sure and finally in a desiccator under reduced pressure; yield 8.4 g.

This colorless amorphous material was acetylated with 5 g. of fused sodium acetate and 100 ml. of acetic anhydride by initiating the reaction at 120° and allowing it to proceed for one hour at 100°. The mixture was cooled, poured with stirring into 900 ml. of ice and water and allowed to stand with occasional stirring for 6 hr. The sirupy mixture was extracted with five 200-ml. portions of chloroform and the extract was washed with cold water until neutral, dried over anhydrous sodium sulfate and finally concentrated by distillation under reduced pressure to a thick sirup which was further dried to an amorphous solid in a vacuum desiccator; yield 13.5 g.

Chromatographic Resolution.—An amount of 6.5 g. of the above mixture of acetylated sugar alcohols was dissolved in 150 ml. of benzene and added at the top of a 265 × 74 mm. (diam.)[15] column of Magnesol–Celite (5:1 by wt.). The chromatogram was developed with 3500 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). An alkaline permanganate streak on the extruded column disclosed four main zones, one at the bottom (A—some of this material had passed into the effluent), a second about the center (B) and two in the top half of the column (C). These were sectioned and eluted with acetone. Solvent removal from the acetone eluate of A left crystalline material. This was combined with that obtained from the effluent on solvent removal and recrystallized from ethanol. It was identified as L-fucitol pentaacetate; yield 3.2 g., m.p. 128–129° unchanged on admixture with authentic L-fucitol pentaacetate synthesized from known L-fucose, $[\alpha]^{23}D$ +21.3° (*c* 2.3, chloroform). These values are in agreement with those reported in the literature for L-fucitol pentaacetate.

The material obtained from the acetone eluate of B crystallized and was recrystallized from ethanol. It was shown to be identical with the material from A by mixed melting point, specific rotation and X-ray diffraction; yield 280 mg.

The sirupy material obtained from C of two such columns was combined and rechromatographed in the same manner by development with 7000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). Two zones were located on the extruded column and were eluted with acetone. The sirup obtained from the lower zone crystallized from ethanol and was shown by melting point (113–157°) to be exceedingly impure; yield 409 mg. This material was rechromatographed but no further separation was obtained. It was then separated by fractional crystallization from ethanol and the acetylated fucobiitol obtained pure. A small amount of a second crystalline substance also was obtained, but this has not yet been characterized. Acetylated fucobiitol; yield 225 mg., m.p. 119–120°, $[\alpha]D^{24}$ −81.5° (*c* 1.08, chloroform).

Anal. Calcd. for $(C_{12}H_{17}O_9)(CH_3CO)_7$: C, 51.48; H, 6.32; (CH_3CO), 11.54 ml. of 0.1 *N* NaOH per 100 mg.; mol. wt., 606. Found: C, 51.42; H, 6.38; (CH_3CO), 11.50 ml.; mol. wt. (Rast), 613.

If left in contact with alcohol for a few days this compound undergoes a change in crystal structure from needles with the above melting point to small prisms which melt at 99–102°.

Preparation of the Crystalline Alditol.—An amount of 255 mg. of the crystalline acetate (m.p. 119–120°) dissolved in absolute methanol (2.6 ml.) was treated with 0.8 ml. of sodium methoxide solution (0.5 g. of sodium in 100 ml. of absolute methanol) for 16 hr. at 5°. The solution was neutralized with dilute acetic acid, diluted with water and deionized by passage through a mixed-bed column of Amberlites IR-120 and IR-4b.[16] Concentration of the solution to dryness by distillation under reduced pressure left a white amorphous solid which was crystallized from ethanol and recrystallized from the same solvent; yield 132 mg., m.p., 190–192°, $[\alpha]^{23}D$ −118° (*c* 0.5, water). Periodate oxidation data on this compound are recorded in Table I.

Hydrolysis.—The above crystalline alditol (15 mg.) was hydrolyzed with 3 ml. of 0.4 *N* hydrochloric acid at 60°. The reaction was followed polarimetrically. The final specific rotation was $[\alpha]^{24}D$ −36.5° which corresponds to an equimolar mixture of L-fucose and L-fucitol. The solution was deionized by passing through a mixed-bed column of Amberlites IR-120 and IR-4b,[16] and concentrated to dryness under reduced pressure. The components were separated by fractional crystallization from ethanol. In this manner there were isolated L-fucitol (4 mg.), m.p. and mixed m.p. with authentic L-fucitol 151–153°, and L-fucose (5 mg.) m.p. 144–145° unchanged on admixture with an authentic specimen.

(15) Adsorbent dimensions.

(16) Products of the Resinous Products Division of Rohm and Haas Co., Philadelphia, Penna.

