[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF FORDHAM UNIVERSITY]

The Action of Borohydrides on Thiamin in Aqueous Medium[1-3]

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Thiamin is reduced by sodium borohydride to a tetrahydrothiamin and a dihydrothiamin. Sodium trimethoxyborohydride converts thiamin chiefly into a lower melting dihydrothiamin from which the higher melting isomer can be obtained. Sulfite cleavage, desulfurization, physical data, hydrolytic deamination and yeast regeneration to thiamin show tetrahydrothiamin to be 4-methyl-5-β-hydroxyethyl-3-(2′-methyl-4′-amino-5′-methylenepyrimidyl)-thiazolidine. Ultraviolet and infrared spectra, potentiometric titration and other considerations suggest that the low melting dihydrothiamin is 4-methyl-5-β-hydroxyethyl-3-(2′-methyl-4′-amino-5′-methylenepyrimidyl)-4-thiazoline and the high melting isomer 8-methyl-3-(2′-methyl-4′-amino-5′-methylenepyrimidyl)-octahydrofurano[1,2-d]thiazole.

The suggested importance[4] of dihydrothiamin in the functioning of thiamin in enzyme systems led us to attempt its synthesis. In confirmation of Karrer, *et al.*,[5] we observed that sodium hydrosulfite caused cleavage of thiamin. From structural considerations it seems that catalytic hydrogenation[4] might also cleave thiamin by hydrogenolysis. The use of lithium aluminum hydride to obtain dihydroheterocycles from the corresponding quaternary salts[6] suggested to us the trial of the borohydrides for the preparation of dihydrothiamin. It was felt that the use of an aqueous medium in which the borohydrides are reasonably stable might offer advantages in increased yield and simpler procedure. In 1950, after part of this work had been reported,[2a] the preparation, in low yield, of dihydrothiamin by the use of lithium aluminum hydride was reported by Karrer and Krishna.[7]

The chief product of the reduction of thiamin (I) by aqueous sodium borohydride[8] was a tetrahydrothiamin (II), characterized as its dihydrobromide and monopicrate. Its ultraviolet absorption spectrum is typical of 4-aminopyrimidines.[9] Sulfite cleavage yielded 2-methyl-4-aminopyrimidyl-5-methanesulfonic acid (III)[10] and a basic product having the composition of 4-methyl-5-β-hydroxyethylthiazolidine (IV), characterized as a picrate. Desthiotetrahydrothiamin (V), obtained by Raney nickel desulfurization[11] of tetrahydrothiamin, had the composition expected in analogy to desthiobiotin methyl ester[12] and desthiobenzylpenicillin.[13] Acid hydrolysis of thiamin results in hydrolytic deamination to yield oxythiamin (IV).[14] The analogous hydrolytic product, *viz.*, tetrahydroöxythiamin (VII), was formed in good yield from tetrahydrothiamin; VII was also obtained by the sodium borohydride reduction of oxythiamin. Sulfite cleavage of tetrahydroöxythiamin yielded 2-methyl-4-oxypyrimidyl-5-methanesulfonic acid.[15] Yeast[16] with added 4-methyl-5-β-hydroxyethylthiazole[17] quantitatively regenerated thiamin[18] from II, as determined by the thiochrome assay.

It seems rather certain from these data that tetrahydrothiamin is 4-methyl-5-β-hydroxyethyl-3-(2′-methyl-4′-amino-5′-methylenepyrimidyl)-thiazolidine (II).

A minor product of the sodium borohydride reduction of thiamin was a dihydrothiamin (IX), m.p. 175°, differing from that of Karrer and Krishna. In order to make a comparative study of the two dihydrothiamins, we repeated the preparation described by Karrer and Krishna. The product obtained, as judged from its composition and ability to reduce silver nitrate to form a mirror, was indeed dihydrothiamin. However, its melting point was 151°. Karrer and Krishna give 138° as the melting point. The same low melting point was reported by these workers[19] for dihydrothiamin prepared by the lithium aluminum hydride reduction of 'thiaminthiazolone.[20] The dihydrothiamin prepared in our laboratory differed again in lacking the absorption band at 320 mμ, reported by Karrer and Krishna. We were unable to see in the structure of dihydrothiamin a chromophore group which might account for absorption at 320 mμ. It was felt that perhaps we had in hand a purer material. This opinion was supported by the preparation of dihydrothiamin (X), m.p. 151°, in 40% yield by the sodium trimethoxyborohydride reduction of thiamin in cold aqueous methanol. This material was identical in all respects, including infrared spectrum, with the preparation obtained by following the Karrer and Krishna procedure.

