[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF FORDHAM UNIVERSITY]

The Action of Fish Tissue of Thiamine. II.[1] Identification of the Pyrimidine Moiety of Icthiamin[2,3,4]

BY JAMES D. BARNHURST[5] AND DOUGLAS J. HENNESSY

Icthiamin is the principal pyrimidine derivative resulting from the action of clam tissue on thiamin. Ultraviolet absorption spectra and results of a potentiometric titration of icthiamin dihydrobromide are shown. These, together with the isolation and identification of 2,5-dimethyl-4-aminopyrimidine and 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid as products of treatment of icthiamin dihydrobromide with Raney nickel and sodium bisulfite, respectively, prove the presence in icthiamin of a 2-methyl-4-aminopyrimidine-5-methylene moiety.

The method of assay of icthiamin reported previously[1] has suggested the presence in icthiamin of a 2-methyl-4-aminopyrimidine-5-methylene moiety. Ultraviolet absorption spectra, potentiometric titration and chemical degradations of icthiamin dihydrobromide, reported here, offer additional proof for the presence of this moiety.

Figure 1 shows the ultraviolet absorption spectra[6] of icthiamin dihydrobromide and, for purposes of comparison, those of 2,5-dimethyl-4-aminopyrimidine. The figure illustrates the dependence of the absorption of ultraviolet light by icthiamin on the *p*H of its solution, and the similarity of its absorption spectra to those of a compound of known structure.

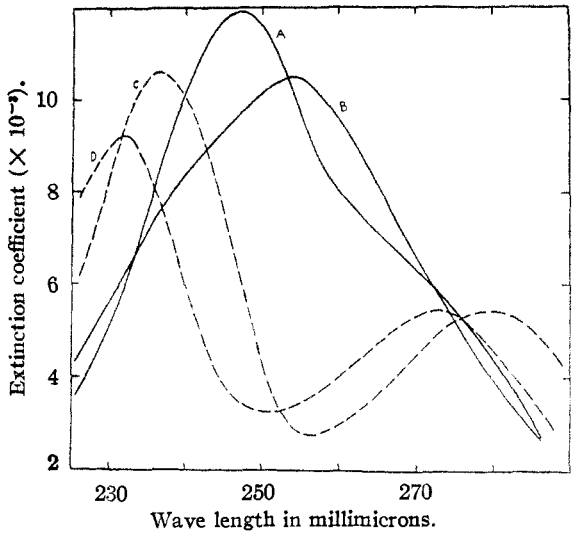


Fig. 1.—Ultraviolet absorption of: A, icthiamin dihydrobromide in 0.001 *N* HCl; B, 2,5-dimethyl-4-aminopyrimidine in 0.001 *N* HCl; C, icthiamin dihydrobromide in 0.005 *N* NaOH; D, 2,5-dimethyl-4-aminopyrimidine in 0.005 *N* NaOH.

Figure 2 shows the results of a potentiometric titration of icthiamin dihydrobromide using a model 3A Coleman *p*H electrometer and the procedure of Williams and Ruehle[7] for the titration of thiamin The breaks in the curve occurring at the points of addition of one and two moles of alkali per mole of salt indicate the presence in icthiamin of two basic groups whose pK_b values have been calculated as 6.4 and 9.1. The weaker basic group approximates in strength the 4-amino group in the pyrimidine moiety of thiamin, the pK_b value of which has been reported to be 9.5.[7]

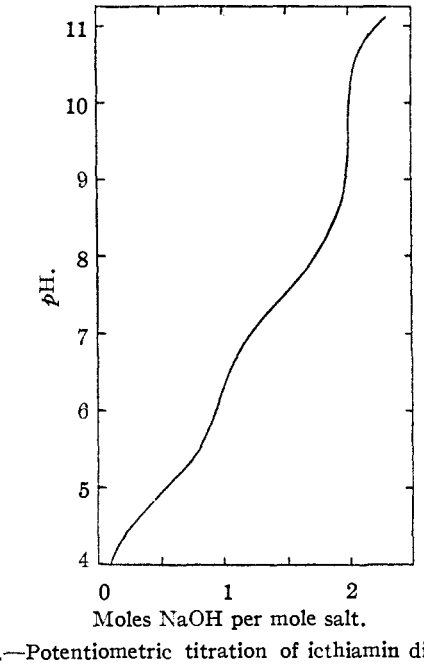


Fig. 2.—Potentiometric titration of icthiamin dihydrobromide.

At *p*H 5–5.5 in an aqueous sodium bisulfite solution at room temperature, icthiamin dihydrobromide $C_8H_{14}N_4O_3S\cdot 2HBr$ undergoes a cleavage and deposits a sparingly soluble crystalline substance identical with the acidic sulfite cleavage product of thiamin, 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid.[8] The evidence which establishes these compounds as identical is the following: (1) The acidic sulfite cleavage product of icthiamin and an authentic sample of 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid, obtained by sulfite cleavage of thiamin, display similar solubilities. They are sparingly soluble in cold water, moderately soluble in hot water from which they may be recrystallized, insoluble in organic solvents, and soluble in dilute alkali and concentrated acid.