HALIFAX, N. S., CANADA

[CONTRIBUTION FROM THE SHELL DEVELOPMENT COMPANY]

Peptide Derivatives Containing Two Trifunctional Amino Acids

BY R. F. FISCHER AND R. R. WHETSTONE[1]

RECEIVED APRIL 12, 1954

A number of derivatives of histidyltyrosine, histidylserine, histidylglycine, seryltyrosine, tyrosylserine, tyrosylhistidine and serylglycylhistidine have been prepared by adaptation of the Curtius azide route. In the course of the work methods for the preparation of L-histidyl peptides employing carbobenzoxy-L-histidine azide or carbobenzoxyglycyl-L-histidine azide were developed.

There have been many peptides prepared containing several of the simpler amino acids, and a moderate number which have included one of the more complex trifunctional amino acids, but relatively few which include more than one of the trifunctional amino acids. This is especially true for peptides of L-serine and L-histidine, and when this work was undertaken no general method for synthesis of L-histidyl compounds had been described in the literature.

Recently, however, Holley and Sondheimer[2] have reported a synthesis of L-histidyl peptides which is identical in most respects with one employed by us as part of our synthesis program. We are able to confirm the authors' general statements and their physical constants for carbobenzoxy-L-histidine hydrazide and carbobenzoxy-L-histidylglycine hydrazide (Ib). For our purposes we found it more convenient to use carbobenzoxyglycyl-L-histidine hydrazide, since this compound could be prepared in 70% over-all yield. The coupling of the glycyl and histidine groups was accomplished *via* an azide reaction, which avoided the use of acyl halides.

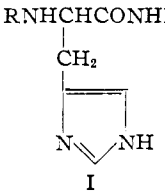
In the normal azide procedure, hydrazides are converted to azides in excess aqueous mineral acid (often with acetic acid present also), whereupon the azides precipitate and are allowed to react with the

(1) Present address: Shell Development Co., Denver, Colo.

(2) R. W. Holley and E. Sondheimer, THIS JOURNAL, **76**, 1326 (1954).

desired amine in a neutral solvent. When, however, the histidine azides were prepared in this way, they remained in solution due to the basic imidazole nucleus. Unexpectedly, adjustment of the *p*H of the solutions to 7 (with potassium bicarbonate) did not precipitate the azides, and only at *p*H 9 (potassium carbonate) were the oily azides liberated. They could then be taken up in chloroform, dried and allowed to react with the desired amino compound also in chloroform. In our work we found that azide formation was satisfactory either in the presence of acetic acid or in its absence, though isolation of the azide was more time-consuming when it was necessary to neutralize a large excess of acetic acid.

The reaction sequences were conducted first with aniline as the amine, giving the anilides, Ia and Ic, and then with glycine ethyl ester to show that the method was also applicable to amino acid esters. Carbobenzoxy-L-histidylglycine ethyl ester was obtained first as an oil which was converted to the hydrazide Ib in 51% over-all yield. By contrast, carbobenzoxyglycyl-L-histidylglycine ethyl ester (Id) separated from the reaction solvent in crystalline form in 65% yield. The improved yield, along with the greater ease of preparation of carbobenzoxyglycyl-L-histidine hydrazide led us to choose this compound as the azide precursor for coupling with esters of serine and tyrosine. Although the yields of carbobenzoxyglycyl-L-histidyl-L-serine methyl ester (Ie) and carbobenzoxyglycyl-L-histidyl-L-tyrosine methyl ester (If) were only 25 and 43%, respectively, the products were obtained in almost pure form directly from the reaction solvent, and further purification was relatively simple.

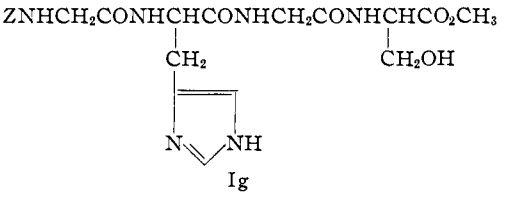


I

Compound	R	R'
a	Z	C_6H_5
b	Z	$CH_2CONHNH_2$
c	$ZNHCH_2CO$	C_6H_5
d	$ZNHCH_2CO$	$CH_2CO_2C_2H_5$
e	$ZNHCH_2CO$	$CH(CH_2OH)CO_2CH_3$
f	$ZNHCH_2CO$	$CH(CH_2C_6H_4OH)CO_2CH_3$

The tyrosine derivative was saponified to the crystalline acid and then decarbobenzoxylated to the tripeptide, glycyl-L-histidyl-L-tyrosine.

