[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

On the Mechanism of Thiamine Action. IV.[1] Evidence from Studies on Model Systems

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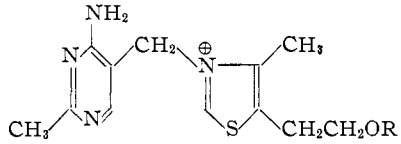
RECEIVED JANUARY 14, 1958

A rather stable thiazolium zwitterion has been detected by deuterium exchange studies, and a mechanism of thiamine action is suggested which involves such a zwitterion. It is shown that the mechanism accommodates the data which have been obtained by study of model systems for biochemical reactions catalyzed by thiamine.

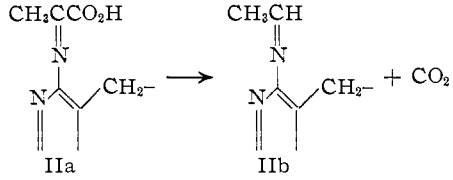
Thiamine (Ia), in the form of its pyrophosphate, cocarboxylase(Ib), is the coenzyme for a number of important biochemical reactions, including the decarboxylation of pyruvic acid to acetaldehyde, the conversion of pyruvic acid to acetoin and the transketolase reaction; it is also involved in the oxidative decarboxylation of pyruvic acid to "active acetate."[2]



Ia, R = H
b, R = P(O)OP(O)OH
OH OH

These reactions all have one common feature in that they can be considered to involve the formation of an intermediate acyl carbanion, R–CO(−), or some stabilized equivalent. This type of intermediate has been called an "active aldehyde."[3]

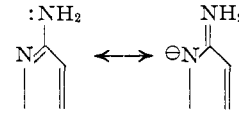
There have been several suggestions as to the possible role of thiamine in facilitating these reactions. One of the earliest proposals was that of Langenbeck, who considered in particular the decarboxylation of pyruvic acid to acetaldehyde. He suggested[4] that the amino group of thiamine condenses with pyruvic acid to form a Schiff base IIa which decarboxylates to the Schiff base of acetaldehyde IIb, this latter compound then hydrolyzing to the free aldehyde and regenerated thiamine.



IIa IIb

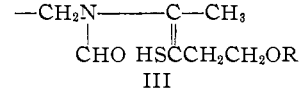
Although his suggestion was based on model studies in which various amines will in fact catalyze the decarboxylation of pyruvic acid *via* Schiff base formation, Stern and Melnick showed[5] that thiamine itself was not effective under the same conditions; these authors concluded that this fact casts doubt on the Langenbeck proposal for the mechanism of thiamine-catalyzed decarboxylations.

In discussing the reason for the failure of thiamine to react under conditions where other primary amines are active, the same authors point out a property of thiamine which is often overlooked. There is a considerable amount of evidence that the amine group of thiamine is quite unreactive, presumably due to resonance contributions of the form



and would not be expected to form a Schiff base readily. A minor objection to the Langenbeck proposal is that it suggests no role for the thiazole ring of thiamine, which is perhaps the most unusual feature of the molecule.

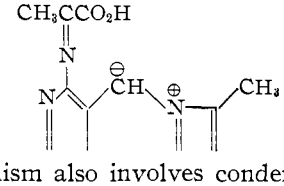
Karrer has suggested[6] that thiamine may act in a form in which the thiazole ring has opened to free a sulfhydryl group (III).



III

In view of the known ring-opening reactions of thiamine, the possibility must of course be kept in mind that an open form of the molecule could be the actual catalytic species.

Valenta and Wiesner have recently suggested[7] that thiamine forms a Schiff base with pyruvic acid, as in Langenbeck's scheme, and that this tautomerizes *via* an ylid of the type



This mechanism also involves condensation on an unreactive amino group of thiamine, but does suggest a role for the thiazole portion of the molecule in helping to stabilize the ylid. Moreover, a very similar type of tautomerism is likely for many of Langenbeck's catalysts, such as the 3-aminooxindoles, although tautomerism cannot explain all of his cases (*e.g.*, catalysis by aniline).

Most of such speculations on the chemical mechanism of thiamine action suffer from the disadvantage that up until recently there has been no good way to examine them experimentally, for the results from biological testing of analogs are diffi-

(1) For the earlier papers of this series, see (a) R. Breslow, *Chemistry & Industry*, B.I.F. Review, R 28 (1956); (b) THIS JOURNAL, **79**, 1762 (1957); (c) *Chemistry & Industry*, 893 (1957).

(2) The functions of thiamine have been reviewed by B. Jansen, *Vitamins and Hormones*, **7**, 83 (1949).

(3) See, for instance, E. Racker in "The Mechanism of Enzyme Action," W. McElroy and B. Glass, ed., Johns Hopkins Press, Baltimore, Md., 1954, p. 470.

(4) W. Langenbeck, "Die Organischen Katalysatoren," Julius Springer, Berlin, 1935, p. 55 ff.

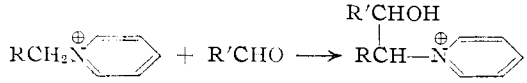
(5) K. Stern and J. Melnick, *J. Biol. Chem.*, **131**, 597 (1939).

(6) P. Karrer, *Bull. soc. chim. France*, 149 (1947).

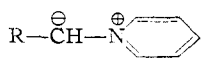
(7) K. Wiesner and Z. Valenta, *Experientia*, **12**, 192 (1956).

cult to interpret, influenced as they are by enzyme specificity. More recently, however, model systems have been found in which the action of thiamine can be studied in the absence of any enzyme.

It has long been known, as the result of extensive work by Kröhnke and his colleagues, that certain pyridinium compounds will condense readily with aldehydes.[8]



Such condensations must occur *via* ylids, of the type



Ugai reasoned that, in view of the similarities[9] between pyridine and thiazole, thiazolium compounds should also condense with aldehydes. However, when he and his co-workers heated an alcoholic solution of N-ethylthiazolium bromide (VII) with benzaldehyde in the presence of one mole of base, the product was not the expected adduct VIII, but benzoin.[10] Thus the thiazolium

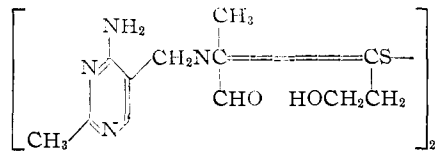
VII VIII

compound had apparently acted as a catalyst for the self-condensation of benzaldehyde to benzoin. A number of thiazolium compounds were found to share this catalytic ability, including thiamine itself. His results are included in Table I.

