[CONTRIBUTION FROM THE MALLINCKRODT LABORATORY OF HARVARD UNIVERSITY]

The Reduction of Thioketones by a Model for a Coenzyme

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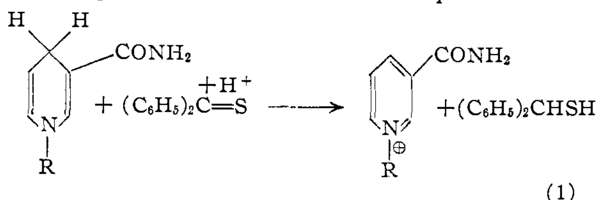
Reduced diphosphopyridine nucleotide or 1-benzyl-1,4-dihydronicotinamide will reduce thiobenzophenone to benzhydryl mercaptan with direct transfer of hydrogen from the dihydropyridine compound to the thioketone. The effects of *p*H, of substituents in the thioketone, of substituents in the dihydro compound, of solvent and of deuterium substitution on the rate of the reaction have been determined. These studies have led to the conclusion that the hydrogen atom is transferred with its electron pair in the rate-controlling step of the oxidation-reduction process. The "model system" has been compared with the enzymatic one.

In many enzymatic reactions involving reduced diphosphopyridine nucleotide (hereafter called DPNH) hydrogen is directly transferred from coenzyme to substrate.[3] 1-Benzyl-1,4-dihydronicontinamide, a "model" for DPNH, will transfer hydrogen directly to malachite green[4] and to thiobenzophenone.[5] This latter process approximates more closely than any other model reaction yet available the enzymatic reduction of ketones by DPNH. This paper reports our tracer and kinetic studies on the reaction shown in equation (1) and presents a mechanism for the process.

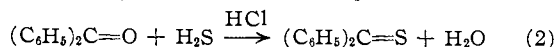


(1)

In the course of this work, a number of new substituted thiobenzophenones were prepared. Moreover, in our hands, the preparative methods recorded in the literature for known thioketones usually failed to give pure products, and the compounds, when finally purified, had physical properties (ultraviolet spectra, melting points) at variance with those recorded earlier. By far the best (and occasionally the only) method of purification of substituted thiobenzophenones discovered in the course of this work consists in chromatography on Florisil, and subsequent elution with carbon tetrachloride, chloroform or methylene chloride.

Experimental

Materials.—**Thiobenzophenone** was prepared by Staudinger's earlier method[6] according to equation 2. His later preparation (presented in ref. 7) consistently gave a mixture of benzophenone and thiobenzophenone; the two compounds proved very difficult to separate by fractional crystallization, especially since thiobenzophenone is sensitive to



$$(C_6H_5)_2C{=}O + H_2S \xrightarrow{HCl} (C_6H_5)_2C{=}S + H_2O \quad (2)$$

air, and the crystallizations therefore had to be conducted in a dry-box under an atmosphere of nitrogen. In a typical preparation, 1 g. of benzophenone was dissolved in 10 ml. of 95% alcohol and cooled in an ice-bath. Slow streams of dry HCl and of dry H_2S were passed into the mixture for about 4 hr. Then the HCl stream was shut off, and the H_2S continued for 8 more hours. The reaction mixture was maintained in an ice-bath during the reaction time. The thiobenzophenone separated from the solution in dark blue crystals and was collected by filtration under nitrogen. The infrared spectrum of the compound is shown in Fig. 1; the carbonyl band for benzophenone at 5.95 μ is almost totally lacking, in marked contrast to products prepared by the method of reference 7, where this band was strong. The bands at 7.95, 8.27, 9.55 and 11.27 μ are not present in the spectrum of benzophenone itself. The compound, in 0.006 *M* solution in 95% alcohol, gave an extinction coefficient at 595 mμ of 167; this figure is about three times that recorded in the early literature[8] and about 25% greater than the figure recently reported by Sartori and Furlani.[9]

Anal. Calcd. for $C_{13}H_{10}S$: S, 16.16. Found: S, 15.88.

***o*-Methoxythiobenzophenone** was prepared by Staudinger's method.[6,9] HCl and H_2S were passed into an alcoholic solution of ketone[10] for 6 hr. The blue thioketone separated from solution and was recrystallized from 95% EtOH; m.p. 70–71°. The compound showed an extinction coefficient of 168 at 600 mμ.

