completely of the branched polymer, amylopectin; whereas tapioca contains 17–19% of the linear polymer, amylose.

The relative lack of dimensional hysteresis as a humidity (Table I), particularly in the case of corn and potato starch, is unusual, especially in view of the known hysteresis of the water sorption as a function of relative vapor pressure. In tapioca starch where a sorption–desorption loop is present, the desorption results in smaller swelling than does absorption.

In the curves of swelling as a function of water content (Fig. 1), an absorption–desorption loop is prominent in the data for all starches, the desorption values lying anomalously below the absorption curve. Similar anomalous "hysteresis" has been reported for the length swelling of cotton[3] and for the length swelling of human hair[4] as a function of humidity, yet the mechanism for such "shrinking" behavior seems obscure.

Assuming the specific volume of starch to be 0.67, as indicated by pycnometric densities in water, and that volumes are additive in the sorption process, the linear expansion for isotropic swelling accompanying a 1% addition of water would be 0.5%. The initial swelling of dry potato and tapioca starch occurs at approximately this rate. The swelling at high moisture contents may also occur at this rate, but the effect of intergranular porosities on increasing the water sorption of bulk starch at high humidities prevents our obtaining the relation between swelling and granule water content in the highest moisture range. Since calculations indicate that significant amounts of water can be held in intergranular porosities only above 99% R.H., this ambiguity exists for only the last 3–5% of the water sorbed.

The greater portion of the swelling curves of the starches reported exhibits smaller or larger rates of swelling than above calculated. A smaller rate of swelling could result from sorption occurring in voids. To account for the small swelling of corn and waxy corn starch at low humidities, however, the voids must extend to molecular dimensions. A rate of swelling larger than above calculated could result from hydration occurring at junction points of a three-dimensional molecular network causing an opening up of the structure beyond the volume of the water introduced. Such an assumed three-dimensional network structure would also explain the oberved anomalous "hysteresis" in the function of swelling *versus* water content in a manner similar to the "ink bottle"[5] explanation of hysteresis in the function of vapor pressure *vs.* water content. Extent of hydration of the junctions of the starch polymer chains would determine the extent of swelling. Then, for the same granule size on absorption, only the junctions would be hydrated, whereas on desorption the junction and its associated void would be filled with water.

Acknowledgment.—The authors are grateful to Dr. Majel M. MacMasters for providing the starch samples for this work.

(3) G. E. Collins, *Textile Inst. J.*, **21**, T311 (1930).

(4) H. T. White and P. B. Stam, *Textile Res. J.*, **19**, 136 (1949).

(5) S. Brunauer, "The Adsorption of Gases and Vapors," Princeton University Press, Princeton, N. J., 1945, p. 398.

PEORIA, ILL. RECEIVED AUGUST 30, 1950

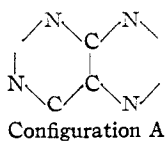
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

Studies in Imidazoles. II.[1] Imidazo[b]pyrazines[2,3]

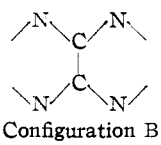
BY EDGAR SCHIPPER[4] AND ALLAN R. DAY

Imidazo[b]pyrazines, members of a previously unknown ring system, were prepared and several derivatives were tested as potential antimetabolites. The synthesis of the imidazo[b]pyrazines was accomplished by reactions of the corresponding diaminopyrazine with ethyl orthoformate, acyl halides and urea, respectively.

In the first paper of this series[1] the synthesis and chemical reactions of imidazo[b]quinoxalines were described. It was pointed out that these compounds possessed the oxamidine moiety (Configuration B) which bears close structural analogy to Configuration A, a grouping present in the ring systems of a variety of essential metabolites.

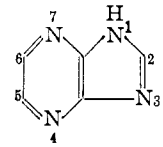


Configuration A



Configuration B

Another type of molecule containing Configuration B is the hitherto unknown ring system of imidazo[b]pyrazine



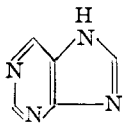
This molecule, as can be seen, is a "cross" between purines and pteridines, retaining the imidazole moiety of the former and the pyrazine ring of the latter. Furthermore, imidazo[b]pyrazines possess an apparent structural relationship to benzimidazoles, one member of which, 5,6-dimethylbenzimidazole, recently has been shown to constitute an

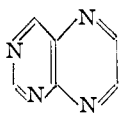
(1) For the first paper in this series see E. Schipper and A. R. Day, THIS JOURNAL, **73**, 5672 (1951).