Mizuhara recognized the fact that in this system thiamine was catalyzing a reaction of the same type as those involved in its biological action, since the benzoin condensation can also formally be regarded as involving $R\text{–}CO^{(-)}$ or some stabilized equivalent, and he reasoned that a modification of the reaction of Ugai would show this fact more clearly. Mizuhara demonstrated that thiamine would act at room temperature in aqueous solution at *p*H 8.4 to decarboxylate pyruvic acid.[12] It also catalyzed the formation of acetoin from pyruvic acid and acetaldehyde, and catalyzed the reaction of biacetyl and acetaldehyde to form acetate and acetoin. These are all reactions which have been observed with enzymatic systems involving thiamine, but in this case no enzyme is used.

Although it is always extremely difficult to be sure that any "model" system for an enzymatic process acts by a mechanism similar to that of the enzyme system, it seems quite likely that this reaction developed by Mizuhara is in fact a good model. Thus reaction occurs at room temperature and in aqueous solution, and the system catalyzes *several* of the reactions known to involve thiamine enzymatically. In addition, it shows a *p*H optimum at 8.4.[12] Although this is higher than normal physiological *p*H's, there is good reason to believe that the *effective* *p*H at an enzyme surface can be higher than that measurable in solution, so that these conditions may duplicate fairly closely the conditions of the biochemical reactions. Thus it seemed very likely that the mechanism of thiamine action in an enzymatic system is closely related to the mechanism of action of this non-enzymatic system; studies on the latter accordingly became of considerable interest.

Mizuhara had found that thiamine pyrophosphate (Ib) was also effective as a catalyst, but that thiamine disulfide (IX) was not.



IX

He showed that the reaction exhibits a very pronounced optimum at *p*H 8.4, which is near the *pK* of pseudo-base formation by thiazolium compounds, and proposed an unusual and rather unlikely mechanism involving the *pseudo*-base of thiamine.

TABLE I

CATALYSIS OF THE BENZOIN CONDENSATION

	Catalyst	Method[a]	Result
A	3-Benzylthiazolium bromide	a	–
B	3-Ethylthiazolium bromide	a	–
C	3-Ethylthiazolium iodide	a	–
D	3-Benzyl-4-methylthiazolium bromide	a,d	+
E	Thiamine chloride hydrochloride	a,c	+
F	3-Ethyl-4-methylthiazolium iodide	a	+
G	3-Ethyl-4-methylthiazolium bromide	c	+
H	3,4-Dimethylthiazolium bromide	d	+
I	3-(β-Hydroxy-β-phenylethyl)-4-methylthiazolium bromide	c,d	+
J	3-Benzyl-2,4-dimethylthiazolium bromide	a	–
K	3-Phenacylthiazolium bromide	a,d	–
L	3-*p*-Bromophenacylthiazolium bromide	a	–
M	3-Benzyl-4-phenylthiazolium bromide	a	–
		d	+
N	3-Phenyl-4-methylthiazolium perchlorate	a	–
O	3-Isopropyl-4-methylthiazolium iodide	c	–
P	3-Methylbenzothiazolium iodide	b,d	–
Q	2,3-Dimethylbenzothiazolium iodide	b	–
R	1,3,4-Trimethylimidazolium iodide	b	+
S	1,3-Dimethylbenzimidazolium iodide	b,d	+
T	1,2,3-Trimethylbenzimidazolium iodide	b	+
		d	–
U	3-Methylbenzoxazolium iodide	b	–
V	2,3-Dimethylbenzoxazolium iodide	b	–
W	2,3-Dimethylbenzoselenazolium iodide	b	–
X	1,2-Dimethylpyrazolium iodide	b	–
Y	Thiazole	a	–

[a] a, Ugai, *et al.*, ref. 10. b, Ref. 11. c, Present author, benzoin test, see Experimental. d, Furoin test, see Experimental.

(8) See F. Kröhnke, *Angew. Chem.*, **65**, 605 (1953), for a review with references.

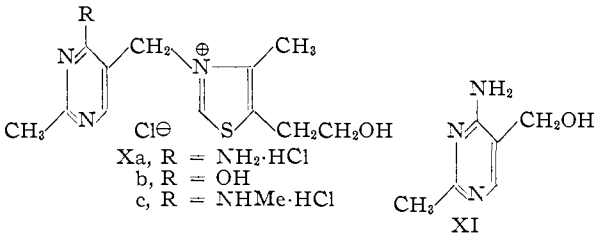
(9) *Cf.* R. Rambaud in V. Grignard, "Traite de Chimie Organique," Vol. XXI, Masson et Cie, Paris, 1953, p. 445.

(10) T. Ugai, S. Tanaka and S. Dokawa, *J. Pharm. Soc. Japan*, **63**, 269 (1943).

(11) T. Ugai, T. Dokawa and S. Tsubokawa, *ibid.*, **64**, 7A, 3 (1944).

(12) S. Mizuhara, R. Tamura and H. Arata, *Proc. Japan Acad.*, **27**, 302 (1951); see also S. Mizuhara and P. Handler, THIS JOURNAL, **76**, 571 (1954).

In the present work a number of additional compounds have been tested as catalysts for the acyloin reaction. It has been found that pyruvic acid was not necessary, as thiamine will catalyze the self-condensation of acetaldehyde to acetoin, again as in the biological systems, but the yield of acetoin is somewhat higher with the pyruvic acid–acetaldehyde mixture, so Mizuhara's conditions were used. The results are shown in Table II.



XI

TABLE II

Catalyst	Micrograms acetoin (Mean)
Thiamine chloride hydrochloride (Xa)	300
Oxythiamine chloride (Xb)	15
N-Methylthiamine chloride hydrochloride (Xc)	200
N-Benzyl-4-methylthiazolium chloride	100
N-Benzyl-4-methylthiazolium bromide	90
N-Allyl-4-methylthiazolium bromide	50
N-Ethyl-4-methylthiazolium bromide	15
N-Propyl-4-methylthiazolium bromide	20
4-Amino-5-hydroxymethyl-2-methylpyrimidine (XI)	15
No catalyst	15

On the basis of such data we have suggested[1a] a mechanism of thiamine action in which pyruvic acid first condenses on the N-methylene group of the catalyst by analogy with the reactions of pyridinium compounds, but Westheimer and Ingraham have reported[13] that deuterium incorporation studies on thiamine rule this mechanism out. To check their conclusions, we have synthesized N-benzyl-(α-d_2)-thiazolium bromide and employed it as catalyst for the furoin condensation. As deuterium is not lost during the reaction, it is clear that catalysis does not involve condensation on the N-methylene group, in corroboration of the conclusions of Westheimer and Ingraham.