(1) Paper I, J. D. Barnhurst and D. J. Hennessy, THIS JOURNAL, **74**, 353 (1952).

(2) This work was aided by a grant from the Williams-Waterman Fund.

(3) Presented before the Division of Biological Chemistry, American Chemical Society, 117th Meeting, Philadelphia, April, 1950, and 118th Meeting, Chicago, September, 1950.

(4) This paper is based on a portion of a thesis submitted by J. D. Barnhurst to the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(5) Wallace and Tiernan Co., Inc., Belleville, N. J.

(6) All ultraviolet absorption spectra were measured with a model DU Beckman spectrophotometer and ultraviolet accessories.

(7) R. R. Williams and A. E. Ruehle, THIS JOURNAL, **57**, 1856 (1935).

(8) H. Grewe, *Z. physiol. Chem.*, **243**, 89 (1936).

(2) The elementary analysis agrees with that calculated for the insoluble sulfite cleavage product of thiamin as shown in Table I.

TABLE I

ELEMENTARY ANALYSIS OF ICTHIAMIN DEGRADATION PRODUCTS

	C	H	S
Acidic sulfite cleavage product			
Calcd. for $C_6H_8N_2O_3S$	35.46	4.46	15.78
Found	35.62	4.18	15.32
The oxysulfonic acid			
Calcd. for $C_6H_8N_2O_4S$	35.27	3.95	15.71
Found	34.58	3.65	15.16

(3) The ultraviolet absorption spectra[8] of the compounds in acidic and alkaline media are practically identical, as shown in Fig. 3.

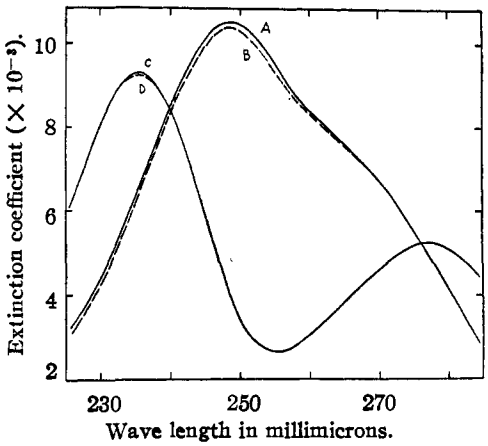
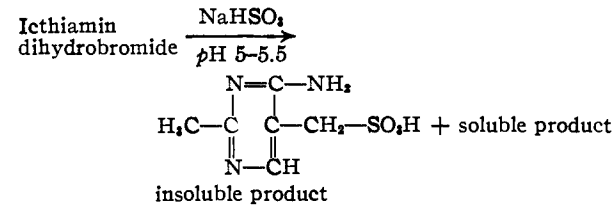


Fig. 3.—Ultraviolet absorption of: acidic sulfite cleavage product of icthiamin, A, in 0.001 *N* HCl, C, in 0.005 *N* NaOH; 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid, B, in 0.001 *N* HCl, D, in 0.005 *N* NaOH.

(4) The insoluble sulfite cleavage products from thiamin and icthiamin, when treated in sealed tubes with concentrated hydrochloric acid yield the same product as established by elementary analysis (Table I), and by the similarity of their ultraviolet absorption spectra, as shown in Fig. 4. Cline, *et al.*,[9] in their work on the structure of thiamin, have shown this product to be 2-methyl-4-oxypyrimidine-5-methylenesulfonic acid.

Thus the sparingly soluble sulfite cleavage product of icthiamin is 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid, and the cleavage of icthiamin by sulfite may be represented as



Icthiamin dihydrobromide is hydrogenolyzed by adjusting its aqueous solution to *p*H 7.5–8 and refluxing it for two hours with Raney nickel. From the reaction mixture, 2,5-dimethyl-4-aminopyrimidine can be isolated by removing the Raney nickel, adjusting the filtrate to *p*H 9, evaporating it to dryness *in vacuo*, and subliming the dry residue.

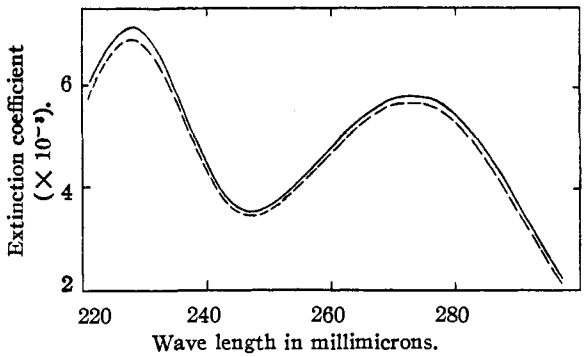


Fig. 4.—Ultraviolet absorption in water of: —, oxysulfonic acid derived from icthiamin; — —, 2-methyl-4-oxypyrimidine-5-methylenesulfonic acid.

