

temperature of separation within a half a degree. Each determination was made in triplicate and the averaged results appear in Table I.

TABLE I  
CRITICAL SOLUTION TEMPERATURES FOR SULFUR AND BUTYL PHTHALATE

Mole % dibutyl phthalate	Mole % sulfur (S <sub>8</sub> )	Critical soln. temp., °C.
97.54	2.46	61.1
94.98	5.02	91.1
91.45	8.55	127.8
87.26	12.74	161.1
87.14	12.86	164.4
84.02	14.98	197.8

OLD OCEAN, TEXAS

RECEIVED JUNE 15, 1950

### Preparation of Oxythiamine Diphosphate and Oxythiamine Monophosphate<sup>1</sup>

BY LEOPOLD R. CERECEDO AND ALBERT J. EUSEBI

In a previous paper,<sup>2</sup> oxythiamine was shown to be a potent thiamine antagonist. These findings have been confirmed and extended by us and by other workers.<sup>3,4,5,6</sup> In contrast, experiments carried out in this Laboratory<sup>7,8</sup> with chloro- and bromoxythiamine, in which the hydroxyl group of the thiazole moiety of oxythiamine was replaced by halogen, showed these substances to be not toxic to mice, even when administered at high levels. This observation suggested that the hydroxyl group was necessary for the anti-thiamine effect of oxythiamine. Furthermore, since it is well known that thiamine functions *in vivo* as the diphosphate, the idea suggested itself that oxythiamine exerted its anti-thiamine effect in the form of the diphosphate. In this paper, we wish to report the preparation of the diphosphate and the monophosphate of oxythiamine.

**Phosphate Salt of Oxythiamine Diphosphate.**—This substance was prepared according to a modification of the method which Karrer and Viscontini<sup>9</sup> used for the preparation of thiamine diphosphate.

Five grams of 85% phosphoric acid was heated in a small beaker to 300–310°, or until crystals appeared on its walls. The acid was allowed to cool and then placed in an oil-bath previously heated to 135–140°. After temperature equilibration, 4 g. of oxythiamine was added all at once and kept at this temperature for 20 minutes with constant stirring. The fused mass was allowed to cool somewhat and then washed twice with 50 ml. of acetone before it solidified. The solid was dissolved in 50 ml. of water containing 4 drops of concentrated phosphoric acid, and precipitated with 150 ml. of acetone. After standing overnight, the dark brown oil was dissolved in 15 ml. of water plus 4 drops of concentrated phosphoric acid, and decolorized with Norit A, filtered, washed with water, and brought to a

volume of 75 ml. The solution was treated with 150 ml. of acetone to give an initial dense cloudiness. It was then placed in the refrigerator for 6 hours, after which the turbid supernatant was decanted and discarded. The yellowish oil which remained was taken up in 15 ml. of phosphoric acid and decolorized a second time with a small amount of Norit A, filtered, washed and brought to a volume of 25 ml. The material was then precipitated with 125 ml. of acetone and kept in the refrigerator overnight. After removing the supernatant, the colorless oil was dissolved in 20 ml. of phosphoric acid, and precipitated with 100 ml. of acetone. A further fractionation was then carried out by dissolving the oil in 25 ml. of phosphoric acid and precipitating with 50 ml. of acetone. After removal of the supernatant, the glassy appearing material was taken up in 10 ml. of phosphoric acid and precipitated with an equal mixture of absolute alcohol and acetone. The precipitate was treated with 50 ml. of absolute alcohol and placed in the refrigerator. After two days, with occasional scratching, the material became crystalline and could be filtered. It was washed with absolute alcohol, acetone and ether, and then dried *in vacuo* over sulfuric acid for 2 days; yield 1.7 g. (27.5%). The compound started to brown at 190° and decomposed at 245–255°.