Although insoluble in cold water, X dissolved in boiling water but did not reappear upon cooling, even when seeded. Extraction of the solution

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

(2) Presented before the Division of Biological Chemistry, American Chemical Society, (a) 117th Meeting, Philadelphia, Pa., April, 1950, and (b) 122nd Meeting, Atlantic City, N. J., September, 1952.

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

(4) F. Lipmann and G. Perlmann, THIS JOURNAL, **60**, 2574 (1938).

(5) P. Karrer, W. Graf and J. Schukri, *Helv. Chim. Acta*, **28**, 1523 (1945); P. Karrer and M. Viscontini, *ibid.*, **29**, 711 (1946).

(6) H. Schmidt and P. Karrer, *ibid.*, **32**, 964 (1949).

(7) P. Karrer and H. Krishna, *ibid.*, **33**, 555 (1950).

(8) All hydrides were obtained from Metal Hydrides, Inc., Beverly, Mass.

(9) R. R. Williams, E. R. Buchman and A. E. Ruehle, THIS JOURNAL, **57**, 1093 (1935).

(10) R. R. Williams, R. E. Waterman, J. C. Keresztesy and E. R. Buchman, *ibid.*, **57**, 536 (1935).

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

(12) V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *J. Biol. Chem.*, **146**, 475 (1942).

(13) E. Kaczka and K. Folkers, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, Chapt. IX, p. 243.

(14) H. N. Rydon, *Biochem. J.*, **48**, 383 (1951).

(15) J. K. Cline, R. R. Williams, A. E. Ruehle and R. E. Waterman, THIS JOURNAL, **59**, 530 (1937).

(16) Low thiamin yeast, generously furnished by Standard Brands, Inc., New York, N. Y.

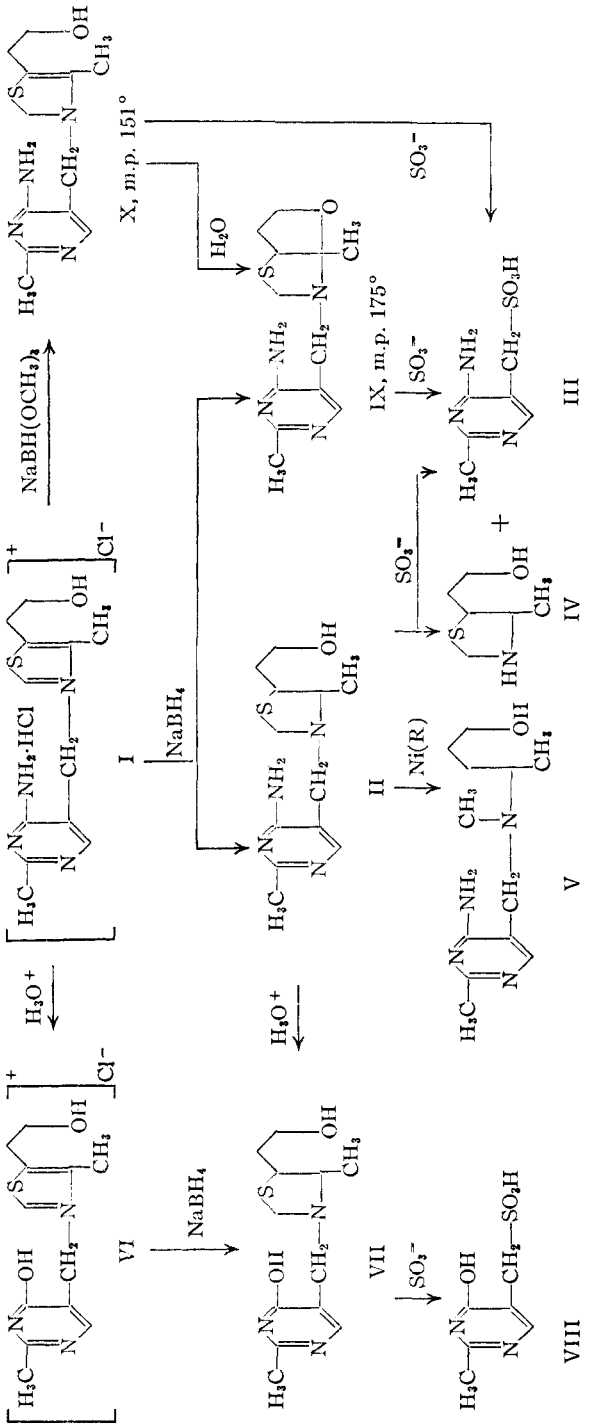
(17) Generously furnished by Merck and Co., Inc., Rahway, N. J.

