was carried out in tightly stoppered bottles containing very little free air space, in an incubator set at 40°. After 4 days the precipitate which formed was filtered and washed successively with water, 2% sodium bicarbonate, 0.1 *N* HCl, and again with water. The anilide was dissolved in boiling dimethylformamide (DMF), treated with Norit, and the solution filtered. On cooling, the anilide precipitated as a white powder which decomposed slightly but did not melt below 315°, yield 10.0 g. (52%).

Anal. Calcd. for $C_{18}H_{18}O_4N_4$: C, 61.0; H, 5.12; N, 15.8. Found: C, 60.9; H, 5.14; N, 16.2.

Preparation of N,N'-Oxalylbis-(L-alanine anilide).—To 25 ml. of 0.5 *M* acetate buffer, *p*H 4.7, were added 0.3 g. of oxalylbis-(L-alanine), 0.5 g. of aniline, 0.08 g. of cysteine hydrochloride and 1.5 ml. of the papain extract. After incubation for 4 days at 40°, the precipitated anilide was removed by filtration, washed, and recrystallized from DMF–water, m.p. 305° dec., $[\alpha]^{25}D$ −11.5° (*c* 1.0, DMF).

It was somewhat more convenient to start with the ester instead of the free acid. Thus 0.90 g. (0.003 mole) of oxalylbis-(L-alanine ethyl ester) was shaken with 20 ml. of 0.7 *N* sodium hydroxide until solution was complete. The *p*H was then adjusted to 4.7 with glacial acetic acid and to the solution were added 0.96 g. (0.01 mole) of aniline, 0.15 g. of cysteine hydrochloride, and 4 ml. of the papain extract. After the addition of enough water to fill the vessel (a 40-ml. centrifuge tube) and stoppering tightly, the incubation was carried out at 35° for 4 days. In this case, the yield of anilide was 0.225 g. (19%), m.p. 305° dec.

The same product was obtained when the crude mixture of isomers of oxalylbis-(*dl*-alanine) was used as the substrate. A mixture of 1.5 g. (0.0065 mole) of the substrate, 2.4 g. (0.026 mole) of aniline, 0.15 g. of cysteine hydrochloride, and 3 ml. of the papain extract in 40 ml. of the acetate buffer after 2 days at 40°, yielded 0.225 g. (9%) of the anilide, m.p. 305° dec., $[\alpha]^{25}D$ −11.3° (*c* 1.0, DMF).

Anal. Calcd. for $C_{20}H_{22}O_4N_4$: C, 62.9; H, 5.80; N, 14.7. Found: C, 63.1; H, 5.69; N, 14.6.

Admixtures of this product and those obtained above from the L-isomer showed no depression in m.p.

When oxalylbis-(D-alanine) was subjected to the same reaction conditions, no precipitate was obtained.

Similarly, when the insoluble *meso*-oxalylbis-(alanine) was subjected to the same reaction conditions, no precipitate was obtained. However, after removal of this first fraction from the crude mixture of isomers, subsequent crops isolated by concentrating the mother liquor always produced some anilide under the above reaction conditions, demonstrating the presence of oxalylbis-(L-alanine), and therefore the racemate, in these more soluble fractions.

HOUSTON 25, TEXAS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY]

The Action of Fish Tissue on Thiamin. III.[1] The Further Elucidation of the Structure of Icthiamin[2-4]

BY EDWARD E. KUPSTAS AND DOUGLAS J. HENNESSY

RECEIVED FEBRUARY 27, 1957

Chromatographic studies show that hypotaurine (2-aminoethanesulfinic acid) is formed in the reaction of icthiamin with hydroxide and with bisulfite in the presence of hydroquinone. Exhaustive drying indicates that icthiamin dihydrobromide is a monohydrate. These facts together with infrared absorption characteristic of the sulfone group and earlier information suggest that icthiamin is 4-amino-5-(2-aminoethanesulfonyl)-methyl-2-methylpyrimidine.