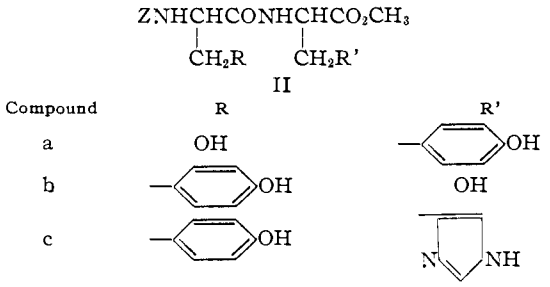
In order to prepare a compound in which the histidyl group was followed by two amino acids, it was necessary to modify the general procedure somewhat. Carbobenzoxyglycyl-L-histidylglycine azide was found to be insoluble in the solvents commonly employed for azide couplings. However, it reacted satisfactorily with L-serine methyl ester in a pyridine-chloroform slurry, giving carbobenzoxyglycyl-L-histidylglycyl-L-serine methyl ester, Ig.



Ig

In the course of other attempts to prepare Ig, carbobenzoxyglycyl-L-serine methyl ester was prepared. This compound has apparently not been described previously in the literature, and its preparation and physical constants are recorded in the experimental section.

Continuing the preparation of peptide derivatives containing two trifunctional amino acids, the azide route was again used exclusively, since its advantages in the L-histidine syntheses also are evident in the L-serine and L-tyrosine series. Although carbobenzoxy-L-serine azide[3a] and carbobenzoxy-L-tyrosine azide[3b] have been used successfully, the reactions, especially in the L-serine series, have been confined to relatively simple peptides. We have extended the series to include the carbobenzoxy methyl esters of L-seryl-L-tyrosine (IIa), L-tyrosyl-L-serine (IIb) and L-tyrosyl-L-histidine (IIc).

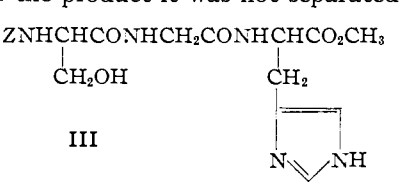


II

Compound	R	R'
a	OH	OH
b	OH	OH
c	OH	NH

Azide formations in these cases also were conducted both in the presence and absence of acetic acid. No significant yield differences were noted, but work-up was again somewhat more difficult when acetic acid was present, since it was necessary to remove it from the azide solution with several bicarbonate washes. The crude yields were only fair (22 to 55%), and purification was relatively difficult; repeated crystallization yielded about half the original quantities of pure esters, two of which (IIa and IIc) were saponified to the crystalline acids. Carbobenzoxy-L-seryl-L-tyrosine was also decarbobenzoxylated to the free dipeptide, L-seryl-L-tyrosine.

Early in the study, a mixture of the diastereoisomeric carbobenzoxy-D- and L-serylglycyl-L-histidine methyl esters (III) was prepared from carbobenzoxy-DL-serylglycine azide and L-histidine methyl ester, but due partly to the hygroscopic nature of the product it was not separated into its



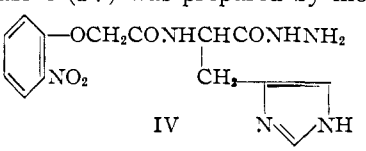
III

(3) (a) J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942); (b) C. R. Harington and R. V. Pitt Rivers, *Biochem J.*, **38**, 417.

stereoisomers, and subsequent work was confined to the pure L-amino acids.

Throughout the course of this work it was necessary to prepare amino acid esters from their hydrochlorides. While the usual reagents (potassium carbonate or sodium methoxide) are fairly satisfactory, we found the dry ammonia procedure described by Bailey[4] to be very useful, especially for those esters which have considerable water solubility. By this method, L-histidine methyl ester, previously obtained only as an oil, has been isolated as a crystalline, though hygroscopic and unstable, compound with m.p. 64–66° (crystallized from ethyl acetate).

A number of other approaches to the desired compounds were also tried, and while these did not prove fruitful some new amino acid derivatives have been prepared and several discrepancies with the literature noted. Phthaloylhistidine was prepared by the method of Keil,[5] and was found to melt at 294° rather than 188° as reported. Its methyl ester hydrochloride melted at 221–23° rather than at 238–240°, but the melting point of the free ester (187°) agreed with that reported by Keil. Phthaloylglycine azide was prepared as a crystalline solid, stable for several months at room temperature. It reacted immediately with the esters of L-serine and L-histidine to give phthaloylglycyl esters of L-serine and L-histidine in excellent yields. The ease of preparation of these compounds made them attractive as potential intermediates, but in a preliminary study, we were not able to remove the phthaloyl group without causing cyclization to the mixed diketopiperazines, and the phthaloyl intermediates were then abandoned in favor of the carbobenzoxy compounds. Another possible intermediate, *o*-nitrophenoxyacetyl-L-histidine hydrazide (IV) was prepared by modification



IV

of the general method of Holley and Holley,[6] but the difficulties encountered in a small-scale synthesis made its preparation in larger amounts unattractive.

