double salt also forms solid solutions. Although the internal structure of $Li_2SO_4 \cdot H_2O$ has been determined,[22] it is unfortunate that the structure of $LiNH_4SO_4$ has not. It would be interesting to see whether the role of NH_4^+ ion in $LiNH_4SO_4$ is the same as that of the combination Li^+–H_2O in $Li_2SO_4 \cdot H_2O$.

(22) G. E. Ziegler, *Z. Krist.*, **89**, 456 (1934).

DEPARTMENT OF CHEMISTRY
ST. JOHN'S UNIVERSITY
BROOKLYN, NEW YORK

COMMUNICATIONS TO THE EDITOR

MECHANISM OF THE REACTION OF NITROMETHANE WITH BASES

Sir:

We wish to report a mechanism for the reaction of nitromethane with bases in aqueous solutions which accounts for both the initial reaction to form the salt of the aci form of nitromethane, and the subsequent slower reaction to form the salt of methazonic acid.

$$CH_3NO_2 + OH^- \rightleftharpoons CH_2NO_2^- + H_2O \quad (1)$$

$$2\ CH_2NO_2^- \xrightarrow{k_2} O_2NCH_2CHNO^- + OH^- \quad (2)$$

We have found that basic solutions containing methazonate ion show very strong absorption of light at 2,980 Å. while acid solutions show none. We have also observed that freshly prepared mixtures of dilute solutions of nitromethane and bases react at relatively slow rates to form the methazonate ion which we can follow by observing the increasing absorption at 2,980 Å. with time. The identification of the absorption peak with the methazonate ion has been made by preparing ammonium methazonate by a well established procedure (3) and establishing the absorption spectra pattern of this material.[8]

Our studies at 25.6° show that the initial rate of formation of methazonate ion is second order with respect to the initial concentration of nitromethane over the range of *p*H from 9.5 to 12.5. The order with respect to hydroxide ion concentration is second order at *p*H 9.5, and decreases asymptotically to almost zero order as we increase the *p*H to 12.5. It should be noted that the *p*H remains constant during the course of any single experiment.

On the assumption that the equilibrium in reaction (1) is established very rapidly relative to the velocity of reaction (2), an expression can be derived for the over-all rate from equations (1) and (2) as shown

$$r = \frac{k_2 K^2 X^2 (OH^-)^2}{[1 + K(OH^-)]^2} \quad (3)$$

where

r = rate of formation of methazonate ion
k_2 = specific rate constant for reaction (2)
K = equilibrium constant for reaction (1)
X = concentration of nitromethane plus aci nitromethane.

The kinetic data obtained are in complete agreement with equation (3).

This relation enables us to estimate K at higher temperatures than has been heretofore possible, and we will also be able to obtain the ΔH for reaction (1) and the activation energy for reaction (2). It should be noted that the evaluation of K is not our primary purpose. Rather we wish to show that known values of K_N are entirely consistent with our proposed mechanism.

This work supports the Pedersen mechanism[1] for pseudo acid behavior of nitro paraffins and gives good agreement with published data[2,3] on the ionization constant of nitromethane, K_N. This constant is related through the water equilibrium constant K_W to our equilibrium constant K in the following way

$$K = K_N/K_W$$

(1) J. K. Pedersen, *Det. Kgl. Videnak. Selskab., Math-fys, Medd.*, **12**, 1-16 (1932) (in English).
(2) D. Turnbull and S. H. Maron, THIS JOURNAL, **65**, 212 (1943).
(3) W. R. Dunstan and E. Goulding, *J. Chem. Soc. Trans.*, **2**, 1262 (1900).

PHYSICS DIVISION CHARLES M. DREW
RESEARCH DEPARTMENT JAMES R. MCNESBY
U. S. NAVAL ORDNANCE TEST STATION
INYOKERN, CHINA LAKE, CALIFORNIA ALVIN S. GORDON

RECEIVED MARCH 18, 1954

CARBON DIOXIDE FIXATION IN HEART EXTRACTS BY β-HYDROXYISOVALERYL COENZYME A[1]

Sir:

