CONCERNING HISTIDINE AND CARNOSINE. THE SYNTHESIS OF CARNOSINE.*

By LOUIS BAUMANN and THORSTEN INGVALDSEN.

(*From the Chemical Research Laboratory, Department of Internal Medicine, State University of Iowa, Iowa City.*)

(Received for publication, June 13, 1918.)

HISTORICAL.

Our knowledge of carnosine is largely due to the admirable investigations of Gulewitsch. The discovery of the base in beef muscle extract was first reported by Gulewitsch and Amiradžibi[1] in 1900. Later Kutscher[2] isolated a substance from beef muscle extract, which he named ignotine, but Gulewitsch[3] showed that ignotine and carnosine were identical. In the same volume, he[4] showed that hydrolysis of the base with barium hydroxide liberated the amino-acid, histidine. 5 years later, he[5] established the identity of the other component, namely β-alanine. This amino-acid had been found in beef extract by Engeland,[6] as a result of the hydrolysis of carnosine during the process of isolation. Thus 11 years after its discovery, Gulewitsch showed that carnosine consisted of but two amino-acids, probably joined to form a dipeptide. Either one of two linkages was possible. Thus far no conclusive evidence had been advanced in favor of either formula.

On the whole little has been done to establish the relative distribution, biological significance, and fate of carnosine in the

* The data are taken from a dissertation submitted by Thorsten Ingvaldsen to the Graduate Faculty of the State University of Iowa, as a partial requirement for the degree of Doctor of Philosophy.

[1] Gulewitsch, W., and Amiradžibi, S., *Z. physiol. Chem.*, 1900, xxx, 565; *Ber. chem. Ges.*, 1900, xxxiii, 1902.

[2] Kutscher, F., *Z. Untersuch. Nahrungs- u. Genussmittel*, 1905, x, 528.

[3] Gulewitsch, W., *Z. physiol. Chem.*, 1906–07, l, 204.

[4] Gulewitsch, *Z. physiol. Chem.*, 1906–07, l, 535.

[5] Gulewitsch, *Z. physiol. Chem.*, 1911, lxxiii, 434.

[6] Engeland, R., *Z. Untersuch. Nahrungs- u. Genussmittel*, 1908, xvi, 658.

body. Gulewitsch prepared the base by precipitation of muscle extract with phosphotungstic acid, followed by purification by the silver-barium fractionation method of Kossel and Kutscher. The nitrate of carnosine was thus obtained in crystalline form. Later Smorodinzew[7] precipitated carnosine with mercuric sulfate in sulfuric acid solution. This author also demonstrated the absence of carnosine in the liver. Dietrich[8] recommended the addition of large quantities of alcohol to accelerate the precipitation with mercuric sulfate.

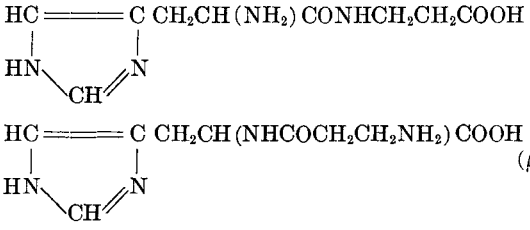
Skworzow,[9] it appears, first attempted the quantitative determination of carnosine by estimating the nitrogen content of the carnosine silver fraction. He found that 35 and 51 per cent of the nitrogen precipitated by phosphotungstic acid from calf and beef muscle extracts, respectively, appeared in the carnosine silver fraction, but did not attribute all of this to carnosine itself. He actually isolated an amount of carnosine nitrate corresponding to 0.176 per cent of the free base from calf muscle extract. This value agrees with those obtained by other investigators. Von Fürth and Schwarz[10] found that carnosine accounted for from 30 to 44 per cent of the total extractive nitrogen of the skeletal muscle of the horse and dog. Buglia and Costantino[11] and von Winiwarter[12] applying similar methods concluded that about one-third of the extractive nitrogen contained in skeletal muscle was in the form of carnosine. Miss Mauthner[13] unsuccessfully attempted the quantitative isolation of carnosine as the copper salt, which had been prepared previously by Gulewitsch. She next hydrolyzed the carnosine silver fraction and was able to isolate from 70 to 90 per cent of the theoretical quantity of histidine as the picrolonate (assuming that carnosine wholly accounted for the nitrogen content of this fraction). Miss Mauthner also succeeded in precipitating 80 per cent of the nitrogen contained in the carnosine silver fraction, as the sodium salt of carnosine dipicrolonate.