Anal. Calcd. for $C_{14}H_{12}OS$: C, 73,69; H, 5.26; S, 14.04. Found: C, 73.27; H, 5.39; S, 13.77.

***p*-Hydroxythiobenzophenone** was similarly prepared from recrystallized *p*-hydroxybenzophenone (Eastman Tech.). In our hands, the published preparative method[9] failed to give a pure product; even after several recrystallizations, the material still showed a small amount of ketonic impurity (infrared spectrum). The melting point (115°), although in agreement with the literature,[9] was not sharp. The pure thioketone could be obtained, however, by chromatography. The impure reaction product was dissolved in methylene chloride, placed on a column filled with Florisil (Floridin Co., Tallahassee, Florida) and the column developed with more methylene chloride. The resulting blue solution, on evaporation, gave a red solid, m.p. 140°, with an extinction coefficient of 193 at 580 mμ in 95% EtOH; the carbonyl band was almost totally absent from the infrared spectrum.

Anal. Calcd. for $C_{13}H_{10}OS$: S, 14.96. Found: S, 14.96.

***o*-Hydroxythiobenzophenone** was prepared from *o*-hydroxybenzophenone[11] by the method outlined above for thiobenzophenone itself. The stream of H_2S was passed through the alcoholic solution of the ketone and HCl for 2 days. The solution turned dark red; when the alcohol was evaporated, a viscous red oil was obtained. Infrared spectra showed that this oil was a mixture which contained approximately equal quantities of thioketone and starting material. The compounds could be separated easily and quantitatively by chromatography of a carbon tetrachloride solution of the mixture on Florisil. When the chromatogram was developed with carbon tetrachloride, the ketone formed a yellow band at the top of the column, which separated

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(3) B. Vennesland and F. H. Westheimer, in McElroy and Glass, "The Mechanism of Enzyme Action," Johns Hopkins Press, Baltimore, Md., 1954, p. 357; B. Vennesland, *Discussions of Faraday Soc.*, **20**, 240 (1955).

(4) D. Mauzerall and F. H. Westheimer, THIS JOURNAL, **77**, 2261 (1955).

(5) R. H. Abeles and F. H. Westheimer, *Federation Proc.*, **15**, 675 (1956).

(6) H. Staudinger and H. Freudenberg, *Ber.*, **61**, 1576 (1928).

(7) H. Gilman, "Organic Syntheses," Vol. II, John Wiley and Son, Inc., New York, N. Y., p. 573.

(8) A. E. Gillam and E. S. Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," Edward Arnold, London, 1954.

(9) G. Sartori and C. Furlani, *Ann. Chim. (Rome)*, **44**, 95 (1954).

(10) R. Stoermer and E. Friderici, *Ber.*, **41**, 324 (1908).

(11) P. Pfeiffer and W. Loewe, *J. prakt. Chem.*, **147**, 293 (1936).

cleanly from the red band of thioketone which moved down the column more rapidly. Evaporation of the carbon tetrachloride left behind a red oil, which gave an infrared spectrum (Fig. 1) almost devoid of the carbonyl band. The extinction coefficient for the ketone in CCl_4 solution was 220 at 550 mμ.

Anal. Calcd. for $C_{13}H_{10}OS$: C, 72.84; H, 4.70; S, 14.96. Found: C, 72.64; H, 4.67; S, 14.68.

p-Methoxythiobenzophenone.—*p*-Methoxybenzophenone was prepared from the sodium salt of *p*-hydroxybenzophenone and dimethyl sulfate; the compound[12] melted at 61–62°. It was converted to the thioketone with H_2S and HCl by the same method as that used for thiobenzophenone and purified by chromatography over Florisil. The extinction coefficient for the blue oil at 585 mμ is 252.

Anal. Calcd. for $C_{14}H_{12}OS$: S, 14.04. Found: S, 13.82.

p-Chlorothiobenzophenone was prepared from *p*-chlorobenzophenone (Harvard Research stores) with H_2S and HCl and purified by chromatography over Florisil. The extinction coefficient of the blue oil is 186 at 600 mμ in 95% EtOH.

Anal. Calcd. for $C_{13}H_9ClS$: S, 13.78. Found: S, 13.67.

p-Nitrobenzoate of Benzhydryl Thiol.—Benzhydryl thiol was prepared[13] from diphenyldiazomethane and H_2S and purified by vacuum distillation. A gram of the thiol reacted readily with 1.5 g. of *p*-nitrobenzoyl chloride; the procedure parallelled that of Wertheim.[14] The thioester, after crystallization from ethanol, melted at 85°.