(18) J. D. Barnhurst and D. J. Hennessy, THIS JOURNAL, **74**, 355 (1952).

(19) P. Karrer and H. Krishna, *Helv. Chim. Acta*, **35**, 459 (1952).

(20) R. R. Williams and O. Zima, *Ber.*, **73**, 941 (1940).

yielded only the dihydrothiamin IX, m.p. 175°. Any studies carried out on aqueous solution of X would then actually be studies on IX. When such solutions are subjected to sulfite cleavage, III is ob-



tained but no pure basic product could be isolated. Yeast regeneration of thiamin from solutions of IX or X with added 4-methyl-5-β-hydroxyethylthiazole was quantitative as determined by thiochrome assay.[19,21] The ultraviolet absorption spectra of alcoholic solutions of these dihydrothiamins, although not identical, are both characteristic of the 4-aminopyrimidine system. The change brought about by the reductions is therefore in the thiazole portion of the molecule.

Since the ultraviolet absorption spectra of their alcoholic solutions differ and since both IX and X on repeated crystallization from alcohol remain unchanged, their difference cannot be attributed to polymorphism. Examination of their infrared spectra (Fig. 1) discloses the absence of the characteristic OH band just below 3 μ in the case of IX. This band is present in the spectra of thiamin (I), tetrahydrothiamin (II) and dihydrothiamin (X). The appearance of new bands for IX at 8.23, 9.10 and 9.16 μ may be attributed to a C–O–C grouping. The potentiometric titration curves and the ultraviolet absorption for aqueous solutions of IX and X are practically indistinguishable as expected if X changes to IX in water. The close similarity of pK_1 and pK_2 of II, IX and X is significant in view of the work of Adams and Mahan[22] in which analogs of X were shown to be considerably more basic than analogs of II.

Rearrangement of X to IX would account for the loss of the characteristic OH band in the infrared, the appearance of bands attributed to C–O–C and for decreased basicity resembling that of II. A similar rearrangement has been reported by Witkop[23] for the conversion of cinchonamine to quinamine. The existence of two mutually interconvertible S-methylthiamins has been attributed to this same isomerism.[24] The loss of aromaticity by the ring to which the β-hydroxyethyl group is attached precedes the tetrahydrofuran ring closure in each of the cases cited.

An instance in which aromaticity is gained at the expense of the opening of the tetrahydrofuran ring is to be found in the synthesis of thiamin from 2-methyl-2-ethoxy-3-chlorotetrahydrofuran and 2-methyl-4-amino-5-thioformylaminomethylpyrimidine.[25] The fact that solutions of dihydrothiamin IX or X will reduce silver nitrate rapidly to form a mirror only if heated is a further indication that dihydrothiamin in aqueous solution does not have the structure usually attributed to α-dihydroheterocycles.

Structure IX is therefore proposed for the higher melting dihydrothiamin. This isomerization somewhat complicates the interpretation of the results of studies in biological systems such as those reported by Schopfer, *et al.*,[26] and by Lenthardt and Nielsen[27] where presumably, since it is in aqueous solution, dihydrothiamin would exist as IX rather than X.

