SYNTHESIS AND SOME PROPERTIES OF S-BENZOYL-THIAMINE DIPHOSPHATE

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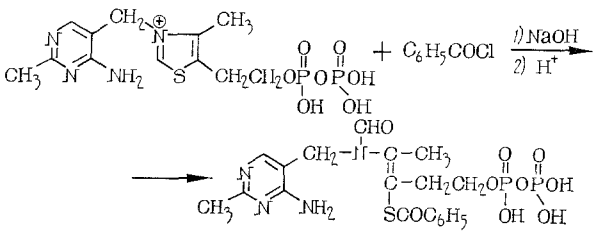
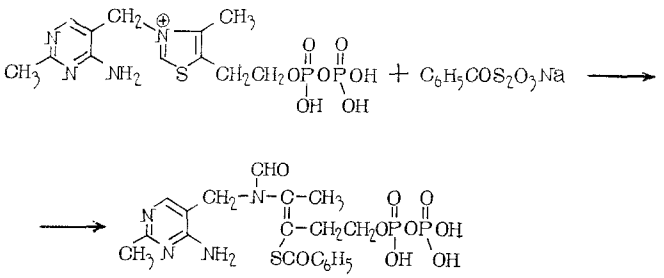
UDC 615.356:577.164.11

Thiamine is known to have a low rate of absorption and to be inactivated by specific enzymes in the intestine. Its conversion to the active coenzyme form is slow. This has provided the motivation for work on the controlled synthesis of biologically more active thiamine derivatives. These include acyl derivatives of thiamine [1] such as S-benzoylthiamine monophosphate [2]. S- and O-acyl derivatives of thiamine are more easily assimilated by the organism than is thiamine and can maintain a higher vitamin B_1 level in the blood [3].

To examine the biological activity of one such compound we have repeated the syntheses of S-benzoylthiamine diphosphate [3, 4] by acylation with the acid chloride:



or by reaction with the sodium acylthiosulfate prepared in turn by reaction of sodium thiosulfate with benzyl chloride [5]:



We used the Schotten–Baumann reaction for the benzoylation of thiamine diphosphate with benzoyl chloride while carefully monitoring the course of the reaction by thin-layer chromatography. The end of the reaction was determined from the negative thiochrome test, which demonstrates the absence of the starting thiamine diphosphate. We isolated the reaction product by acidification of the reaction mixture to pH 3.5-4.0 and purification by ion-exchange chromatography on KU-23 (H^+) cation-exchange resin. By careful adherence to these experimental conditions we were able to prepare S-benzoylthiamine diphosphate in 63-69% yield, which is much higher than that reported earlier [4].

We were unable to repeat the synthesis of S-benzoylthiamine diphosphate by the second route – acylation with the sodium acylthiosulfate [5].

We examined the biological activity of S-benzoylthiamine diphosphate in the Pharmacology Laboratory of the All-Union Scientific-Research Vitamin Institute [6]. We measured the pyruvate content of the blood and the thiamine content of the liver, and the transketolase activity in the blood and organs; the transketolase activity was assayed in vitro. We found that on peroral administration the preparation is more effective not only than thiamine but also more effective than thiamine diphosphate. On intramuscular administration under

All-Union Scientific-Research Vitamin Institute, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 7, pp. 58-61, July, 1979. Original article submitted January 31, 1979.

0091-150X/79/1307-0727$07.50

the conditions of dietary vitamin deficiency S-benzoylthiamine diphosphate, relative to thiamine and thiamine diphosphate, enhances the transketolase activity by a factor of 1.3-1.5 in the blood, and by a factor of two in the heart; the enzyme activity in the liver is roughly doubled.

After preliminary administration of oxythiamine, S-benzoylthiamine diphosphate causes a considerable increase in transketolase activity in the blood and heart; moreover, its activity in the heart is restored almost to normal whereas thiamine and thiamine diphosphate do not activate the enzyme under these conditions.