Experimental[7a,b]

Carbobenzoxy-L-histidine hydrazide and carbobenzoxy-L-histidylglycine hydrazide (Ib) were prepared by methods not significantly different from those described by Holley and Sondheimer.[2] Melting points were 172–174° and 180–181°, respectively.

Carbobenzoxy-L-histidine anilide (Ia) prepared similarly melted at 172° after recrystallization from 50% methanol.

Anal. Calcd. for $C_{20}H_{20}N_4O_3$: C, 65.92; H, 5.53; N, 15.38. Found: C, 65.7; H, 5.6; N, 15.0.

Carbobenzoxyglycyl-L-histidine Hydrazide.—Carbobenzoxyglycine hydrazide was prepared by the method of Erlanger and Brand[8] and in quantitative yield by the esterification of carbobenzoxyglycine[9] with 5–10 parts of 0.5 *N* methanolic hydrochloric acid, followed by removal of the solvent and treatment with hydrazine.

The hydrazide (12 g.) was converted to the azide[8] which was dissolved in chloroform and added to 11.9 g. of L-histidine methyl ester also in chloroform. After 20 hours the chloroform was removed *in vacuo*, leaving a viscous oil. This was taken up in 100 ml. of methanol, 3 g. of 95% hydrazine was added and the solution was allowed to stand overnight. There was obtained 13.5 g. (70%) of carbobenzoxyglycyl-L-histidine hydrazide, m.p. 199–202°. After recrystallization from methanol it melted at 200–202°.

Anal. Calcd. for $C_{16}H_{20}N_6O_4$: C, 53.32; H, 5.59; N, 23.32. Found: C, 53.4; H, 5.5; N, 23.0.

Carbobenzoxyglycyl-L-histidine anilide (Ic) was prepared in a manner similar to that employed for carbobenzoxy-L-histidine anilide (Ia). From 3.6 g. (0.01 mole) of the hydrazide there was obtained 1.0 g. (24%) of the anilide, melting at 200–201° after crystallization from methanol (mixed m.p. with starting hydrazide, 184–189°).

Anal. Calcd. for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.5; H, 5.4; N, 16.1.

Carbobenzoxyglycyl-L-histidyl-L-tyrosine Methyl Ester (If).—Carbobenzoxyglycyl-L-histidine azide was formed from the hydrazide (2.4 g., 0.0067 mole) as in the preparation of the anilide Ia. However, neutralization of excess acid was accomplished by means of ice-cold saturated potassium carbonate solution, and 150 ml. of chloroform was stirred into the partly frozen mixture to take up the azide as liberated. The dried chloroform layer was added to 1.3 g. of L-tyrosine methyl ester[10] in 200 ml. of chloroform and allowed to stand overnight. The crystalline precipitate weighed 1.5 g. (43%), m.p. 190–198°. After four crystallizations from 50% methanol, the melting point was constant at 208°.

Anal. Calcd. for $C_{26}H_{29}N_5O_7$: C, 59.64; H, 5.58; N, 13.38. Found: C, 59.0; H, 5.3; N, 13.0.

Carbobenzoxyglycyl-L-histidyl-L-tyrosine.—A solution of 1.3 g. (0.0025 mole) of the ester If in 52 ml. of 0.1 *N* sodium hydroxide (2 equiv.) was allowed to stand for one hour and then neutralized and evaporated to dryness. The organic material was taken up in three portions of boiling ethanol, the solvent was removed, the organic residue was taken up in 0.3 *N* hydrochloric acid (10–15 ml.), the *p*H was adjusted to 6.5, and the solution was allowed to stand overnight. The crystalline product (0.8 g.) was recrystallized twice from water to constant decomposition point at 223–224°. For analysis it was dried at 100° *in vacuo* over P_2O_5.

Anal. Calcd. for $C_{25}H_{27}N_5O_7 \cdot {}^1/_2H_2O$: C, 57.91; H, 5.44; N, 13.51. Found: C, 58.3; H, 5.3; N, 13.4.

Glycyl-L-histidyl-L-tyrosine was prepared by hydrogenation of the carbobenzoxy acid over palladium-on-barium sulfate until evolution of carbon dioxide was complete. Addition of methanol to the filtered solution induced crystallization; m.p. 215° dec. For analysis it was dried at 100° *in vacuo* over P_2O_5.

Anal. Calcd. for $C_{17}H_{21}N_5O_5 \cdot 2H_2O$: C, 49.63; H, 6.12; N, 17.02. Found: C, 49.9; H, 5.8; N, 16.4.