We are thus forced to look elsewhere in the molecule for a site of potential reactivity.

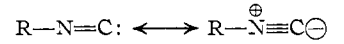
It is well known that anions on triply-bonded carbon are relatively stable. The acidity of acetylene and of hydrogen cyanide furnish familiar examples, and have been ascribed to increased s-character of the C–H bond compared with that at a saturated carbon.[14] In the anions there is also opportunity for resonance with a "carbene" form, which may be a significant factor; *e.g.*, $\overset{\ominus}{C}\equiv CH \longleftrightarrow :C=\overset{\ominus}{C}H$. That hydrogen cyanide is more acidic than is acetylene is presumably due to the greater electronegativity of nitrogen compared with carbon, which should help stabilize the anion, both by induction and by placing the negative charge of the carbene form on an electronegative element.

There is good evidence that anions at doubly-bonded carbon can also be relatively stable. Thus, Hammick has shown[15] that α-picolinic acid decarboxylates rather readily. His studies on the influence of *p*H on the reaction suggest that the intermediate is not the simple anion XII but rather the dipolar ion XIII. Moreover, quaternization of the nitrogen leads to compounds which decarboxylate especially easily, and it seems that stabilization of an anion by the double bond is markedly assisted by the inductive effect of a positively-charged nitrogen (or alternatively, by the fact that the "carbene" resonance form is neutral).

XII XIII

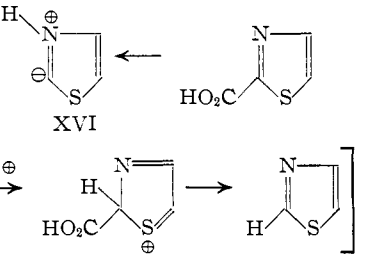
It seems that similar factors explain the decarboxylation of those Schiff bases of α-ketoacids in which tautomerism is not possible, such as the Schiff bases formed with aniline. Here again an anion on a doubly-bonded carbon is presumably involved, the protonated nitrogen furnishing a big inductive boost to the stabilization. That α-ketoacids themselves do not decarboxylate so readily probably is due to the lesser tendency for oxygen to protonate, so that decarboxylation may lead to a stable zwitterion.

Similarly, one should expect that quaternization of the cyanide ion would increase its stability. This is the case, for isonitriles are structural hybrids[16] of a carbene form (XIV) and an ionic form (XV), and are thus quaternary derivatives of cyanide ion; they are of course much less basic than is cyanide ion.



XIV XV

Thiazole-2-carboxylic acid is much more readily decarboxylated than is the pyridine-2-carboxylic acid, decomposing at an appreciable rate in quinoline even at room temperature.[17] The decarboxylation is much slower in acid, and thus probably goes *via* the zwitterion XVI, rather than by an alternative prior protonation at C-2



XVI

It is interesting that 1-benzyl-2-carboxyimidazole (XVII) also decarboxylates readily,[18] although

(13) (a) L. Ingraham and F. Westheimer, *Chemistry & Industry*, 846 (1956); (b) K. Fry, L. Ingraham and F. Westheimer, THIS JOURNAL, **79**, 5225 (1957).

(14) G. Wheland, "Resonance in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1955, pp. 221 and 350.

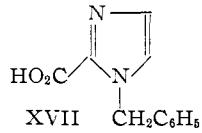
(15) B. Brown and D. Hammick, *J. Chem. Soc.*, 659 (1949).

(16) L. Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1944, p. 199.

(17) H. Schenkel and M. Schenkel-Rudin, *Helv. Chim. Acta*, **31**, 924 (1948).

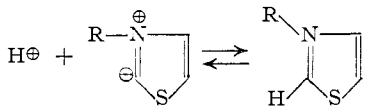
(18) R. Jones, THIS JOURNAL, **71**, 383 (1949).

kinetic evidence is not available to rule out the acid-catalyzed mechanism in this case.



XVII

These considerations all suggested that a species such as XVIII might be stable enough to be formed by loss of a proton from a thiazolium salt under relatively mild conditions.



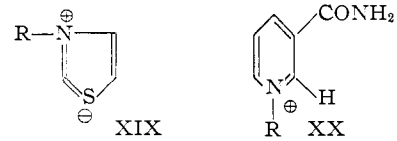
XVIII

This is indeed the case. Thus, after standing for a few hours in neutral D_2O at room temperature, 3,4-dimethylthiazolium bromide and 3-benzyl-4-methylthiazolium bromide are found to have exchanged one proton for deuterium, and thiamine chloride hydrochloride exchanges five protons (including the OH and NH_3^+ groups). That the hydrogen at C-2 is exchanging is shown by infrared and nuclear magnetic resonance evidence.

All three compounds show the development of an intense band at 4.5 μ in the infrared, suggesting[19] incorporation of deuterium at a highly polarizable double bond rather than at a saturated carbon (the absorption by the saturated 3-benzyl-(αd_2)-4-methylthiazolium bromide is much less intense). Also, all three lose a band near 11.0 μ which is probably ascribable[20] to out-of-plane bending of the hydrogen at C-2 of a thiazolium ring. Finally, nuclear magnetic resonance studies on 3,4-dimethylthiazolium iodide in D_2O as solvent confirm this conclusion. The spectrum initially contains four peaks, two of equal intensity at low field, which can be assigned to the protons at C-2 and at C-5, and two others of triple the magnitude at higher field which can be assigned to the protons on the methyl groups. The peak at lowest field must be due to the proton at C-2, which should have the greatest chemical shift[21] due to its proximity to the positive nitrogen, and it is this peak which disappears on standing in D_2O. This disappearance is slowed by acid and accelerated by base, but in neutral D_2O it has a half-time of about twenty minutes, which means that the rate of base-catalyzed ionization is the same order as that for malonic ester.[22]

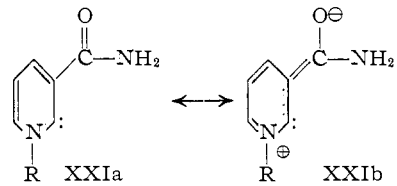
The role of the sulfur atom in stabilizing the anion is not obvious.[23] Although it seems geometrically unlikely that valence expansion by sulfur to furnish a contribution of XIX to the structure of the anion would be important, it is possible. The same type of geometrical problem is present in benzyne, in which overlap of the two *ortho* sp^2-orbitals has been suggested.[24] It is not clear how effective such bonding is, however, and consequently it is hard to estimate how much a structure such as XIX should contribute in our case.[25]

It is interesting that an example has been reported of base-catalyzed exchange of the hydrogen at C-2 of a pyridinium ring which bears a carboxamide group at C-3. San Pietro has found[26] that diphosphopyridine nucleotide (XX) exchanges at C-2, but not at C-6, on standing at room temperature for two hours at *p*H 11.0.