Experimental[28]

Tetrahydrothiamin (II).—A 20% solution of thiamin chloride was cooled to 0° (ice–salt-bath) and treated with 2 molar

(21) These determinations were made by Dr. R. J. Moshy of this laboratory.

(22) R. Adams and J. E. Mahan, THIS JOURNAL, **64**, 2588 (1942).

(23) B. Witkop, *ibid.*, **72**, 2311 (1950).

(24) E. DiBella, Thesis, Fordham University, 1953.

(25) Hoffmann–LaRoche, British Patent 500,519.

(26) W. H. Schopfer, M. L. Bein and G. Besson, *Z. Vitaminforsch.*, **23**, 36 (1951).

(27) F. Lenthardt and H. Nielsen, *Helv. Chim. Acta*, **35**, 1207 (1952).

(28) Melting points were taken on a Fisher–Johns melting point apparatus. Microanalyses were performed by Dr. F. A. Bubler, Mr. A. A. Sirotenko and Mr. J. F. Alicino.

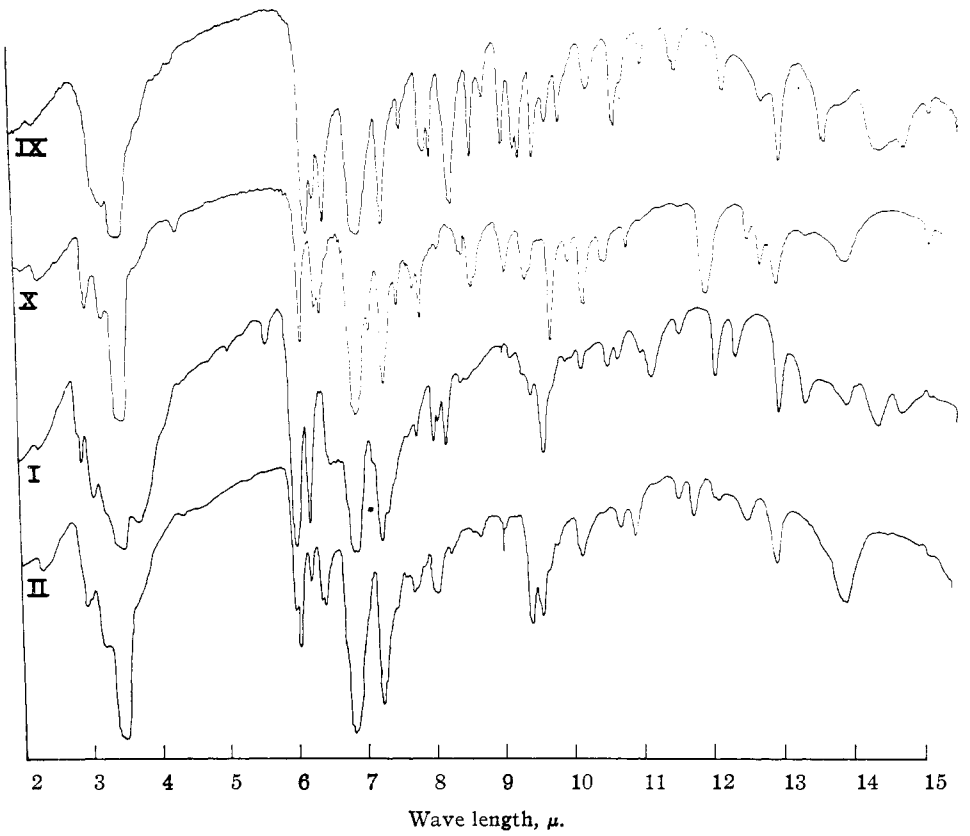


Fig. 1.—The infrared absorption spectra of thiamin (I), tetrahydrothiamin (II) and the dihydrothiamins IX and X.

equivalents of *N* sodium hydroxide. This solution was kept at 0° and stirred constantly during the dropwise addition of a cold 2 *M* aqueous solution containing one molar equivalent of sodium borohydride. After all the reducing agent was added, the reaction mixture was permitted to reach room temperature. Filtration yielded II, m.p. 145° after several recrystallizations from hot water, yield 70%.

