the β-aminocampholic acid hydrochloride in just the same manner as the α-camphidone was prepared from the α-aminocampholic acid. A melting point of 234–235° was found and a specific rotation $[\alpha]_D^{24°}$ = 63.2°; 0.25 g. in 5 cc. of alcohol solution. Rupe and Splittgerber[1] report a melting point of 225° and its specific rotation $[\alpha]_D$ = 66.5° for a 10% solution in benzene. An analysis of the compound was made.

Calc. for $C_8H_{14}\begin{matrix}CH_2\\ \diagup\quad\diagdown\\ CO\end{matrix}$:N = 8.38; found: N = 8.37.

Nitroso Derivative of β-Camphidone, $C_8H_{14}\begin{matrix}CH_2\\ \diagup\quad\diagdown\\ CO\end{matrix}$N NO.—The β-camphidone was dissolved in dilute hydrochloric acid (1 : 4) and a sodium nitrite solution was added. The yellow precipitate was filtered and recrystallized from alcohol two times, when a melting point of 164–165° is given. A portion recrystallized a third time showed the same melting point.

$[\alpha]_D^{23°}$ = 103°; 0.25 g. in 10 cc. of solution.

Summary.

It is shown in this paper that the specific rotations of some amino derivatives of camphoric acid are consistent with the view that those amino acids which can form cyclic salts containing quinquivalent nitrogen and a ring of six atoms form salts having the general formula $R\begin{matrix}CO\\ \diagup\quad\diagdown\\ NH_3\end{matrix}O$ in aqueous solutions. Amino acids which would give a ring of seven atoms in forming a cyclic salt appear to exist in solution as compounds of the form, $R\begin{matrix}CO_2H\\ \diagdown\quad\diagup\\ NH_2\end{matrix}$. These relations furnish strong evidence that nitrogen is in reality quinquivalent in ammonium salts and that the hydrogen of the acid combines with the nitrogen instead of remaining combined with the acid radical, as Werner has supposed.

Several new compounds have been prepared and the specific rotations of a number of known compounds have been determined.

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[FROM THE RESEARCH LABORATORY OF PARKE, DAVIS & CO.]

ON THE PRESENCE OF HISTIDINE-LIKE SUBSTANCES IN THE PITUITARY GLAND (POSTERIOR LOBE).

BY T. B. ALDRICH.

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In 1896, A. Kossel discovered among the cleavage products of sturine

[1] *Ber.*, 40, 4313 (1907).

a basic compound[1] to which he gave the name histidine. Later this compound was found among the cleavage products of complex proteins when these were subjected to acid hydrolysis[2] or to tryptic digestion.[3]

According to Pauly,[4] a solution of histidine made alkaline with sodium carbonate gives a very beautiful diazo reaction with diazobenzene sulfonic acid, which is deep cherry-red in dilutions of 1 : 20,000 and still markedly red in 1 : 100,000. Furthermore it is stated that, with the exception of tyrosine, no other cleavage product from protein, among a large number tested, gives such a color reaction, and that not only can histidine be detected by means of this reaction when mixed with other cleavage products, but also when in the original protein before cleavage, where it is united with other groups, providing the tyrosine is removed or changed so as not to react with this reagent.

As far as investigated by Pauly no other proteid cleavage product outside of histidine and tyrosine gives this reaction. Nearly all give a lemon yellow color in soda solution; such color being given, according to Pauly, by glycocol, alanine, leucine, valine, serine, lysine, ornithine, arginine, asparagine, glutamic acid, cystine, and hippuric acid, while with pyrolidine carboxylic acid and tryptophane no reaction is given. Glucosamine, phenylalanine and oxypyrolidine carboxylic acid, as well as the cleavage products obtained by Skraup[5] from caseine were not available to Pauly, but he states that it is not to be supposed that an exception would be found among these products.[6]

In the diazo-reaction we have, therefore, a positive means of determining whether protein or protein cleavage products contain histidine or tyrosine, especially as these bodies give a positive reaction where Millon's test is practically negative. The greatest importance seems, however, to lie in the fact that this reaction shows the presence of histidine and tyrosine in protein-like combination. Whenever a protein substance gives Pauly's reaction, then either tyrosine or histidine has been demonstrated among the hydrolytical cleavage products of this protein; while in other proteins where the diazo-reaction was negative these bodies were absent.

Pauly's method[7] of preparing diazobenzene sulfonic acid (which should be prepared fresh every time) and his directions for applying the test are as follows:

[1] *Z. physiol. Chem.*, **22**, 182 (1896).

[2] Hedin, *Ibid.*, **22**, 191 (1896).

[3] Kutscher, *Ibid.*, **25**, 195 (1898).

[4] *Ibid.*, 42, 513 (1904).

[5] *Ber.*, **37**, 3 (1896).

[6] My experience with Pauly's histidine test has shown me that other bodies besides tyrosine and histidine respond to this test, giving a reddish color that might be confusing. Among the bodies tested may be mentioned *p*-oxyphenylethylamine and *β*-iminazolyl-ethylamine.