[7] Smorodinzew, J., *Z. physiol. Chem.*, 1914, xcii, 214.
[8] Dietrich, M., *Z. physiol. Chem.*, 1914, xcii, 212.
[9] Skworzow, W., *Z. physiol. Chem.*, 1910, lxviii, 26.
[10] von Fürth, O., and Schwarz, C., *Biochem. Z.*, 1911, xxx, 413.
[11] Buglia, G., and Costantino, A., *Z. physiol. Chem.*, 1912, lxxxi, 120.
[12] von Winiwarter, A., *Arch. Gynäk.*, 1913, c, 530.
[13] Mauthner, *Monatsh. Chem.*, 1913, xxxiv, 883.

An article by Gulewitsch[14] now appeared in which he pointed out that a levorotatory substance may be precipitated with carnosine, which interferes with its crystallization, depresses its melting point, and specific rotation. In this article the physical constants of pure carnosine and its nitrate are recorded. Von Fürth and Hryntschak[15] next attempted to determine carnosine colorimetrically. The intensity of the blue color of a solution of the copper compound or that of the red of the diazo compound, was estimated. The average value found by these methods was 0.27 gm. of carnosine per 100 gm. of muscle. These authors also attempted to determine quantitatively carnosine as the copper salt. In most cases the values were considerably lower than those obtained by the colorimetric methods. In one instance, however, they isolated 0.26 gm. and found 0.298 gm. by the colorimetric method. As to the carnosine content of other organs little is known. Buglia and Costantino,[11] by estimating the nitrogen content of the silver fraction, found 0.045 per cent of carnosine nitrogen in heart and 0.036 per cent in smooth muscle. Von Winiwarter[12] employed the same method and obtained 0.045 per cent for human and 0.026 per cent for equine uterine muscle. Suzuki, Joshimura, Jamakawa, and Irie[16] found as much as 0.2 gm. of carnosine in 100 gm. of fresh fish muscle. Finally, Drummond[17] isolated carnosine from chicken sarcoma.

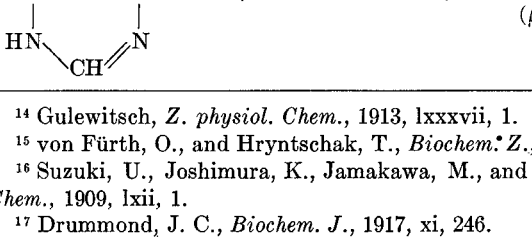
DISCUSSION.

The chief object of the present investigation was to determine the constitution of carnosine. Either one of two formulas was possible; viz.,



(histidyl-β-alanine)

or



(β-alanyl-histidine)

[14] Gulewitsch, *Z. physiol. Chem.*, 1913, lxxxvii, 1.

[15] von Fürth, O., and Hryntschak, T., *Biochem. Z.*, 1914, lxiv, 172.

[16] Suzuki, U., Joshimura, K., Jamakawa, M., and Irie, Y., *Z. physiol. Chem.*, 1909, lxii, 1.

[17] Drummond, J. C., *Biochem. J.*, 1917, xi, 246.

Two ways of solving the problem seemed evident. One, the more conclusive, was to synthesize the base; the other, to prepare deaminocarnosine, hydrolyze it, and then isolate one of its cleavage products.

For the synthesis of carnosine relatively large quantities of histidine were needed. The amino-acid was obtained from dried blood (Armour) by a modification of Fränkel's[18] method.

At first an attempt was made to prepare natural carnosine by the method of Smorodinzew,[7] but this procedure did not lead to the desired result. Only syrupy products were obtained. After several trials, we resorted to precipitation with mercuric acetate after preliminary purification with copper acetate and milk of lime. By this method the extract from several hundred pounds of horse muscle was precipitated. The product thus obtained was purified by the silver process.

Before proceeding to prepare deaminocarnosine, it was advisable to determine the amino nitrogen (Van Slyke) of carnosine and β-alanine. In both cases the analyses agreed well with the calculated amounts for one atom of free amino nitrogen.

Deaminocarnosine was prepared by treating the base with barium nitrite and sulfuric acid. After almost complete deamination had occurred (only 2 per cent of the free amino nitrogen remained), the product was hydrolyzed with acid, without attempting to isolate it. A 70 per cent yield of histidine was obtained, thus indicating that β-alanyl-histidine properly expressed the mode of linkage in the carnosine molecule.