Previous isotopic studies have indicated that isovaleric acid, an intermediate in leucine metabolism, yields acetoacetate in liver tissue.[2,3,4,5,6] Carbons 1 and 2 furnish "acetate" for the well-recognized acetoacetate condensation, and the carbons of the isopropyl group yield acetoacetate by a carbon dioxide-fixing reaction. The intermediate steps in this metabolic pathway have recently been investigated in heart and liver extracts in this laboratory, and the following series of reactions is proposed to account for the results obtained

$$\text{Isovaleryl CoA} \longrightarrow \text{Senecioyl CoA}^7 \quad (1)$$

$$\text{Senecioyl CoA} + H_2O \longrightarrow \text{HIV CoA} \quad (2)$$

$$\text{HIV CoA} + CO_2 \xrightarrow{\text{ATP}} \text{HMG CoA} \quad (3)$$

$$\text{HMG CoA} \longrightarrow \text{Acetoacetate} + \text{Acetyl CoA} \quad (4)$$

(1) Supported by grants from the National Science Foundation and the United States Public Health Service.
(2) K. Bloch, *J. Biol. Chem.*, **155**, 255 (1944).
(3) M. J. Coon and S. Gurin, *ibid.*, **180**, 1159 (1949).
(4) I. Zabin and K. Bloch, *ibid.*, **185**, 117 (1950).
(5) M. J. Coon, *ibid.*, **187**, 71 (1950).
(6) G. W. E. Plaut and H. A. Lardy, *ibid.*, **192**, 435 (1951).
(7) Abbreviations: acyl Coenzyme A derivatives, acyl CoA; β-hydroxyisovaleryl CoA, HIV CoA; β-hydroxy-β-methylglutaryl CoA, HMG CoA; adenosine triphosphate, ATP; tris-(hydroxymethyl)-aminomethane, Tris.

The oxidation of isovaleryl CoA to the α,β-unsaturated thiol ester (Reaction 1) is postulated by analogy to the corresponding reaction of the CoA derivatives of straight chain fatty acids.[8,9] The conversion of senecioyl CoA to HIV CoA in heart extracts, due to the presence of crotonase,[10,11] and the fixation of radioactive carbon dioxide into the carboxyl carbon of acetoacetate in the presence of either of these synthetically prepared substrates have been described.[12]

It has recently been found that brief heating at 60° at *p*H 7.4 destroys the crotonase present in a dialyzed potassium chloride–ethanol extract of fresh pig heart tissue. With the use of this heated enzyme preparation HIV CoA has been established as the substrate for acetoacetate synthesis and the reaction has been shown to require the presence of ATP as well as bicarbonate (Table I).

TABLE I

ENZYMATIC SYNTHESIS OF ACETOACETATE

The test system contained 500 μM. Tris buffer, *p*H 8.1, 20 μM. $MgCl_2$, 26 μM. cysteine, 10 μM. ATP, 200 μM. $KHCO_3$, 2 μM. CoA ester (prepared by the general method of Wieland and Rueff[13]), pig heart enzyme fraction free of crotonase (6 mg. protein in Expts. 1–3, 32 mg. in Expts. 4–6), and, where indicated, 0.01 mg. crystalline liver crotonase[14]; volume 4.2 ml.; incubation, 60 minutes at 38°.

Expt.	System	Substrate	μM. acetoacetate formed[15]
1	Complete	HIV CoA	0.26
2	Complete	Senecioyl CoA	0.02
3	Complete + crotonase	Senecioyl CoA	0.20
4	Complete	HIV CoA	0.23
5	ATP omitted	HIV CoA	0
6	$KHCO_3$ omitted	HIV CoA	0.05

TABLE II

ENZYMATIC CLEAVAGE OF β-HYDROXY-β-METHYLGLUTARYL COENZYME A

3 μM. HMG CoA[16] was incubated 60 minutes at 38° with 200 μM. Tris buffer, *p*H 8.1, 13 μM. cysteine, 20 μM. $MgCl_2$, and 26 mg. heart enzyme protein, final volume 3.0 ml. After deproteinization with trichloroacetic acid the *p*H was adjusted to 7.4 and aliquots were taken for independent determination of acetoacetate[15] and acetyl CoA (by citrate formation[17] in the presence of oxalacetate and crystalline citrate condensing enzyme[14]).

Total μM. acetoacetate	1.13
Total μM. acetyl CoA	0.85[a]
Ratio, acetyl CoA/acetoacetate	0.75

[a] The lability of acetyl CoA at *p*H 8.1 during the initial incubation at 38° accounts for a value less than that obtained for acetoacetate.