XIX XX

It seems likely that the effect of the carboxamide group is purely inductive, but there is the additional factor that the carbene form of the anion XXIa has a new possibility for resonance (XXIb) and this



XXIa XXIb

should tend to favor ionization.[27] It is not obvious how big an effect this latter type of factor can have.

The Mechanism.—Given that thiazolium salts are in equilibrium with anions at C-2 under mild conditions, however, it seems that this fact can account for the catalyses which have been observed. The thiazolium zwitterion XVIII is structurally similar to the cyanide ion, and thiazolium salts catalyze reactions which are related to the benzoin condensation, for which cyanide can be a catalyst. It is tempting to conclude that catalysis by thiazolium salts occurs *via* the zwitterion XVIII in a fashion analogous to catalysis by cyanide ion.

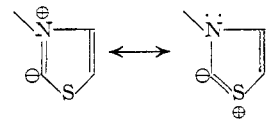
(19) The relationship of intensity of absorption to the change in dipole moment induced by the associated motion is well known. In this case, C–D stretching at C-2 would lead to an increased contribution of a form of the molecule which can be represented as D^+ associated with XVIII. This results in a major change in the charge structure of the molecule, so the high intensity is not surprising.

(20) See, for instance, R. Jones and C. Sandorfy in A. Weissberger "Techniques of Organic Chemistry," Vol. IX, Interscience Publishers, Inc., New York, N. Y., 1956, Chap. IV.

(21) *Cf.* B. P. Dailey and J. Shoolery, THIS JOURNAL, **77**, 3977 (1955).

(22) R. P. Bell, *Trans. Faraday Soc.*, **39**, 253 (1943).

(23) Downes and Sykes (ref. 31) suggest that the anion is stabilized by resonance of the type



Clearly this is so, but such resonance is of course present in the thiazolium ring whether the proton at C-2 has ionized or not. For this reason the resonance they mention will not contribute to the acidity of the C-2 proton, as it will not favor the anion *relative* to its conjugate acid.

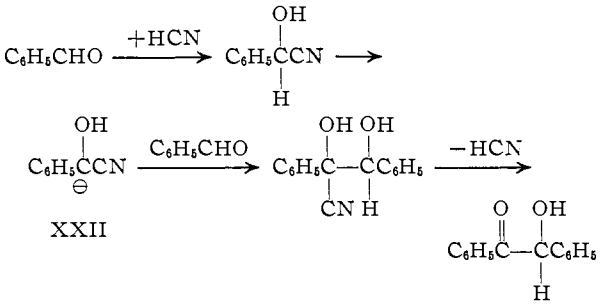
(24) J. Roberts, D. Semenow, H. Simmons, Jr., and L. Carlsmith, THIS JOURNAL, **78**, 601 (1956). For another relevant case, see W. von E. Doering and L. Levy, *ibid.*, **77**, 509 (1955).

(25) Since 1,3-dimethylbenzimidazolium iodide also undergoes rapid deuterium exchange (see Experimental) it seems probable that in the thiazolium zwitterions sulfur is not playing any special role involving valence expansion, but is simply furnishing inductive stabilization to the anion.

(26) A. San Pietro, *J. Biol. Chem.*, **217**, 589 (1955).

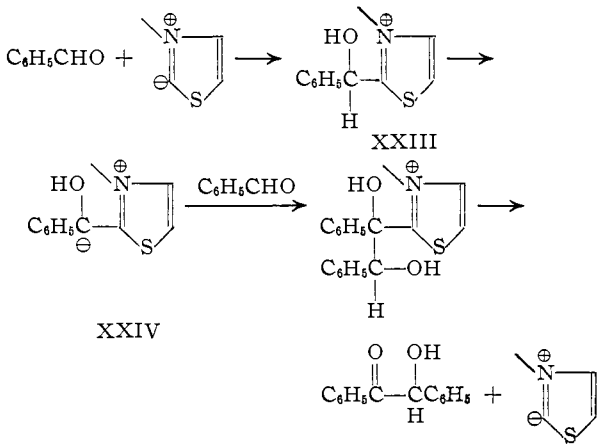
(27) This is equivalent to the idea that the anion at C-2 can participate in carbene-type resonance with both the C=N group and the unsaturated amide group, and is thus doubly stabilized.

The Lapworth mechanism[28] for the cyanide-catalyzed benzoin condensation is by now classic.

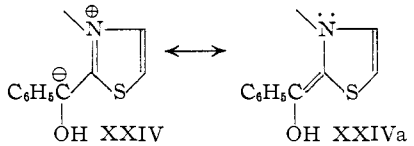


Compound XXII is what one might call an "active aldehyde" in biochemical terms. The proton of the aldehydic group has been lost, forming a "stabilized acyl carbanion," *i.e.*, the corresponding cyanohydrin. The carbanion is stabilized by being adjacent to the cyano group, because of resonance with a structure $C_6H_5C(OH)=C=N^-$.

In an exactly analogous way, a thiazolium zwitterion should be able to catalyze the benzoin condensation.



Here it is species XXIV which is the active aldehyde[29]; again it is stabilized by resonance, in this case with a form XXIVa.

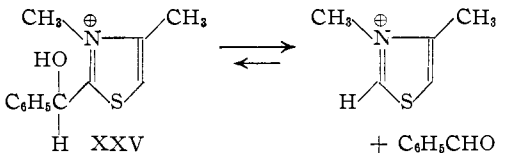


Although we have tried to isolate an intermediate such as XXIII from the reaction of benzaldehyde and thiazolium salts, we have obtained only benzoin and the recovered catalyst. This suggests, in terms of the proposed mechanism, that compounds such as XXIII are unstable under reaction conditions. As a test of this specific prediction we have synthesized XXV and examined its stability.