[7] Pauly, *Loc. cit.*

1. **Preparation of Diazobenzenesulfonic Acid.**—Two grams of finely powdered sulfanilic acid are mixed with 3 cc. of water and 2 cc. concentrated hydrochloric acid, forming a thick paste. To this is added in small portions, in less than a minute, cooling after each addition, a gram of potassium nitrite dissolved in 1–2 cc. of water. The sulfanilic acid, for the most part, passes rapidly in solution and there is formed a thick, white crystalline precipitate of diazobenzene sulfonic acid which, after a few minutes, is filtered off by suction and washed with a little cold water. Any unchanged sulfanilic acid does not influence the reaction.

2. **Reaction with Histidine.**—To the solution to be tested, having demonstrated the absence of tyrosine by Millon's reagent, an excess of sodium carbonate (preferred to caustic soda) is added, and then 3–5 cc. of an alkaline carbonate solution of a few centigrams of the diazobenzene sulfonic acid prepared at the time of testing. Within three minutes at the longest, usually immediately, a dark cherry-red color appears, which even by dilution with many times its volume of water, retains its red color and does not shade into yellow. By acidulating, the color passes into a pure orange color.

Quite recently, K. Inouye,[1] working in Kossel's laboratory, published a method for detecting histidine in the presence of tyrosine, employing the above reaction. According to this investigator, the reaction is also given even when histidine is in combination with the protein molecule, and were it not for the fact that tyrosine, free or attached to protein, gives a very similar red color reaction with diazobenzene sulfonic acid, it would be a comparatively simple matter to detect histidine, just as tyrosine and tryptophane are recognized by color tests.

Since tyrosine gives with diazobenzene sulfonic acid, in alkaline solution, a color reaction that can not be distinguished from that of histidine, Inouye modified the test so as to eliminate the tyrosine from the reaction. This modification was brought about by benzoylating in alkaline soda solution.

If one shakes a solution of tyrosine with a few drops of benzoylchloride until the odor of the chloride has disappeared, after making alkaline with soda solution, the addition of diazobenzene sulfonic acid to the filtrate does not produce the characteristic color. If, on the other hand, one benzoylates histidine by the same process, the color reaction persists.

It was expected, according to Inouye, that this reaction for recognizing histidine would be applicable when histidine was united with protein or other complexes; but this expectation was not realized; for, after benzoylating such bodies, the color reaction could not be obtained, and it was found necessary to hydrolyze these substances either with the help of acids or by digestion with trypsin in order to establish the presence of histidine.

[1] *Z. physiol. Chem.*, 83, 79 (1912).

Having observed several years ago that pituitary preparations, obtained from the posterior lobe, gave a marked histidine reaction, I was lead to infer either the presence of histidine or some such body, but at that time, no method had been worked out to recognize histidine in the presence of tyrosine. On the appearance of Inouye's communication referred to above, I concluded to use his method for determining the presence of histidine in this lobe, the results of which investigations are given below:

Experimental.

The material employed was the desiccated defatted posterior lobe of the pituitary gland (consisting for the greater part of protein) prepared in the usual way, and a purified product soluble in water. (Three tests were made in all.)

Hydrolysis by Acid.—(1) 0.5 g. of the desiccated product was boiled for 5 hrs. (employing a return condenser) with 100 cc. of water, to which was added 2.5 cc. of concentrated hydrochloric acid. It was then evaporated on the steam bath to a syrupy consistency, taken up in water, an excess of lead oxide added, and the mixture warmed on the steam bath. After cooling it was made alkaline with sodium carbonate solution, filtered, and brought up to 80 cc.

(*a*) 10 cc. of the above were taken (representing 63 mg. of the original powder), 0.5 cc. saturated sodium carbonate solution and then 3 drops of benzoyl chloride were added and the mixture was agitated until the odor of the chloride had disappeared. It was then filtered and Pauly's test applied as follows:

5 cc. (32 mg.) + 4 drops of sodium carbonate saturated solution + 30 mg. of diazobenzene sulfonic acid either in solid form or dissolved in water. *Color reaction very pronounced.*

(*b*) 10 cc. + 1 1/2 cc. saturated sodium carbonate solution + 15 drops of benzoyl chloride, etc. *Reaction pronounced but not as strong as by* (*a*).

(2) 0.5 g. of the perfectly soluble product, treated as by (1). Made up to 80 cc.

(*a*) 10 cc. taken + 1 1/2 cc. of saturated sodium carbonate solution + 15 drops of benzoyl chloride, etc., etc.

Pauly's reaction pronounced, but less so than the original product.

Without Hydrolysis.—(3) 0.5 g. (perfectly soluble product) dissolved in 25 cc. H_2O, 3 cc. of saturated sodium carbonate solution added then 20 drops of benzoyl chloride. The resulting solution worked up in the usual way, *gave a strong Pauly's reaction.*

(4) Repeated (3) *Pauly's reaction pronounced.*

Hydrolysis by Trypsin.—(1) 0.2 g. pancreatin was agitated with 40 cc. of a 0.5% sodium carbonate solution.

(*a*) To 20 cc. of the above was added 0.5 g. of desiccated posterior lobe powder, and a few drops of chloroform. Placed in incubator for about 2 days.