Anal. Calcd. for $C_{20}H_{15}NO_3S$: S, 9.18. Found: S, 9.01.

N-Benzyl-1,4-dihydronicotinamide and 4-deutero-1-benzyl-1,4-dihydronicotinamide were prepared as in our earlier work[4]; the latter showed on analysis 0.9 atom of deuterium per molecule. **N-Tetraacetylglucosyl-1,4-dihydropyridine** (m.p. 151°) was prepared according to Karrer.[15] The "70%" ethanol for the kinetic experiments was prepared by adding water to 350 ml. of absolute alcohol (Gold Shield) to bring the total volume to 500 ml.

Buffers were prepared by dissolving the proper amounts of amine and amine hydrochloride in "70%" ethanol. Tetrahydrofuran, refluxed with and distilled from KOH, was redistilled from $LiAlH_4$ immediately before use. Other chemicals were of reagent grade.

Tris-(hydroxymethyl)-aminomethane (Sigma 7-9, Sigma Chemical Co.) was recrystallized from methanol-water. "Tris"-hydrochloride was prepared by completely dissolving 50 g. of "tris" in 50 ml. of concentrated hydrochloric acid; the crystals which separated on cooling were washed generously with acetone, ether and dried over $CaCl_2$ and NaOH *in vacuo*. Potentiometric titration for hydrogen ion and Volhard titration for chloride ion of the prepared 1:1 "tris" buffer solution showed 0.996 meq./ml. by both methods.

2,6-Lutidine purified by the method of Brown, *et al.*,[16] had $n^{26}D$ 1.4966, b.p. 145° (lit.[16] $n^{25}D$ 1.4953, b.p. 143.4° (740 mm.)). The authors are indebted to Dr. Martin Saunders (Yale University) for a vapor phase chromatogram of the lutidine; the compound showed only a single peak. Addition of anhydrous hydrogen chloride to an ethereal lutidine solution precipitated lutidine hydrochloride, which was washed with ether and dried *in vacuo* over $CaCl_2$ and KOH.

Products.—A solution of 222 mg. of thiobenzophenone (77% purity by spectrophotometric analysis) and 202 mg. of N-benzyl-1,4-dihydronicotinamide in 6 cc. of 95% ethanol was added to 1 cc. of a 4 *M* aqueous acetate buffer at *p*H 4.9. A slow stream of nitrogen was passed through the reaction mixture. After 2.5 hr., an additional 98 mg. of the dihydro compound was added. After 3 more hr. at room temperature, the intense blue color of the thioketone had faded to a yellowish green; 12 hr. later the solution was yellow. The solution was filtered to remove about 6 mg. of solid (the disulfide; see below) and the filtrate evaporated to dryness. The residue was extracted with ether to give a solution, Q, and a residue, R. The ether was evaporated from Q, and the resulting yellow oil dissolved in alcohol and oxidized with a 0.2 *M* alcoholic solution of iodine; 43 mg. (0.34 millimole) of iodine was required to reach the end-point. During and after the oxidation a white solid (81.4 mg. or 0.21 millimole of disulfide) separated from the solution. The thiobenzhydryl disulfide (m.p. 151–152°) proved identical (infrared spectrum) with an authentic sample.[6] The benzhydryl mercaptan from Q was also identified as its *p*-nitrobenzoate, m.p. 83–84°.

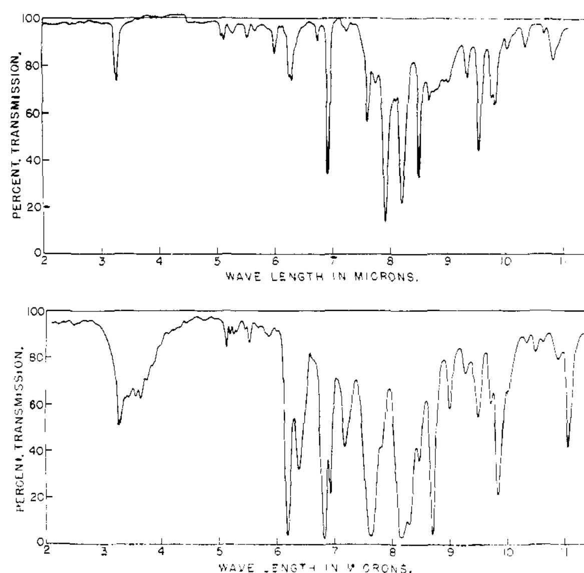


Fig. 1.—Upper spectrum, thiobenzophenone, lower spectrum *o*-hydroxythiobenzophenone.