The presence in icthiamin of a (4-amino-2-methyl-5-pyrimidyl)-methyl moiety was reported in an earlier publication from this Laboratory.[1b] Evidence for the identity of an aliphatic fragment formed by a nucleophilic cleavage of icthiamin is now presented together with a structure for icthiamin.

In a preliminary report, Hennessy and Warner[5] had assigned the formula $C_8H_{16}N_4O_3S \cdot 2HCl$ to icthiamin dihydrochloride. Barnhurst and Hennessy later assigned the formula $C_8H_{14}N_4O_3S \cdot 2HX$ to the dihydrohalides despite the better agreement of the hydrogen analyses with the earlier formula. Support for the latter formula included the almost exact correspondence of the analytical data obtained on the dipicrate with that calculated for $C_8H_{14}N_4O_3S \cdot 2C_6H_3N_3O_7$ and the lack of any reasonable structure which could be assigned to the formula of Hennessy and Warner.

The isolation of taurine and 4-amino-2-methyl-5-pyrimidine-methanesulfonic acid from the bisulfite cleavage of icthiamin[6] at first suggested I or II, each having the formula $C_8H_{14}N_4O_3S$, as possible structures for icthiamin.

The failure of taurine to effect the destruction of thiamin in the presence of dialyzed clam tissue while many other nucleophilic reagents were quite effective seemed to militate against I.

When the dissociation constants of the conjugate acids of the aliphatic amino group of I and II were calculated using ammonia as the reference base according to a method described by Branch and Calvin,[7] pK_b values of 7.2 and 5.6, respectively, are obtained as compared to the value of 6.6 actually observed for icthiamin.[6] Better agreement, *i.e.*, 7.0, is found when the pK_b is calculated for the aliphatic amino group of a compound having structure III, whose hydrate agrees with the formula of Hennessy and Warner, $C_8H_{16}N_4O_3S$.

However, the failure of the icthiamin salts to lose water of hydration under mild conditions of dehydration and the synthesis[6] of what was believed to be 4-amino-5-(2-aminoethanesulfonyl)-methyl-2-methylpyrimidine (III) which was not identical with icthiamin appeared to eliminate this as a possible structure for icthiamin.

Icthiamin dihydrohalides when exhaustively

(1) Papers I and II, J. D. Barnhurst and D. J. Hennessy, (a) THIS JOURNAL, **74**, 353 (1952); (b) **74**, 356 (1952).

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

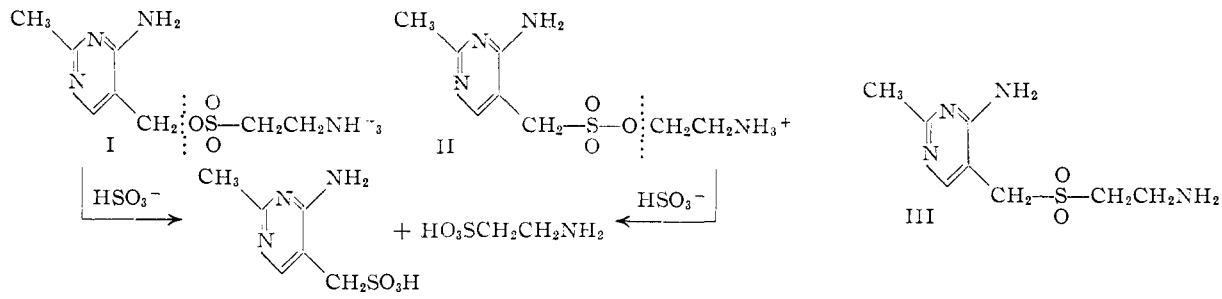
(3) Presented in part before the Division of Biological Chemistry, American Chemical Society, 126th Meeting, New York, September, 1954.

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

(5) D. J. Hennessy and S. Warner, Abstracts, 109th Meeting, American Chemical Society, Atlantic City, N. J., April 1946.

(6) J. D. Barnhurst, Thesis, Fordham University, 1951.

