Carbinols	$n^{20}D$	d^{20}_4	Reaction		Yield, %
2-Methylhexanol-2	1.4186	0.8146	BuMgBr	Me_2CO[a]	80
2-Methyloctanol-2	1.4280	.8210	MeMgCl	Me-*n*-hexyl ketone	74
3-Ethylheptanol-3	1.4360	.8429	EtMgBr	*n*-Valeryl chloride[b]	66
3-Methylnonanol-3	1.4358	.8311	EtMgBr	Me-*n*-hexyl ketone	76
4-Methyldecanol-4	1.4375	.8296	PrMgBr	Me-*n*-hexyl ketone	56

[a] Acetone was purified by fractional distillation from solid potassium permanganate and freshly prepared calcium oxide.

[b] *n*-Valeryl chloride (b. p. 126° (730 mm.); $n^{20}D$ 1.4200) was prepared from the acid by treatment with thionyl chloride in 84% yield. The valeric acid (b. p. 86° (18 mm.), $n^{20}D$ 1.4080) was prepared in 81% yield by treating *n*-butylmagnesium bromide with carbon dioxide. The flask containing the Grignard reagent was cooled to 0°, solid lumps of carbon dioxide were dropped into the solution and the material allowed to stand overnight. Secondary and tertiary alcohols may be formed if the addition of carbon dioxide gas is slow. Gilman, *Rec. trav. chim.*, **49**, 1172 (1930).

PROPERTIES OF THE HYDROCARBONS

Hydrocarbons	B. p. °C.	B. p. Mm.	B. p. °C.[a]	B. p. Mm.	n^{20}_4	$n^{20}D$	F. p., °C.	Yield, %
2-Methylhexane	89.1	735	90.3	760	0.6794	1.3851	−120.3	23.7
2-Methyloctane	141.6	736	142.8	760	.7132	1.4030	[b]	48.8
3-Ethylheptane	141.9	736	143.1	760	.7272	1.4090	[b]	38.3
3-Methylnonane	166.3	732	167.6	760	.7319	1.4123	−90.0	30.1
4-Methyldecane	186.8	739	188.1	760	.7422	1.4177	−92.9	27.8

[a] The boiling points were taken at 760 mm. with a Cottrell b. p. apparatus and a barostat.

[b] Refer to Frank C. Whitmore and H. A. Southgate, THIS JOURNAL, **60**, 2571 (1938).

with water, twice with 25% sodium hydroxide solution, and dried over sodium. It was carefully fractionated[5] from sodium–potassium alloy, washed with concentrated sulfuric acid, water, 25% sodium hydroxide, dried over sodium, and refractionated from sodium–potassium alloy to give a constant index material that gave no halide test with copper wire and no olefin test with bromine in carbon tetrachloride.

(5) Whitmore and Lux, THIS JOURNAL, **54**, 3451 (1932).

Summary

A new synthesis of tertiary hydrocarbons has been described, and applied to five aliphatic hydro carbons.

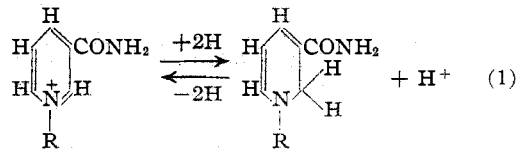
STATE COLLEGE, PENNA. RECEIVED AUGUST 16, 1938

[CONTRIBUTION FROM THE BIOLOGICAL INSTITUTE OF THE CARLSBERG FOUNDATION, COPENHAGEN]

Hydrogenation of Vitamin B_1 and Other Quaternary Thiazoles[1]

BY FRITZ LIPMANN AND GERTY PERLMANN

Dehydrogenation of various substrates in the cell is effected by quaternary pyridine compounds—nicotinic acid derivatives[2,3]—acting as hydrogen-transporting systems between the respective substrates and other hydrogen carriers.[2,4] The catalytic action of these pyridinium compounds is due to alternate hydrogenation and dehydrogenation at the double bond adjoining the quaternary nitrogen.



