PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

O-Benzoylthiamine Disulphide and its preparation

We, TANABE SEIYAKU CO., LTD., a corporation organized under the laws of Japan, of 21, Doshomachi 3-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

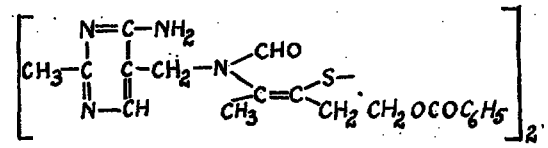
This invention relates to O-benzoylthiamine disulphide which possesses vitamin B_1-like activity, and to its preparation.

It is known that acid addition salts of thiamine have many disadvantages such as unpleasant odour, low stability, solubility in water and limited absorption upon oral ingestion.

It is also known that some people have thiaminase, thiamine destroying enzyme in their intestines and, therefore, no effect is produced by the oral administration of thiamine acid-addition salts.

We have found that O-benzoylthiamine disulphide is free of the disadvantages mentioned above. The O-benzoylthiamine disulphide of this invention occurs as colourless and odourless crystals melting at 146° to 147°C.

Its structural formula is as follows:



It has been found that O-benzoylthiamine disulphide is sparingly soluble in water. The solubility in distilled water at various temperature is as follows:

TABLE 1

Temperature (°C.)	Solubility (%) by weight/volume
20	0.0060
30	0.0082
40	0.0097
50	0.0110

The solubility may be increased to more than 50% by the addition of an equimolar amount of an acid (pH 3.0—3.2).

It is known that thiamine acid-addition salts such as the hydrochloride are generally unstable in alkaline media.

However it has been found that O-benzoylthiamine disulphide is quite stable, even in alkaline media. The following Tables II and III illustrate residual rate (%) of both thiamine hydrochloride (B_1 HCl) and O-benzoylthiamine disulphide (BTDS) in alkaline media on accelerated ageing tests.

[*Price 4s. 6d.*]

TABLE II

Residual rate (%) in aqueous solutions (2 mg/cc.) at 100° C.

pH	After 30 minutes		After 60 minutes		After 120 minutes	
	BTDS (%)	B_1 HCl (%)	BTDS (%)	B_1 HCl (%)	BTDS (%)	B_1 HCl (%)
5.0	96.5	96.5	96.5	96.0	94.5	94.5
6.0	97.0	94.5	96.0	86.5	84.5	83.5
7.0	91.5	67.0	78.0	45.5	51.0	20.0
8.0	83.5	60.0	65.0	37.5	49.0	10.0
9.0	56.0	9.0	26.5	4.5	15.0	2.0

pH 5.0 — 7.0 : MacIlvain's buffered solution

pH 8.0 — 9.0 : Clark-Lubus' buffered solution

TABLE III

Residual rate (%) in calcium carbonate powder (200 mg/g.)

Relative humidity 82% at room temperature

Substance \ Days	After 5 days	After 25 days	After 40 days
B_1 HCl	59	12	5
B T D S	100	94	94

It has been found that O-benzoylthiamine disulphide is more resistant to enzymatic degradation with thiaminase than thiamine hydrochloride, by the test described below.

A mixture of 1 ml. of substrate solution containing 20 γ of B_1 HCl or 22.8 γ of BTDS (equivalent to 20 γ of B_1 HCl), 1 ml. of buffered solution and 3 ml. of an enzymatic solution prepared from a cultural medium of *Bacillus thiaminolyliticus* (M.M.) or *Bacillus aneurinolyticus* (K.A.) was incubated for 1 hour at 37°C., and the degradation rate of each of the substances was assayed by the thiochrome method.

The results are as follows:

TABLE IV

Substance	Degradation Rate (%)			
	Bacillus M. M., After 5 days		Bacillus K. A., After 3 days	
	× 5*	× 10*	× 30*	× 60*
B_1 HCl	89.8	46.8	81.4	61.3
B T D S	15.0	4.7	2.7	2.0

* Dilution of enzymatic solution.

It has also been found that the compound of this invention possesses vitamin B_1-like activity on tests on both animals and human beings. Moreover, when the compound of this invention is orally administered, the blood level of total vitamin B_1 rises more rapidly, and remains at a high level for a longer period as compared with thiamine hydrochloride, and a larger quantity of free vitamin B_1 is excreted in urine as compared with thiamine hydrochloride. These results indicate a better absorbability of the compound from the intestines than that of thiamine hydrochloride.