(7) G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, p. 203.



dried *in vacuo* over phosphorus pentoxide are found to lose weight equivalent to one mole of water. The rapid recovery of this water of hydration when the anhydrous icthiamin salt is exposed to the atmosphere may account for the failure to detect its presence in the earlier work. The obtention of hypotaurine, 2-aminoethanesulfinic acid, when icthiamin is treated with barium hydroxide, or sodium bisulfite in the presence of hydroquinone, and the infrared spectrum of icthiamin which indicates the presence of a sulfone function by the absorption at 7.71 and 8.62 μ[8] lead us to propose the formula $C_8H_{14}N_4O_2S\cdot 2HX\cdot H_2O$ for the icthiamin salts and III as the structure of the free base.

The oxidation of hypotaurine to taurine in the presence of air and bisulfite explains the obtention of taurine as an artifact rather than as the primary product of the reaction of icthiamin and bisulfite. The prevention of this apparent autoxidation by the inclusion of hydroquinone during the reaction, the successful oxidation of hypotaurine to taurine by bisulfite and air, and the formation of hypotaurine and 4-amino-2-methyl-5-hydroxymethylpyrimidine by the alkaline scission of icthiamin all indicate that hypotaurine and not taurine is the primary aliphatic product of the cleavage of icthiamin by bisulfite.

Chromatographic analysis of the alkaline cleavage products of icthiamin using paper buffered at *p*H 12 and a buffered phenol solvent, following McFarren's[9] procedure, gave rise to a violet spot, R_f 0.49, when developed with 0.1% ninhydrin in acetone. Similar chromatography of the sulfite cleavage products of icthiamin gave a taurine spot, R_f 0.29. Assuming that the product from the alkaline cleavage was closely related to taurine, its greater R_f value in this solvent would be compatible with the weaker acidity found in the sulfinic or sulfenic analogs of taurine. Subsequent chromatographic analyses of the alkaline cleavage products of icthiamin which had been submitted to oxidation by bromine water revealed the characteristic taurine spot.

The formation of taurine as a secondary reaction in the bisulfite cleavage was then confirmed chromatographically. Acting on the hypothesis that autoxidation involving the bisulfite ion[10] and hypotaurine accounted for the formation of taurine, the sulfite cleavage was run in the presence of an antioxidant, 0.1% hydroquinone solution. The spot at R_f 0.49 was clearly observed with only a faint spot at R_f 0.29. Bromine water oxidation of the hydroquinone-protected material once again yielded the material with an R_f value of 0.49. Ethyl alcohol was found to be less effective as an antioxidant. When the bisulfite cleavage was conducted in the presence of ethyl alcohol, chromatographic analysis of the reaction mixture over a period of three days showed a gradual disappearance of the spot at R_f 0.49 and a corresponding increase in the intensity of the spot at R_f 0.29. The cleavage run in the presence of hydroquinone showed only a faint spot at R_f 0.29 at the end of this time.

Chromatographically pure hypotaurine as isolated from the barium hydroxide cleavage of icthiamin and synthetic hypotaurine had an R_f value of 0.49; both were oxidized to taurine by either bromine water or by bisulfite in the absence but not in the presence of hydroquinone. Hypotaurine, 2-aminoethanesulfinic acid, had been detected chromatographically by Chatagner and Bergeret[11] as a result of enzymatic decarboxylation of L-cysteinesulfinic acid. The products from the cleavage of icthiamin which have been designated as taurine and hypotaurine give R_f values in the phenol–water solvent system used by Bergeret and Chatagner which are in close agreement with those reported by these authors for these same compounds.[12] Table I summaries the chromatographic data on the aliphatic cleavage fragment.