The residue, R, was water soluble and showed an ultraviolet spectrum identical with that of nicotinamide N-benzoyl chloride. From the intensity of the absorption at 265 mμ, it was calculated that 0.54 millimole of the cation was present. No attempt was made to isolate the salt, but it was directly reduced[4] with $Na_2S_2O_4$ to give 69 mg. (0.32 millimole) of N-benzyl-1,4-dihydronicotinamide, identified by comparison of its melting point and infrared spectrum with those of an authentic sample.

Deuterium Transfer.—The isolation experiment outlined above was repeated with 4-deutero-N-benzyl-1,4-dihydronicotinamide. The disulfide so obtained was burned and the resulting water converted to hydrogen for mass-spectrometric analysis of deuterium.[4,17] The resulting disulfide showed 0.17–0.18 atom of deuterium per molecule. The fractionation of hydrogen and deuterium during the reduction was about the same as that previously encountered for the reduction of malachite green; the rate factor k_H/k_D is about 4 to 5.

The isolation experiment was then carried out with ordinary N-benzyl-1,4-dihydronicotinamide in D_2O as solvent. The resulting disulfide showed only 0.006 atom of D per molecule.

Reductions with DPNH.—Thiobenzophenone (0.0042 g.) and DPNH (0.030 g., Sigma Co.) were dissolved in 3 ml. of 60% alcohol containing a 1-1 tris buffer. The blue color of the thiobenzophenone disappeared. at room temperature, with a half-time of 25 minutes; the rate was therefore greater than, but comparable to, that with N-benzyldihydronicotinamide.

Kinetic Method.—The thioketones were stored at −20° under nitrogen in small ampoules. For each series of experiments (conducted on the same day) the contents of a freshly opened ampoule were dissolved in "70"% ethanol in a 10-ml. volumetric flask swept with nitrogen and fitted with a rubber serum cap. Standard solutions in the same solvent were prepared from N-benzyldihydronicotinamide, freshly recrystallized from alkaline ethanol. The absorption cells of 6-ml. capacity were constructed from square

(12) *Cf.* E. H. Rennie, *J. Chem. Soc.*, **41**, 220 (1882).

(13) H. Staudinger and J. Siegwert, *Ber.*, **49**, 1918 (1916).

(14) E. Wertheim, THIS JOURNAL, **51**, 3661 (1929).

(15) P. Karrer, B. H. Ringier, J. Buchi, H. Fritzsche and U. Solmssen, *Helv. Chim. Acta*, **20**, 55 (1937).

(16) H. C. Brown, S. Johnson and H. Podall, THIS JOURNAL, **76**, 5556 (1954).

(17) R. B. Alfin-Slater, S. M. Rock and M. Swislocki, *Anal. Chem.*, **22**, 421 (1950).

Pyrex tubing fitted with $^7/_{15}$ ₮ joints, which would accommodate either a ₮ stopper or a rubber serum cap.

For a typical experiment, the buffer, N-benzyldihydronicotinamide and other components (except thioketone) were pipetted into the cell and the volume made up to 4 ml. The cell was repeatedly swept with nitrogen and shaken, fitted with a serum cap and allowed to come to a temperature in the thermostated cell compartment of a Beckman model DU quartz spectrophotometer. To initiate a "run," 1 ml. of thioketone solution (at thermostat temperature) was rapidly injected with a 2 ml. syringe through the serum cap into the absorption cell; mixing was essentially instantaneous and complete. The reaction was followed by measuring, at stated time intervals, the optical density at the wave length of maximum absorption in the visible (595 mμ for thiobenzophenone). The results of a typical experiment are shown in Table I.

TABLE I

TYPICAL KINETIC EXPERIMENT

[Dihydronicotinamide]$_0$ = 0.039 M, T = 30.15°, [Thiobenzophenone]$_0$ = 0.0042 M, μ = 0.10

Time, min.	Optical dens., 595 mμ	Time, min.	Optical dens. 595 mμ
0	0.710 (extrap.)	12	0.363
1	.673	14	.327
2	.636	16	.296
3	.600	19	.252
4	.565	22	.217
6	.502	25	.186
8	.453	29	.153
10	.406		

Control experiments were conducted identical with those described above except that the N-benzyldihydronicotinamide was omitted; no diminution of the thioketone concentration was observed. Control experiments with N-benzyldihydronicotinamide but without thioketone showed that the acid-catalyzed decomposition of the dihydro compound[18] was negligible in the "tris" buffers and less than 6% (during two half-lives) in the lutidine buffer.