Carbobenzoxyglycyl-L-histidyl-L-serine Methyl Ester (Ie).—Carbobenzoxyglycyl-L-histidine azide was prepared from 3.6 g. (0.01 mole) of the hydrazide and taken up in chloroform as in the preparation of carbobenzoxyglycyl-L-histidyl-L-tyrosine methyl ester (If). The solution was added to 1.8 g. (0.015 mole) of L-serine methyl ester in 50 ml. of chloroform and allowed to stand overnight. The flocculent precipitate was collected with the aid of Filter-Cel and recrystallized from methanol; yield 1.1 g. (25%), m.p. 156–160°. After two further recrystallizations from methanol the melting point was constant at 163–165°.

Anal. Calcd. for $C_{20}H_{25}N_5O_7$: C, 53.68; H, 5.63; N, 15.69. Found: C, 53.5; H, 5.7; N, 15.2.

Carbobenzoxyglycyl-L-histidylglycine Ethyl Ester (Id).—The azide was prepared from 3.6 g. of carbobenzoxyglycyl-L-histidine hydrazide (0.01 mole) and isolated as in the preparation of carbobenzoxyglycyl-L-histidyl-L-tyrosine methyl ester (If). The solution was added to 2 g. (0.019 mole) of glycine ethyl ester in chloroform and allowed to stand overnight. The product weighed 2.8 g. (65%) and melted at 160–165°. Two recrystallizations from ethanol raised the melting point to 172–173°.

(4) J. L. Bailey, *J. Chem. Soc.*, 3461 (1950).

(5) W. Keil, *Z. physiol. Chem.*, **208**, 70 (1932).

(6) R. W. Holley and A. D. Holley, THIS JOURNAL, **74**, 3069 (1952).

(7) (a) Melting points are corrected; (b) nitrogen analyses are by micro-Kjeldahl, except on hydrazides, which are by micro-Dumas.

(8) B. F. Erlanger and E. J. Brand, THIS JOURNAL, **73**, 3508 (1951).

(9) H. E. Carter, R. L. Frank and H. W. Johnston, *Org. Syntheses*, **23**, 13 (1943).

(10) E. Fischer and W. Schrauth, *Ann.*, **354**, 34 (1907).

Anal. Calcd. for $C_{20}H_{25}N_5O_6$: C, 55.67; H, 5.84; N, 16.23. Found: C, 55.9; H, 5.6; N, 16.0.

Carbobenzoxyglycyl-L-histidylglycine hydrazide was prepared from the ethyl ester by refluxing in ethanol solution with hydrazine for two hours. After several recrystallizations from 90% methanol it melted at 199–201°.

Anal. Calcd. for $C_{18}H_{23}N_7O_5$: C, 51.67; H, 5.54; N, 23.44. Found: C, 51.5; H, 5.4; N, 23.5.

Carbobenzoxyglycyl-L-histidylglycyl-L-serine Methyl Ester (Ig).—A solution of 1.4 g. (0.0034 mole) of carbobenzoxyglycyl-L-histidylglycine hydrazide in a mixture of 25 ml. of water and 5 ml. of 12*N* hydrochloric acid was cooled to −10°. To it was added all at once, a chilled solution of 0.30 g. (0.0043 mole) of sodium nitrite in 10 ml. of water. The reaction mixture was allowed to stand for 20 minutes. Keeping the temperature of the solution at −5°, a cold solution of 9 g. of potassium carbonate in 50 ml. of water was then added. A viscous, yellowish oil precipitated. After it proved to be insoluble in chloroform or ethyl acetate, it was gathered on a spatula and added to a solution of 2.5 g. (0.021 mole) of L-serine methyl ester in 100 ml. of chloroform, about 5 ml. of pyridine being used to aid in the transfer. After standing overnight with occasional shaking, the partly oily precipitate was filtered and dried; yield, 1.2 g. Careful washing with 20 ml. of ice-water left 0.5 g. (29%) of colorless granules, m.p. 178–181°. The product was crystallized from methanol–ether and then from methanol-ethyl acetate; m.p. 184–185°. The ester was quite soluble in water and more readily so in dilute acid.

Anal. Calcd. for $C_{22}H_{28}N_6O_8$: C, 52.38; H, 5.59; N, 16.66. Found: C, 52.3; H, 5.5; N, 16.1.

Carbobenzoxyglycyl-L-serine methyl ester was prepared from carbobenzoxyglycine azide and L-serine methyl ester, following the general procedure of Erlanger and Brand.[8] After working up in the usual manner, the crude product was obtained in 41% yield, m.p. 86–89°. Three recrystallizations from benzene raised the m.p. to 92–93°.

Anal. Calcd. for $C_{14}H_{18}N_2O_6$: C, 54.18; H, 5.85; N, 9.03. Found: C, 53.8; H, 5.7; N, 8.9.

