The preparation of 2-(*o*-methoxyphenyl)-tropone is reported here because work with this compound has been stopped.

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Experimental[9]

2-Phenyltropone (II) from Copper Tropolone (I).—An ethereal solution of phenyllithium from 3.78 g. of bromobenzene and 0.666 g. of finely divided lithium was transferred under nitrogen to another flask leaving unreacted lithium behind. I (0.300 g.) was then added slowly. The mildly exothermic reaction was terminated after 20 min. by adding ether and then dilute sulfuric acid until the aqueous phase was acidic. The ether layer was separated, washed with water and then with 5% aqueous sodium hydroxide and finally concentrated to a residue, sublimation of which yielded 0.320 g. (89%) of yellow needles, m.p. 70–75°. One recrystallization from isoöctane yielded II as long yellow needles, m.p. 83–84°; reported m.p. 83.5–84°[5b] and 83–84°.[6]

α-(*o*-Methoxyphenyl)-tropone.—An ethereal solution of *o*-methoxyphenyllithium, prepared from 2.48 g. of *o*-bromoanisole and 0.370 g. of finely divided lithium, was transferred to another flask under nitrogen leaving behind unreacted lithium and treated with 0.085 g. of copper tropolone at room temperature. After 30 min., the reaction mixture was diluted with ether, washed with dilute sulfuric acid, and dried over anhydrous magnesium sulfate. Removal of the ether left an oily residue which was evaporatively distilled at 64° and 0.5 mm. until all of the anisole was removed. Sublimation of the resulting solid residue at 110° and 0.5 mm. yielded a yellow material recrystallization of which from isohexane afforded 0.075 g. (63%) of pale yellow crystals, m.p. 93.5–94.5°.

Anal. Calcd. for $C_{14}H_{12}O_2$: C, 79.2; H, 5.7. Found: C, 79.2; H, 5.7.

7-Bromo-2-phenyltropone (III).—To a stirred solution of 0.310 g. of II in 50 ml. of dry carbon tetrachloride containing 0.300 g. of anhydrous sodium carbonate, 0.091 ml. (1 equiv.) of bromine in 3.2 ml. of carbon tetrachloride was added over a period of 2 hr., after which stirring was continued for 17 hr. The clear solution remaining after centrifuging all solid material was then treated with 0.272 g. of dry pyridine and refluxed for 1 hr. The pyridine hydrobromide was centrifuged, and the organic layer was washed successively with dilute sulfuric acid, water and 5% sodium bicarbonate, and concentrated. Sublimation of the residue yielded, first a colorless crystalline solid which was removed, and then at 110° and 0.5 mm. a yellow oil which afforded 0.155 g. (35%) of yellow crystals, m.p. 68–73°, on triturating with isohexane. Recrystallization from isohexane gave about 0.12 g. of fluffy yellow needles of III, m.p. 80–81°. Further crystallization afforded an analytical sample, m.p. 83.5–84.0°; reported[5a] m.p. 82–83°.

Anal. Calcd. for $C_{13}H_9BrO$: C, 59.8; H, 3.4; Br, 30.7. Found: C, 60.0; H, 3.6; Br, 30.5.

Alkaline Rearrangement of III.—III (20 mg.) was dissolved in 2 ml. of freshly prepared 10% alcoholic potassium hydroxide and refluxed for 6 hr. The cooled, pale yellow solution was acidified and extracted with chloroform. Several extractions of the chloroform solution with 5% sodium bicarbonate were combined, acidified and extracted with chloroform. Removal of the solvent yielded 4.6 mg. (30%) of *o*-phenylbenzoic acid, which on recrystallization gave 4.0 mg. of the acid, m.p. 111–112°, m.p. 111–112° on admixture with an authentic sample of m.p. 111–112°. The infrared absorption spectra of the two samples were identical.