Fischer and Cone[19] had prepared leucyl-histidine by the interaction of bromoisocapronyl chloride and histidine methyl ester, followed by hydrolysis of the resulting ester and amination of the bromo acid. The procedure which was adopted in the present investigation was more convenient. The acid chloride was combined with free histidine in aqueous solution in the presence of an excess of barium hydroxide. The barium was easily removed and the soluble reaction product was aminated without attempting to isolate it. At first we prepared β-iodopropionyl-α-alanine by this method, then β-chloropropionyl chloride was combined with histidine and the reaction product aminated by heating with

[18] Fränkel, S., *Monatsh. Chem.*, 1904, xxiv, 229.

[19] Fischer, E., and Cone, L. H., *Ann. Chem.*, 1908, cccIxiii, 107.

ammonia in a sealed tube. A few crystals were obtained which had the characteristic appearance and optical property of carnosine but the amount was too small for analysis. However, when the product resulting from the reaction of β-iodopropionyl chloride with histidine, was aminated, a substance was formed with properties, optical rotation, and analyses identical with those of natural carnosine.

In order to obtain some preliminary knowledge concerning the fate of carnosine in the body, a study of its behavior toward organ ferments was made. The extracts of dog liver and muscle failed to hydrolyze carnosine. The activity of these extracts had been demonstrated on another dipeptide, glycyl-tryptophane.

The preparation of histidyl-β-alanine or its derivatives was also attempted. Fischer and Cone[19] had tried to prepare histidyl chloride but failed. Their attempt to prepare the chloride of formyl-histidine led to no definite result. We attempted to prepare the chloride of benzoyl-histidine which was easily obtained in a 75 per cent yield by the method of Pauly,[20] but without success. Pauly[21] has also described the preparation of di-β-naphthalene-sulfonyl-histidine. Our attempts to prepare this compound were unsuccessful. α-Naphthalene-sulfonyl chloride, purified by distillation in high vacuum (0.3 mm.) was then combined with histidine according to Fischer and Bergell.[22] However, the properties and analysis of the resulting substance indicated that it was a salt of naphthalene-sulfonyl-histidine with naphthalene sulfonic acid and not the diacyl compound. Naphthalene-sulfonyl-histidine was easily prepared from this salt. Our endeavors to make the chloride of this compound were entirely futile. In this connection it is interesting to note that Pyman[23] failed to prepare the chloride of glyoxaline carboxylic acid, though a number of methods were tried.

Another attempt to synthesize a derivative of histidyl-β-alanine may be mentioned. The main reactions involved in the well known Erlenmeyer[24] synthesis for amino-acids may be expressed as follows:

[20] Pauly, H., *Ber. chem. Ges.*, 1910, xliii, 2243.
[21] Pauly, *Z. physiol. Chem.*, 1904, xlii, 508.
[22] Fischer, E., and Bergell, P., *Ber. chem. Ges.*, 1902, xxxv, 3779.
[23] Pyman, F. L., *J. Chem. Soc.*, 1916, cix, 186.
[24] Erlenmeyer, E., Jr., *Ann. Chem.*, 1893, cclxxv, 1, 8, 13.

$$C_6H_5CONHCH_2COOH + RCHO \longrightarrow C_6H_5CONHC(:CHR)COOH \xrightarrow{H_2} C_6H_5CONHCH(CH_2R)COOH$$

By analogy, hippuryl-β-alanine might react with glyoxaline aldehyde[23] thus leading to the benzoyl derivative of histidyl-β-alanine.

$$C_6H_5CONHCH_2CONHCH_2CH_2COOH + C_3H_3N_2CHO \longrightarrow C_6H_5CONHC(:CHC_3H_3N_2)CONHCH_2CH_2COOH \xrightarrow{H_2} C_6H_5CONHCH(CH_2C_3H_3N_2)CONHCH_2CH_2COOH$$

We accordingly coupled hippuryl chloride with β-alanine and obtained a beautiful crystalline product which was purified without difficulty. Our attempts to condense it with the easily accessible benzaldehyde were unsuccessful. We abandoned the project at this point.

All our efforts, therefore, to prepare histidyl derivatives of amino-acids have been without avail.

EXPERIMENTAL.

Preparation of Histidine.

Histidine was prepared from dried blood essentially as described by Steudel.[25] The method was altered by the use of litharge to make the removal of hydrochloric acid more complete than could be accomplished by distillation alone.

