cm.$^{-1}$, accompanying the strong maximum at 1703 cm.$^{-1}$ (0.01 *M* soln. in CCl_4)[7] corresponding to "non-interacted" conformations of I. By contrast, under the same conditions, tetrahydro-4H-1-thiapyran-4-one (II)[8] and 1-thiacycloheptan-4-one (III)[9] exhibited single maxima at 1716 and 1711 cm.$^{-1}$, respectively. The dipole moment of the eight-membered ring compound (I), 3.81 *D* in benzene, was higher than that of the seven-membered ring compound (III) (3.04 *D*; 1.73 *D* for II).[2b] It is important to note that S–C_{CO} interaction occurs to a lesser extent than N–C_{CO} transannular interaction in the electronic *ground state* by comparison (infrared especially) of 1-thiacycloöctan-5-one with 1-methyl-1-azacycloöctan-5-one.[7]

Finally, the ultraviolet absorption maxima of I in cyclohexane, at 226 mμ (ϵ 2445) and ~232 mμ (ϵ 2150), are associated with *excitation* of the interacting S–C_{CO} system (λ^{III}_{max} 223 mμ (ϵ 695), λ^{II}_{max} 233 mμ (ϵ 507).[10]

(7) N. J. Leonard, M. Ōki, J. Brader and H. Boaz, THIS JOURNAL, **77**, 623 (1955).

(8) E. A. Fehnel and M. Carmack, *ibid.*, **70**, 1813 (1948).

(9) C. G. Overberger and A. Katchman, *ibid.*, **78**, 1965 (1956).

(10) E. Fehnel and M. Carmack (*ibid.*, **71**, 84 (1949)) have suggested earlier that the difference between the ultraviolet spectrum of tetrahydro-4H-1-thiapyran-4-one (II) and those of its acyclic analogs is attributable to direct interaction between the 1,4-atoms in the excited state. (See also V. Georgian, *Chemistry and Industry*, 1480 (1957).) If this is correct, the four- to five-fold increase in intensity for the eight membered ring over the six-membered ring may be regarded as manifestation of the greater contribution of transannular interaction in the medium-ring compound.

(11) Sinclair Refining Co. Fellow in Organic Chemistry, 1957–1958. Work done under the sponsorship of the Sinclair Research Laboratories, Inc.

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ON THE MECHANISM OF OXIDATIVE DECARBOXYLATION OF PYRUVATE

Sir:

Extracts of *Escherichia coli* contain an enzyme system which catalyzes an oxidative decarboxylation of pyruvate represented by reaction 1.[1,2,3]

$$\text{Pyruvate} + \text{DPN}^{+}[4] + \text{CoA} \xrightarrow[(RS_2)]{(TPP)} \text{acetyl CoA} + CO_2 + \text{DPNH} + H^{+} \quad (1)$$

We have obtained highly purified preparations (250-fold purification) of this system from extracts of the Crookes strain. It is apparently an enzyme complex,[5] and sediments in the ultracentrifuge (1 to 2 hr. at 144,000 × *g*) as a dark yellow, fluorescent pellet. The complex contains a flavin which has been tentatively identified as FAD. Release of the flavin by precipitation of the enzyme complex with ammonium sulfate at *p*H 3.6 resulted in a decrease in the enzymatic activities: dihydrolipoic dehydrogenase, DPN reduction (reaction 1), pyruvate dismutation, and reduction of free lipoic acid (reaction 2).[3,6] These activities were restored by

$$CH_3COCO_2H + \text{LipS}_2 + HPO_4^{=} \xrightarrow[(CoA)]{(TPP,RS_2)} CH_3COOPO_3^{=} + \text{Lip(SH)}_2 + CO_2 \quad (2)$$

addition of FAD, but not of FMN (Table I). The dihydrolipoic transacetylase activity of the preparation was not affected by removal of flavin.

TABLE I

REACTIVATION OF SPLIT PYRUVATE DEHYDROGENATION SYSTEM WITH FAD

Assay system	Before splitting	Specific activities[a] After splitting: Without FAD	With FAD[g]	With FMN[g]
Lipoic DeH[b]	870	214	544	228
Dismutation[c]	870	214	486	144
Reaction 1[d]	156	20	62	20
Reaction 2[e]	90	32	64	32
Lip. transac.[f]	112	100	110	100

[a] Expressed as μmoles/hr./mg. protein based on assays described previously. [b] Ref. 7, *p*H 7. [c] Ref. 3. [d] Ref. 8. [e] Ref. 6, *p*H 7, 5 μmoles DL-lipoamide employed. [f] Ref. 8. [g] Aliquots of split complex incubated 10 min. at 30° with FAD or FMN before assay. Final concentration of added flavin in assays was 10^{-5} to 10^{-6} *M*.