α-Phenyltropolone (IV).—A solution of 0.038 g. of III in 3 ml. of glacial acetic acid, 3 ml. of 48% hydrobromic acid and 4 ml. of water (1.75 *M* in hydrobromic acid) was heated in a sealed tube at 160° for 6 hr. The reaction mixture was extracted with chloroform until test portions of the extracts no longer gave a green coloration with a 1% alcoholic ferric chloride solution. The combined chloroform extracts were shaken vigorously with an equal volume of saturated aqueous cupric acetate for about 5 min. and separated. The aqueous layer was extracted several times with warm chloroform until a negative ferric chloride test was obtained. Removal of the chloroform from these extracts gave 0.026 g. (77%) of the green copper salt of IV, m.p. 299–300° (dec.).

When 22.5 mg. of the copper salt dissolved in about 10 ml. of warm chloroform was treated with hydrogen sulfide, a precipitate of copper sulfide formed quickly. Mixing with Super-Cel and filtration through a Super-Cel mat gave a clear, almost colorless solution. Distillation of the solvent yielded 18 mg. (93%) of yellow crystalline material. Recrystallization from isohexane afforded 14 mg. of yellow needles of α-phenyltropolone, m.p. 116–116.5°.

Anal. Calcd. for $C_{13}H_{10}O_2$: C, 78.8; H, 5.1. Found: C, 79.0; H, 5.1.

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(9) All melting points are corrected.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF MEDICINE, STATE UNIVERSITY OF NEW YORK]

Synthesis of Carnosine and Related Peptides by the Phthaloyl Method[1]

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Condensation of phthaloyl-β-alanyl chloride with L-histidine in the presence of triethylamine at low temperature has yielded phthaloyl-β-alanylhistidine. After detachment of the phthaloyl group with hydrazine, carnosine was isolated. Several related peptides have also been prepared by the present method.

The phthaloyl synthesis of peptides, reported almost simultaneously by King and Kidd,[2] and by Sheehan and Frank,[3] appears especially useful for the synthesis of those peptides whose N-terminal amino acids are either glycine or β-alanine, for in such cases there is no question of racemization during the preparation of the phthaloylamino acid.[4]

The condensation of phthaloyl-β-alanyl chloride (I) with L-histidine by the method of Sheehan and Frank[3] succeeded, but the product, phthaloyl-β-alanyl-L-histidine (phthaloylcarnosine) (II), was not isolable, for it could not be crystallized from water or alcohol when magnesium chloride, formed from the magnesium oxide added at the start of the

(1) This investigation was supported in part by a grant from the National Science Foundation.

(2) F. E. King and D. A. A. Kidd, *Nature*, **162**, 776 (1948); *J. Chem. Soc.*, 3315 (1949).

(3) J. C. Sheehan and V. Frank, THIS JOURNAL, **71**, 1856 (1949).

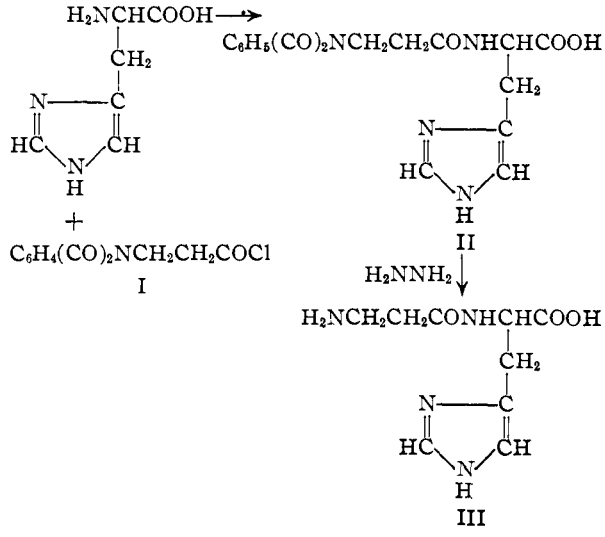
(4) In unpublished research the author has shown that phthaloylhistidine is prepared in good yield only when histidine and phthalic anhydride are fused above 200° (oil-bath temperature), which is higher than the usual temperature (170°) for preparing phthaloylamino acids. The phthaloylhistidine isolated was completely racemized. Recently, Sheehan, Chapman and Roth, THIS JOURNAL, **74**, 3822 (1952), have shown that racemization of phenylalanine and of leucine may be avoided by fusion at 150°, but it would seem that these amino acids are special cases.