The action of vitamin B_1 (which hereafter in this paper will be referred to as "thiamin") as codehydrase for pyruvic acid dehydrogenation was made highly probable by the work of Peters and co-workers[5] with avitaminotic tissues (see also Lipmann[6]). Using the pyruvic acid dehydrogenase of *Bacterium Delbrückii*, the codehydrase function of thiamin—as thiamin pyrophosphate, Lohmann's cocarboxylase[7]—could be shown definitely.[8]

The presence of a quaternary thiazole in thiamin,[9] the well-known similarity between thiazoles and pyridines together with the codehydrase function of thiamin suggested a similar mode of action for the pyridine and thiazole codehydrases.

(1) This investigation was supported by a grant from the Ella Sachs Plotz Foundation.

(2) (a) O. Warburg and W. Christian, *Biochem. Z.*, **287**, 291 (1936); (b) O. Warburg, *Ergebnisse Enzymforschung.*, **7**, 210 (1938).

(3) P. Karrer, G. Schwarzenbach, F. Benz and U. Solmsson, *Helv. Chim. Acta*, **19**, 811 (1936).

(4) H. v. Euler, *Ergebnisse Physiol.*, **38**, 1 (1936).

(5) R. A. Peters, *Biochem. J.*, **31**, 2240 (1937).

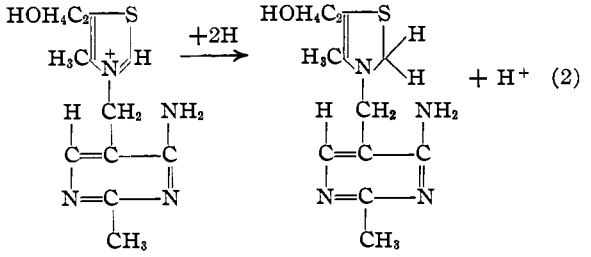
(6) F. Lipmann, *Skand. Arch. Physiol.*, **76**, 255 (1937).

(7) K. Lohmann and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).

(8) F. Lipmann, *Enzymologia*, **4**, 65 (1937).

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

In a previous short communication[10] the dehydrogenation of thiamin with sodium hyposulfite ($Na_2S_2O_4$) was described. The reaction was followed by the use of Warburg's manometric method,[11,12] which measures bicarbonate decomposition due to oxidation of neutral hyposulfite to acid sulfite ($Na_2S_2O_4 + R + 2NaHCO_3 = 2Na_2SO_3 + H_2R + 2CO_2$). It was found that for each mole of thiamin approximately 3 moles of bicarbonate was decomposed. In accordance with the findings of Warburg[2b] and Karrer[3] with the quaternary pyridines (equation 1), the appearance of one extra equivalent of acid was interpreted as being due to the change from strong to weak basicity on reduction. This led to the conclusion that the hydrogenation of thiamin takes place at the double bond adjoining the quaternary nitrogen in the thiazole part of the molecule



This interpretation is supported by the recent findings of Erlenmeyer[13] that thiazole-5-carboxylic ester methiodide is hydrogenated with hyposulfite in exactly the same manner as the corresponding pyridine compound, both yielding one extra equivalent of acid on reduction.

The results mentioned above made it desirable to study the hydrogenation of thiamin and other quaternary thiazoles more in detail.

Throughout the work, the hydrogenation with hyposulfite was measured by the manometric method of Warburg.[11,12] Hydrogenation with platinum black and hydrogen was measured in Warburg manometers. The platinum black was prepared according to the directions of Willstätter and Waldschmidt-Leitz.[14]

For all the experiments carried out with thiamin, the synthetic product of the I. G. Farbenindustrie was used, for a large gift of which we are greatly indebted to the staff of the I. G. Farbenindustrie.

(10) F. Lipmann, *Nature*, **138**, 1097 (1936).

(11) O. Warburg, W. Christian and A. Griese, *Biochem. Z.*, **282** 157 (1935).

(12) E. Haas, *ibid.*, **285**, 368 (1936).

(13) H. Erlenmeyer, A. Epprecht and H. von Meyenburg, *Helv. Chim. Acta*, **20**, 514 (1937).