Considerable quantities of *l*-leucine were obtained by evaporating the mother liquor of the histidine mercury compound, after removal of the metal with hydrogen sulfide.

Preparation of Carnosine.

Fresh hashed horse muscle was extracted in 10 pound portions by covering with tap water and gradually heating to the boiling point. The coagulum was separated by filtration through a cotton bag, and washed with boiling water. About 75 liters of water were used for 100 pounds of meat. The extract was evaporated in shallow pans to about 15 liters, filtered, and treated with 1 kilo of copper acetate and enough milk of lime to make the solution strongly alkaline to litmus. The voluminous precipitate was filtered and thoroughly washed. The filtrate was slightly acidified with sulfuric acid and precipitated with mercuric acetate and

[25] Steudel, H., in Abderhalden, E., *Handb. Biochem. Arbeitsmethoden*, Berlin and Vienna, 1910, ii, 505.

sodium carbonate. The reaction was kept slightly acid. The mercury compound was washed, suspended in water, and decomposed with hydrogen sulfide. The solution was freed of hydrogen sulfide by aeration and treated with a small excess of silver nitrate. Barium hydroxide was added until the reaction was distinctly alkaline to litmus. After a few hours the precipitate was removed and the filtrate saturated with barium hydroxide. The carnosine silver was thoroughly washed with dilute barium hydroxide solution and decomposed with hydrogen sulfide. After removal of the barium with carbon dioxide and sulfuric acid the solution was neutralized with nitric acid and concentrated. About 50 gm. of crude carnosine nitrate were obtained from 100 pounds of horse muscle. The free base was prepared from the nitrate by precipitation with phosphotungstic acid or preferably, by precipitation with silver nitrate and barium hydroxide. The carnosine thus obtained melted at 254° (uncorrected). Its properties were similar to those described by Gulewitsch. It analyzed as follows:

0.0548 gm. substance required 9.6 cc. 0.1 N sulfuric acid.
0.0215 " " gave 0.00148 gm. N according to Van Slyke (micro method).

	Calculated for carnosine $C_9H_{14}O_3N_4$:	Found:
Total nitrogen	24.77	24.52
Amino "	6.2	6.8

Preparation and Hydrolysis of Deaminocarnosine.

4 gm. of carnosine dissolved in 75 cc. of water were deaminated by the addition of 3.2 gm. of barium nitrite and 20 cc. of 0.1 N sulfuric acid. After 5 hours an additional 6.5 cc. of the acid were added. The solution was placed in the refrigerator until the next morning. After heating on the water bath and aerating, it was filtered and diluted to 200 cc. 2 cc. of the solution gave 0.095 cc. of nitrogen gas at 743.5 mm. pressure and 25°, when analyzed according to Van Slyke for amino nitrogen. This was equivalent to but 2.08 per cent of the amino nitrogen originally present. The solution was hydrolyzed by boiling 4 hours with 5 N sulfuric acid. The acid was then removed with barium hydroxide, and the filtrate evaporated in vacuum, and then treated with alcohol. 1.66 gm. of flaky crystals were obtained, which analyzed as follows:

0.0868 gm. substance required 16.5 cc. 0.1 N sulfuric acid (Kjeldahl).

	Calculated for histidine $C_6H_9O_2N_3$:	Found:
N	27.08	26.61

The substance was converted into its hydrochloride, which melted at 255°. Pure histidine hydrochloride melts at the same temperature. When both compounds were mixed there was no change in the melting point. Another portion of the substance was converted into its dihydrochloride by evaporating in a desiccator over sodium hydroxide with concentrated hydrochloric acid. The melting point of the dihydrochloride was 244–245°, that of pure histidine dihydrochloride was 245°. Mixing both compounds did not depress the melting point.

0.0801 gm. substance required 10.6 cc. 0.1 N sulfuric acid (Kjeldahl).

	Calculated for histidine dihydrochloride $C_6H_{11}O_2N_3Cl_2$:	Found:
N	18.4	18.5

The total quantity of histidine obtained from deaminocarnosine was 70 per cent of the theoretical.

β-Iodopropionyl-Alanine ($ICH_2CH_2CONHCH(CH_3)COOH$).