Anal. Calcd. for $C_{12}H_{20}N_4OS$: C, 53.73; H, 7.52; N, 20.89; S, 11.94. Found: C, 53.40; H, 7.60; N, 20.10; S, 11.65.

Tetrahydrothiamin Dihydrobromide.—To 0.5 g. (0.002 mole) of tetrahydrothiamin dissolved in 0.8 ml. (0.007 mole) of 48.5% hydrobromic acid, 15 ml. of absolute alcohol was added, and the solution was allowed to stand overnight. The filtered solid was recrystallized by dissolving it in the minimum amount of water and diluting the solution with 10 times its volume of absolute alcohol to yield the pure hydrobromide, m.p. 226°.

Anal. Calcd. for $C_{12}H_{22}N_4OSBr_2$: C, 33.49; H, 5.11; N, 13.02; Br, 37.21. Found: C, 33.22; H, 4.69; N, 13.00; Br, 37.38.

Tetrahydrothiamin Monopicrate.—Tetrahydrothiamin (0.5 g., 0.002 mole) and picric acid (0.5 g., 0.002 mole) were each dissolved in 50 ml. of hot water. The hot solutions were mixed. On standing, the picrate separated, m.p. 167° after recrystallization from hot water.

Anal. Calcd. for $C_{18}H_{23}N_7O_8S$: C, 43.46; H, 4.67; N, 19.72. Found: C, 43.85; H, 4.19; N, 19.41.

Sulfite Cleavage of Tetrahydrothiamin (II).—To 1.6 g. (0.0037 mole) of tetrahydrothiamin dihydrobromide in 20 ml. of water was added 2.0 g. (0.019 mole) of sodium bisulfite. The solution was adjusted to *p*H 5–5.5 with 5% sodium hydroxide and allowed to stand at room temperature. Two days later the white precipitate was collected, washed with cold water and dried to yield 0.7 g. (92%) of crude **2-methyl-4-aminopyrimidyl-5-methanesulfonic acid (III)**, which was recrystallized from hot water.

Anal. Calcd. for $C_6H_9N_3O_3S$: C, 35.44; H, 4.46; S, 15.76. Found: C, 35.69; H, 4.07; S, 16.10.

The above cleavage reaction was repeated on a 5.0-g. sample of tetrahydrothiamin. The filtrate from removal of the sulfonic acid was adjusted to *p*H 8 with solid sodium carbonate and extracted with several small portions of ether. The dried (sodium sulfate) extract was evaporated. The viscous residue was dissolved in the minimum amount of hot absolute alcohol. Slow crystallization yielded 2.2 g. (80%) of **4-methyl-5-hydroxyethylthiazolidine (IV)**, m.p. 108°.

Anal. Calcd. for $C_6H_{13}NOS$: C, 48.97; H, 8.84; N, 9.52. Found: C, 48.42; H, 8.42; N, 10.06.

To 200 mg. of IV in 10 ml. of alcohol was added 10 ml. of a saturated alcoholic solution of picric acid. After 2 days the crude picrate was collected, m.p. 138–140° after recrystallization from 1:1 alcohol–ether.

Anal. Calcd. for $C_{12}H_{16}N_4O_8S$: C, 38.30; H, 4.25. Found: C, 38.10; H, 4.11.

Desthiotetrahydrothiamin (V).—Tetrahydrothiamin (4.0 g.) was refluxed with *ca.* 25 g. of Raney nickel[11,29] in 125 ml. of absolute alcohol for 2.5 hr. The nickel was removed by hot filtration and the filtrate evaporated *in vacuo*. The residue was dissolved in 30 ml. of hot water and the filtered solution allowed to cool slowly to room temperature. Repetition of this procedure afforded 1.2 g. (34%) of white crystals, m.p. 134°.

Anal. Calcd. for $C_{12}H_{22}N_4O$: C, 60.50; H, 9.24; N, 23.53. Found: C, 60.31; H, 9.30; N, 23.42.