From the above facts, the use of O-benzoylthiamine disulphide of our invention is more advantageous than the use of thiamine hydrochloride for the treatment of vitamin B_1-deficiency. It is also useful as a fortifying agent suitable for use in those instances where a fortifying agent of low water-solubility is desired, for instance, in the enrichment of cereals subjected to rinsing before being cooked.

The present invention also provides processes for preparing the O-benzoylthiamine disulphide. The O-benzoylthiamine disulphide may be prepared by reacting thiamine disulphide with an acylating agent derived from benzoic acid such as the chloride, bromide or anhydride in the presence of an acid acceptor such as sodium hydroxide, sodium carbonate, potassium hydroxide, potassium carbonate or pyridine.

As the starting material, an oxidation mixture of thiamine in an alkaline solution in which the thiamine disulphide is formed may also be used. Alternatively, O-benzoylthiamine disulphide may be prepared by treating O-benzoylthiamine with an oxidizing agent such as iodine, hydrogen peroxide or potassium ferricyanide, while maintaining the reaction mixture alkaline. The starting material, O-benzoylthiamine may be prepared according to the process set forth in an article reported by K. Vematsu in The Annual Report of Takamine Laboratory (Japan), vol. 11, pages 42—45, 1959.

It has been found that the reaction proceeds most satisfactorily at a pH within the range of 10.0 to 12.0 in an aqueous medium.

The invention is further illustrated by the following examples. The compound thiamine disulphide, used in Examples 1 and 2, has been reported by Nesbitt and Sykes in J. Chem. Soc. (1954), pages 4581—4587.

EXAMPLE 1

10 ml. of benzoyl chloride were dripped into a stirred suspension of 20 g. of thiamine disulphide in 20 ml. of absolute pyridine at room temperature. The mixture was stirred for an additional 3 hours at room temperature and was allowed to stand overnight. The reaction mixture was concentrated under reduced pressure to remove pyridine and, after adding small amounts of a 20% aqueous solution of sodium hydroxide, the residue was extracted with chloroform. The extract was washed with small amounts of aqueous solution of sodium bicarbonate and water successively and was dried with sodium sulphate. Then the chloroform was removed by evaporation and 20 ml. of benzene were added to the residue whereby O-benzoylthiamine disulphide crystallized out. Yield, 20 g., colourless prisms melting at 146°—147°C. after recrystallized from 99% ethanol.

Analysis: Calculated for $C_{38}H_{42}O_6N_8S_2$
C: 59.21; H: 5.49; N: 14.54

Found: C: 59.39; H: 5.79; N: 14.31

Di-hydrochloride: Colourless prisms melting at 180 — 181° C. (decomp.)

Analysis: Calculated for $C_{38}H_{42}O_6N_8S_2.2\ HCl\ .\ 2\ H_2O$

C: 51.88; H: 5.50; N: 12.74

Found: C: 52.02; H: 5.57; N: 12.74

EXAMPLE 2

20 g. of thiamine disulphide were suspended in 200 ml. of water and 3.5 g. of concentrated hydrochloric acid were added to the suspension to dissolve the particles. The mixture was made alkaline with sodium hydroxide. 10 ml. of benzoyl chloride were dripped into the mixture with stirring on an ice bath, while maintaining the mixture alkaline with a 20% aqueous solution of sodium hydroxide. The mixture was stirred for an additional 3 hours at room temperature. After being adjusted to pH 7, the mixture was extracted with chloroform and the extract was treated as described in Example 1. 18 g. of O-benzoylthiamine disulphide were obtained.

EXAMPLE 3

The procedure described in Example 1 was followed except that 20 g. of benzoic anhydride were used instead of benzoyl chloride. 16 g. of O-benzoylthiamine disulphide were obtained.