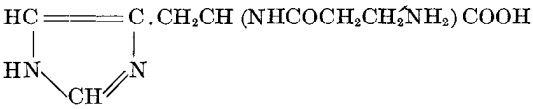
To a solution of 10 gm. of α-alanine dissolved in 300 cc. of water 15 gm. of crystalline barium hydroxide were added. The mixture was cooled with a freezing mixture and agitated with a mechanical stirrer. 21.3 gm. of β-iodopropionyl chloride diluted with petrolic ether were added in the course of 1 hour. In order to maintain an alkaline reaction, an additional 28 gm. of barium hydroxide were required. The excess of barium was removed with carbon dioxide. The chlorine and the remainder of the barium were removed with silver sulfate and sulfuric acid, respectively. After concentration of the solution 18.5 gm. of the reaction product were obtained. The melting point was 153–155°. The substance was dried in vacuum over sulfuric acid.

0.3420 gm. substance required 12.8 cc. 0.1 N sulfuric acid (Kjeldahl).

	Calculated for β-iodopropionyl-alanine $C_6H_{10}O_3NI$:	Found:
N	5.17	5.27

The compound was more soluble in alcohol and acetone than in water, soluble with difficulty in chloroform, and almost insoluble in benzene and petrolic ether.

Synthesis of Carnosine.



40 gm. of free histidine were dissolved in 500 cc. of water and cooled with a freezing mixture. 59 gm. of β-iodopropionyl chloride,[26] diluted to 150 cc. with petrolic ether, were added in the course of 1½ hours. Crystalline barium hydroxide, 140 gm. in all, was added at intervals to maintain alkalinity. Stirring was continued for 2½ hours. The barium was quantitatively removed with carbon dioxide and ammonia. The filtrate was cooled with a freezing mixture and saturated with ammonia. After 48 hours at 37°, the solution was evaporated to 150 cc. and again saturated with ammonia in the cold. Further amination was carried out in sealed tubes at 100°. Owing to breakage of the glass tubes the contents of two were lost. The remainder was evaporated to dryness in vacuum at 45°. The residue was dissolved in water and fractionally precipitated with silver nitrate and barium hydroxide. The precipitate which first appeared, when the solution was made distinctly alkaline, was gummy in nature and care was taken to separate this from the carnosine fraction itself. The precipitate which appeared upon complete saturation with barium hydroxide was washed with dilute baryta solution, decomposed with sulfuric acid, treated with hydrogen sulfide, and filtered. The last trace of sulfuric acid was removed and the solution concentrated. The crystals which soon appeared resembled carnosine in every detail. The yield was about 3 gm. After two recrystallizations the substance melted at 250–251° (corrected). Crystallization was carried out by dissolving in the least amount of warm water and adding pure absolute alcohol. (As carnosine is a strong base it must not be exposed to an atmosphere containing carbon dioxide for too long a time.)

[26] Jacobs, W. A., and Heidelberger, M., *J. Biol. Chem.*, 1915, xxi, 467.

0.0866 gm. substance gave 0.1511 gm. carbon dioxide and 0.0538 gm. water.

0.1222 gm. substance required 21.3 cc. 0.1 N sulfuric acid (Kjeldahl).
0.1044 " " " 18.25 " 0.1 N " " "

	Calculated for carnosine $C_9H_{14}O_3N_4$:	Found:	
C	47.78	47.59	
H	6.19	6.21	
N	24.77	24.40	24.47

The specific rotation of the substance in aqueous solution was + 21.5°, which corresponds with the value recorded for pure natural carnosine.

$$[\alpha]_D^{20} = \frac{4.5550 \times 2.68°}{0.5490 \times 1.034} = +21.5°$$

Action of Liver and Muscle Extracts on Carnosine.

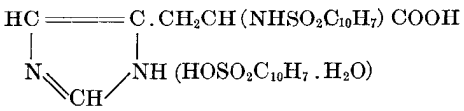
A dog was bled from the carotid artery under ether anesthesia and perfused with 0.9 per cent sodium chloride solution until most of the blood had been removed from the organs. Portions of the liver and muscle were ground with clean sea-sand and extracted with the saline solution. Both extracts hydrolyzed glycyltryptophane after 24 hours at 37°. A solution of carnosine containing 10.18 mg. of amino nitrogen was neutralized with acetic acid and diluted to 10 cc. with Henderson's phosphate solution. To 4 cc. of this solution, 1 cc. of the organ extract and 1 cc. of toluene were added. The control determinations were prepared in a similar manner without the carnosine. After 24 hours at 40°, the contents of the tubes were acidified with dilute acetic acid, coagulated by heat, filtered, washed, and diluted to 10 cc. Amino nitrogen (Van Slyke) was determined in 2 cc. portions of the solution. The temperature of the room was 27° and the barometric pressure 742.5 mm.