TABLE I

R_f VALUES OF THE ALIPHATIC CLEAVAGE FRAGMENTS[a,b]

Reaction products chromatogrammed	Phenol buffered at *p*H 12[c]	Phenol:water (4:1)
Icthiamin + $NaHSO_3$	0.29	
Icthiamin + $NaHSO_3$ (in 0.1% hydroquinone soln.)	.49	
Icthiamin + $Ba(OH)_2$	.49	
Taurine (control)	.29	0.39[d]
Hypotaurine (isolated from icthiamin)	.49	.67
Hypotaurine + $NaHSO_3$	.29	
Hypotaurine + $NaHSO_3$ (in 0.1% hydroquinone soln.)	.49	
Hypotaurine + bromine water	.29	.38
Synthetic hypotaurine (control)	.49	.67
Synthetic hypotaurine + bromine water	.29	.39
Hypotaurine (from the enzymic decarboxylation of L-cysteinesulfinic acid)[12]		.68[d]

[a] The R_f values given are the average values. Actual values usually varied ±0.02 from the average values. [b] All spots were developed by ninhydrin treatment. [c] The paper for these chromatograms was buffered at *p*H 12 and dried before using. [d] Values reported by Bergeret and Chatagner.

(8) K. C. Schreiber, *Anal. Chem.*, **21**, 1168 (1949).

(9) E. F. McFarren, *ibid.*, **23**, 168 (1951).

(10) W. A. Waters, "The Chemistry of Free Radicals," 2nd edition, Oxford, Clarendon Press, 1948, p. 234.

(11) F. Chatagner and B. Bergeret, *Comp. rend.*, **232**, 448 (1951).

(12) B. Bergeret and F. Chatagner, *Biochim. Biophys. Acta*, **14**, 543 (1954).

The pyrimidine fragment obtained from the alkaline cleavage was identified on separate chromatograms run in four different alcoholic solvents. The pyrimidine moiety, which developed no color with ninhydrin, was located by scanning the chromatograms before an ultraviolet light.[13] In each of the four solvent systems used, the pyrimidine fragment gave a spot with an R_f value close to or identical with that for 4-amino-2-methyl-5-hydroxymethylpyrimidine. The complete results are given in Table II.

TABLE II

IDENTIFICATION OF THE PYRIMIDINE MOIETY

Solvent system	Av. R_f values[a] Cleavage fragment	4-Amino-2-methyl-5-hydroxymethylpyrimidine
1. Ethyl alc.–water (4:1)	0.58	0.59
2. Ethyl alc.–water–formic acid (80:20:1)	.86	.85
3. Ethyl alc.–water–ammonium hydroxide (75:24:1)	.88	.89
4. Isopropyl alc.–water–acetic acid (7:2:1)	.83	.83

[a] Actual values were generally within ±0.02 of the average values given.

Alkaline cleavage of aliphatic sulfones to sulfinic acids is known to take place at higher temperatures.[14,15] More readily, aromatic *o*-hydroxysulfones undergo a reversible rearrangement when in alkaline medium.[15–18] The reactive 5-methylene carbon atom would leave icthiamin even more susceptible to alkaline cleavage. This reaction shown to yield a sulfinic acid, hypotaurine, is entirely compatible with structure III.

The elementary analyses for icthiamin when recalculated for the monohydrates of 4-amino-5-(2-aminoethanesulfonyl)-methyl-2-methylpyrimidine salts agree well with the reported values.[1a]

	C	H	N	S	Halogen
	Icthiamin dihydrobromide				
Calcd. for $C_8H_{14}N_4O_2S\cdot 2HBr\cdot H_2O$	23.42	4.42	13.66	7.81	38.97
Found	23.55	4.21	13.74	7.93	39.41
	Icthiamin dihydrochloride				
$C_8H_{14}N_4O_2S\cdot 2HCl\cdot H_2O$	29.91	5.64	17.44	9.98	22.08
Found	30.00	5.55	17.58	10.17	21.84
	Icthiamin dipicrate				
$C_8N_{14}N_4O_2S\cdot 2C_6H_3N_3O_7\cdot H_2O$	33.99	3.13	19.82	4.54	
Found	34.10	2.83	19.91	4.53	

We wish to express our appreciation to Sister Marguerite Miriam Casco and Miss Lilia Beauchamp for performing the infrared analyses and to Merck and Co., Inc., Rahway, N. J., for their gift of 4-amino-5-ethoxymethyl-2-methylpyrimidine.