(14) R. Willstätter and E. Waldschmidt-Leitz, *Ber.*, **54**, 113 (1921).

The Hydrogenation of the "Cleavage Products."—To exclude the possibility that hydrogenation takes place upon the pyrimidine part, the split products obtained by the sulfite cleavage of Williams[15] were tried separately. As shown by Williams, thiamin is split into pyrimidine-sulfonic acid and thiazole by the action of acid sulfite

$$C_{12}H_{16}N_4OS + H_2SO_3 \longrightarrow C_6H_9N_3SO_3 + C_6H_9NOS$$

Thereby the quaternary thiazole is converted into a tertiary. The methiodide of the thiazole part was prepared according to Buchman, Williams and Keresztesy.[16]

Hydrogenation with Hyposulfite.—The results of the manometric measurements are assembled in Table I.

TABLE I

HYDROGENATION OF THE "CLEAVAGE PRODUCTS" WITH HYPOSULFITE: MANOMETRIC MEASUREMENTS

No.	Substance	Moles CO_2	Atoms hydrogen	Extra acid
1	Pyrimidine sulfonic acid	0	0	..
2	Thiazole part (tertiary)	0	0	..
3	Thiazole part (quaternary)	3.1	2	1.1
4	Thiamin	2.75	2	0.75

It appears from the table that none of the split products (Nos. 1 and 2) reacts with hyposulfite but that the thiazole methiodide (No. 3) reacts in the same manner as thiamin (No. 4), but more slowly (see last column and Fig. 1). In both

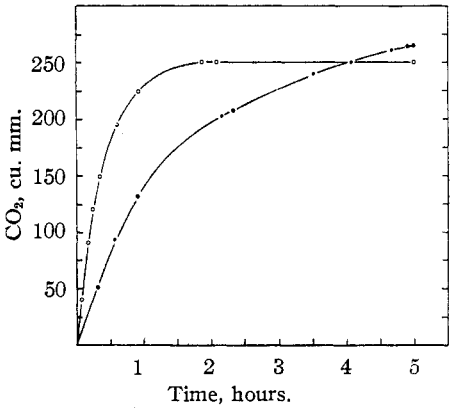


Fig. 1.—Manometric experiment with 0.405×10^{-2} millimole of thiamin (upper curve) and 0.383×10^{-2} millimole of 4-methyl-5-hydroxyethylthiazole methiodide (lower curve); temperature 30°.

(15) R. R. Williams, R. E. Waterman, J. C. Keresztesy and E. R. Buchman, THIS JOURNAL, **57**, 536 (1935).

(16) E. R. Buchman, R. R. Williams and J. C. Keresztesy, *ibid.*, **57**, 1849 (1935).

cases nearly one equivalent of extra acid appears on reduction. Since the pyrimidine part does not and the thiazole part, when quaternary, does, react as the whole, evidence is thus obtained that the hydrogenation of thiamin with hyposulfite takes place in the thiazole part of the molecule.

Hydrogenation with Platinum Black–Hydrogen.—Though only the hydrogenation with hyposulfite can be taken as significant as a model reaction, reduction with Pt–H_2 was also measured for comparison. The results are assembled in Table II. As mentioned in the first publication,[9] thiamin absorbs nearly one mole of hydrogen (Nos. 7 and 8). The reactivity of the pyrimidine part depends on *p*H (or buffer system). In

TABLE II

HYDROGENATION OF THE "CLEAVAGE PRODUCTS" AND QUATERNARY THIAZOLES WITH PLATINUM BLACK AND HYDROGEN

No.		Substance	*p*H	Moles H_2	Time, hours
1		Pyrimidine sulfonic acid	10.5	0.12	4
2		Pyrimidine sulfonic acid	8	1.88	20
3		Thiazole part tertiary	10.5	0.1	4
4	a	Thiazole part quaternary	8	1.0	1.5
	b			1.97	20
5		Thiamin	8	0.92	4
6		Thiamin	10.5	1.07	8
7		4-Methyl-5-carboxylic ester thiazole methiodide	8	1.2	18
8		4-Methylthiazole methiodide	8	1.8	18