(*b*) The remaining 20 cc. of (5) was also placed in incubator, a little chloroform being added.

Both (*a*) and (*b*) were agitated from time to time.

At the end of 2 days both solutions were evaporated (after neutralizing with hydrochloric acid) to a small volume, made alkaline, then benzoylated and eventually filtered. Both gave pauly's reaction, but the color given by (*a*) was more intense.

It might be stated at this point that both powders gave Millon's test before as well as after hydrolysis; but that after benzoylating, this reaction was always negative.

In the above experiments I have purposely used a large excess of benzoyl chloride and sufficient sodium carbonate solution to maintain an alkaline reaction. Inouye states that he used only a few drops; but in every case after thorough benzoylating I found even with a large excess of benzoyl chloride that the solution gave a positive reaction. In some instances the color was not as pronounced as one would expect, but this might be accounted for by the larger amount of benzoyl chloride used, or the fact that all was not decomposed, or that the presence of benzoic acid or its salts influenced the same.

One would conclude from the above, if Inouye's observations are correct, that histidine is present in the above preparations, prepared from the pituitary gland, were it not for the fact that the solutions before or after hydrolysis or after benzoylating failed to give in my hands Weidel's reaction as modified by Fisher or Knoop's bromine reaction. Whether we are justified, however, in drawing this conclusion is questionable, for it may be assumed that other bodies are present which interfere with the reaction or that histidine is present in too small an amount.

Under hydrolysis by trypsin the presence of a small amount of histidine in the *control* is explained by its presence in the pancreatin employed.

From (3) without hydrolysis, it would seem that at least one of the histidine-like substances in the pituitary gland (posterior) is not united to protein, for after benzoylating, employing an excess of sodium carbonate, the filtrate gave a strong Pauly's reaction. It is quite probable that we have a free and possibly a combined histidine or histidine-like compound. We are also confronted by the possibility of several substance of this nature being present. This latter view having support from the work of Fühner[1] who claims to have isolated four distinct substances from the posterior lobe of the pituitary gland, all of which give Pauly's reaction.

What compound or compounds we have to deal with here is suppositional at present, although evidence seems to be accumulating that points not to histidine but to some histidine-like compounds similar to histamine.[2] This view is also shared by Fühner[3] who says: "According to the work of others and myself I am led to conclude that histamine and the active principle of the posterior lobe of the gland are probably not identical; but pharmacologically directly related."

Conclusions.

1. It would seem that histidine (or some such compound or compounds) is contained in the desiccated posterior lobe of the pituitary gland.

2. These substances are probably in a more or less free state, or in some combination other than protein.

[1] *Deutsche Med. Wochschr.*, **39**, 491 (1913).

[2] Histamine is the commercial name for β-Iminazolylethylamine.

[3] *Münch. Med. Wochschr.*, **599**, 852 (1913).

3. The compounds giving Pauly's reaction are probably not histidine, since Weidel's reaction, as modified by Fischer, or Knoop's reaction with bromine were both negative.

4. Pauly's reaction is not a specific reaction for histidine, unless other compounds, such as tyrosine, *p*-oxyphenylethylamine, *β*-iminazolylethylamine, adrenaline, etc., are removed; but a general reaction for a class of compounds yet to be determined.

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TRI-AMMONIUM CITRATE.

BY ROBERT A. HALL.

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The ammonium salts of citric acid were seemingly first investigated by Heldt[1] in 1843, although in Berzelius' Lehrbuch der Chemie IV the statement is made that these compounds are very difficult to prepare and that no analysis of them had been made. Heldt's method of preparation was to neutralize a solution of citric acid with ammonia and by evaporation crystallize out the salts. His efforts to obtain the monoammonium citrate were unsuccessful, as his thick syrupy liquid always gave star-shaped groups of crystals containing varying amounts of ammonia. With the diammonium citrate he was more successful, as he obtained this salt in two separate, distinct crystalline forms. From a strong, concentrated solution after standing some hours, there separated out a mass of interlaced rhomboidal prisms; but upon slow evaporation in the cold winter air, the salt separated at the bottom of the vessel as a ring of massed oblique rhomboidal prisms with rectangular sides and semi-circular back-sides. In their composition both salts were identical and afforded a very interesting example of dimorphic organic compounds. Both crystalline forms rapidly absorbed moisture from the air and were soluble in boiling alcohol from which, upon cooling, they separated as oily drops. Their taste is agreeably acid with a cooling, bitter after effect. They were dried by pressing between filter paper and an elementary analysis made of their hydrogen and carbon content; the nitrogen was determined by precipitation as platinum ammonium hydrochloride. Both crystalline forms were found to have the composition represented by the formula $(NH_4)_2C_6H_6O_7$, that is, both were the diammonium citrate. Both salts were stable on heating to 100°.

Heldt's efforts at preparing the triammonium citrate were unsuccessful. He attempted to prepare this salt by evaporating a citric acid solution saturated with ammonia, but obtained the diammonium citrate. A

[1] Heldt, *Lieb. Ann.*, 47, 167 (——).