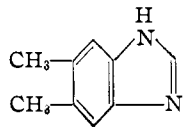
(2) From a thesis submitted February, 1951, by E. Schipper to the Department of Chemistry and Chemical Engineering of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) Presented in part at the 118th Meeting of the American Chemical Society in Chicago, Ill., September 8, 1950.

(4) National Institutes of Health Predoctoral Research Fellow 1949–1950.


purine


pteridine


5,6-dimethylbenzimidazole

essential part of vitamin B_{12}.[5] Because of this, our efforts were concentrated on the preparation of 5,6-dimethylimidazo[b]pyrazine (I) and several derivatives.

2,3-Diamino-5,6-dimethylpyrazine (II), the ultimate intermediate in the reaction scheme leading to the synthesis of the desired imidazo[b]pyrazines was prepared by a combination of the methods of Jones[6] and McDonald and Ellingson.[7] This pathway is somewhat more efficient than the previous procedure for preparing (II).[8] Because of the considerable number of steps involved in the synthesis of the pyrazine diamine it became necessary to prepare aminomalonic ester (the precursor of aminomalonamide (IV)) on a relatively large scale. Most of the published procedures for the preparation of aminomalonate by the reduction of isonitrosomalonic ester[9] were not applicable to larger runs. It was found that isonitrosomalonate, freed of N-oxides by washing with a solution of urea, could be reduced readily and in good yield by a low pressure catalytic hydrogenation.

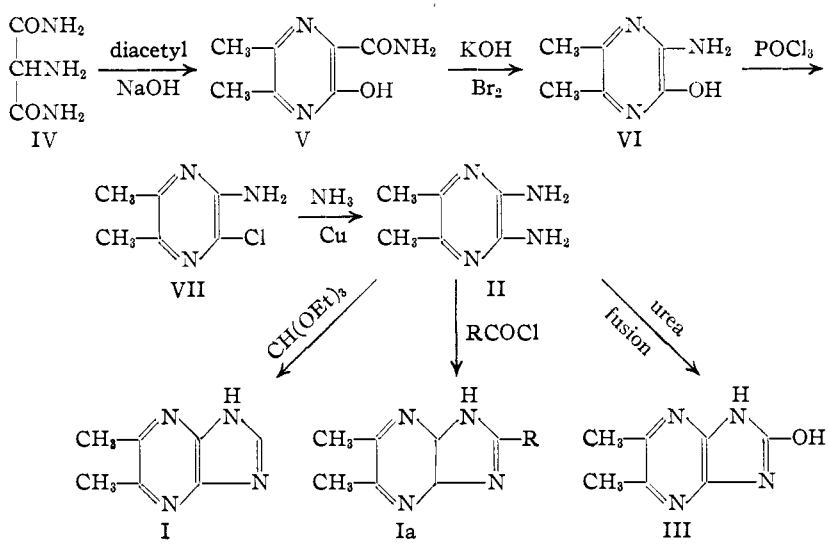


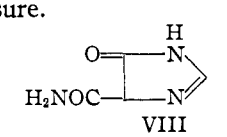
Fig. 1.

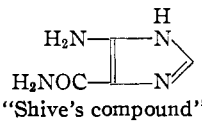
5,6-Dimethylimidazo[b]pyrazine (I), and a few derivatives with substituents in the 2-position as well as the corresponding imidazolone (III) were prepared from 2,3-diamino-5,6-dimethylpyrazine (II) by methods described for the synthesis of imidazo[b]quinoxalines.[1] The conditions of the Phillips procedure,[10] as in the analogous case of 2,3-diaminoquinoxaline,[1] caused hydrolysis of the pyrazine diamine (II) to 2-hydroxy-3-amino-5,6-dimethylpyrazine (VI) and thus could not be applied to the synthesis of imidazo[b]pyrazines.