(13) "Mineralight" sold by the Will Corporation, New York, with a transmittance peak at 254 mμ, kindly loaned to us by Dr. L. R. Cerecedo.

(14) G. W. Fenton and C. K. Ingold, *J. Chem. Soc.*, 3127 (1928).

(15) G. W. Fenton and C. K. Ingold, *ibid.*, 705 (1930).

(16) A. R. Levy, H. C. Rains and S. Smiles, *J. Chem. Soc.*, 3264 (1931).

(17) B. A. Kent and S. Smiles, *ibid.*, 422 (1934).

(18) R. R. Coats and D. T. Gibson, *ibid.*, 422 (1940).

Experimental

Water of Hydration.—Samples of icthiamin dihydrobromide weighing approximately 100 mg. were dried 24 hours at room temperature over phosphorus pentoxide at 1 mm.

Av. wt. loss (3 samples)	4.44%
Wt. loss calcd. for $C_8H_{14}N_4O_2S\cdot 2HBr\cdot H_2O$	4.39%

The entire weight loss was regained within 8 hours after the samples were removed from the drying pistol.

Barium Hydroxide Cleavage of Icthiamin.—Icthiamin dihydrobromide (4 mg.), barium hydroxide (8 mg.) and 0.5 ml. of water were sealed in a 3 in. ignition tube and heated for 8 hours at 100°. After cooling, the tube was opened and the solution adjusted to *p*H 5 with 0.5 *N* sulfuric acid. After centrifuging, the centrifugate was filtered through a thin layer of filter-aid before spotting on a chromatogram.

Sulfite Cleavage of Icthiamin.—(a) Icthiamin dihydrobromide (4 mg.) and sodium bisulfite (4 mg.) were dissolved in 0.5 ml. of water and let stand 24 hours at room temperature, before spotting on a chromatogram.

(b) When it was desired to inhibit the bisulfite autoxidation, a 0.1% solution of hydroquinone or 10% aqueous ethyl alcohol was substituted as the solvent and the reaction kept under nitrogen.

Isolation of Hypotaurine.—Icthiamin dihydrobromide (100 mg.) and barium hydroxide (200 mg.) were heated with 10 ml. of water in a sealed 6 in. Pyrex ignition tube for 8 hours at 100°. After cooling, the tube was opened and the contents adjusted to *p*H 5 with 0.5 *N* sulfuric acid. The barium sulfate was removed by centrifuging and filtering the centrifugate through a thin layer of filter-aid. After reducing the filtrate to dryness *in vacuo*, the residue was triturated with 5 ml. of absolute methanol, then filtered. Absolute ether was added to the filtrate until a slight precipitate formed. After standing several hours, the solid material was removed by filtering and ether added to the filtrate until a faint cloudiness appeared. Small portions of ether were added over a period of several hours until an amorphous precipitate settled out. The solid material was collected on a filter and dried *in vacuo* to yield 15 mg. of chromatographically pure hypotaurine. No detectable oxidation to taurine had occurred after one year when stored in a desiccator over anhydrous calcium chloride.

Synthesis of Hypotaurine. (a) 2-Phthalimidoethanesulfonhydrazide.—2-Phthalimidoethanesulfonyl chloride[19] (546 mg.) was dissolved in 15 ml. of hot chloroform. This was cooled to 40° and 15 ml. of absolute ethanol containing 0.24 ml. of 85% hydrazine hydrate was added. This was stirred for a few minutes and then placed in an ice-bath for an hour. The solid material was collected on a filter; wt. 290 mg. The filtrate was reduced to 8 ml. on the steam-bath. On cooling, an additional 210 mg. of material settled out. The combined precipitates were recrystallized several times from hot methanol to yield 255 mg. of silvery-white flakes of 2-phthalimidoethanesulfonhydrazide, m.p. 158.5–160.5° (with frothing).

Anal. Calcd. for $C_{10}H_{11}N_3O_4S$: C, 44.60; H, 4.11; N, 15.65; S, 11.90. Found: C, 44.45; H, 4.01; N, 15.10; S, 11.65.