Tetrahydroöxythiamin (VII). (a) By Acid Hydrolysis of Tetrahydrothiamin (II).—A solution of 5.0 g. of tetrahydrothiamin in 50 ml. of 20% hydrochloric acid was refluxed for 10 hr. and then evaporated to dryness. The residue was redissolved in 20 ml. of water. The residue obtained on evaporation again was dissolved in 20 ml. of water. Saturation of this solution with solid sodium carbonate yielded a precipitate which was dried, powdered and then refluxed for 0.5 hr. in chloroform to dissolve any unreacted tetrahydro-

(29) Welght was estimated as suggested by H. Adkins, "Reactions of Hydrogen," The University of Wisconsin Press, Madison, Wis., 1946, p. 22.

thiamin. The insoluble material was recrystallized from hot water to yield 4.0 g. (80%) of VII, m.p. 217°.

(b) **By Reduction of Oxythiamin Chloride VI.**—Oxythiamin chloride, reduced with sodium borohydride by the method used above on thiamin, gave VII which melted at 217° and did not depress the melting point of a sample prepared by method a. The infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{12}H_{19}N_3O_2S$: C, 53.53; H, 7.06. Found from (a): C, 53.56; H, 6.82. From (b): C, 53.54; H, 6.75.

Sulfite Cleavage of Tetrahydroöxythiamin (VII).—To 2.0 g. of tetrahydroöxythiamin dissolved in 3 ml. of concentrated hydrochloric acid, 10 ml. of water was added, followed by 4.0 g. of sodium bisulfite. The solution was adjusted to *p*H 5–5.5 with 5% sodium hydroxide. After standing for 2 days in a stoppered flask, the solution was made alkaline and extracted with chloroform. Treatment of the aqueous layer according to the method used[16] in isolating the oxysulfonic acid obtained from 2-methyl-4-oxy-5-ethoxymethylpyrimidine gave 1.2 g. (80%) of **2-methyl-4-oxypyrimidyl-5-methanesulfonic acid (VIII)**, m.p. 325°.

Anal. Calcd. for $C_6H_8N_2O_4S$: C, 35.29; H, 3.29; N, 13.72. Found: C, 35.10; H, 3.72; N, 13.40.

Dihydrothiamin (IX).—The reaction filtrate obtained in the above preparation of tetrahydrothiamin was saturated with solid sodium carbonate and extracted several times with chloroform. The combined extracts were evaporated and the residue leached with water. The insoluble tetrahydrothiamin was collected and recrystallized from hot water. The aqueous filtrate was saturated with solid sodium carbonate and extracted several times with chloroform. The residue obtained by reduced-pressure evaporation of the dried (over potassium carbonate) extract was recrystallized from absolute alcohol–petroleum ether (b.p. 30–60°) to yield IX, m.p. 175°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.24; H, 6.81; N, 20.77.

Dihydrothiamin (X). (a) **By Reduction of Thiamin with Sodium Trimethoxyborohydride.**—A solution of 5.0 g. (0.015 mole) of thiamin chloride in 15 ml. of water at 0° was treated with 15 ml. (0.015 mole) of ice-cold *N* sodium hydroxide and 20 ml. of methanol. This solution was maintained at −12° during addition of 2.4 g. (0.019 mole) of sodium trimethoxyborohydride in small portions with mechanical stirring over a period of 0.5 hr. The cooling bath was removed and the mixture allowed to reach room temperature while still being stirred. Filtration afforded 2.1 g. (54%) of solid. This was recrystallized from 15 ml. of hot absolute alcohol to yield 1.6 g. (40%) of pure X, m.p. 151°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.27; H, 6.91; N, 20.82.