A considerable number of experiments was carried out with lower precision; here only the approximate half-times are available. These experiments were conducted in stoppered Corex cells which had been flushed with nitrogen. Each cell contained 1 ml. of 1 M acetate buffer "pH" 5.2. The reactants were added in 2 ml. of 95% ethanol. The final solutions were 0.017 M in N-benzyl-1,4-dihydronicotinamide; the solvent contained 70% ethanol. The rate of disappearance of the thioketone was followed in the Beckman spectrophotometer.

Oxygen Effect.—In order to determine the effect of oxygen on the oxidation–reduction process, the reaction was conducted in vacuum. The apparatus is shown in Fig. 2; bulb C was constructed of square Pyrex tubing to fit the spectrophotometer cell-holder. The solutions of buffer and dihydro compound in bulb C and the solvent in bulb B were degassed in high vacuum. Then thiobenzophenone from bulb A was distilled into the solvent. The apparatus was sealed, detached from the vacuum line and thermostated; the reaction was begun by mixing the solutions. The rate in high vacuum was essentially the same as that in a parallel control "run."

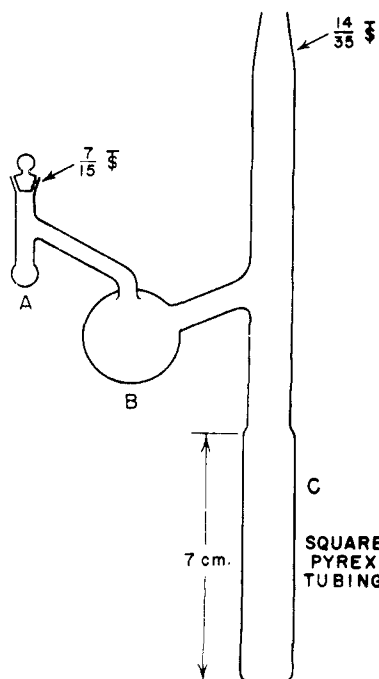


Fig. 2.—Vacuum apparatus.

(18) P. Karrer, G. Schwartzenbach, F. Benz and U. Solmssen, *Helv. Chim. Acta*, **19**, 811 (1936).

Results

The kinetics for the reduction of thiobenzophenone by N-benzyl-1,4-dihydronicotinamide are presented in Table II.

The rates of reduction of other thioketones are given in Table III.

A comparison of the reducing properties of N-benzyl-1,4-dihydronicotinamide and of N-tetraacetyl-1,4-dihydropyridine is presented in Table IV.

Discussion

The mechanism for the "model reaction" can be deduced from the available data. The reaction is strictly second order, as can be seen from Table II. The original concentrations of the thioketone and of the dihydro compound have been varied separately by factors of five without appreciably changing the second-order rate constants. The rate of the reaction is unaffected by excluding oxygen from the solutions, and it is unaffected by the variations in oxygen concentration, from run to run, which must necessarily occur when only the simplest precautions are taken to remove oxygen from the spectrophotometer cells. Further, typical free-radical chain breakers are without effect upon the reaction rate. In all probability, then, the reaction does not involve a free-radical chain process.

The reaction is largely unaffected by changes in pH, as is shown in Table II. The "pH" here recorded refers to the reading on the glass electrode of a Beckman pH meter and therefore refers to a comparison of two half-cells, one in water and the other in 70% ethanol as solvent. However, the "pH" readings were standardized against 0.001 M HCl in the 70% ethanol; this solution was assigned a "pH" of 3.00. The readings may not be accurate, but they should at least serve for internal comparison with moderate precision. The data show that no additional proton is present in the activated complex for the reaction.

Although there is no pH effect, the reaction clearly proceeds more rapidly in the more polar solvents. Chloroform or tetrahydrofuran as replacement for ethanol, slows the reaction down. The activated complex is therefore more polar than the reactants.