(b) Hypotaurine.—2-Phthalimidoethanesulfonhydrazide (269 mg.) was heated with 30 mg. of 85% hydrazine hydrate in 2 ml. of water at 80°. A simultaneous reduction of the sulfonhydrazide to a sulfinic acid[20] and removal of the phthaloyl group occurs.[21] When the evolution of nitrogen had ceased and no more precipitate seemed to form (usually 30 minutes), the mixture was cooled in an ice-bath for an hour and the insoluble phthalhydrazide removed by filtering. After taking the filtrate to dryness *in vacuo*, the residue was warmed with 1 ml. of water and filtered. To the filtrate 2 ml. of ethanol was added, then ether until an amorphous precipitate began to form. This was let stand overnight in the refrigerator. The precipitate was collected and dissolved in a minimum of warm water before treating with Norite and filtering. Warm ethyl alcohol (1 ml.) was

(19) R. Winterbottom, J. W. Clapp, W. H. Miller, J. P. English and R. O. Roblin, Jr., THIS JOURNAL, **69**, 1397 (1947).

(20) A. T. Dann and W. Davies, *J. Chem. Soc.*, 1050 (1929).

(21) (a) R. Radenhausen, *J. prakt. Chem.*, [2] **52**, 433 (1895); (b) H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348 (1926); (c) D. A. Kidd and F. A. King, *Nature*, **162**, 776 (1948); (d) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

added to the filtrate, followed by ether until faint cloudiness set in. This was let stand overnight at room temperature before placing in the refrigerator for 12 hours. On filtering, 30 mg. of hygroscopic, needle-like crystals were collected, m.p. 173–175°. Melting points between 170 and 177° have been reported by different authors.[22-24] R_f values of the product were identical with hypotaurine isolated from ichthiamin and from the enzymic decarboxylation of cysteinesulfinic acid (see Table I). Oxidation with potassium permanganate gave an equivalent weight of 52.5; calculated for $C_2H_7NO_2S$, 54.5.

Chromatographic Analysis of Alkaline Cleavage Products. 1. **Identification of the Aliphatic Moiety.**—(a) The solution from the barium hydroxide cleavage of icthiamin was spotted on strips of Whatman No. 1 filter paper which had been sprayed previously with a *p*H 12 phosphate buffer[9] and dried. The chromatograms were developed in a phenol solvent buffered at *p*H 12[9] for 15 hours. After being dried thoroughly, the chromatograms were sprayed with or dipped in a 0.1% ninhydrin solution in acetone and dried in an oven for 5 minutes at 90°. A violet spot appeared at R_f 0.49. Synthetic hypotaurine had R_f 0.49. (b) A portion of the alkaline cleavage solution was treated with several drops of bromine water before spotting and developing as above. A violet spot appeared at R_f 0.29. Synthetic hypotaurine treated as above gave a spot at R_f 0.29. Taurine control had R_f 0.29. (c) Several drops of the alkaline cleavage solution were let stand overnight with 1 mg. of sodium bisulfite before spotting and developing. A violet spot appeared at R_f 0.29. Synthetic hypotaurine treated in this way also gave a spot at R_f 0.29. (d) The solution from the alkaline cleavage reaction was let stand overnight with 1 mg. of sodium bisulfite with 0.1% hydroquinone added. On spotting and developing, a single spot appeared at R_f 0.49.

2. **Identification of the Pyrimidine Moiety.**—The solution from the alkaline cleavage was spotted on strips of unbuffered Whatman No. 1 filter paper and developed in four different alcoholic solvent systems (Table II) until the solvent front had travelled 20–24 cm. The dried chromatograms were scanned before an ultraviolet lamp[12] to locate the pyrimidine compounds which appeared as purplish spots. The results are given in Table II.

Chromatographic Analysis of the Aliphatic Fragment from the Bisulfite Cleavage of Icthiamin.—(a) The solution from the bisulfite cleavage reaction was spotted on strips of Whatman No 1 filter paper previously buffered at *p*H 12, and developed in a buffered phenol solvent as before. Treatment with 0.1% ninhydrin in acetone revealed a single spot with R_f 0.29. Taurine control had R_f 0.29.