(28) A. Lapworth, *J. Chem. Soc.*, **83**, 995 (1903).

(29) This is a specific formulation of "active benzaldehyde." Precisely similar structures can be written for the other "active aldehydes" involved in the various model reactions, as well as the biochemical reactions involving thiamine pyrophosphate as coenzyme. As the only conceptual problem has been the formulation of a structure for these "active aldehyde" intermediates, the mechanisms involving them will not be detailed here. One such scheme, for pyruvic acid decarboxylation, has been presented elsewhere (ref. 1c).

The compound was obtained by treating 2-(α-hydroxybenzyl)-4-methylthiazole with methyl iodide; as predicted, it was unstable, decomposing to benzaldehyde and 3,4-dimethylthiazolium iodide under very mild conditions.



More evidence for the mechanism outlined above can be derived from consideration of the effectiveness of various analogs of thiamine as catalysts in the model reactions. The data are summarized in the tables.

Thus, in Table I, we see that many thiazolium salts (A–I), but not all (J–Q), are catalysts for the benzoin-type condensation. A thiazolium salt with hydrogen at C-2 is active (D) while the related compound with a methyl group at C-2 is not (J); this result is certainly required in terms of the mechanism. The failure of the phenacylthiazolium salts (K and L) to catalyze the reaction is apparently due to their destruction by side reactions under the model conditions.[30] The 3-benzyl-4-phenylthiazolium bromide (M) proves to be active in our hands, and a 3-phenyl-4-methylthiazolium salt (N) has been found[31] to have some activity in the acetoin test. Thus there is nothing fundamentally wrong with these compounds as catalysts, although factors such as steric hindrance may be decreasing their effectiveness. Certainly, steric hindrance at C-2 seems a reasonable explanation for the failure of a 3-isopropylthiazolium salt (O) to catalyze the benzoin condensation.

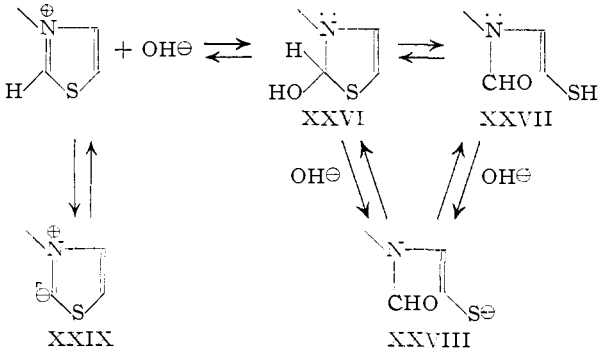
Imidazolium compounds also catalyze the reaction (R and S). In view of our finding that 1,3-dimethylbenzimidazolium iodide has an active hydrogen just as do the thiazolium salts this is especially significant. It would be striking indeed if this correlation of catalytic activity with zwitterion formation were fortuitous. The reported activity of 1,2,3-trimethylbenzimidazolium iodide (T), on the other hand, is impossible in terms of our mechanism, as the necessary C-2 position is blocked. For this reason we have re-examined the compound in some detail, and find that it is completely devoid of catalytic activity in our hands. Other compounds blocked at C-2 all fail to catalyze the reaction (Q, V and W).

At first sight it is not clear why 3-methylbenzothiazolium iodide is ineffective (P), especially considering the activity of a benzimidazolium compound (S). We find that it does exchange with D_2O under neutral conditions, but we have also confirmed its lack of catalytic activity. The explanation seems to involve a subtle feature of the interaction of thiazolium salts with base.

It is well known that, in base, thiazolium salts are in equilibrium with their pseudo-bases and with products of ring opening (and, as we have shown, with zwitterions).

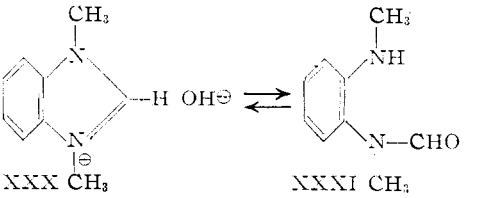
(30) Unpublished results of E. McNelis.

(31) J. Downes and P. Sykes, *Chemistry & Industry*, 1095 (1957).

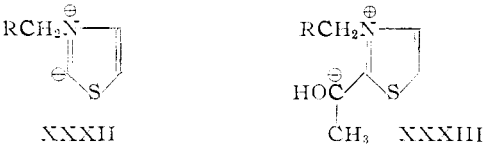


Compounds XXVI, XXVII and XXIX are different forms of the first conjugate base, and XXVIII is the second conjugate base of a thiazolium salt. One of the obvious predictions based on our mechanism is that there should be a *p*H optimum, below which the concentration of zwitterion should be too low for observable catalysis, and above which the increasing conversion of the first conjugate base into XXVIII should prevent catalysis. This has been found.[12] Another prediction is that compounds in which XXVIII is especially stable relative to XXIX will be poor catalysts (as will thiazolium salts in which XXVI or XXVII are favored relative to XXIX). Now *XXIX is unique among these forms in retaining the aromatic thiazolium ring*, and it is presumably this feature which permits it to compete. In a benzothiazolium salt this aromaticity will be less important, as fusion of aromatic rings in general diminishes their stability,[32] and one would thus expect that ring opening should be easier, and should compete effectively with zwitterion formation so as to prevent catalysis. It is reported[33] that benzothiazole methiodide reacts with two moles of alkali without any break in the titration, but the average pK_a has apparently not been determined. Accordingly, we have titrated the compound, and find that two moles of base react, without inflection, with an average pK_a of 6.35. By contrast, Metzler reports[34] an average pK_a for simple thiazolium salts of 10.3. The difference suggests that ring opening is much easier in benzothiazolium salts, and supports our explanation of their lack of catalytic activity. It is not surprising that in neutral D_2O exchange can occur, but when the *p*H is raised in order to increase the concentration of the zwitterion and obtain detectable catalysis the ring opening reaction takes place instead.