borate at *p*H 10.5 a very sluggish absorption takes place (No. 1). In phosphate at *p*H 8 approximately two moles of hydrogen were taken up at a medium rate. The thiazole part (tertiary) absorbs virtually no hydrogen (No. 3). The quaternary thiazole absorbs a total of two moles. The absorption curve shows a break after one mole is taken up (No. 4 a and b). Besides, two other quaternary thiazoles were tried: 4-methyl-5-carboxylic ester thiazole methiodide and 4-methylthiazole methiodide, both prepared according to Clarke and Gurin.[17] The ester thiazole, like thiamin, takes up approximately one mole at a rapid rate, the methylthiazole takes approximately two moles; the first at a rapid, the second at a slower, rate.

The reactivity of the second double bond in quaternary thiazoles depends apparently to a large extent on the nature of the substituents. When reacting, it always went more slowly than the first. It is to be noted that the pyrimidine-sulfonic acid is hydrogenated with Pt–H_2. That the one mole of hydrogen taken up by the thiamin goes to the same place as on hydrogenation with hyposulfite seems quite probable, since it was found that with Pt–H_2 also one mole of acid appears on reduction (by titration of a larger sample before and after reduction, in Na_2HPO_4 with thymolphthalein as indicator).

The Colored Intermediate Hydrogenation Product.—It was observed that a transient yellowish-green color appears on addition of hyposulfite to neutral thiamin solutions.[18] Experiments with various thiazoles (see next paragraph) have shown that the greenish colored intermediate appears generally on hydrogenation of quaternary thiazoles with hyposulfite. In the special cases, the intensity of the color depends on the rate of reaction. With the fast-reacting ester thiazole (Table III, No. 3) a bright color appears in very dilute solution; with a slow reacting substance as 4-methyl-5-ethoxythiazole methiodide only concentrated solutions give a coloration. To compare the described phenomenon with the corresponding one found by Karrer and Benz[19] and Adler, Hellström and von Euler[20] with quaternary pyridines and cozymase, nicotinic acid amide ethiodide[21] was prepared. The course of events is exactly the same with the two classes of compounds. With the pyridines the color is more orange.

On reduction of thiamin with zinc and normal hydrochloric acid, a very similar transient color appears. After the disappearence of the color vigorous hydrogen sulfide formation sets in, indicating that here hydrogenation is followed by profound degradation.

The most obvious explanation for the transient coloration, proposed already for the pyridines,[19] is that hydrogenation occurs in two steps.[22] The half reduced colored compound, of a semiquinone type, is decolorized by further reduction. As the two-step oxidation or reduction is very common with the oxido–reduction catalysts of the cell (flavin, pyocyanine, pyridine coferments, etc.), it seems of importance that it occurs also with thiamin.

The Hydrogenation of Various Quaternary Thiazoles with Hyposulfite.—We have studied the reaction with 4-methyl-5-carboxylic acid

(17) H. T. Clarke and S. Gurin, THIS JOURNAL, **57**, 1876 (1935).

(18) F. Lipmann, *Nature*, **140**, 849 (1937).

(19) P. Karrer and F. Benz, *Helv. Chim. Acta*, **19**, 1028 (1936).

(20) E. Adler, H. Hellström and H. v. Euler, *Z. physiol. Chem.*, **242**, 225 (1936).

(21) P. Karrer and F. J. Stare, *Helv. Chim. Acta*, **20**, 418 (1937).

(22) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935).

ethyl ester methiodide, a compound similar to that studied by Erlenmeyer.[13] From the ester the corresponding acid amide was prepared, following a procedure used by Karrer[3] to prepare nicotinic acid amide. The similarity of constitution of the two acid amides made the study of this thiazole interesting. Benzothiazole methiodide was prepared according to Möhlau and Krohn.[23] This substance has been studied by Mills and co-workers.[24] Their work, to be discussed later, made the inclusion of it desirable.