The reaction of the pyrazinediamine (II) with acyl halides led to imidazole formation only when a high boiling solvent such as xylene was used. In the presence of pyridine, monoacylation of the diamine occurred but no ring closure could be observed.

Imidazo[b]pyrazines are colorless compounds possessing a very high degree of water solubility (with the exception of the 2-phenyl derivative). They can best be recrystallized from absolute alcohol and, like the imidazo[b]quinoxalines,[1] are easily sublimed. Table I lists the compounds prepared during this study. The ultraviolet spectra of (I) and its 2-ethyl derivative are shown in Fig. 2.

When aminomalonamide (IV) was treated with an excess of ethyl orthoformate, 5-imidazolone-4-carboxamide (VIII), the oxygen analog of "Shive's compounds,"[11] was obtained. This reaction emphasizes both the orthodiamine character of α-amino acid amides and the advantage of orthoformate as a ring forming reagent. The attempt of Johnson and Nicolet[12] to prepare (VIII) by the action of formic acid on aminomalonamide only led to the formation of the formyl derivative and under no circumstances could these workers effect ring closure.


VIII


"Shive's compound"

Pharmacology.—5,6-Dimethylimidazo[b]pyrazine (I), 2-ethyl-5,6-dimethylimidazo[b]pyrazine and 2-hydroxy-5,6-dimethylimidazo[b]pyrazine (III) were tested by the assay method of Skeggs, *et al.*, for vitamin B_{12}.[13] None of the above compounds exhibited growth inhibitory or stimulatory action at concentration levels of 0.05–5 gammas.

Acknowledgment.—The authors are indebted to Dr. Paul Gyorgy of the Medical School of the University of Pennsylvania for the microbiological assays and to Mrs. Sara Woods for the analytical data reported in this paper.

Experimental

Ethylaminomalonate.—Isonitrosomalonate was prepared by a modification of the method described by Snyder and Smith.[14] The modification consisted in extending the

(5) N. G. Brink and K. Folkers, THIS JOURNAL, **71**, 2951 (1949); N. G. Brink, F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill and K. Folkers, *ibid.*, **72**, 1866 (1950).

(6) R. G. Jones, *ibid.*, **71**, 78 (1949).

(7) F. G. McDonald and R. C. Ellingson, *ibid.*, **69**, 1034 (1947).

(8) R. C. Ellingson and R. L. Henry, *ibid.*, **70**, 1257 (1948)

(9) O. Piloty and J. Neresheimer, *Ber.*, **39**, 514 (1906); N. J. Putochin, *ibid.*, **56**, 2213 (1923); P. A. Levene and A. Schormuller, *J. Biol. Chem.*, **106**, 595 (1934).

(10) M. A. Phillips, *J. Chem. Soc.*, 2393 (1928).

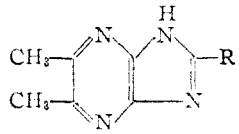
(11) W. Shive, W. W. Ackermann, M. Gordon, M. E. Getzendaner and R. E. Eakin, THIS JOURNAL, **69**, 725 (1947).

(12) T. B. Johnson and B. H. Nicolet, *ibid.*, **36**, 355 (1914).

(13) H. R. Skeggs, H. M. Nepple, K. A. Valentik, J. W. Huff and L. D. Wright, *J. Biol. Chem.*, **184**, 211 (1950).

(14) H. R. Snyder and C. W. Smith, THIS JOURNAL, **66**, 350 (1944).

TABLE I



R	Yield, %	M.p., °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
H	52	209	$C_7H_8N_4$	56.74	56.75	5.44	5.28	37.82	38.00
CH_3	65	259	$C_8H_{10}N_4$	59.24	58.93	6.22	6.46	34.54	34.69
C_2H_5	71	256	$C_9H_{12}N_4$	61.34	61.32	6.87	7.06	31.79	31.75
n-C_3H_7	42	227	$C_{10}H_{14}N_4$	63.13	63.22	7.42	7.34	29.45	29.67
n-C_4H_9	54	186	$C_{11}H_{16}N_4$	64.65	64.54	7.90	7.76	27.45	27.47
C_6H_5	53	310	$C_{12}H_{12}N_4$	69.62	69.41	5.40	5.41	24.98	24.76
OH	59	418	$C_7H_8ON_4$	51.21	51.30	4.91	5.11	34.14	34.04

period of addition of sodium nitrite to two hours and continuing the stirring after completed addition for six to eight hours while a gentle stream of air was passed over the reaction mixture. The final purification prior to hydrogenation consisted of several washings with a 20% aqueous urea solution; yield 80–85%.

