THE OXIDATION OF WHEAT FLOUR
III. The Isolation of Thioctic Acid[1]

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ABSTRACT

Thioctic acid, a coenzyme, was isolated from wheat flour. The compound was identified by its sulfur content, by the melting point of its S-benzyl thiuronium derivative, by its R_f value, and by its ultraviolet spectrum.

It is estimated that the compound is present in amounts of approximately 1 to 10 p.p.m., although less than this amount was recovered with the isolation procedure used. Wheat germ, as might be expected, contains a much greater amount of thioctic acid than flour.

In a recent paper, Dahle and Sullivan (2) reported the presence of thioctic acid in the acid-hydrolyzed extract of wheat flour. The compound was identified by the absorption maximum at 334 $m\mu$ and by chromatography. This paper will describe the isolation and further identification of thioctic acid from wheat flour.

Materials and Methods

A straight-grade, unbleached, spring wheat flour of 0.43% ash and 13.0% protein was used as the source material. All chemicals were analytical reagent grades.

The isolation procedure was as follows: A total of 8.9 kg. of flour was hydrolyzed in eight batches of 1,000 g. each and one batch of 900 g. Two liters of water and 65 ml. of concentrated hydrochloric acid were mixed with 1,000 g. of flour and autoclaved for 1 hour at 126°C. The suspension was cooled and centrifuged at 1,700 r.p.m. for 25 min-

[1]Manuscript received March 30, 1961. Contribution from the Research Laboratories of Russell Miller-King Midas Mills, Minneapolis, Minnesota.

utes. The water-soluble layer was extracted with benzene, using a ratio of one part benzene to three parts of the water extract. The extract was washed twice with small amounts of benzene. During the extraction with benzene, emulsions formed occasionally; they were broken by centrifugation. The benzene extract was then extracted with 5% sodium bicarbonate, using about one part of sodium bicarbonate solution to four parts of the benzene extract. The water extract, containing the sodium salts of any acids present, was acidified with 6*N* hydrochloric acid until the pH was less than 1.0. The solution changed from a brown to a yellow color and was allowed to stand for 15 to 30 minutes until the carbon dioxide escaped. The combined acid solutions from all batches (2 parts) were then extracted with ethyl ether (1 part) and the ether solution evaporated to about 2 ml. This solution was introduced into a silica-powder-1% ether column about 4 cm. in length and 1.5 cm. in diameter. The ether solution and subsequent eluting solutions were forced through the column with slight air pressure. Fatty acids were eluted with three washings of 1% ethyl ether in petroleum ether—first 4 ml., then 2 ml. and, finally, 1 ml. Thioctic acid and a quinone-type pigment were eluted with two washings of ethyl ether and concentrated to about 1 ml. The crude thioctic acid was esterified by refluxing 1 hour with 75 ml. of ethanol and a drop of hydrochloric acid. After cooling, the ethanol solution was diluted with twice its volume of water and extracted with ethyl ether. The ether solution was washed with cold 5% sodium bicarbonate to remove the pigment, then with 0.1*N* hydrochloric acid and, finally, with water. The ester was then hydrolyzed to the acid by gentle refluxing with 0.1*N* hydrochloric acid and the acid extracted with chloroform and, finally, recrystallized from petroleum ether. A few milligrams of yellow crystals were isolated.

Identification of Thioctic Acid

The following means were used to identify the compound as thioctic acid.

1. *Ascending Strip Chromatography.* A benzene extract was applied by micropipet in two or three drops of 10 to 15 μl. each to a strip of Whatman No. 1 filter paper (17 cm. by 3 cm.) using 1% glacial acetic acid as the solvent. After 1 hour, the paper was immersed in 0.1*N* potassium permanganate, withdrawn, and, after 1 minute, rinsed in a large quantity of distilled water. A brown spot having an R_f value of 0.70 was found. The same value was obtained using pure dl-thioctic acid.

2. *Ultraviolet Spectrum.* The yellow extract in ether gave a maximum absorbance at 334 mμ. Synthetic thioctic acid (1) and the isolated

compound gave identical absorbance maxima.

3. *S-Benzyl Thiuronium Derivative.* This derivative was made according to the procedure of Donleavy (3). Ether was evaporated from the solution of thioctic acid isolated from flour by the above procedure. Sodium bicarbonate (2 mg.), water (10 ml.), and 1 drop of 1.0*N* hydrochloric acid were added. This solution was added to a boiling solution of 7.2 mg. of S-benzyl thiuronium chloride in 10 to 15 ml. of ethanol. After evaporation to 1 to 2 ml. and cooling in an ice bath, crystals were obtained showing a melting point of 140°C. The derivative made in a similar manner from pure dl-thioctic acid and recrystallized from absolute methanol gave a melting point of 140°C. The only reference in the literature is that of Segre, Viterbo, and Parisi (4), who reported a melting point of 152° to 154° of the S-benzyl thiuronium derivative crystallized from absolute alcohol and another value of 132° to 134° observed once. They indicated that this wide discrepancy might be due to crystallization and drying.

4. *Analysis of Thioctic Acid.* The material isolated from flour was found to contain 31.87% sulfur. The theoretical sulfur content of thioctic acid, $C_8H_{14}O_2S_2$, is 31.1%. Since less than 1 mg. was used for analysis, the agreement between the theoretical and the sulfur as found was considered adequate.

Wheat germ, as might be expected, contains much more thioctic acid than flour; our present estimate is 40 to 50 times the amount in flour.

Discussion

Thioctic acid is a coenzyme generally considered to be bound to a protein; hence there is need of an acid hydrolysis to release it. Recent work in the development of a method for the measurement of thioctic acid indicates that the acid hydrolysis conditions employed may have caused significant losses. Present methods would indicate that flour contains from 1 to 10 p.p.m. of thioctic acid and wheat germ about 200 to 300 p.p.m.

Further work is needed on the best conditions for hydrolysis and reduction before accurate figures can be given.

Literature Cited

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