which were recrystallized from a small quantity of cold water. The peptide was washed with alcohol and ether, and dried. The substance contained 1 mole of water which was removed by drying over phosphorus pentoxide *in vacuo* at 100°; yield 63%, m. p. 261–262° (dec.).

Anal. Calcd. for $C_{15}H_{22}O_3N_2$: C, 64.72; H, 7.91; N, 10.07. Found: C, 64.43; H, 7.79; N, 9.89.

d-Alanyl-*d*-leucine

N-Carbobenzoxy-*d*-alanine.—In a similar manner, *d*-(−)-alanine was converted to carbobenzoxy-*d*-alanine which crystallized from ether–petroleum ether in clusters of needles, m. p. 84–85°; yield 77%, $[\alpha]^{24}D$ +13.5° (*c* 8.5, glacial acetic acid).

Anal. Calcd. for $C_{11}H_{13}O_4N$: N, 6.28. Found: N, 6.09.

The above compound was treated with phosphorus pentachloride to yield the acid chloride which was coupled with *d*-leucine methyl ester, in a manner similar to that described above, to yield the ester (sirup) which was saponified and hydrogenated to the free dipeptide. The peptide crystallized from water in small prisms, which were washed with alcohol and ether, and dried. The peptide contained 1 mole of water which was removed over phosphorus pentoxide *in vacuo* at 100°, m. p. 254–255° (dec.).

Anal. Calcd. for $C_9H_{18}O_3N_2$: C, 53.46; H, 8.91; N, 13.86. Found: C, 53.21; H, 8.80; N, 13.68.

Glycyl-*d*-leucine

N-Carbobenzoxy-glycyl-*d*-leucine.—Carbobenzoxy-glycine was converted to the acid chloride and coupled with *d*-leucine methyl ester. A sirup resulted which on saponification yielded carbobenzoxy-glycyl-*d*-leucine. On recrystallization from ethyl acetate–petroleum ether, long prisms were obtained; m. p. 101–102°.

Anal. Calcd. for $C_{16}H_{22}O_5N_2$: N, 8.70. Found: N, 8.86.

This compound was hydrogenated to glycyl-*d*-leucine which crystallized from water in long prisms; yield 91%; m. p. 234° (yellow), 242–243° (dec.); $[\alpha]^{25}D$ +35.7° (*c* 3, water).

Anal. Calcd. for $C_8H_{16}O_3N_2$: C, 51.06; H, 8.51; N, 14.89. Found: C, 50.89; H, 8.41; N, 14.74.

N-Carbobenzoxy-*d*-leucyl Hydrazide.—In attempts at other syntheses in this field, N-carbobenzoxy-*d*-leucyl hydrazide was prepared by Bergmann's[5] general method. On recrystallization from ethyl acetate–petroleum ether, clusters of needles melting at 121° were obtained in 84% yield. Bergmann[5] reported the same m. p. for the *l*-form.

Anal. Calcd. for $C_{14}H_{21}O_3N_3$: N, 15.06. Found: N, 15.01.

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Summary

Derivatives of amino acids of the *d*-series, especially the *d,d*-dipeptides, have been prepared by the Bergmann carbobenzoxy method. In view of their recent interest, possible applications of these types of compounds in the fields of enzyme and protein chemistry have been discussed.

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