Carbobenzoxy-L-seryl-L-tyrosine Methyl Ester (IIa).—Carbobenzoxy-L-serine azide was prepared from 2.5 g. (0.01 mole) of the hydrazide by the method of Fruton,[3a] treated with 2.5 g. (0.01 mole) of L-tyrosine methyl ester[10] in 200 ml. of 50% dioxane–ethyl acetate, and allowed to stand overnight. The solution was evaporated to dryness, taken up in ethyl acetate, washed with water, 1 *N* hydrochloric acid and water, and then dried over magnesium sulfate. After concentration *in vacuo*, the addition of petroleum ether caused slow crystallization of 2.3 g. of product, m.p. 108–115°. Repeated crystallization from ethyl acetate–petroleum ether did not narrow the melting point range, but after one crystallization from methanol–water, the product could be recrystallized from ethyl acetate alone, and after three such recrystallizations it melted sharply at 112–113°.

Anal. Calcd. for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.80; N, 6.73; O, 26.90. Found: C, 60.4; H, 5.6; N, 6.2; O (direct), 27.3.

Carbobenzoxy-L-seryl-L-tyrosine.—Carbobenzoxy-L-seryl-L-tyrosine methyl ester (1.6 g., 0.00386 mole) was dissolved in 58 ml. of 0.10 *N* sodium hydroxide (1.5 eq.) and allowed to stand at room temperature for one hour. The *p*H was adjusted to 2, and the solution was evaporated to dryness. The organic material was taken up in several portions of boiling methanol, and evaporation of the solvent left a viscous residue which could not be induced to crystallize. It was then dissolved in 5% potassium bicarbonate, washed with ethyl acetate, and acidified. The crystals which separated over a period of several days weighed 0.4 g., m.p. 185–188°, which was raised to 187–188° after recrystallization from water.

Anal. Calcd. for $C_{20}H_{22}N_2O_7$: C, 59.69; H, 5.51; N, 6.96. Found: C, 59.8; H, 5.5; N, 6.8.

L-Seryl-L-tyrosine.—Hydrogenation of the above compound was accomplished in aqueous methanol over palladium-on-barium sulfate. Evolution of carbon dioxide was complete in three hours, and the solution was filtered, evaporated to dryness, and again taken up in a few ml. of water. Addition of 10–15 volumes of methanol caused separation of an oil which slowly crystallized; the solid charred gradually in the range 200–260°. The product was somewhat hygroscopic and slowly turned yellow when exposed to the light.

Anal. Calcd. for $C_{12}H_{16}N_2O_5 \cdot {}^1/_2H_2O$: C, 51.96; H, 6.0; N, 10.10. Found: C, 52.2; H, 6.0; N, 9.9.

Carbobenzoxy-L-tyrosyl-L-serine Methyl Ester (IIb).—Carbobenzoxy-L-tyrosine azide was prepared from 6.6 g. (0.02 mole) of the hydrazide[11] by the method of Bergmann and Fruton[12] and added to a solution of L-serine methyl ester in 40 ml. of ethyl acetate. After working up as for IIa, a yellow oil was obtained which after Norit treatment crystallized from ethyl acetate. There was obtained 1.8 g. (22%) of pale yellow crystals, m.p. 142–146°. After five recrystallizations from ethyl acetate, the melting point was constant at 151–152°.

Anal. Calcd. for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.80; N, 6.73; O, 26.90. Found: C, 60.3; H, 5.6; N, 6.3; O (direct), 27.3.

Carbobenzoxy-L-tyrosyl-L-histidine Methyl Ester (IIc).—Carbobenzoxy-L-tyrosine azide prepared as above from 10 g. (0.03 mole) of the hydrazide and dissolved in 100 ml. of chloroform was added to 6 g. (0.035 mole) of L-histidine methyl ester in 100 ml. of chloroform. A brown oil separated within a few hours, which crystallized only when shaken with water; yield 10 g., m.p. 125–135°. Crystallization from 50% methanol raised the melting point to 150–160°, and several further recrystallizations gave 4 g. (29%) of a white powder, m.p. 165–166° (dried *in vacuo*, room temperature), $[\alpha]^{24}D$ −14° (1%, methanol).

Anal. Calcd. for $C_{24}H_{26}N_4O_6 \cdot H_2O$: C, 59.50; H, 5.83; N, 11.56; O, 23.13. Found: C, 59.9; H, 5.7; N, 11.1; O (direct) 23.3.

The product would crystallize only from aqueous solvents, and samples dried less or more rigorously invariably melted lower and less sharply. On attempted crystallization from boiling *n*-butyl alcohol racemization evidently occurred since the product which had the same solubilities as the starting material melted at 137–139°; on standing the melting point dropped to 125°.