*Anal.* Calcd. for C<sub>12</sub>H<sub>20</sub>O<sub>12</sub>N<sub>2</sub>P<sub>2</sub>S: C, 27.53; H, 3.82; N, 8.03; free P, 5.93; hydrolyzable P, 11.87; total P, 17.80. Found: C, 27.83; H, 4.06; N, 7.77; free P, 5.65; hydrolyzable P, 11.62; total P, 17.70.

**Oxythiamine Monophosphate.**—Thiamine diphosphate, on treatment with nitrous acid gases in an aqueous medium, loses one phosphoric acid group quite rapidly. The second phosphate group is more difficult to remove. The deamination and dephosphorylation of thiamine diphosphate was carried out according to the procedure used in the preparation of oxythiamine from thiamine.<sup>2</sup>

Four grams of cocarboxylase phosphate salt, prepared according to the method of Karrer and Viscontini,<sup>9</sup> was dissolved in 50 ml. of water, and nitrous acid gases passed into the solution for four hours. The mixture was allowed to stand overnight, and the process repeated on the second and on the third day. The excess acid was removed by passing the solution through a column of the resin Amberlite IR-4B<sup>10</sup> which was kept cold. The effluent was acidified with *N* HCl and concentrated to an oil *in vacuo* at room temperature. The oil was taken up in alcohol, precipitated with an excess of ether, and left in the refrigerator. As no precipitate formed, the alcoholic solution was treated with an excess of ether. After standing for five hours in the refrigerator, the slightly turbid supernatant was decanted, the precipitate dissolved in 10 ml. of 0.1 *N* HCl, the solution decolorized with Norit A, the Norit washed, and the volume brought to 20 ml. with the washings. The solution was treated with 175 ml. of acetone and kept in the refrigerator overnight. The resultant precipitate was taken up in absolute alcohol, and hydrogen chloride gas passed in at ice-bath temperature to saturation. The solution was then treated with ether to give a dense cloudiness. After standing overnight, the precipitate was treated with 20 ml. of absolute alcohol to aid crystallization; yield 1.1 g. The compound was very hygroscopic and melted at 185–196°.

*Anal.* Calcd. for C<sub>12</sub>H<sub>18</sub>O<sub>8</sub>N<sub>2</sub>PSCl<sub>2</sub>: C, 34.45; H, 4.31; free P, 0; hydrolyzable P, 0; total P, 7.41. Found: C, 35.05; H, 4.49; free P, 0, hydrolyzable P, 0; total P, 7.18.

The analysis indicated that the product probably contained a very small amount of free oxythiamine. However, it was sufficiently pure for use with certain enzyme systems and for experiments *in vivo*. The results of these studies and of those with oxythiamine diphosphate will be reported in a separate paper.

DEPARTMENT OF BIOCHEMISTRY  
FORDHAM UNIVERSITY  
NEW YORK 58, N. Y.

RECEIVED JUNE 19, 1950

(10) Supplied by Rohm and Haas Company, Philadelphia, Pa. This technique for the removal of excess acid has been used with success by Mr. G. Bonvicino of the Organic Chemistry Department in the preparation of oxythiamine from thiamine.

(1) This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

(2) M. Soodak and L. R. Cerecedo, *THIS JOURNAL*, **66**, 1988 (1944).

(3) A. J. Eusebi and L. R. Cerecedo, *Science*, **110**, 162 (1949).

(4) C. E. Frohman and H. G. Day, *J. Biol. Chem.*, **180**, 93 (1949).

(5) J. H. Jones, C. Foster and W. Henly, *Proc. Soc. Exptl. Biol. Med.*, **69**, 454 (1948).

(6) L. Daniel and L. C. Norris, *ibid.*, **72**, 165 (1949).

(7) M. Soodak and L. R. Cerecedo, *Federation Proc.*, **6**, Part II, 293 (1947).

(8) A. J. Eusebi and L. R. Cerecedo, *ibid.*, **9**, Part I, 169 (1950).

(9) P. Karrer and M. Viscontini, *Helv. Chim. Acta*, **29**, 711 (1946).