The sublimate was identified as 2,5-dimethyl-4-aminopyrimidine by its melting point, 199°, by a mixed melting point with an authentic sample of 2,5-dimethyl-4-aminopyrimidine in which no depression was observed, by the melting point of the picrate of the sublimate, 224°, and by a mixed melting point of the picrate with an authentic sample of 2,5-dimethyl-4-aminopyrimidine picrate, again no depression. The melting points reported in the literature[10] for 2,5-dimethyl-4-aminopyrimidine and its picrate are 201–202° and 222°, respectively.

The structure of the precursor of icthiamin, *viz.*, thiamin, the fact of the reconversion of icthiamin to thiamin by the action of the thiazole moiety of the vitamin in the presence of live yeast, and the similarity of the ultraviolet absorption spectra of icthiamin and 2,5-dimethyl-4-aminopyrimidine suggest the presence in icthiamin of a 2-methyl-4-aminopyrimidine-5-methylene moiety. Since one product of sulfite cleavage of icthiamin, 2-methyl-4-aminopyrimidine-5-methylenesulfonic acid, and one product of Raney nickel hydrogenolysis of thiamin, 2,5-dimethyl-4-aminopyrimidine, contain a 2-methyl-4-aminopyrimidine-5-methylene moiety, icthiamin, it would seem, must also contain it.

Experimental

Sulfite Cleavage of Icthiamin Dihydrobromide.—Into 10 ml. of a 2.5 *M* aqueous solution of sodium bisulfite, was introduced 450 mg. of icthiamin dihydrobromide. The solution was adjusted to *p*H 5–5.5 with 10% aqueous sodium hydroxide and allowed to stand at room temperature for 40 hours. The white precipitate which formed was then collected, washed with a few drops of cold water and dried *in vacuo;* wt. 180 mg. The combined mother liquor and washings were then concentrated to a volume of about 6 ml., readjusted to *p*H 5–5.5, and allowed to stand at room temperature for an additional 40 hours, whereupon more precipitate formed. This was collected, washed and dried as above, wt. 40 mg. The combined precipitates were recrystallized by dissolving them in a minimum of boiling water and allowing the solution to cool. The recrystallized material did not melt below 300°.

Acid Hydrolysis of the Acidic Sulfite Cleavage Product of Icthiamin.—The aminosulfonic acid obtained by sulfite cleavage of icthiamin dihydrobromide, 130 mg., was converted to the oxysulfonic acid according to the procedure of

(9) J. K. Cline, R. R. Williams, A. E. Ruehle and R. E. Waterman, THIS JOURNAL, 59, 580 (1937).

(10) R. R. Williams, A. E. Ruehle and J. Finkelstein, *ibid.*, 59 526 (1937).

Cline, *et al.*[9]; yield of recrystallized oxysulfonic acid 115 mg. It did not melt below 300°.

Raney Nickel Hydrogenolysis of Icthiamin Dihydrobromide.—Icthiamin dihydrobromide, 420 mg., was dissolved in 25 ml. of water, adjusted to *p*H 7.5–8 with 10% sodium hydroxide and refluxed for two hours with 2.2 ml. of settled Raney nickel catalyst prepared from the alloy by the method of Mozingo, *et al.*[11] The Raney nickel was then removed by filtering the hot suspension. The clear filtrate was adjusted to *p*H 9 with 10% sodium hydroxide and then evaporated to dryness *in vacuo*. The well-powdered residue was then sublimed in a Craig still at 12 mm. over a temperature range of 150–185°. The colorless sublimate weighed 48 mg., m.p. 199°. The picrate of the sublimate was prepared in the usual manner in water; m.p. 224°. The melting points of the sublimate and its picrate were not depressed when mixed, respectively, with authentic 2,5-dimethyl-4-aminopyrimidine and 2,5-dimethyl-4-aminopyrimidine picrate.

(11) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

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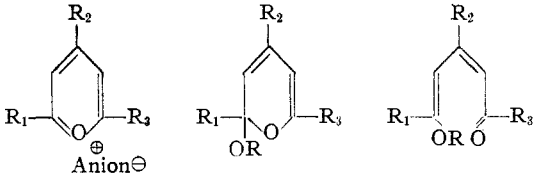
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTHERN CALIFORNIA]

Ring-Chain Tautomerism of Pyrylium Pseudo-bases[1]

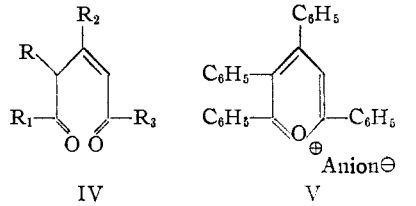
BY JEROME A. BERSON

The ultraviolet and infrared spectra of some triphenylpyrylium pseudo-bases indicate strongly that the predominant species in solutions of these substances are to be represented by the open-chain 1,5-diketone formula. The results are in accord with the infrared spectrum of triphenylpyrylium pseudo-base and with the observed and previously reported chemical behavior of the pseudo-bases.