	Amino nitrogen.	
	cc.	*mg.*
(*a*) Liver extract	0.55	0.295
(*b*) " " plus carnosine	2.03	1.088
(*c*) Muscle "	0.27	0.145
(*d*) " " plus carnosine	1.80	0.965

The amino nitrogen added to the extracts, in the form of carnosine, was 0.814 mg. Deducting *a* from *b* and *c* from *d* 0.793 mg.

and 0.820 mg., respectively, are obtained from the liver and muscle mixtures. Hydrolysis did not occur in either case. Abderhalden and Fodor[27] have shown that β-alanine is deaminated in alkaline solution. To see if the slightly alkaline Henderson's phosphate solution decomposed β-alanine, the following experiment was carried out. A quantity of β-alanine equivalent to 1.2490 mg. of amino nitrogen per cc. was dissolved in Henderson's phosphate solution and incubated for 24 hours at 40°. At the end of the period 1.269 mg. were found; hence the alkalinity of the phosphate solution is not sufficient to decompose the amino-acid.

α-Naphthalene-Sulfonyl-Histidine-Naphthalene-Sulfonate.



To a solution of 1 gm. of histidine hydrochloride in 10 cc. of water, 5 cc. of 10 per cent sodium hydroxide and 3 gm. of α-naphthalene-sulfonyl-chloride, dissolved in ether, were added. This mixture was shaken on the machine for 4 hours. During this time 9 cc. of 10 per cent alkali were added in three equal portions. The ether was now removed and the liquid extracted with fresh portions of ether three times. After adding 10 cc. of 5 N hydrochloric acid, an oil appeared which crystallized at room temperature but not in the refrigerator. The crystals are best described as hexagonal plates. The melting point was 146°. Repeated crystallization from water raised the melting point to 155°. On further heating, the fused substance solidified but melted again at 220°. The weight of the substance remained constant when heated over boiling toluene in vacuum for 6 hours in the presence of phosphorus pentoxide.

0.1237 gm. substance gave 0.0975 gm. barium sulfate (sodium peroxide fusion method).

0.1227 gm. substance required 6.8 cc. 0.1 N sulfuric acid (Kjeldahl).

0.1227 " " were neutralized by 4.35 cc. 0.1 N sodium hydroxide solution. Calculated, 4.30 cc.

[27] Abderhalden, E., and Fodor, A., *Z. physiol. Chem.*, 1913, lxxxv, 112.

0.3167 gm. substance gave 0.6359 gm. carbon dioxide and 0.1338 gm. water.

	Calculated for $C_6H_{25}O_8N_3S_2$:	Found:
C	54.6	54.7
H	4.4	4.7
N	7.35	7.76
S	11.2	10.8

In order to remove the crystal water it was necessary to heat the substance to 150° over phosphorus pentoxide in a vacuum. 0.5114 gm. lost 0.0191 gm. of water and became darker. Calculated for one molecule of crystal water, 3.1 per cent. Found, 3.7 per cent. The discrepancy was accounted for by the slight decomposition.

The substance was also prepared by adding naphthalene-sulfonic acid to naphthalene sulfonyl-histidine. The crystal form of the compound prepared in this manner was similar to that already described. The melting point and solubilities also indicated that both compounds were identical. Owing to difficulties of purification and analysis we were unable to obtain acceptable analytical figures.

0.2085 gm. substance gave 0.4200 gm. carbon dioxide and 0.0900 gm. water.

	Calculated for $C_{26}H_{25}O_8N_3S_2$:	Found:
C	54.6	55.45
H	4.4	4.8

The substance was soluble, with difficulty, in water; more easily soluble in alcohol. When dissolved in boiling methyl alcohol containing a few drops of glacial acetic acid and cooled, long prismatic rods appeared. The melting point was 222° (uncorrected). The substance was dried by heating in a vacuum over boiling toluene for 6 hours in the presence of phosphorus pentoxide.

0.2089 gm. substance gave 0.4339 gm. carbon dioxide and 0.0809 gm. water.

0.1038 gm. substance gave 0.0870 gm. barium sulfate (sodium peroxide fusion method).