We examined the coenzyme activity of S-benzoylthiamine diphosphate by comparison with thiamine diphosphate in tests with crystalline transketolase. We found that S-benzoylthiamine diphosphate displays coenzyme activity in the transketolase system.

In the context of the theory of thiamine biocatalysis, in which the 2-C position of the thiazolium ring of thiamine diphosphate has an important role, the appearance of coenzyme activity of S-benzoylthiamine diphosphate, a form of cocarboxylase that lacks the thiazolium ring as such, is extremely interesting. We can suggest several possible reasons for this: Either the activity of this compound is due to some mechanism other than the conventional one or else the benzoyl group first rapidly dissociates and the resulting thiol form then gives the usual thiamine diphosphate.

A possible solution stems from our work on the hydrolytic properties of the compound, which were not reported earlier. Firstly the isolation of the reaction product by acidification of the mixture to pH 3.5-4.0 is accompanied by partial hydrolysis of the $S-COC_6H_5$ group and the subsequent cyclization of the open form of thiamine, demonstrated by the positive thiochrome test. Moreover, we were able to isolate thiamine diphosphate hydrochloride after purification on the ion-exchange resin. Secondly, when S-benzoylthiamine diphosphate is heated in aqueous ethyl alcohol partial alcoholysis of the $S-COC_6H_5$ group also occurs and thiamine diphosphate and ethyl benzoate are formed. Thirdly, prolonged contact (about 20 h) of the reaction product with the KU-23 (H^+) resin causes its decomposition via several pathways. We verified the formation of S-benzoylthiamine diphosphate, thiamine diphosphate, and thiamine under these conditions by chromatography and electrophoresis. After prolonged standing (for 20 h and more) of aqueous and aqueous alcoholic solutions of S-benzoylthiamine diphosphate decomposition can also be detected.

Thus in aqueous acidic and neutral solutions S-benzoylthiamine diphosphate is unstable at room and higher temperatures. This applies equally to S-, O-, and S,O-dialkoxy and S-acyl derivatives of thiamine [7]. Thus, these compounds decompose in neutral and acidic aqueous solutions. The S-substituted thiamines are less stable than the O-derivatives; in the case of the S,O-diacyl derivatives (with the same acid group) the thio ester bond is preferentially hydrolyzed. We found that S-benzoylthiamine decomposes when heated in acidic solution at pH 5.0 and to a lesser extent at pH 2.0-3.0 to thiamine and O-benzoylthiamine.

Our results demonstrate the generally low stability of solutions of S-benzoylthiamine diphosphate, which seems to support our second suggestion regarding the origin of its transketolase activity. Equally its instability in solution will impede the development of a pharmaceutical preparation based on this compound, despite the interesting properties revealed in the pharmacological tests.

EXPERIMENTAL

S-Benzoylthiamine Diphosphate. Thiamine diphosphate hydrochloride (10 g, 0.02 mole) was dissolved in water (200 ml). The solution was cooled to 10-15°C and 10% aqueous sodium hydroxide was added dropwise with stirring to pH 11.0. The mixture was left for 15-20 min. At the same temperature a solution of benzoyl chloride (10 ml, 0.06 mole) in dry chloroform (50 ml) was added dropwise with vigorous stirring (over a period of 1.5-2 h), while the solution pH was maintained throughout at 11.0 by adding alkali from a dropping funnel; the total consumption of sodium hydroxide solution was 70-90 ml. The reaction was monitored by the thiochrome test; a negative thiochrome test indicated completion of the reaction. After the reaction the mixture was stirred at 10-15°C for 30 min, while its pH was monitored, and then transferred to a separating funnel. The lower chloroform layer was separated and the aqueous layer was extracted two or three times with chloroform (20-25 ml each time) to remove benzoic acid. The aqueous layer was acidified to pH 3.5 with hydrochloric acid (1 : 1), the precipitated benzoic acid was filtered off, and the filtrate was evaporated to dryness on a rotary evaporator. The solid residue was dissolved in water (50-60 ml), the insoluble suspension was filtered off, and the filtrate was applied to a column (diameter 2.5 cm, length 48-50 cm) packed with KU-23 (H^+) resin and eluted with distilled water. The eluate at pH 1.0 contained mainly thiamine diphosphate hydrochloride formed by hydrolysis of the reaction product during acidification of the reaction mixture. The eluate at pH 2.0-3.0 contained S-benzoylthiamine diphosphate (500-250 ml). Evaporation of this on a rotary evaporator (bath temperature 30°C) gave a crystalline product with mp 169-170°C (decomposition), yield 62.8-69.2%; R_f