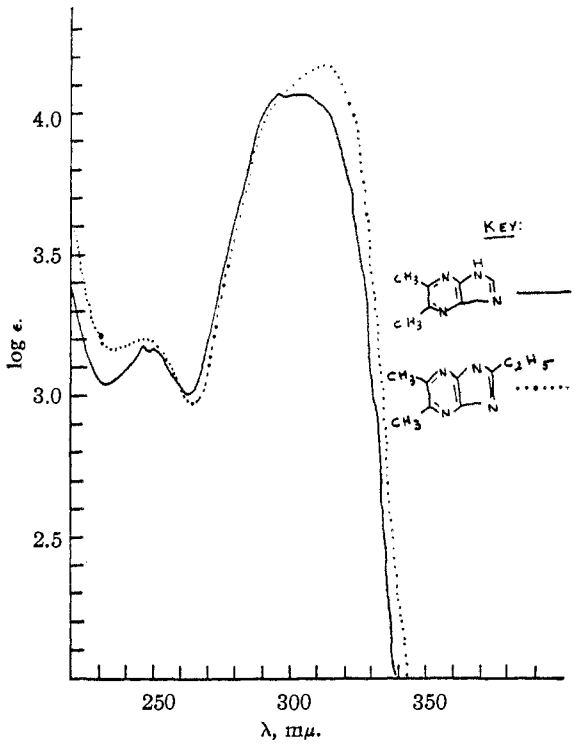


Fig. 2.

One hundred grams of the isonitroso ester dissolved in 50 ml. of absolute alcohol was hydrogenated rapidly at room temperature and at 40–45 lb. of pressure using 4 g. of palladium-on-charcoal[15] or alumina[16] catalyst. The catalyst was filtered off and the alcohol removed under reduced pressure. Ethyl aminomalonate was distilled at pressures not exceeding 1 mm.; yield 79 g. (85%, based on the isonitroso ester), b. p. 75–77° at 0.4 mm.

Aminomalonamide (IV).—This compound was prepared by the procedure of Cerchez,[17] yield 86%, m. p. 197°.

3-Hydroxy-5,6-dimethylpyrazine-2-carboxamide (V).—The procedure of Jones[9] was followed. It may be pointed out that in the absence of base catalysis, contrary to Jones' findings, none of the desired compound was formed. In the presence of NaOH, pyrazine formation took place, but in considerably lower yield (75%) than reported in Jones' paper. Various modifications of Jones' procedure failed to improve the yield of (V), m.p. 232–234°.

2-Hydroxy-3-amino-5,6-dimethylpyrazine (VI).—This compound was prepared by the procedure of McDonald and Ellingson[7] for the synthesis of 2-hydroxy-3-aminopyrazine; yield 86%, m.p. 300–303°. For analytical purposes a sample was sublimed *in vacuo* (1 mm.).

Anal. Calcd. for $C_6H_9ON_3$: C, 51.78; H, 6.51; N, 30.20. Found: C, 51.55; H, 6.42; N, 30.34.

2-Chloro-3-amino-5,6-dimethylpyrazine (VII).—The procedure of McDonald and Ellingson[7] for the preparation of 2-chloro-3-aminopyrazine was followed. It was found advantageous to employ twice as much phosphorus oxychloride as was used by the above authors; yield 70%, m.p. 98°. An analytical sample was prepared by vacuum sublimation (1 mm.).

Anal. Calcd. for $C_6H_8N_3Cl$: C, 45.72; H, 5.11; N, 26.67. Found: C, 45.81; H, 5.15; N, 26.88.

2,3-Diamino-5,6-dimethylpyrazine (II).—The directions of Ellingson and Henry[8] were employed using as starting material 2-chloro-3-aminopyrazine (VII) instead of the corresponding 2-bromo compound; yield 63%, m. p. 215–216°.