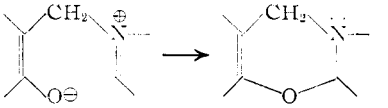
The benzimidazolium compound could also ring open, but the open form has an amino group, not a sulfhydryl group, so it will not form a stable second conjugate base. It is reported[35] that the first conjugate base exists, in solution, largely in the closed (XXX) rather than open (XXXI) form. Zwitterion formation can thus compete with ring opening.



The results which have been obtained in the acetoin test (Table II) also can be explained on the basis of our mechanism. Thus we find that, in addition to thiamine, simple thiazolium salts such as N-benzyl-4-methylthiazolium bromide are effective catalysts. On the other hand, N-ethyl-4-methylthiazolium bromide is not effective, although it catalyzes the benzoin condensation (Table I). The increased effectiveness of the benzyl compound, compared to the ethyl one, as catalyst for acetoin formation must be due to an inductive effect by the aromatic ring. Anions, both in the zwitterion XXXII and in the "active acetaldehyde" XXXIII would be favored by an



electron-withdrawing R group such as phenyl or vinyl; while these factors should also affect catalysis of the benzoin condensation, the latter test is apparently not sensitive enough to respond to a small change in activity of the catalyst. Oxythiamine chloride fails to catalyze the acetoin reaction, although it has an aromatic ring, but under the reaction conditions it will be ionized[36] to an oxy-anion XXXIV which should certainly prevent zwitterion formation at C-2 by adding to the thiazolium ring XXXV.



Further work on the acetoin test has been reported recently by Sykes.[31] He finds that otherwise effective catalysts are completely ineffective when they bear a methyl group at C-2 in place of the hydrogen, and he points out that this fact offers strong support for our mechanism.

Thus it seems that the model reactions involve a novel "zwitterion" which, both in structure and in mechanism of catalysis, resembles cyanide ion. Although it appears likely that they reflect the processes involved in biochemical reactions, only further work will reveal whether or not the simple chemical model systems have furnished a true insight into the mechanism of thiamine action.

Acknowledgment.—The author wishes to acknowledge the valuable assistance of Mr. Edward McNellis and Mr. Rudi Winter, and generous financial support by the Research Corporation.

(32) This phenomenon is well grounded in both theory and experiment. One example is the higher reactivity of naphthalene than of benzene.

(33) W. Mills, L. Clark and J. Aeschlimann, *J. Chem. Soc.*, **123**, 2353 (1923).

(34) G. Maier and D. Metzler, THIS JOURNAL, **79**, 4386 (1957).

(35) C. Tinkler, *J. Chem. Soc.*, **101**, 1246 (1912).

(36) A. Albert, D. Brown and G. Cheeseman, *ibid.*, 474 (1951), give *p*Ka 8.59 for 4-hydroxypyrimidine.

Experimental[37]

Materials.—Thiamine chloride hydrochloride, N-methylthiamine chloride hydrochloride, oxythiamine chloride, 3-benzyl-4-methylthiazolium chloride and 4-amino-5-hydroxymethyl-2-methylpyrimidine were generously supplied by Dr. Peter Sykes. The other thiazolium salts were prepared from the appropriate thiazoles and alkyl halides.

3-Allyl-4-methylthiazolium bromide was crystallized from methanol–ether, m.p. 132–134°. *Anal.* Calcd. for $C_7H_{10}BrNS$: C, 38.19; H, 4.58; N, 6.36. Found: C, 38.35; H, 5.00; N, 6.51.

3-Ethyl-4-methylthiazolium bromide was crystallized from ethanol, m.p. 170–171°. *Anal.* Calcd. for $C_6H_{10}BrNS$: C, 34.62; H, 4.84; N, 6.73. Found: C, 34.80; H, 4.46; N, 6.73.

3-Propyl-4-methylthiazolium bromide was crystallized from methanol–ether, m.p. 139–141°. *Anal.* Calcd. for $C_7H_{12}BrNS$: C, 37.84; H, 5.44; N, 6.30. Found: C, 38.06; H, 5.06; N, 6.11.

3-Isopropyl-4-methylthiazolium iodide was crystallized from methanol–ether, m.p. 136–137°. *Anal.* Calcd. for $C_7H_{12}INS$: C, 32.44; H, 4.67; N, 5.41. Found: C, 32.22; H, 4.64; N, 5.47.

3,4-Dimethylthiazolium bromide was crystallized from ethanol, m.p. 155–157°. *Anal.* Calcd. for C_5H_8BrNS: C, 30.94; H, 4.15; N, 7.23. Found: C, 31.08; H, 3.87; N, 6.95.

3-(β-Hydroxy-β-phenylethyl)-4-methylthiazolium bromide was crystallized from ethanol, m.p. 182.5–183°. *Anal.* Calcd. for $C_{12}H_{14}BrNOS$: C, 48.01; H, 4.70; N, 4.66. Found: C, 48.48; H, 5.16; N, 4.42.

Test for Acetoin Formation.—Mizuhara's procedure[12] was followed. Aqueous solutions of pyruvic acid (1.0 ml., 0.1 *M*), acetaldehyde (0.3 ml., 1.0 *M*) and catalyst (0.5 ml., 0.1 *M*) were mixed in a Thunberg tube and the mixture was adjusted to *p*H 8.4 with dilute NaOH. After evacuation the tube was incubated at 34° for 40 hr. Then 20 ml. of distilled water was added, and 7-ml. aliquots were analyzed for acetoin by the method of Westerfeld[38] (in which the acetoin is oxidized to biacetyl, which is distilled and assayed by a color reaction).

Most reactions were run in duplicate, although key compounds were tested more than twice. In no case was any significant variation observed between the results of different runs. The average results of the tests are listed in Table II.

The above test also was conducted with the omission of pyruvic acid. In this case acetaldehyde solution (0.4 ml., 1.0 *M*) and thiamine chloride hydrochloride solution (0.5 ml., 0.1 *M*) were adjusted to *p*H 8.4 and incubated in an evacuated Thunberg tube. Analysis revealed that 20% of the usual amount of acetoin was formed under these conditions.

Test for Benzoin Formation.—A modification of Ugai's method[10] was used. To a 5% solution of benzaldehyde in methanol (2 ml.) was added 0.05 mmole of catalyst and 0.02 mmole of NaOH. After evacuation of the tube and incubation for 24 hr. at 34°, the solution was tested for benzoin by the method of Corson and McAllister.[39] Under these conditions, 3-ethyl-4-methylthiazolium bromide catalyzed the formation of more than ten times the minimum detectable amount of benzoin. The results are included in Table I.