TABLE I

PEPTIDES SYNTHESIZED BY THE PHTHALOYL METHOD

Peptide	Yield,[a] %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Phthaloyl-β-alanyl-DL-alanine	65	209–212	57.93	58.27	4.87	4.81		
Phthaloyl-β-alanyl-L-asparagine	60	185–187	54.05	54.32	4.54	4.17		
Phthaloyl-β-alanyl-L-histidine	50	221–224	57.30	57.26	4.54	4.54		
Phthaloyl-β-alanyl-L-leucine	40	113–117					8.43	8.28
Phthaloyl-β-alanyl-DL-serine	55	195–196	54.90	55.13	4.61	4.36		
Phthaloyl-β-alanyl-L-tyrosine	40	193–194					7.33	7.33
Phthaloylglycyl-L-histidine	40	258–262	56.14	55.24	4.12	4.22	16.37	15.53
Phthaloylglycyl-DL-phenylalanine	80	194–197[b]						
Phthaloylglycyl-L-tyrosine	45	241–244	61.95	61.86	4.38	3.85	7.60	7.75
β-Alanyl-L-histidine	85	253–256	47.78	47.64	6.24	5.97		
β-Alanyl-L-leucine[c]	80	259–260	53.45	53.72	8.98	8.81		
β-Alanyl-DL-serine	80	209–210	40.90	41.18	6.86	7.16		
Glycyl-L-histidine	60	175–176[d]						

[a] Refers to the amount of pure substance isolated. [b] Reported (ref. 3) 197.0–198.5°. [c] The m.p. 245° and $[\alpha]^{26}D$ −31° (1.5% in water) have been reported by H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948); M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, **15**, 108 (1950), have reported $[\alpha]^{25}D$ −27.4° (3.3% in water). [d] M. Hunt and V. du Vigneaud, *J. Biol. Chem.*, **127**, 43 (1939), reported the m.p. 175° for the hydrochloride hydrate.



condensation, was present. The *formation* of phthaloylcarnosine was proved by distillation of the solvents from the reaction mixture and esterification of the vitreous residue with methanol, whence **phthaloylcarnosine methyl ester** was isolated. However, saponification of the ester in the usual way did not yield phthaloylcarnosine; the product, which was possibly the related phthalamic acid, was not investigated further. The ester was identified by analysis and by comparison with a sample prepared by esterification of phthaloylcarnosine, which is described below.

The rather low yield of phthaloylcarnosine methyl ester as well as the difficulties in its isolation made evident the need for a method of condensation that would diminish the hydrolysis of the phthaloyl-β-alanyl chloride during the course of reaction and would permit isolation of phthaloylcarnosine directly. It has been found that when phthaloyl-β-alanyl chloride is condensed with L-histidine in the presence of triethylamine at low temperature, phthaloylcarnosine is readily isolable from the reaction mixture in good yield. Detachment of the phthaloyl group by means of hydrazine[5] at room temperature led to the partly crystallized phthaloylhydrazide salt of carnosine, which was converted into a precipitate of phthaloylhydrazide and a solution of carnosine (III) by means of acetic acid.[2] After evaporation of the solvents, carnosine was isolated as the crystalline base upon the addition of alcohol.

Carnosine has been synthesized in small yield by Barger and Tutin[6] and by Baumann and Ingvaldsen[7]; it has been synthesized in 65% yield (based on histidine) by Sifferd and du Vigneaud,[8] who employed the carbobenzoxy method. Recently, Hanson and Smith[9] have reported improvements in the carbobenzoxy synthesis. The present method has the advantages of manipulative ease and requiring only a small amount of time. Kroll has recently announced that carnosine hydrochloride may be isolated as an amorphous salt after condensation of phthaloyl-β-alanyl azide with histidine sodium salt, and subsequent hydrazinolysis.[10]

The present method has extended the phthaloyl synthesis to peptides of histidine. Several peptides related to carnosine such as glycylhistidine have been prepared. The present method is especially useful when the phthaloyl peptide is quite soluble or is not isolable in the presence of magnesium salts; it lends itself readily to the synthesis of peptides of phenylalanine and tyrosine for the reason that when triethylamine is added to an aqueous suspension of these amino acids, the solid dissolves at once, so that the amino acid is in solution during the condensation. In some instances the reaction mixture crystallized partly as the temperature fell below 0°. This could be relieved or prevented by addition of acetone.