5,6-Dimethylimidazo[b]pyrazine (I).—Two grams of 2,3-diamino-5,6-dimethylpyrazine (II) was heated for three hours at 140–145° in an open vessel with 15 ml. of ethyl orthoformate. The volume of the reaction mixture was kept constant by occasional additions of ethyl orthoformate. At the end of the reaction period the bath temperature was raised gradually to 200° until all of the excess ethyl orthoformate had evaporated. The dark residue was taken up in hot 1 *N* sodium hydroxide, decolorized with Darco and filtered. Upon acidification of the filtrate with glacial acetic acid fine white needles appeared. They were recrystallized from a small quantity of absolute alcohol. An analytical sample was sublimed *in vacuo* (1 mm.) at 150–160°.

The Acyl Halide Procedure for 2-Substituted Imidazo[b]-pyrazines (1).—The following procedure was applied generally to the preparation of 2-substituted imidazo[b]pyrazines: 2,3-Diamino-5,6-dimethylpyrazine (II) and a 20% excess (based upon the diamine) of the acyl halide suspended in a tenfold excess of xylene were refluxed for six hours. The xylene was removed under reduced pressure and the residue was worked up according to the procedure described above for the preparation of 5,6-dimethylimidazo[b]pyrazine. The final purification of all samples consisted in vacuum sublimation.

(2) **2-Acetylamino-3-amino-5,6-dimethylpyrazine.**—This compound was prepared by applying the preceding procedure to acetyl chloride in the presence of pyridine as solvent; yield 25%, m. p. 191–193°.

Anal. Calcd. for $C_8H_{12}ON_4$: C, 53.32; H, 6.72; N, 31.45. Found: C, 53.52; H, 6.98; N, 31.50.

2-Hydroxy-5,6-dimethylimidazo[b]pyrazine (III).—Two grams of 2,3-diamino-5,6-dimethylpyrazine (II) was ground together with 3 g. of urea. The mixture was fused at 160° for four hours and then at 200° for an additional four hours. The residue was dissolved in hot NaOH solution and treated with Darco. After filtration the filtrate was neutralized with glacial acetic acid. The amorphous white

(15) *Org. Syntheses*, **26**, 31 (1946).
(16) Purchased from Baker and Co., Inc.
(17) M. V. Cerchez, *Bull. soc. chim., France*, **47**, 1287 (1930).

precipitate was purified by redissolving in concentrated ammonia and subsequent reprecipitation with glacial acetic acid. An analytical sample was prepared by vacuum sublimation.

5-Imidazolone-4-carboxamide (VIII).—Two grams of aminomalonamide (IV) and 25 ml. of ethyl orthoformate were heated for two hours at 145°. The excess ortho ester was removed under reduced pressure and the bluish-green residue was recrystallized several times from aqueous alcohol, using Darco as decolorizing agent. The grayish crystals possessed no sharp melting point but decomposed between 270 and 275°; yield 1.7 g. (78%).

Anal. Calcd. for $C_4H_6O_2N_2$: C, 37.79; H, 3.97; N, 33.07. Found: C, 37.91; H, 3.79; N, 33.09.

PHILADELPHIA, PENNA. RECEIVED JULY 16, 1951

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF FORDHAM UNIVERSITY]

The Action of Fish Tissue on Thiamin. I. The Isolation of Icthiamin[1,2,3]

BY JAMES D. BARNHURST[4] AND DOUGLAS J. HENNESSY

Thiamin is inactivated by a constituent of clam tissue. The principal pyrimidine derivative formed thereby is icthiamin of empirical formula $C_8H_{14}N_4O_3S$. A method of isolation of icthiamin as its dihydrobromide is presented, utilizing ion exchange, precipitation with silico-tungstic acid and precipitation with Ag^+. An assay method is presented for the estimation of icthiamin during the isolation.

It has been known for a number of years that the raw tissues of several species of aquatic fauna are able to inactivate thiamin. For example, Green, Carlson and Evans[5,6] have reported the occurrence of a thiamin avitaminosis in foxes fed on a diet containing 10% or more of raw carp. Woolley[7] and Sealock, *et al.*,[8] have described the thiamin-inactivating activity of raw carp *in vitro*.