Test for Furoin Formation.—A mixture of 1.0 mmole of catalyst, 1.0 ml. of furfural (freshly distilled) and 1.0 ml. of ethanol was warmed to 70°. Then 0.1 ml. of 40% aqueous KOH was added and the temperature maintained at 70° for 1 hr. Cooling and dilution with water precipitated any furoin formed which was collected and identified. The results are included in Table I.

Deuterium Exchange Experiments.[40]—Benzyl bromide (α-d_2) was prepared by reduction of ethyl benzoate with $LiAlD_4$ and reaction of the resulting benzyl alcohol (α-d_2) with 48% aqueous HBr. The bromide was allowed to react with 4-methylthiazole in the usual fashion, yielding 3-benzyl-(α-d_2)-4-methylthiazolium bromide (found: 2.0 atoms of deuterium), m.p. 183.5–184°, undepressed on mixture with unlabeled 3-benzyl-4-methylthiazolium bromide. The compound (237 mg., 0.87 mmole) was submitted to the furoin test (*vide supra*), and the solvent ethanol (1 ml.) was then collected by vacuum transfer and analyzed for deuterium[41]; found: 0.048 atom % excess; calcd. for complete exchange: 1.73 atom % excess. The furoin formed was collected and crystallized from ethanol; 430 mg. (263% based on catalyst), m.p. 135–137°.

3,4-Dimethylthiazolium bromide was dissolved in D_2O at room temperature, and after 20 hr. the solvent was removed by vacuum transfer. After vacuum drying (over P_2O_5) the compound was analyzed for deuterium (found: 1.1 atoms). In the infrared the compound (KBr pellet) showed a strong band at 4.5 μ, but none at 4.0 μ (a band at 4.0 μ appeared when a little D_2O was added as control). The strong band at 11.0 μ of the undeuterated compound had disappeared. A solution of the undeuterated compound, in D_2O, showed four peaks in the nuclear magnetic resonance spectrum; a small pair, of equal area, appeared at −108 and −47 cycles/sec., and a pair, of triple the area, appeared at +66 and +114 cycles/sec. (Varian V-4012A magnet, 7050 gauss field, 30 megacycles/sec. probe; frequencies referred to benzene capillary and increasing field). On standing, the peak at −108 cycles/sec. disappeared, the half-life being about 20 min. The disappearance is markedly slowed by a trace of acid, and accelerated by a trace of base.

3-Benzyl-4-methylthiazolium bromide contains 1.2 atoms of deuterium after the twenty-four exchange, as above, and also shows the strong band at 4.5 μ in the infrared and the absence of a band at 4.0 μ. Here too a band at 11.0 μ had disappeared. **Thiamine chloride hydrochloride** incorporates 5.2 atoms of deuterium under these conditions, and shows, in the infrared, the new band at 4.5 μ and the loss of a band at 11 μ.

1,3-Dimethylbenzimidazolium iodide (m.p. 200–201°, reptd.[42] 144°) was prepared, as reported,[42] both by direct methylation of benzimidazole with methyl iodide and by the stepwise methylation *via* N-methylbenzimidazole.

Anal. Calcd. for $C_9H_{11}IN_2$: C, 39.43; H, 4.05. Found: C, 39.55, 39.60; H, 4.17, 3.91.

On treatment with alkali the compound was converted to its pseudo-base (m.p. 70°, reptd.[42] 74°).

On standing in D_2O for 12 hr., followed by vacuum drying, the iodide showed, in its infrared spectrum, development of a strong band at 4.5 μ and almost complete loss of a former very intense band at 11.7 μ, indicating that exchange was virtually complete. Similar treatment of **benzothiazole methiodide** led to exchange, as evidenced by the development of a strong band at 4.5 μ in the infrared.

Synthesis of the Intermediate XXV.—Attempts to synthesize 2-(α-hydroxybenzyl)-4-methylthiazole by decarboxylation of 2-carboxy-4-methylthiazole[43] in benzaldehyde medium failed. Thus the following route was adopted.

Mandelonitrile benzoate[44] (10 g.) was dissolved in 70 ml. of ethanol containing 1 ml. of diethylamine. The solution, in a pressure bottle, was saturated with H_2S at −60° and atmospheric pressure. The bottle was sealed, heated at 100° for 5 hr., cooled, and the solvent was removed *in vacuo*. The product, O-benzoylmandelic acid thioamide, was crystallized from benzene: 9.7 g. (86%), m.p. 142–143°.

Anal. Calcd. for $C_{15}H_{13}N_2OS$: C, 66.02; H, 4.82; N, 5.16. Found: C, 65.85; H, 4.75; N, 5.08.

The thioamide (18 g.) was refluxed with chloroacetone (6.5 g.) in 50 ml. of ethanol for 1.5 hr., and the solvent was then removed *in vacuo*. The residue was directly hydrolyzed with 10 g. of KOH in 30 ml. of ethanol at reflux (N_2) for 3 hr. Dilution and ether extraction yielded **2-(α-hydroxybenzyl)-4-methylthiazole** as an oil which, after distillation (125–130° (0.1 mm.)), was crystallized from ether–petroleum.

Anal. Calcd. for $C_{11}H_{11}NOS$: C, 64.36; H, 5.40; N, 6.82. Found: C, 64.39; H, 5.67; N, 6.66.

(37) Melting points were taken on a Fisher–Johns apparatus, and are uncorrected.

(38) W. Westerfeld, *J. Biol. Chem.*, **161**, 495 (1945).

(39) B. Corson and R. McAllister, THIS JOURNAL, **51**, 2822 (1929).

(40) Deuterium was determined by the method of J. Graff and D. Rittenberg, *Anal. Chem.*, **24**, 878 (1952), with the assistance of Miss Laura Ponticorvo.

(41) A control experiment with deuterated ethanol showed that no loss of deuterium occurs during this procedure.

(42) O. Fischer and E. Fussenegger, *Ber.*, **34**, 936 (1901).

(43) H. Schenkel, E. Marbet and H. Erlenmeyer, *Helv. Chim. Acta*, **27**, 1437 (1944).

(44) F. Francis and O. Davis, *J. Chem. Soc.*, **95**, 1403 (1909).

A portion of the thiazole was heated for 3 hr. with excess methyl iodide in butanone. On standing, crystals separated, m.p. 117–119°, undepressed by authentic **3,4-dimethylthiazolium iodide**[45] (m.p. 119–120°) and identical in the infrared (KBr pellet).