Anal. Found: C, 60.0; H, 5.8; $[\alpha]^{20}D$ −1.9° (1%, methanol).

Carbobenzoxy-L-tyrosyl-L-histidine.—Two grams (0.0041 mole) of the above ester (IIc) was dissolved in 50 ml. of methanol, 17.2 ml. of 0.5 *N* sodium hydroxide was added, and the solution was allowed to stand 40 minutes. It was then neutralized with 17.2 ml. of 0.5 *N* hydrochloric acid, evaporated to dryness, taken up in 5% potassium bicarbonate solution, washed twice with ethyl acetate and adjusted to *p*H 6.5. The product crystallized overnight, m.p. 196–199°; three further crystallizations sharpened the melting point at 199°; yield 1.0 g. (53%).

Anal. Calcd. for $C_{23}H_{24}N_4O_6 \cdot {}^1/_2H_2O$: C, 59.86; H, 5.46; N, 12.14. Found: C, 59.5; H, 5.5; N, 11.9.

Another sample independently prepared and dried at 100° *in vacuo* had identical analytical values.

Carbobenzoxy-DL-serine hydrazide was prepared by slight modifications of the method of Fruton[3a] who prepared the corresponding L-serine derivative. The yield was 66%, and the melting point after several recrystallizations from methanol was 160–161°.

Anal. Calcd. for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59. Found: C, 52.4; H, 5.9; N, 16.7.

Carbobenzoxy-DL-serylglycine ethyl ester was also prepared following the directions of Fruton[3a] for the L-compound. The yield was 60% of a material of m.p. 70–76°. Two recrystallizations from tetrahydrofuran–petroleum ether raised the m.p. to 86–88°.

Anal. Calcd. for $C_{15}H_{20}N_2O_6$: C, 55.54; H, 6.21; N, 8.64. Found: C, 55.4; H, 6.1; N, 8.3.

Carbobenzoxy-DL-serylglycine Hydrazide.—A solution of 8.3 g. of the crude ester prepared above was refluxed for two hours with 1.8 g. of 95% hydrazine in 75 ml. of ethanol. There was obtained 7.3 g. (91%) of fluffy needles, m.p. 187–189°; recrystallization from ethanol did not raise the melting point.

Anal. Calcd. for $C_{13}H_{18}N_4O_5$: C, 50.32; H, 5.85; N, 18.06. Found: C, 50.1; H, 5.4; N, 17.9.

(11) Mann Research Laboratories, 136 Liberty Street, New York 6, N. Y.

(12) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 413 (1937).

Carbobenzoxy-D- and L-serylglycyl-L-histidine Methyl Ester (III).—This product was prepared by modification of the general method of Braud and associates[13] for carbobenzoxytripeptide esters. The intermediate azide was water soluble, but was extracted with chloroform. The combined dried extracts were then added to a solution of 1.9 g. (0.011 mole) of L-histidine methyl ester in 50 ml. of chloroform. A yellow oil began to separate in a few minutes, and the mixture was allowed to stand overnight. The chloroform was decanted and replaced by dry ether. Rubbing under this solvent gave a pale yellow powder; yield 3.4 g. (96%). The product was quite hygroscopic and very soluble in water and in alcohols. For analysis it was dissolved in dry methanol and poured with stirring into 10–15 volumes of dry ether, and then filtered quickly and stored over phosphorus pentoxide. It was dried for several hours at 36° *in vacuo* over phosphorus pentoxide immediately prior to analysis; m.p. 37–55°.

Anal. Calcd. for $C_{20}H_{25}N_5O_7$: C, 53.68; H, 5.62; N, 15.69. Found: C, 53.3; H, 5.7; N, 16.4.

Insoluble precipitates were obtained with mercuric chloride and silver nitrate; the mercuric chloride complex was formed by addition of excess mercuric chloride to an aqueous solution of the peptide derivative. The precipitate was washed successively with water, methanol and ether and then dried *in vacuo;* it slowly decomposed in the range 70–160°.

Anal. Calcd. for $C_{20}H_{25}N_5O_7 \cdot 2HgCl_2$: N, 7.07; Cl, 14.32. Found: N, 7.1; Cl, 14.2.

Phthaloylhistidine was prepared in accordance with the directions of Keil.[5] The product, obtained repeatedly in 80% yield, melted at 294–296° rather than 188° as reported.[5] For analysis it was recrystallized from water.

Anal. Calcd. for $C_{14}H_{11}N_3O_4$: C, 58.94; H, 3.89; N, 14.73. Found: C, 58.9; H, 3.8; N, 14.5.