The intermediate product predicted as a result of carbon dioxide fixation (Reaction 3), HMG CoA, has been prepared and has been shown to furnish acetoacetate and acetyl CoA in almost equimolar amounts upon incubation with the enzyme system (Table II). As anticipated, acetyl CoA was found to yield no acetoacetate under these conditions.[18,19] The cleavage (Reaction 4) requires the presence of cysteine (or glutathione), but, unlike the carboxylation reaction, is not dependent upon the addition of ATP and bicarbonate.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA 4, PA.
BIMAL K. BACHHAWAT
WILLIAM G. ROBINSON[20]
MINOR J. COON

RECEIVED MAY 3, 1954

(8) W. Seubert and F. Lynen, THIS JOURNAL, **75**, 2787 (1953).
(9) D. E. Green, S. Mii and H. R. Mahler, *J. Biol. Chem.*, **206**, 1 (1954).
(10) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953).
(11) H. Beinert, *et al.*, *ibid.*, **75**, 4111 (1953).
(12) W. G. Robinson, B. K. Bachhawat and M. J. Coon, *Federation Proc.*, **13**, 281 (1954).
(13) T. Wieland and L. Rueff, *Angew. Chem.*, **65**, 186 (1952).
(14) Kindly furnished by Dr. Joseph R. Stern.
(15) Estimated by a modification of the method of S. S. Barkulis and A. L. Lehninger, *J. Biol. Chem.*, **190**, 339 (1951).
(16) β-Hydroxy-β-methylglutaric acid was synthesized by the method of H. J. Klosterman and F. Smith, THIS JOURNAL, **76**, 1229 (1954). The acid has been shown to occur in liver by J. R. Rabinowitz and S. Gurin, *J. Biol. Chem.*, in press (1954).
(17) J. R. Stern and S. Ochoa, *J. Biol. Chem.*, **191**, 161 (1951).
(18) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).
(19) D. E. Green, D. S. Goldman, S. Mii and H. Beinert, *J. Biol. Chem.*, **202**, 137 (1953).
(20) Postdoctoral Research Fellow, U. S. Public Health Service.

5-HEPTYL-2-THIOHYDANTOIN, A NEW ANTITUBERCULAR AGENT

Sir:

In the course of screening compounds for antitubercular activity it was found that 5-hexyl-2-thiohydantoin, prepared some years ago in these laboratories[1] and tested as an anti-thyroid agent, exhibited a significant therapeutic effect when tested in mice infected with *M. tuberculosis* H37Rv. This result stimulated an extensive program designed to exploit this lead.

At the outset it was found that small structure changes profoundly affected the antitubercular activity. Maximum activity in the 5-alkyl-2-thiohydantoin series was attained with 5-*n*-heptyl-2-thiohydantoin (m.p. 132.8–134.0°. *Anal.* Calcd. N, 13.07; S, 14.96. Found: N, 12.93; S, 14.89). The effectiveness fell precipitously as the 5-alkyl chain was lengthened; the *n*-octyl homolog (m.p. 132.8–134.0°. *Anal.* Calcd.: N, 12.27; S, 14.31. Found: N, 11.90; S, 14.33) was only slightly suppressive and the *n*-decyl-2-thiohydantoin (m.p. 133.8–134.8°. *Anal.* Calcd.: N, 10.93; S, 12.50. Found: N, 10.62; S, 12.45) was an inactive drug. Many isomers of 5-*n*-heptyl-2-thiohydantoin were without effect. The 5-(2-methylhexyl)-, (m.p. 190.2–190.4°. *Anal.* Found: N, 13.22; S, 14.96), 5-(3-methylhexyl)-, (m.p. 124.5–125.4°. *Anal.* Found: N, 13.05; S, 15.02) and 5-(4-methylhexyl)-2-thiohydantoin (m.p. 143–144°. *Anal.* Found: N, 13.20; S, 14.94), were inactive for all practical purposes. Only 5-(5-methylhexyl)-2-thiohydantoin (m.p. 152.1–153.3°. *Anal.* Found: N, 13.14; S, 15.02) showed moderate suppressive activity. The closely related 5-(2-nonenyl)- (m.p. 123.1–125.3°, *Anal.* Calcd.: N, 11.66; S, 13.34 Found: N, 11.78; S, 13.62) and 5-(2-hexenyl)-2-thiohydantoin (m.p. 130.3–133.9°. *Anal.* Calcd.: S, 16.17. Found: S, 15.97) were without appreciable antitubercular activity.

A similar state of affairs was encountered when the thiohydantoin moiety was varied. For example, both 5-heptylhydantoin[2] and 5-hexylhydantoin[2] were inactive. This was also true for 5-*n*-hexyl-2,4-

(1) M. Jackman, *et al.*, THIS JOURNAL, **70**, 2884 (1948).
(2) P. Gagnon, *Can. J. Research*, **27B**, 742 (1949).