0.23-0.26 (methanol–aqueous ammonia, 3:1), 0.37-0.41 (isopropyl alcohol–water–phosphate buffer solution, pH 5.8, 3:1:1).

The S-benzoylthiamine diphosphate was purified by dissolving it (1 g) in water (10 ml) and pouring the solution slowly with stirring into acetone (300 ml) cooled to 0°C. After standing overnight in a refrigerator the crystals were then filtered, washed with acetone, and dried to give a compound with mp 187-189°C (decomposition). Found, %: C 41.79; H 4.57; N 9.96. $C_{19}H_{24}N_4O_9P_2S$. Calculated, %: C 41.76; H 4.43; N 10.25.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2,3-DIFUNCTIONAL 1,2,4-TRIAZINE DERIVATIVES

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UDC 615.31:547.873

Triazine derivatives have been reported to have a broad spectrum of biological activity [1, 2].

We have continued the search for biologically active compounds among substituted cyclic azines with work on the synthesis and some reactions of 2,3-difunctional 1,2,4-triazine derivatives and examination of their antibacterial activity.

We have shown [3] that 3-amino-5,6-diphenyl-1,2,4-triazine (I) and other 3-amino-1,2,4-triazine derivatives react with α-haloketones under mild conditions to form 2-acylmethyl derivatives of 2,3-dihydro-3-imino-1,2,4-triazine, which very readily cyclize to imidazo[1,2-b]-1,2,4-triazine derivatives. We decided to survey the alkylation of (I) with β-halo alcohols and 1,2-dihaloalkanes, isolate the 2,3-disubstituted dihydro-1,2,4-triazines, and examine their chemical reactions.

Reaction of (I) with ethylene bromohydrin in anhydrous DMF under reflux gave 2,3-dihydro-3-imino-2-(2-hydroxyethyl)-5,6-diphenyl-1,2,4-triazine (II). Use of ethylene chlorohydrin as starting component in this reaction considerably reduced the yield of (II). We verified the structure of (II) from its IR spectrum, which has the characteristic associated NH and OH bands in the 2970-3320 cm^{-1} region. The mass spectrum of (II) contains the intense molecular ion (M^+) at m/e 372, which is consistent with the calculated value.

When refluxed in excess thionyl chloride, (II) gave, without isolation of the intermediate 2,3-dihydro-2-(2-chloroethyl)-3-imino-5,6-diphenyl-1,2,4-triazine, 2,3-diphenyl-6,7-dihydroimidazo[1,2-b]-1,2,4-triazine (IV). This compound has been synthesized by a longer route, though its precise physical constants were not quoted [4].

The reaction of (I) with 1,2-dihaloalkanes could form both the disubstituted 1,2,4-triazines (III), or the products of their subsequent intramolecular cyclization (IV), and substituted 1,2-bis(1,2,4-triazin-2-yl)alkanes. Prolonged refluxing of (I) with 1,2-dibromoethane in DMF gave the bicyclic compound (IV). We did not detect the formation of any other products. Use of 1,2-dichloroethane and cyclizing agent considerably reduced the yield of the final product (IV). We characterized the bicyclic compound (IV) as the base, hydrobromide, and picrate.

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 7, pp. 61-63, July, 1979. Original article submitted September 26, 1978.