The peptides prepared by the present method are summarized in Table I. The products obtained through methylation of phthaloyl-carnosine will be reported in another publication.

(5) H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348 (1926).

(6) G. Barger and F. Tutin, *Biochem. J.*, **12**, 402 (1918).

(7) L. Baumann and T. Ingvaldsen, *J. Biol. Chem.*, **35**, 263 (1918).

(8) R. H. Sifferd and V. du Vigneaud, *ibid.*, **108**, 753 (1935).

(9) H. T. Hanson and E. L. Smith, *ibid.*, **179**, 789 (1949).

(10) H. Kroll, Abs. Meeting Am. Chem. Soc., Sept., 1952, p. 44C.

Experimental

Phthaloyl-β-alanine was prepared by fusion of equimolar quantities of phthalic anhydride and β-alanine at 175° for 25 minutes; m.p. of recrystallized product (97%), 152–153°; reported[11] 150–151°.

Phthaloyl-β-alanine Methyl Ester (Methyl β-Phthalimidopropionate).—The ester was prepared from phthaloyl-β-alanyl chloride[12] and methanol, crystallizing as fine needles from benzene–heptane; m.p. 72–74°, reported[13] 73–75°.

Anal. Calcd. for $C_{12}H_{11}O_4N$: N, 6.45. Found: N, 6.40.

Phthaloyl-β-alanylhistidine Methyl Ester.—A solution of 4.74 g. of phthaloyl-β-alanyl chloride in 45 ml. of dioxane was added dropwise during 24 minutes to a stirred suspension of 4.20 g. of histidine hydrochloride hydrate and 2.42 g. of magnesium oxide in 75 ml. of water and 15 ml. of dioxane, cooled to −8°. Stirring was continued as the temperature was allowed to rise to 10° during 30 minutes.

The following day the mixture, acidified with 2 *N* hydrochloric acid, was distilled to dryness *in vacuo* and esterified with anhydrous methanol and hydrogen chloride. After evaporation of the solvent the residue was dissolved in 20 ml. of water and made slightly alkaline by the gradual addition of 50 ml. of 4% sodium carbonate solution. Filtration of the colorless plates which appeared yielded 2.40 g., m.p. 70°. They were identified as **methyl β-phthalimidopropionate** by the melting point of a mixture with the sample described above. The filtrate was treated with 25 ml. more of the carbonate solution, whereupon a new crystallizate formed. After an hour in the cold it was filtered and washed with water; yield 2.84 g., m.p. 180–185°. During further storage the filtrate yielded 0.45 g. more, of lower m.p. Unlike methyl β-phthalimidopropionate, the new substance was insoluble in ethyl acetate. Recrystallization was performed by solution in 0.5 *N* hydrochloric acid and, after charcoal treatment, neutralization with 4% sodium carbonate solution. The filtered phthaloyl-β-alanylhistidine methyl ester was washed with water; yield 1.50 g., m.p. 190–191°.

Anal. Calcd. for $C_{18}H_{18}O_5N_4$: C, 58.37; H, 4.90; N, 15.13. Found: C, 58.25; H, 4.85; N, 15.46.