More than a dozen other pertinent papers have appeared in the literature in most of which the problem of the nature of the inactivation has been attacked by studying the behavior of the active principle responsible for the inactivation.

To our knowledge, only two reports have appeared in which the problem has been attacked through a study of the structures of the products into which thiamin is converted by such inactivation. The first of these reports is that of Krampitz and Woolley[9] who isolated from carp-inactivated thiamin, 2-methyl-4-amino-5-hydroxymethylpyrimidine and 4-methyl-5-β-hydroxyethylthiazole. The second report is that of Hennessy and Warner[10] who isolated in low yield from clam-inactivated thiamin, the crystalline dihydrochloride of a base of undetermined structure which they named "icthiamin" and to which they assigned the empirical formula $C_8H_{16}N_4O_3S\cdot 2HCl$. These latter workers showed also that one of the products of inactivation of thiamin by carp tissue extract and by smelt tissue extract is similar to icthiamin in its reaction with sodium bisulfite and with the thiazole moiety of thiamin in the presence of live yeast.

Our report describes a procedure for the routine isolation of icthiamin in quantities large enough to allow a determination of its structure.

Icthiamin is formed by allowing thiamin to react with a finely ground aqueous suspension of clam tissue. The icthiamin thus formed is then isolated by a method patterned after the procedure of Cerecedo and Hennessy[11] for the isolation of thiamin from rice polishings. The bulk of the clam tissue is removed by causing it to coagulate by *p*H adjustment and heat. The resulting solution is then passed through an ion-exchange column which takes up the icthiamin. Elution of icthiamin from the column is followed by precipitation with silicotungstic acid. After decomposition of the silicotungstate precipitate with barium hydroxide, icthiamin is further purified by fractionation with silver ion, with which it forms a sparingly soluble salt. The silver salt is then decomposed with hydrobromic acid and icthiamin is finally isolated as its dihydrobromide. The details of the isolation procedure are described in the experimental part.

In order to determine the efficiency of each step of the isolation, an assay for icthiamin was employed, the basis for which is the following[12]: (1) Icthiamin and 2-methyl-4-amino-5-hydroxymethylpyrimidine, when subjected to the conditions of the thiochrome method for the assay of thiamin, yield no fluorescent products.

(2) Icthiamin and 2-methyl-4-amino-5-hydroxymethylpyrimidine, with an equivalent or excess of the thiazole moiety of thiamin, added to live yeast in the presence of a suitable nutrient, yield thiamin quantitatively equivalent to the amount of pyrimidine derivatives used, as determined by thiochrome assay.

(3) If icthiamin is treated in aqueous solution with sodium bisulfite at *p*H 5, it yields a sulfited product which cannot be converted to thiamin by the treatment with the thiazole moiety of thiamin and yeast.

(4) 2-Methyl-4-amino-5-hydroxymethylpyrimi-

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

(2) Presented before the division of Biological Chemistry, American Chemical Society, 117th Meeting, Philadelphia, Penna., April, 1950.

(3) 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.

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

(5) R. G. Green, W. E. Carlson and C. A. Evans, *J. Nutrition*, **21**, 243 (1941).

(6) R. G. Green, W. E. Carlson and C. A. Evans, *ibid.*, **23**, 165 (1942).

(7) D. W. Woolley, *J. Biol. Chem.*, **141**, 997 (1941).

(8) R. R. Sealock, A. H. Livermore and C. A. Evans, THIS JOURNAL, **65**, 935 (1943).

(9) L. O. Krampitz and D. W. Woolley, *J. Biol. Chem.*, **152**, 9 (1944).

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

(11) L. R. Cerecedo and D. J. Hennessy, THIS JOURNAL, **59**, 1617 (1937).

(12) In order to simplify the interpretation of the results of the assay, the assumption is made that the only thiamin-regenerable pyrimidine derivatives formed in significant amounts by the action of clam tissue on thiamin are icthiamin which is sulfite sensitive and 2-methyl-4-amino-5-hydroxymethylpyridmidine which is sulfite insensitive.