The pseudo-bases derived by treatment of triphenylpyrylium salts (I) with mild alkali have been formulated[2a,b,c,d] as II, III and IV.



I II III



IV V

Ia: R_1, R_2, R_3 = C_6H_5
Ib: R_1 = *p*-$CH_3OC_6H_4$; R_2, R_3 = C_6H_5
Ic: R_1, R_3 = C_6H_5; R_2 = *p*-$CH_3OC_6H_4$
IIa, IIIa, IVa: R_1, R_2, R_3 = C_6H_5; R = H
IIb, IIIb, IVb: R_1 = *p*-$CH_3OC_6H_4$; R_2, R_3 = C_6H_5; R = H
IIc, IIIc, IVc: R_2 = *p*-$CH_3OC_6H_4$; R_1, R_3 = C_6H_5; R = H
IId, IIId, IVd: R_1, R_2, R_3 = C_6H_5; R = CH_3

The isolation and interconversion[2d] of two discrete forms (probably a diketone and an enol) of the pseudo-base of tetraphenylpyrylium salts (V) have effectively demonstrated the existence of a mobile equilibrium between at least two such desmotropes in a related case. As a part of a study on pyrylium compounds in progress in this Laboratory, we have undertaken an investigation aimed at determining which, if any, of the hypothetically tautomeric modifications (II, III or IV) predominates in solutions of the triphenylpyrylium pseudo-bases. While this work fails to demonstrate the presence of more than one tautomer, the findings are strongly in favor of the open-chain diketone structure (IV) as the predominant desmotrope.

While the ultraviolet absorption characteristics of desmotropes IIa or IIIa are not easily predictable in the absence of suitable model substances, it is clear that IVa would be expected, as a first approximation, to exhibit absorption due to two insulated chromophoric types, namely, acetophenone and benzalacetophenone. The ultraviolet spectrum of the pseudo-base (IVa) of triphenylpyrylium salts (Ia) (Fig. 1) shows two maxima, λ_{max} 247, 298 mμ, which correspond almost exactly in position and intensity to the principal maxima of acetophenone (λ_{max} 247 mμ[3]) and benzalacetophenone (λ_{max} 299 mμ[4]). Further, a summation of the acetophenone and benzalacetophenone spectra (Fig. 1) corresponds very closely to the spectrum of the pseudo-base.

On the assumption that the long wave length maximum (λ 298 mμ) results from an essentially independent benzalacetophenone-type chromophore in IVa, one would anticipate a strong bathochromic shift of this peak in the spectrum of the pseudo-base (IVc) derived from 2,6-diphenyl-4-*p*-anisylpyrylium salts (Ic) as a consequence of the extension of the conjugated benzalacetophenone system through the methoxyl group.[5] Little or no effect on the position of the short wave length maximum, which is presumably due to the insulated acetophenone chromophore, is to be expected. These conclusions are verified by the absorption curve (Fig. 1): λ_{max} 245, 339 mμ. Moreover, the now anticipated bathochromic shift of the short wave length maximum due to extension of the acetophenone chromophore[6] in the pseudo-base (IVb) derived from 2-*p*-anisyl-4,6-diphenylpyrylium salts (Ib) is observed (Fig. 1): λ_{max} 285–290 mμ. The absorption due to the unchanged benzalacetophenone chromophore is apparently strongly overlapped and remains only as the sloping shoulder at 300–310 mμ.

(1) Presented in part at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September 10–13, 1951.

(2) (a) W. Dilthey, *J. prakt. Chem.*, **94**, 53 (1916); (b) *ibid.*, **95**, 107 (1917); (c) *ibid.*, **101**, 177 (1920); (d) W. Dilthey and T. Böttler, *Ber.*, **52B**, 2040 (1919).

(3) H. Mohler and J. Polya, *Helv. Chim. Acta*, **19**, 1222 (1936).

(4) N. H. Cromwell and W. R. Watson, *J. Org. Chem.*, **14**, 411 (1949).

(5) H. Stobbe and A. Hensel, [*Ber.*, **59**, 2255 (1926)], report λ_{max} 350 mμ for *p*-anisalacetophenone.

(6) Ramart-Lucas and Rabaté [*Compt. rend.*, **196**, 1493 (1933)] report λ_{max} 275 mμ for *p*-methoxyacetophenone.