Phthaloyl-β-alanylhistidine (Phthaloylcarnosine).—Into a three-neck flask equipped with a mechanical stirrer, a dropping funnel and a thermometer was introduced a solution of 4.20 g. of L-histidine hydrochloride hydrate in 40 ml. of water. After addition of 2.9 ml. of triethylamine the solution was cooled to about −8° with the aid of a Dry Ice-bath. A solution of 5.20 g. of phthaloyl-β-alanyl chloride in 25 ml. of dioxane was added slowly in two equal portions; the first was added to the histidine solution during 25 minutes while the temperature was maintained at about −10°. Following addition of 2.9 ml. of triethylamine, the second portion of the dioxane solution was added in the same way as the first. When at the end of the period of addition a test showed that the reaction mixture was not alkaline, a little triethylamine was added. The mixture was stirred as it was allowed to warm to room temperature, and then distilled to dryness *in vacuo*. After addition of 20 ml. of *n*-propyl alcohol the distillation was repeated. The residue was warmed with 4.0 ml. of water to dissolve the gummy particles, treated with 25 ml. of *n*-propyl alcohol, and left in the cold. The filtered product was washed with propanol; yield 4.30 g. of colorless crystals which melted at 215–219° (dec.) on the hot-stage after previous emollescence. Recrystallization from 4.0 ml. of water and 20 ml. of methanol yielded 3.60 g. (50%); decomposition point 221–224°, unchanged by further crystallization; $[\alpha]^{22}D$ 21.5° (1% in water).

Anal. Calcd. for $C_{17}H_{16}O_5N_4$: C, 57.30; H, 4.54. Found: C, 57.26; H, 4.55.

Esterification with anhydrous methanol and hydrogen chloride afforded **phthaloyl-β-alanylhistidine** methyl ester, identical with the sample described above.

Carnosine.—A solution of 3.21 g. of phthaloyl-β-alanylhistidine in 12 ml. of water was treated with 3.0 ml. of a 5 *M* solution of hydrazine hydrate in ethanol. After two days the mixture of the crystallized phthaloylhydrazide salt of carnosine and solution was diluted with 25 ml. of water. Acidification with 0.8 ml. of glacial acetic acid caused the formation of a dense white precipitate of phthaloylhydrazide, which was filtered and washed well with water. The slightly acidic filtrate was evaporated to dryness *in vacuo*. After solution of the residue in 10-ml. portions of water the evaporation was twice repeated. A warm solution of the residue in 3.0 ml. of water was made slightly alkaline with concd. ammonia solution and treated with 20 ml. of hot absolute ethanol. The filtered product weighed 1.75 g. (86%), m.p. 250–253° (dec.) after previous darkening, and unchanged upon admixture with an authentic specimen of carnosine. After recrystallization the m.p. was 253–256°, $[\alpha]^{22}D$ 21.7° (1.1% in water).

Anal. Calcd. for $C_9H_{14}O_3N_4$: C, 47.78; H, 6.24. Found: C, 47.64; H, 5.97.

The m.p. 260° (capillary) and $[\alpha]^{25}D$ 20.5° (2% in water) have been reported.[8]

Phthaloyl-β-alanyl-DL-alanine.—The following procedure was employed in the preparation of *acidic* phthaloyl peptides.[14] A solution of 2.67 g. of DL-alanine and 6.3 ml. of triethylamine in 20 ml. of water was cooled to −15°. Sufficient acetone was added to prevent the mixture from freezing. Half of a solution of 7.85 g. of phthaloyl-β-alanyl chloride in 50 ml. of dioxane was added during 25 minutes, and following the addition of 2.8 ml. of triethylamine, the second half was added. When the stirred mixture reached room temperature, it was distilled to dryness *in vacuo*, and then redistilled similarly after addition of 25 ml. of *n*-propyl alcohol. The residue was dissolved in 50 ml. of water, filtered, and acidified to congo red with hydrochloric acid. The product was then left to crystallize in the cold. After filtration it was recrystallized from aqueous alcohol.

Acknowledgment.—It is a pleasure to acknowledge the interest and encouragement of Prof. Vincent du Vigneaud, in whose laboratory this investigation was begun.

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(11) S. Gabriel, *Ber.*, **38**, 630 (1905).

(12) Prepared by the method described for phthaloylglycyl chloride; *cf.* ref. 3.

(13) V. M. Rodionov and N. G. Yartseva, *Bull. acad. sci. U.S.S.R., classe sci. chim.*, 251 (1948); *C. A.*, **42**, 4942 (1948).

(14) Phthaloylcarnosine is a neutral peptide.