In Table III all our manometric experiments with quaternary thiazoles are summarized. For comparison the nicotinic acid amide ethiodide is included. In all the cases approximately one mole of extra acid appears in addition to the two moles originating from the transfer of two hydrogens. The rate of reaction (see last column) is greatest for derivatives of the acid, nearly approaching that of the pyridine compound. It is intermediate with thiamin and slowest with the methiodide of the cleavage product.

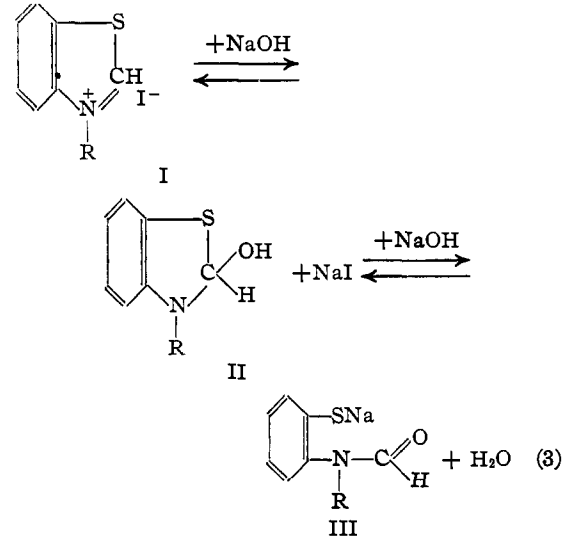
TABLE III

HYDROGENATION OF QUATERNARY THIAZOLES WITH HYPOSULFITE: MANOMETRIC MEASUREMENTS

No.	Substance	Moles CO_2	Atoms H	Extra acid	50% Reduction time, min.
1	Nicotinic acid amide ethiodide	3.1	2	1.1	1.5
2	Thiamin	2.8	2	0.8	15
3	4-Methyl-5-ethoxy thiazole methiodide	3.1	2	1.1	57
4	4-Methyl-5-carboxylic acid ethyl ester thiazole methiodide	2.9	2	0.9	3
5	4-Methyl-5-carboxylic acid amide thiazole methiodide	2.88	2	0.88	5

The behavior of benzothiazole methiodide was somewhat different. Mills and co-workers have described the reduction of this compound with zinc and hydrochloric acid. The α-dihydrobenzothiazole, a water-insoluble substance of characteristic odor, was isolated in good yield. They described also the reversion of the dihydrothiazole to thiazole methiodide by oxidation with iodine in alcoholic solution.

When benzothiazole methiodide, freshly dissolved in bicarbonate, is treated at once with hyposulfite, we found that the bright greenish color of the intermediate appeared, the solution became cloudy and a characteristic odor, the same as on reduction with Zn–HCl, developed. The experiment showed that benzothiazole methiodide is reduced by hyposulfite in the same manner as other quaternary thiazoles. Therefore, it was surprising to find that the manometric experiment did not show any reaction, when carried out in the usual manner—that is to dissolve the thiazole in bicarbonate, equilibrate the vessel in the thermostat and to tip the hyposulfite into the solution. The explanation is found in the fact that benzothiazole methiodide reacts rapidly with bicarbonate. In about fifteen minutes at 30° two moles of bicarbonate was decomposed. It is known from the work of Mills and of Williams that by the action of strong alkali the pseudobase is formed first and then the thiazole ring is opened in the following manner



Mills called attention to the strong acidity of the thiophenol derived from benzothiazole. It must be an appreciably stronger acid than carbonic acid. Therefore in bicarbonate at the end of the reaction practically only molecule III is present in the solution. Molecule I, which reacts with hyposulfite, has disappeared.

The amide reacts similarly, but only incompletely. The equilibrium can be driven back in the direction of molecule I by saturation with carbon dioxide. Therefore the manometric experiment with the amide was carried out with carbon dioxide in the gas phase. The other thiazoles did not decompose bicarbonate.

The Question of Reversibility. Discussion

At an early stage of this investigation, attempts were made to isolate the hydrogenated thiamin,

(23) R. Möhlau and C. W. Krohn, *Ber.*, **21**, 59 (1888).

