[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

BIOCHEMISTRY OF SULFUR. II. THE ISOLATION OF ERGOTHIONEINE FROM ERGOT OF RYE

BY BLYTHE A. EAGLES[1]

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In the previous paper from this Laboratory, it was shown by Eagles and Johnson[2] that sympectothion[3,4,5] and thiasine[6,7] from pig's blood were identical with ergothioneine from ergot of rye. It has also been reported and shown independently by Newton, Benedict and Dakin[8,9] that thiasine is identical with ergothioneine.

A renewed interest in the biochemistry of organic sulfur combinations has been created as a result of these researches. Eagles and Johnson isolated ergothioneine from ergot of rye for purposes of comparison with that obtained from pig's blood. The method adopted for the isolation and final purification of this sulfur compound from ergot of rye differed from the original method of C. Tanret[10] or the later one of G. Tanret.[11] Any improvement in technique that can be recommended for the separation and isolation of this substance from natural sources deserves especial attention. The original procedure of Tanret was followed up to the separation of the compound as the mercuric chloride salt. Instead of isolating the compound as the hydrochloric acid salt at this stage, the procedure of Hunter and Eagles[4,5] for its isolation from blood was then used and the substance isolated in the pure state as the free base.

The procedure will be described in detail.

Experimental

One kg. of finely ground ergot of rye[12] was extracted in a Soxhlet apparatus with 90% alcohol for four hours; 300 cc. of alcohol per 100 g. of powder was used. The residues were twice boiled with one-half the volume of alcohol, filtered through cheesecloth and the filtrate combined with the original extract. Alcohol was distilled from

[1] Holder of a Sterling Research Fellowship in Chemistry at Yale University, 1926–27.

[2] Eagles and Johnson, THIS JOURNAL, **49**, 575 (1927).

[3] Bulmer, Eagles and Hunter, *J. Biol. Chem.*, **63**, 17 (1925).

[4] Hunter and Eagles, *ibid.*, **65**, 623 (1925).

[5] Hunter and Eagles, *ibid.*, **72**, 123 (1927).

[6] Benedict, *ibid.*, **64**, 215 (1925).

[7] Benedict, Newton and Behre, *ibid.*, **67**, 267 (1926).

[8] Newton, Benedict and Dakin, *Science*, **64**, 602 (1926).

[9] Newton, Benedict and Dakin, *J. Biol Chem.*, **72**, 367 (1927).

[10] Tanret, *J. pharm. chim.*, [VI] **30**, 145 (1909).

[11] Tanret, *Bull. soc. chim.*, **31**, 444 (1922).

[12] For the supply of ergot of rye necessary for the isolation of ergothioneine, we wish to express our thanks to the Upjohn Company of Kalamazoo, Michigan, and Sharp & Dohme of Baltimore, Maryland.

the extract, water added and the separated fats and resins were removed by filtration. The volume of solution was then 2200 cc. Considerable coloring matter was removed with barium hydroxide, leaving the solution acid to litmus. Basic lead acetate[13] was then added to maximum precipitation (94 cc. required) and the precipitate centrifuged. Excess lead was removed from the filtrate by sulfuric acid. It was then made alkaline to phenolphthalein with 2.5 *N* sodium hydroxide for the extraction of alkaloids with chloroform. The solution was then acidified with acetic acid and warm 8% aqueous mercuric chloride added to maximum precipitation. This corresponds to the mercuric chloride precipitation used by Hunter and Eagles in the isolation of ergothioneine from pig's blood. The mercury salt of ergothioneine was well washed with 0.25% aqueous mercuric chloride, suspended in 100 cc. of water and the mercury removed with hydrogen sulfide. After the separation of mercuric sulfide, Tanret then isolated ergothioneine as the hydrochloric acid salt. On account of the high solubility of this salt, it was thought advisable to continue further fractionation of the product and attempt its isolation as the free base. The technique used by Hunter and Eagles for its isolation from blood was followed. The filtrate from mercuric sulfide was freed from hydrogen sulfide by aeration and subjected to the lead acetate-sodium hydroxide precipitation. The volume of filtrate was 146 cc. and required for practically complete precipitation of ergothioneine 61 cc. of 20% sugar of lead, 27.5 cc. of 2.5 *N* sodium hydroxide and 8 cc. of 10% sodium chloride. After freeing from its lead salt with sulfuric acid, it was precipitated with phosphotungstic acid and then isolated as the free base; 0.65 g. of pure ergothioneine was obtained.

This method of isolation may be considered an improvement on those of C. and G. Tanret in that it consists in the addition to those methods of two precipitations which serve to separate ergothioneine from impurities present and has the further advantage that the compound is obtained directly as the free base rather than as its hydrochloric acid salt. The yield is much higher than any previously recorded.

I wish to express my great indebtedness to Professor Treat B. Johnson for his kindly advice and help throughout this work.

Summary

An improved method of isolating ergothioneine from ergot of rye has been detailed.

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[13] Goulard's Extract, United States Pharmacopeia IX, Philadelphia, 1916, 249.