0.1100 gm. substance required 4.00 cc. 0.1 N sodium hydroxide solution for neutralization. Calculated, 3.97 cc.

0.1812 gm. substance required 10.10 cc. 0.1 N sulfuric acid (Kjeldahl).

	Calculated for $C_{26}H_{23}O_7N_3S_2$:	Found:
C	56.42	56.64
H	4.19	4.30
N	7.60	7.76
S	11.57	11.51

The analytical data indicated that we were dealing with the anhydrous form of the compound already described.

α-Naphthalene-Sulfonyl-Histidine.

The alcoholic solution of the preceding compound was treated with an amount of 0.1 N sodium hydroxide slightly in excess of neutrality. The solution was evaporated to dryness after the addition of acetic acid and the residue crystallized from methyl alcohol. A further crop of crystals was obtained by diluting the mother liquor with water. Recrystallization was effected by dissolving in dilute ammonia and precipitating with dilute acetic acid. The pure compound appeared in the form of hexagonal blocks, melting at 236° (uncorrected.) The substance lost 5.4 per cent of its weight when heated over boiling toluene in vacuum in the presence of phosphorus pentoxide. Calculated for one molecule of crystal water, 4.98 per cent.

0.2097 gm. substance gave 0.4296 gm. carbon dioxide and 0.0825 gm. water.

0.1225 gm. substance required 10.65 cc. 0.1 N sulfuric acid (Kjeldahl).

0.1082 " " " 3.1 " 0.1 N sodium hydroxide for neutralization. Calculated, 3.13 cc.

0.1082 gm. substance gave 0.0732 gm. barium sulfate (sodium peroxide fusion method).

	Calculated for naphthalene-sulfonyl-histidine $C_{16}H_{15}O_4N_3S$:	Found:
C	55.61	55.87
H	4.47	4.38
N	12.17	12.17
S	9.27	9.29

The substance was very slightly soluble in water, more so in alcohol. It readily dissolved in dilute ammonia or in alcohol containing naphthalene-sulfonic acid. The specific rotation of the sodium salt was determined in aqueous solution.

$$[\alpha]_D^{24} = \frac{3.6852 \times -6.72°}{1.020 \times 0.1272} = -190.8°$$

The rotation of the salt of naphthalene-sulfonic acid with naphthalene-sulfonyl-histidine was also determined. An amount of the substance corresponding to 0.0841 gm. of the sodium salt of naphthalene-sulfonyl-histidine was dissolved in the dimolecular equivalent of sodium hydroxide solution.

$$[\alpha]_D^{24} = \frac{4.8228 \times -3.39°}{1.02 \times 0.0841} = -190.8°$$

The presence of sodium naphthalene sulfonate did not influence the optical activity of the histidine compound. At first we hoped that naphthalene-sulfonyl-histidine would be well adapted for the isolation and characterization of histidine, but this does not seem likely as the yield was only 50 per cent, when pure histidine was used.

Hippuryl-β-Alanine.

3 gm. of β-alanine dissolved in an equivalent of sodium hydroxide solution were combined with hippuryl chloride in the usual manner. 42 cc. of sodium hydroxide (N solution) were added in the course of 30 minutes. Upon acidifying with 9 cc. of N hydrochloric acid, long tyrosine-like needles appeared. The yield was 50 per cent. The melting point was 183–185° (uncorrected). The substance was dried at 100° in vacuum over phosphorus pentoxide.

0.1028 gm. substance required 4.2 cc. 0.1 N sodium hydroxide for neutralization. Calculated, 4.1 cc.

0.1028 gm. substance required 8.5 cc. 0.1 N sulfuric acid (Kjeldahl).

	Calculated for hippuryl-β-alanine $C_{12}H_{14}O_4N_2$:	Found:
N	11.20	11.57

The substance is soluble with difficulty in water and ethyl acetate, but readily in alcohol. It is more soluble in cold water than hippuric acid.

SUMMARY.

The constitution of carnosine has been determined in two ways: (1) By the hydrolysis of deaminocarnosine and isolation of histidine; (2) by the synthesis of carnosine.

The behavior of carnosine toward liver and muscle has been determined.

Attempts to synthesize histidyl-β-alanine were unsuccessful.

CONCERNING HISTIDINE AND CARNOSINE. THE SYNTHESIS OF CARNOSINE

Louis Baumann and Thorsten Ingvaldsen

J. Biol. Chem. 1918, 35:263-276.