(24) W. H. Mills, L. M. Clark and J. A. Aeschlimann, *J. Chem. Soc.*, **123**, 2353 (1923).

but met with great difficulties, which it has not been possible to overcome up to now. On extraction of the more or less alkaline solutions with chloroform or ether, the greater part of the products of reaction remained always in the water phase. The brownish oily residue from chloroform or ether was obviously inhomogeneous. Furthermore, it was not possible to reoxidize the product of reduction to thiochrom with ferricyanide and alkali. Only a comparatively feeble blue fluorescence appeared. Therefore it was not surprising to find that the biological activity was lost after hydrogenation.

A similar behavior was found with the ester thiazole and the acid amide thiazole methiodides. The latter substance required attention because the work of Karrer[25] showed that the acid amide group has a stabilizing effect on the equally unstable dihydropyridines. The only thiazole which yielded with hyposulfite a stable hydrogenated product, was benzothiazole. The identity of this product with benzodihydrothiazole obtained on reduction with Zn–HCl by Mills and co-workers, though still to be established, can scarcely be doubted. With this compound the reversibility of dehydrogenation had been shown before. As our experience makes it quite probable that the primary process of reduction is the same in all cases, it might be concluded that, primarily a reversible hydrogenation product, the 2-dihydrothiazole is always formed which subsequently undergoes irreversible rearrangements.

If the codehydrase action of thiamin or, better, thiamin pyrophosphate should—as was suggested in the beginning of this paper—be due to a reversible hydrogenation of the thiazole, the hydrogenated thiamin must be stabilized in the enzyme (codehydrase–protein compound). This could possibly be effected by the combination with the specific protein. It has been described elsewhere[8] and has been confirmed recently[26] that in pyruvic acid dehydrogenase the codehydrase is strongly bound to the protein and most probably does not dissociate in the cell. Thus the thiamin enzyme differs from the pyridine enzymes, in which the pyridine–protein dissociates easily.

We wish to thank Dr. Albert Fischer, Director of this Laboratory, for the kind support he has given to the above work. We also wish to express our gratitude to Dr. Stig Veibel for his help in obtaining materials for the preparation of some of the compounds used.

Experimental

4-Methyl-5-carboxylic Acid Amide Thiazole Methiodide.—One and one-half ml. of 4-methyl-5-carboxylic ester thiazole[17] was dissolved in 3 ml. of methanol and saturated with ammonia at −18°. The mixture was heated in a bomb tube for twelve hours to 150°. On cooling crystals separated from the methanolic solution. The methanol and ammonia were distilled off. From the brownish, mostly crystalline residue, the amide was extracted by boiling three times with benzene. The substance crystallized from the benzene in only slightly discolored crystals, yield 0.75 g. *Anal.* Calcd. for $C_5H_6N_2OS$: N, 19.67. Found: N (Dumas), 18.81. Without further purification the product was treated with methyl iodide. It was dissolved in 2 ml. of absolute alcohol mixed with 7 ml. of methyl iodide and boiled gently on the reflux condenser for twelve hours. After removal of the methyl iodide, the methiodide was crystallized twice from absolute alcohol.

Anal. Calcd. for $C_6H_9N_2OSI$: N, 9.84. Found: N (Dumas), 9.73.

4-Methyl-5-carboxylic Acid Ethyl Ester Thiazole Methiodide.—The ester was mixed with an excess of methyl iodide and warmed in a sealed tube to 40° for twenty-four hours. A brown oil separated on the surface. After removal of the methyl iodide, the solid, partly crystalline residue was dissolved in absolute alcohol. On careful addition of ether, the methiodide crystallized in large light yellow needles, m. p. 140°.

Summary

The dehydrogenation of vitamin B_1 (thiamin) and other quaternary thiazoles is studied in connection with the codehydrase function of thiamin.

(25) P. Karrer, F. W. Kahnt, R. Epstein, W. Jaffe and T. Ishii, *Helv. Chim. Acta*, **21**, 223 (1938).

(26) Unpublished experiments.