Phthaloylhistidine methyl ester hydrochloride was prepared following the directions of Keil.[5] The hydrochloride melted at 221–223° in a capillary, or 209–211° on an aluminum block. Keil[5] gives the melting point as 238–240° (uncor.). For analysis the product was recrystallized from methanol.

Anal. Calcd. for $C_{15}H_{13}N_3O_4 \cdot HCl$: C, 53.66; H, 4.20; N, 12.51. Found: C, 53.2; H, 4.1; N, 12.2.

Phthaloylhistidine methyl ester, liberated by means of potassium carbonate, melted at 187°; Keil reports 187° (uncor.).[5]

Phthaloylglycine azide was prepared from phthaloylglycyl chloride[14] and sodium azide in aqueous acetone by application of the general method described by Smith.[15] Yields of crude product, m.p. 97–99° dec., were essentially quantitative. The compound was thermally unstable, but some purification was effected by precipitating it from benzene solution by the addition of petroleum ether.

Anal. Calcd. for $C_{10}H_6N_4O_3$: C, 52.18; H, 2.62; N, 24.34. Found: C, 53.2; H, 2.3; N, 23.6.

Phthaloylglycyl-L-serine methyl ester was prepared in 68% yield from equimolar portions of the azide and L-serine methyl ester in tetrahydrofuran; m.p. after crystallization from methanol, 221–222°.

Anal. Calcd. for $C_{14}H_{14}N_2O_6$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.9; H, 4.6; N, 8.9.

Three hours of refluxing with one equivalent of 1 *N* methanolic hydrazine followed by filtration of the phthaloylhydrazide and evaporation of the solution gave quantitative yields of a crystalline residue, melting at 215–217° after crystallization from methanol. Abderhalden and Bahn[16] report 218–220° as the melting point of the DL-serylglycine anhydride.

Anal. Calcd. for $C_5H_8N_2O_3$: C, 41.66; H, 5.60; N, 19.44. Found: C, 42.1; H, 5.6; N, 19.2.

Phthaloylglycyl-L-histidine methyl ester was prepared in 76% yield from equimolar portions of phthaloylglycine azide and L-histidine methyl ester in chloroform. After crystallization from methanol it melted at 199–201°.

Anal. Calcd. for $C_{17}H_{16}N_4O_5$: C, 57.30; H, 4.53; N, 15.72; O, 23.45. Found: C, 57.0; H, 4.4; N, 15.0; O (direct), 23.4.

Phthaloylglycyl-L-histidine was prepared in 45% yields from the azide and L-histidine in 50% aqueous dioxane following the general method of Kroll.[17] The product melted at 258° dec. after crystallization from water; Turner[18] reports 258–262°.

***o*-Nitrophenoxyacetyl-L-histidine hydrazide** was prepared by minor modification of the general directions of Holley and Holley[6] from chloroform solutions of 4.8 g. (0.022 mole) of *o*-nitrophenoxyacetyl chloride and 3.7 g. of L-histidine methyl ester (0.022 mole) with 2 g. (0.022 mole) of 2,6-lutidine as the acid acceptor. After filtration of the lutidine hydrochloride the solution was evaporated to dryness. The residue was taken up in 50 ml. of methanol, treated with 1 g. of 95% hydrazine and allowed to stand overnight. There was obtained 3 g. of colorless crystals, m.p. 193–195° (39% yield). Recrystallization from 90% methanol raised the melting point to 201–202° dec.

Anal. Calcd. for $C_{14}H_{16}N_6O_5$: C, 48.27; H, 4.63; N, 24.13. Found: C, 48.7; H, 4.6; N, 22.7.

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EMERYVILLE, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER & COMPANY, INC.]

Magnamycin B, a Second Antibiotic from *Streptomyces halstedii*

BY F. A. HOCHSTEIN AND KOTARO MURAI

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Magnamycin B is a new antibiotic which has been isolated, along with Magnamycin, from fermentation beers of *Streptomyces halstedii*. The new antibiotic resembles Magnamycin in its antibacterial spectrum, its low toxicity, and in its fundamental chemical structure. Spectral studies show it to contain an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl system, in contrast to the α,β-unsaturated carbonyl group of Magnamycin. Several microbiologically active derivatives of Magnamycin B have been prepared.

Magnamycin,[1] an elaboration product of a strain of *Streptomyces halstedii*, has been carefully characterized, and is described in earlier publications.[2–4] We wish to report at this time a second antibiotic Magnamycin B, which is elaborated by this actinomycete.

Solubility analyses and countercurrent distribution studies on samples of crude Magnamycin revealed the presence of small amounts of other antibacterial substances, substances which were more soluble in benzene and alcohols. Examination of mother liquors resulting from the crystallization

(1) Magnamycin is the trade-mark of Chas. Pfizer & Co., Inc., for the antibiotic carbomycin.
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