The effect of thioketone structure on the rate shows that electron-donating substituents slow down the rate, whereas an electron-withdrawing

TABLE II

REDUCTION OF THIOBENZOPHENONE IN 70% ETHANOL AT 30.1 ± 0.1°

Dihydro-compound, mole/l.	Thioketone, mole/l.	Buffer,[a] mole/l.	Other component	Concn., mole/l.	μ	k_2, l./mole sec.
0.0195	0.0041	0.100			0.100	0.0236
.039	.0042	.100			.100	.0232
.0585	.0042	.100			.100	.0232
.117	.0041	.100			.100	.0227
.078	.0045	.100			.100	.0238
.078	.0100	.100			.100	.0220
.078	.0790	.100			.100	.0226
.078	.0350	.100			.100	.0235
.039	.0043	.020			.020	.0229
.039	.0039	.200			.200	.0256
.039	.0043	.400			.400	.0273
.039	.0045	.600			.600	.0291
.039	.0043	.100	$NaClO_4$	0.100	.200	.0256
.039	.0042	.100	$NaClO_4$	.200	.300	.0260
.039	.0043	.100	$NaClO_4$	.400	.500	.0269
.039	.0047	.100	$NaClO_4$	1.00	1.10	.0298
.039	.0048	.100	*p*-$(CH_3)_2NC_6H_4N(CH_3)_2$	0.0006	0.100	.0237
.039	.0042	.100	$(Et_2NCS_2)_2$	.0004	.100	.0234
.039	.0044	.100	*p*-$O_2NC_6H_4NHC_6H_5$	.0002	.100	.0233
.039	.0048	.100	2-Mercaptobenzothiazole	.0002	.100	.0237
.039	.0048	.100	Hydroquinone	.0005	.100	.0231
.039	.0041	.100[b]	(1:10 Tris)		.100	.0232
.039	.0042	.100[c]	(Lutidine)		.100	.0200
.039	.0040	.100	$CHCl_3$	10%	.100	.0119
.039	.0048	.100	$CHCl_3$	20%	.100	.00674
.039	.0048	.100	Tetrahydrofuran	20%	.100	.00974
.039	.0048	.100	Tetrahydrofuran	40%	.100	.00484
	.0048	.100	Deuterated dihydro-	0.039	.100	.0140
	.0049	.100	compound	.078	.100	.0139

[a] 1:1 buffer of "tris" and "tris"-hydrochloride, "*p*H" 7.67. [b] 1:10 buffer of "tris" and "tris"-hydrochloride. [c] 1:1 buffer of 2,6-lutidine and 2,6-lutidine hydrochloride, "*p*H" 5.08.

TABLE III

RATES OF REDUCTION OF SUBSTITUTED THIOBENZOPHENONES AT 30.1 ± 0.1° IN 0.1 *M* LUTIDINE BUFFER, "*p*H" 5.08

Substituent	Concn., mole/l.	Dihydro-compound, mole/l.	μ	k_2, l./mole sec.
p-Hydroxy	0.0033	0.117	0.100	0.00195
p-Methoxy	.0036	.117	.100	.00447
o-Methoxy	.0038	.039	.100	.0154
None (Table II)				.023
o-Hydroxy	.0030	.039	.100	.0539
p-Chloro	.0034	.039	.100	.0603

TABLE IV

RATE OF REDUCTION OF THIOBENZOPHENONE BY DIHYDRO-COMPOUNDS AT 25°

Thioketone, 10^{-2} mole/l.; dihydro-compound, 4.4×10^{-3} mole/l.

Reductant	Estimated half-time, minutes
N-Benzyldihydronicotinamide	100
N-Tetraacetylglucosyldihydropyridine	60

substituent accelerates it. Apparently, an increase in the partial positive charge on the carbon atom of the thiocarbonyl group causes an increase in rate. An exception to this rule is presented by *o*-hydroxythiobenzophenone, which is reduced more rapidly than thiobenzophenone itself. The significance of this finding is discussed below.

The carboxamide group of the dihydropyridine compound is unnecessary to the model reaction. The reduction goes as rapidly with N-tetraacetylglucosyl-1,4-dihydropyridine as with N-benzyl-1,4-dihydronicotinamide. The tetraacetylglucosyl compound was chosen in preference to the benzyl for this experiment because Karrer[19] had found difficulty in preparing the N-alkyl-1,4-dihydropyridines, whereas the tetraacetylglucosyl derivative can be prepared by the same techniques available for the reduction of the nicotinamides.