EXAMPLE 4

24 g. of sodium hydroxide in 140 ml. of water were dripped into a stirred solution comprising 70 g. of thiamine hydrochloride and 100 ml. of water on an ice bath. To the mixture, was added a solution of 25 g. of iodine and 35 g. of potassium iodide in 200 ml. of water, whereby thiamine disulphide was prepared in the reaction mixture. Then 40 ml. of benzoyl chloride were dripped into the mixture at room temperature with stirring, while maintaining the mixture alkaline with sodium hydroxide. After being allowed to stand overnight, the reaction mixture was extracted with chloroform and the extract was treated as described in Example 1. 50 g. of O-benzoylthiamine disulphide were obtained.

EXAMPLE 5

4.5 g. of O-benzoylthiamine in 460 ml. of water were neutralized with 7.5 ml. of 4N sodium hydroxide on an ice bath. Into the solution, was dripped a solution comprising 1.3 g. of iodine, 4 g. of sodium iodide and 100 ml. of water at the same temperature with stirring. As the reaction proceeded, a white precipitate appeared. The mixture was stirred for an additional 10 minutes, and the precipitate was extracted with ethyl acetate.

After the extract was washed with water and dried, ethyl acetate was evaporated at a reduced pressure and 30 ml. of benzene were added to the residue thereby O-benzoylthiamine disulphide crystallised out.

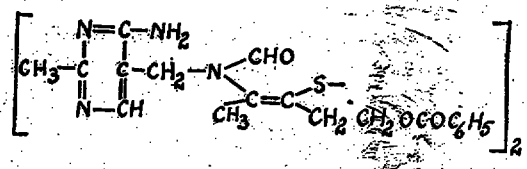
Yield 2.9 g., 75.3% of theory.

EXAMPLE 6

The procedure described in Example 5 was followed except that 0.85 g. of 30% hydrogen peroxide and 40 ml. of water were used instead of iodine-sodium iodide solution, and the pH of the mixture was maintained below 12 with acetic acid during the reaction. 2.8 g. of O-benzoylthiamine disulphide were obtained. In the same way there may be used potassium ferricyanide as the oxidizing agent instead of iodine or hydrogen peroxide.

WHAT WE CLAIM IS:—

1. O-benzoylthiamine disulphide having the structural formula



2. A process for preparing O-benzoylthiamine disulphide which comprises treating thiamine disulphide with a benzoyl halide or benzoic anhydride in the presence of an acid acceptor.

3. A process for preparing O-benzoylthiamine disulphide which comprises treating O-benzoylthiamine with an oxidizing agent in an aqueous medium while maintaining the medium alkaline.

4. A process according to claim 3, wherein the oxidizing agent is iodine, potassium, ferricyanide or hydrogen peroxide.

5. A process according to claim 3 or 4, wherein the aqueous medium is maintained at a pH within the range of 10 to 12.

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EXAMPLE 5

4.5 g. of O-benzoylthiamine in 460 ml. of water were neutralized with 7.5 ml. of 4N sodium hydroxide on an ice bath. Into the solution, was dripped a solution comprising 1.3 g. of iodine, 4 g. of sodium iodide and 100 ml. of water at the same temperature with stirring. As the reaction proceeded, a white precipitate appeared. The mixture was stirred for an additional 10 minutes, and the precipitate was extracted with ethyl acetate.

After the extract was washed with water and dried, ethyl acetate was evaporated at a reduced pressure and 30 ml. of benzene were added to the residue thereby O-benzoylthiamine disulphide crystallised out.

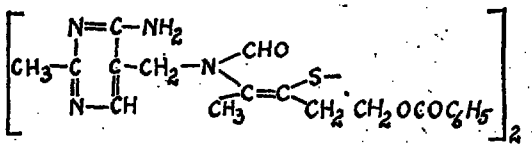
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3. A process for preparing O-benzoylthiamine disulphide which comprises treating O-benzoylthiamine with an oxidizing agent in an aqueous medium while maintaining the medium alkaline.

4. A process according to claim 3, wherein the oxidizing agent is iodine, potassium, ferricyanide or hydrogen peroxide.

5. A process according to claim 3 or 4, wherein the aqueous medium is maintained at a pH within the range of 10 to 12.

* C L M S *

6. The processes for preparing O-benzoylthiamine disulphide substantially as hereinbefore described with reference to the Examples.

STEVENS, LANGNER, PARRY & ROLLINSON,
Chartered Patent Agents,
Agents for the Applicants.

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