(b) **By Reduction of Thiamin with Lithium Aluminum Hydride.**—Ten grams of thiamin chloride was added in small portions, in the course of 0.5 hr., with stirring to a suspension of 4 g. of lithium aluminum hydride in 120 ml. of anhydrous tetrahydrofuran. The reaction mixture was stirred at room temperature for 4 hr. and then treated with 8 ml. of water. The reaction mixture was filtered and the filtrate treated with CO_2 for five minutes and refiltered. The filtrate was evaporated to dryness under vacuum. The solid residue was recrystallized from 10 ml. of hot absolute alcohol to yield 0.8 g. (10%) of X, m.p. 145°. After two additional recrystallizations from absolute alcohol, the product melted sharply at 151°. The infrared spectrum was identical with that of a sample prepared by method a. The melting point of the mixed samples was 151°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.25; H, 6.88; N, 20.56.

Conversion of Dihydrothiamin (X) to its Isomer IX.—Dihydrothiamin (X) (0.5 g.) was dissolved by heating in 5 ml. of water. The cooled solution was saturated with solid sodium carbonate and extracted several times with chloroform. The chloroform extract was dried over anhydrous potassium carbonate and evaporated to dryness. The residue was recrystallized from absolute alcohol–petroleum ether (b.p. 30–60°) to yield IX, m.p. 175°, yield 0.4 g.

Sulfite Cleavage of Dihydrothiamin (X).—Two grams of dihydrothiamin (X) were treated with sodium bisulfite at *p*H 5–5.5 as described in the above procedure for the sulfite cleavage of tetrahydrothiamin (II). The insoluble 2-methyl-4-aminopyrimidyl-5-methanesulfonic acid was recrystallized from hot water. The yield was 1.4 g. (90%).

Anal. Calcd. for $C_6H_9N_3O_3S$: C, 35.44; H, 4.46; N, 20.68; S, 15.76. Found: C, 35.25; H, 4.47; N, 20.76; S, 15.62.

Attempts to isolate the soluble sulfite cleavage product in pure form or as its picrate, picrolonate and hydrochloride were unsuccessful.

Potentiometric Titration.—A Coleman model 3A electrometer with glass electrode was used to follow *p*H changes. Approximately 100-mg. samples of tetrahydrothiamin dihydrobromide and of the dihydrothiamins IX and X were each dissolved in 50 ml. of carbon dioxide-free distilled water and titrated with standardized 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid, respectively, while nitrogen was bubbled into the solution to provide agitation and to exclude carbon dioxide. The basic pK_1 and pK_2 for II were 7.8 and 11.5, while those for IX and X were identical at 7.9 and 11.8.

Spectra.—Infrared spectra were taken by Miss Cecelia Vitiello of Schering Corp., Bloomfield, N. J., on a Perkin-Elmer double beam instrument using Nujol mulls. Ultraviolet spectra were taken on a Cary recording spectrophotometer using 2.0 mg. of solute/100 ml. of solution.

Cpd.	$\lambda_{max}^{H_2O}$, mμ	log ε	λ_{max}^{EtOH}, mμ	log ε
II	236	3.94	235	3.97
	270	3.75	278	3.69
IX	237	3.92	234	3.95
	280	3.81	278	3.70
X	237	3.93	243	3.93
	280	3.81	288	3.83

NEW YORK 58, N. Y.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

The Constituents of *Casimiroa edulis* Llave *et* Lex. III.[1] The Structure of Casimiroin[2]

BY ALEX MEISELS AND FRANZ SONDHEIMER

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Casimiroin, a constituent of the seed and the bark of the tree *Casimiroa edulis* Llave *et* Lex., has been shown through degradation to be 1-methyl-4-methoxy-7,8-methylenedioxycarbostyril (IIIa).

In 1911, Power and Callan[3] described the results of a chemical investigation into the constituents of the seeds of the tree *Casimiroa edulis* Llave *et* Lex. (*Rutaceae*). Six substances (besides benzoic acid) were isolated, namely, casimiroedine (0.043%), casimiroin (0.0076%), casimirolid (0.060%), a "yellow phenolic substance" (0.004%), β-sitosterol β-D-glucoside ("ipuranol") (0.0078%) and β-sitos-

(1) Part II, see J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 4170 (1956).

(2) Presented in part at the 21st Meeting of the Chemical Society of Israel, Jerusalem, April, 1957.

(3) F. B. Power and T. Callan, *J. Chem. Soc.*, **99**, 1993 (1911).