Finally, the experiments with the deuterium analog of the dihydro compound proceed only about 60% as fast as with the hydrogen compound. Since the deuterium compound still contains, at the reactive 4-position of the pyridine ring,[4,20] a hydrogen as well as a deuterium atom, this decrease in rate corresponds to a rate factor k_H/k_D of about 4 to 5. Therefore, a hydrogen (or deuterium) atom is transferred in the rate-controlling step of the reaction.

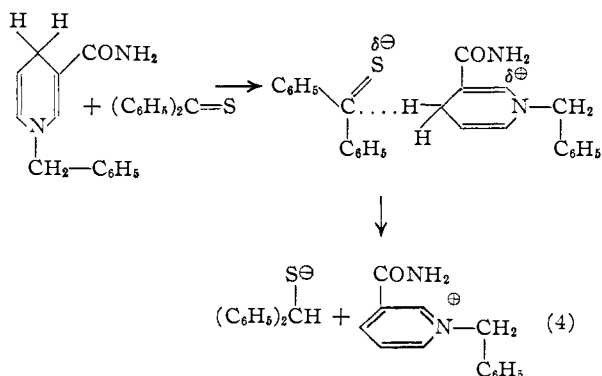
All these data can be explained in terms of the direct transfer of a hydride ion (a hydrogen atom accompanied by its electron pair) from the dihydro compound to the thioketone according to equation 4.

A hydrogen atom is transferred in the rate-controlling step (deuterium effect), and the activated complex is highly polar (solvent effect). The sub-

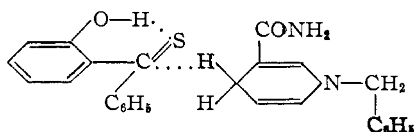
(19) P. Karrer, F. W. Kahnt, R. Epstein, W. Jaffe and T. Ishii, *Helv. Chim. Acta*, **21**, 223 (1938).

(20) M. E. Pullman, A. San Pietro and S. P. Colowick, *J. Biol. Chem.*, **206**, 129 (1954).

stituent effect is generally in the direction predicted; a *p*-methoxy group, for example, stabilizes the reactant (*p*-methoxythiobenzophenone) more than it stabilizes the activated complex. Further, no special role has been assigned to the carboxamide group. The effect of the *o*-hydroxyl group in ac-



celerating the reaction, in contrast to the effect of *o*-methoxy and of *p*-hydroxy groups in slowing it down, can be accounted for in terms of the activated complex



The hydrogen bonding stabilizes the incipient mercaptide anion and thereby overcomes the resonance effect of the *o*-hydroxyl group on the reaction rate.

Several other mechanisms for the reaction were considered and rejected. Mechanisms which required prior attachment of the thioketone group to the pyridine ring cannot be formulated without either adding a proton to the ring or placing a negative charge in the carboxamide group. Neither of these is consistent with the experimental data, since the reaction rate is independent of *p*H and the carboxamide group is not essential to the reaction. The mechanism advanced by Burton and Kaplan[21] for the enzymatic process appears unlikely, since there are no chemical analogs for an internal displacement of oxygen by hydrogen.[22]

Presumably the reason why the reduction has been successful with a thioketone but not (as yet) with a ketone is that the thiocarbonyl group is more polar than is the carbonyl group. Evidence for this greater polarity has been obtained from a preliminary study of the rates of semicarbazone and phenylhydrazone formation from thiobenzophenone and from benzophenone. The reaction with the former is faster by several orders of magnitude.[23]

Of course, there is no assurance as yet that the non-enzymatic and the enzymatic reactions proceed by similar mechanisms. But perhaps the major function of the enzyme is to polarize the carbonyl group of the carbonyl compound and so make it more like the thiocarbonyl group. Since alcohol dehydrogenase contains zinc,[24] the zinc atom may perhaps coördinate at the carbonyl group and provide the needed polarization. This and other possibilities are currently under investigation.

Acknowledgment.—The authors wish to express their appreciation to the Mallinckrodt Fund and to the American Cancer Society for financial assistance in this work.

(21) R. M. Burton and N. O. Kaplan, *J. Biol. Chem.*, **211**, 447 (1954).

(22) The Cannizzaro reaction is not a case in point; the probable mechanism for this reaction, as outlined by Hammett ("Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 350), does not involve any unusual steps.

(23) J. C. Powers, unpublished results.

(24) B. L. Vallee and F. L. Hoch, *Proc. Natl. Acad. Science*, **41**, 327 (1955).

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