(b) The solution from the bisulfite cleavage reaction protected by hydroquinone or ethanol was spotted on buffered Whatman No. 1 filter paper and developed in a buffered phenol solvent as before. Ninhydrin treatment showed a heavy spot with R_f 0.49 and a faint spot at R_f 0.29. The hypotaurine control had R_f 0.49.

NEW YORK 58, N. Y.

(22) L. Eldjarn and A. Sverdrup, *Acta Chem. Scand.*, **9**, 1037 (1955).

(23) E. Bricas, F. Kieffer and C. Fromageot, *Biochim. Biophys. Acta*, **18**, 358 (1955).

(24) D. Cavallini, B. Mondovi and C. DeMarco, *J. Biol. Chem.*, **216**, 577 (1955).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY]

The Action of Fish Tissue on Thiamin. IV.[1] The Synthesis of Icthiamin[2-4]

BY EDWARD E. KUPSTAS AND DOUGLAS J. HENNESSY

RECEIVED FEBRUARY 27, 1957

Icthiamin was synthesized by condensing 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide with 2-phthalimidoethanesulfinic acid, followed by hydrazinolysis of the phthaloyl group. The identity of synthetic phthalylicthiamin and icthiamin dihydrobromide with the corresponding compounds prepared from natural icthiamin was shown by chromatographic, infrared and elementary analyses.

When the structure of icthiamin was proposed as 4-amino-5-(2-aminoethanesulfonyl)-methyl-2-methylpyrimidine,[1c] two routes to its synthesis seemed practical. These were *via* the formation of the corresponding sulfide which could be oxidized to the sulfone, or by the direct formation of the sulfone. The first method had been attempted during the early study of icthiamin by Barnhurst[5] but oxidation of the intermediate 5-(4-amino-2-methylpyrimidyl)-methyl β-phthalimidoethyl sulfide according to the method of Pomerantz and Conner[6] failed to produce phthalylicthiamin.

The formation of sulfones by the reaction of sulfinates with aliphatic halides[7] formed the basis for the successful synthesis.

Zinc 2-phthalimidoethanesulfinate was obtained by reduction of 2-phthalimidoethanesulfonyl chloride with zinc dust in absolute methanol. However, the purification of the zinc or sodium salts of II proved difficult. Reaction of the impure sulfinates with I failed to yield more than traces of phthalylicthiamin (III). The free sulfinic acid II reacts with 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (I) in glacial acetic acid in the presence of anhydrous sodium acetate to give phthalylicthiamin hydrobromide in good yield. Hydroquinone was used in the preparations and reactions of the sulfinates to minimize conversion to sulfonates.

Gabriel and Colman prepared the 2-phthalimidoethanesulfinic acid (II) by two different methods.[8] A simpler procedure which gives a pure product in good yield is described in the Experimental section.

During the synthesis, III was found difficult to prepare analytically pure even with repeated recrystallizations. The extremely insoluble free base

(1) Papers I, II and III (a) J. D. Barnhurst and D. J. Hennessy, THIS JOURNAL, **74**, 353 (1952); (b) **74**, 356 (1952); (c) E. E. Kupstas and D. J. Hennessy, **79**, 5217 (1957).

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

(3) This paper is based on a portion of a thesis submitted by E. E. Kupstas to the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(4) Presented at the Meeting-in-Miniature, American Chemical Society, New York Section, March 16, 1956, and the Division of Biological Chemistry, American Chemical Society, 130th Meeting, Atlantic City, New Jersey, September, 1956.

(5) J. D. Barnhurst, Thesis, Fordham University, 1951.

(6) A. Pomerantz and R. Conner, THIS JOURNAL, **61**, 3386 (1939).

(7) R. Otto, *Ber.*, **13**, 1272 (1880).

(8) S. Gabriel and J. Colman, *ibid.*, **44**, 3628 (1911).