A second attempt to prepare the methiodide of 2-(α-hydroxybenzyl)-4-methylthiazole succeeded, yielding a compound with m.p. 165–167° after repeated crystallization from methanol (cold)–ether.

Anal. Calcd. for $C_{12}H_{14}INOS$: C, 41.50; H, 4.07. Found: C, 40.76; H, 4.43.

The substance was warmed in pyridine for 10 minutes, and then diluted, acidified and treated with dinitrophenylhydrazine reagent. The orange precipitate, m.p. 239–240°, was undepressed in m.p. on mixing with authentic **benzaldehyde 2,4-dinitrophenylhydrazone** (m.p. 240°).

In a separate experiment the methiodide XXV was warmed in pyridine for 10 minutes and the mixture was then cooled and diluted with ether. 3,4-Dimethylthiazolium iodide (m.p., mixed m.p., infrared spectrum) was obtained as the only ether-insoluble material.

***pK* of 3-Methylbenzothiazolium Iodide.**—The compound was titrated with 0.1 *N* NaOH under N_2 using a Beckman model G *p*H meter and allowing 0.5 hour after each addition for *p*H equilibrium to be reached. The *p*H after addition of one equivalent of alkali corresponded to that for minimum slope (dpH/dn) of the titration curve, 6.35, which is thus the pK_{av} for a diprotic acid with no detectable singly dissociated intermediate.

NEW YORK 27, N. Y.

(45) H. Erlenmeyer, H. Baumann and E. Sorkin, *Helv. Chim. Acta*, **31**, 1978 (1948).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE U. S. VITAMIN CORPORATION]

Indanols. I. Preparation and Spectra of Benzylated Indanols[1]

BY SEYMOUR L. SHAPIRO, THEODORE BAZGA, KURT WEINBERG AND LOUIS FREEDMAN

RECEIVED JANUARY 14, 1958

The zinc chloride catalyzed benzylation of 4-indanol and 5-indanol has yielded mono- and dibenzylated products. Group assignments have been made on the basis of the existing literature on phenol chemistry. Benzylation of 4-indanol yielded 7-benzyl-4-indanol and 5,7-dibenzyl-4-indanol, while 5-indanol yielded 6-benzyl-5-indanol and 4,6-dibenzyl-5-indanol. A consideration of the ultraviolet absorption spectra of the indanols along with the corresponding xylenols gave unexpected findings which could not be reconciled with steric factors.

A wide variety of substituted indanols has been prepared in our studies involving derivatives of selected moieties of the pharmacologically active steroids and alkaloids. In addition to the unsubstituted 4-indanol and 5-indanol,[2] their benzylated and chlorobenzylated derivatives[3] were required as initial reactants.

The substituted benzylindanols were prepared by the zinc chloride catalyzed condensation of the required benzyl halide with the indanol following Buu-Hoï and Demerseman.[4] Mono- and dibenzylation products were obtained in each instance. Although the group assignments cannot be made with certainty, it is known that the Friedel–Crafts type of benzylation occurs at the position *para* to the phenolic group whenever that position is free, or otherwise at the *o*-position.[4–6] Consequently, the monobenzylated product from 4-indanol has been designated as the 7-benzyl(or chlorobenzyl)-4-indanol, and the disubstituted derivative, the 5,7-dibenzyl-4-indanol. A similar pattern of substitution has been established for the chlorination of 4-indanol.[7]

In the instance of the benzylated products from 5-indanol, the monosubstituted product would enter the 4- or 6-position. Studies by Dev[8] on the chloromethylation of indan have indicated 75% of the 5-chloromethyl isomer (equivalent to the 6-position) and 25% of the 4-chloromethyl isomer (equivalent to the 7-position). This would indicate that in the presence of equal orientation effects by the alicyclic substituent, steric effects influence a preferred attack at the 6-position.[9–11] Similarly, the presence of a hydroxyl group in the 5-position would indicate that monobenzylation occurs at the 6-position to afford 6-benzyl(or chlorobenzyl)-5-indanol. The disubstituted product was assigned as 4,6-dibenzyl-5-indanol. Conversion in the benzylation ranged from 50 to 79%, monobenzylation being effected in 35 to 55% and dibenzylation in 13 to 31% yields.

In the effort to effect maximal conversion to the monobenzylated product, 3:2 molar ratios of indanol to benzyl (or monochlorobenzyl) halide were used. However, subsequent experiments wherein the dichlorobenzyl halides were condensed with the indanols in equimolar ratios showed no significant alteration of the pattern of mono- and dibenzylation.

The compounds which were prepared have been described in Table I.

The ultraviolet absorption spectra of some of the compounds herein prepared, along with those of the indanols, their methyl ethers and the corresponding xylenols were determined and have been recorded in Table II.

The unsubstituted indanols compared in methanol to their respective methyl ethers reflected the

(1) Presented at the Meeting-in-Miniature, New York Section, American Chemical Society, March, 1958.

(2) These difficultly accessible phenols have become commercially available through Union Carbide Corp., New York, N. Y.,

(3) Enhanced pharmacologic response relative to the unsubstituted phenols has been noted through the use of benzylated derivatives; (a) L. C. Cheney, R. R. Smith and S. B. Binkley, THIS JOURNAL, **71**, 60 (1949); (b) W. B. Wheatley, L. C. Cheney and S. B. Binkley, *ibid.*, **71**, 64 (1949); (c) **71**, 3795 (1949).

(4) Ng. Ph. Buu-Hoï and P. Demerseman, *J. Org. Chem.*, **20**, 1129 (1955).

(5) C. C. Price, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1949, Chapter I.

(6) R. C. Huston and A. L. Houk, THIS JOURNAL, **54**, 1506 (1932).

(7) J. S. Buck, R. A. Cutler, F. C. Nachod, R. G. Powles, R. Rakoczy, T. J. Slauson and B. F. Fuller, *ibid.*, **79**, 3559 (1957).

(8) S. Dev, *J. Indian Chem. Soc.*, **32**, 403 (1955).

(9) R. T. Arnold, THIS JOURNAL, **61**, 1405 (1939).

(10) R. T. Arnold and R. A. Barnes, *ibid.*, **66**, 960 (1944).

(11) R. M. Keefer and L. J. Andrews, *ibid.*, **78**, 5623 (1956).