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(From the Biochemical Journal, Vol. vii, No. 2, March, 1913)

From

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES

BROCKWELL HALL

HERNE HILL

LONDON, S.E.

[From THE BIOCHEMICAL JOURNAL, Vol. VII, No. 2, March, 1913]

XIX. THE IDENTITY OF TRIMETHYLHISTIDINE (HISTIDINE-BETAINE) FROM VARIOUS SOURCES.

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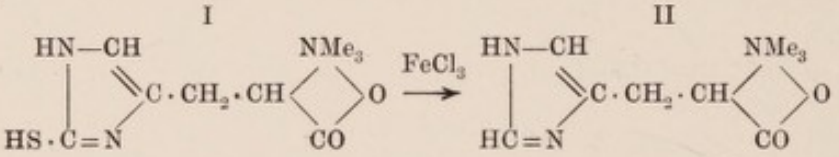
(*Received January 25th, 1913.*)

In a recent paper we showed [Barger and Ewins, 1911] that ergothioneine, a crystalline base containing sulphur, which was isolated from ergot by Tanret [1909], almost certainly possessed the constitution denoted by the formula 1:



Ergothioneine was thus trimethylhistidine (histidine-betaine) containing a sulphur atom attached to a carbon atom of the glyoxaline ring. Further we showed that on oxidation with ferric chloride the sulphur atom was removed as in similar thiolglyoxaline derivatives [Pyman, 1911] and a new base trimethylhistidine or histidine-betaine (II) was produced. We described certain of its salts and pointed out the possibility of its natural occurrence. Kutscher [1910] had previously obtained a crystalline aurichloride of a base from commercial extract of mushrooms, and had stated that this base was possibly trimethylhistidine, since it possessed the formula $C_9H_{15}O_2N_3$ and gave a strong reaction with sodium *p*-diazobenzene sulphonate. As, however, no details were published regarding this salt, we were unable to determine whether our base was identical with Kutscher's.

A short time after our paper there appeared a publication by Reuter [1912] in which he described the isolation of histidine-betaine from *Boletus edulis*, and gave a description of certain of its salts. Among others he characterised two picrates; one, a monopicrate which was analysed, but to which no melting point was assigned, and another, melting at 206° which was not analysed, but which, from a picric acid determination, appeared to

be a dipicrate. The picrate of histidine-betaine obtained by us from ergothioniene melted at 123° and analysed well for the dipicrate. This difference was pointed out by Reuter and for some time we were at a loss for a satisfactory explanation. We now find that the apparent discrepancy was due to the fact that our melting point was determined on the air-dried salt, which contained two molecules of water of crystallisation: our analysis on the other hand was made with an anhydrous specimen of which the melting point was not at the time determined. Quite recently Kutscher [1912] succeeded in synthesising the betaine in question from α-chloro-glyoxaline-propionic acid and trimethylamine at 80°, and by means of the aurichloride established the identity of the synthetic base with the supposed trimethylhistidine previously isolated by him from mushrooms. On repeating Kutscher's synthesis we were able to isolate from the reaction product a small quantity of the picrate of the betaine which melted at 123° as we had found in the case of the base derived from ergothioneine. The two picrates were, indeed, in all respects identical.

At this juncture we communicated with Dr C. Reuter who very kindly supplied us with specimens of the two picrates prepared by him, together with a full description of these salts, for which we offer him our best thanks. We then found that the (anhydrous) dipicrate melting at 205°–206° described by Reuter, crystallised from water with two molecules of water of crystallisation, and, when air dry, melted at 123°–124°, and was then in all respects identical with the picrate obtained by us. The water of crystallisation could be removed by drying *in vacuo* over sulphuric acid, but only with some difficulty. The picrate thus obtained always showed signs of sintering at about 125° (doubtless owing to traces of water still adhering) but did not melt until 205°–206° as described by Reuter. The crystalline anhydrous salt could be readily obtained by recrystallising the picrate (dried *in vacuo*) from absolute alcohol, and then melted at 213°–214°. In a private communication Dr Reuter informed us that a purified specimen of his dipicrate melted at 212°–213°. We carried out determinations of the water of crystallisation present in our picrate (A) and that obtained by recrystallising Reuter's anhydrous dipicrate from water (B) with the following results.

A. 0·2061 (air dry) lost (*in vacuo* over H_2SO_4) 0·0106 $H_2O = 5·14$.
B. 0·1603 ,, ,, ,, ,, 0·0083 $H_2O = 5·17$.

$C_9H_{15}O_2N_3 \cdot (C_6H_3O_7N_3)_2 \cdot 2H_2O$ requires $H_2O = 5·21$ per cent.

The complete identity of the two bases was established by the melting points of the two forms of the dipicrate and of the monopicrate (m.p. 201°–202°) which we prepared from our dipicrate according to Reuter's direction

(treating the aqueous solution of the dipicrate with one molecular proportion of sodium hydrate). The melting points of mixtures in all cases showed no depression.

There can be no doubt, therefore, that the trimethylhistidine (histidine-betaine) obtained by us from ergothioneine is identical with that obtained by Reuter from *Boletus edulis* and with the synthetic base obtained by Kutscher. This result affords a further confirmation of the constitution assigned by us, on other grounds, to ergothioneine.

For the isolation of the betaine of histidine we find that the preparation of the dipicrate is the most convenient method; this salt dissolves in about 25 parts of boiling water and readily crystallises in thin elongated rectangular plates with two molecules of water of crystallisation, and when air dry, melts at 123°–124°.

In our previous paper [1911] the melting point of the aurichloride of the betaine was given as 171°. As the quantity of material at our disposal was at that time very small (a few centigrams only) we were unable to analyse this salt, but now, with a further supply of material, we find that the gold salt when pure melts at 184°, in agreement with the melting point as given by Kutscher and by Reuter.

We also determined the rotation of the base recovered from Reuter's dipicrate with the following result.

Concentration of base (in aqueous solution as hydrochloride) = 0·39 per cent.

Actual rotation measured $\alpha_D = +0{\cdot}40^\circ$ (mean of 6 readings) in 2·2 d.m. tube. Whence $[\alpha]_D = +46{\cdot}5^\circ$.

In conclusion we may point out that the trimethylhistidines obtained from various sources are thus shown to be identical. This substance must be classed with the other naturally occurring betaines from amino-acids, such as those from glycine, proline, oxy-proline, and tryptophane (i.e. ordinary betaine, stachydrine, betonicine and hypaphorine respectively).

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