These data indicate that FAD is an essential component of the enzyme complex, presumably associated with dihydrolipoic dehydrogenase. The data are consistent with the reaction sequence[2,3] shown for oxidative decarboxylation of pyruvate by the enzyme complex.

$$CH_3COCO_2H + \text{TPP} \rightleftharpoons [CH_3CHO\text{–TPP}] + CO_2 \quad (3)$$

$$[CH_3CHO\text{–TPP}] + \begin{matrix}S\\|\\S\end{matrix}\!\!>\!R \rightleftharpoons \begin{matrix}HS\\CH_3COS\end{matrix}\!\!>\!R + \text{TPP} \quad (4)$$

$$\text{CoA–SH} + \begin{matrix}HS\\CH_3COS\end{matrix}\!\!>\!R \rightleftharpoons CH_3COS\text{–CoA} + \begin{matrix}HS\\HS\end{matrix}\!\!>\!R \quad (5)$$

$$\begin{matrix}HS\\HS\end{matrix}\!\!>\!R + \text{FAD} \rightleftharpoons \begin{matrix}S\\|\\S\end{matrix}\!\!>\!R + \text{FADH}_2 \quad (6)$$

R = $-CH_2CH_2\underset{|}{C}H(CH_2)_4CO$-enzyme

The reduced flavoprotein produced in reaction 6 apparently can interact with DPN (*cf.* reaction 1) and free lipoic acid (*cf.* reaction 2).

(1) S. Korkes, *et al.*, *J. Biol. Chem.*, **193**, 721 (1951).

(2) I. C. Gunsalus, in "The Mechanism of Enzyme Action," The Johns Hopkins Press, Baltimore, Md., 1954, p. 545.

(3) L. J. Reed, *et al.*, *J. Biol. Chem.*, **232**, 123, 143 (1958).

(4) Abbreviations: DPN, diphosphopyridine nucleotide; CoA, coenzyme A; TPP, thiamine pyrophosphate; FAD, flavin adenine dinucleotide; LipS_2, free lipoic acid; Lip(SH)_2, free dihydrolipoic acid; RS_2, protein-bound lipoic acid.

(5) R. S. Schweet, *et al.*, *J. Biol. Chem.*, **196**, 563 (1952); D. R. Sanadi, *et al.*, *ibid.*, **197**, 851 (1952).

(6) I. C. Gunsalus, *Federation Proc.*, **13**, 715 (1954).

(7) L. P. Hager and I. C. Gunsalus, THIS JOURNAL, **75**, 5767 (1953).

(8) L. P. Hager, Thesis, University of Illinois, 1953.

(9) During this investigation Dr. V. Massey, *Biochim. et biophys. acta*, **30**, 205 (1958) communicated to us his significant finding (ref. 9) that highly purified diaphorase exhibited strong dihydrolipoic dehydrogenase activity.

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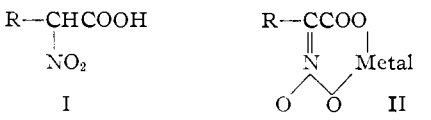
RECEIVED NOVEMBER 25, 1958

CHELATION AS A DRIVING FORCE IN SYNTHESIS. A NEW ROUTE TO α-NITRO ACIDS AND α-AMINO ACIDS

Sir:

Dibasic α-nitro acids (I) are converted in weakly basic media to acid salts which rapidly decarbox-

ylate.[1] In stronger alkali the acids form divalent salts such as dipotassium nitroacetate,[2] which does not decarboxylate readily, and which forms typically ionic crystals.[3] The chelate salts (II) have not been characterized, although their existence



was suggested by the effect which many polyvalent metal ions have upon the rate of decarboxylation of nitroacetic acid.[1,4] We have now succeeded in preparing the aluminum and magnesium salts of nitroacetic acid, and have utilized the stability of magnesium salts to provide a remarkably simple synthesis of α-nitro acids from primary nitroalkanes and carbon dioxide.

Aluminum nitroacetate precipitates when aluminum isopropoxide and nitroacetic acid are mixed in ether solution. The white powder has the composition $Al_2(C_2HNO_4)_3 \cdot (solvent)_x$,[5] is insoluble in the common solvents, and is decomposed by aqueous hydrochloric acid to free nitroacetic acid. Magnesium nitroacetate has not been obtained crystalline, but may be prepared in methanol (λ_{max} 272 mμ, log ϵ 4.05) and shown spectroscopically to be a 1:1 complex by the method of continuous variation.[6]

Magnesium methyl carbonate[7] may be prepared by saturating a solution of magnesium methoxide in dimethylformamide with dry carbon dioxide. Treatment of nitromethane with 4 molar equivalents of this solution (2 molar) at 50° for 4–5 hours resulted in quantitative conversion to magnesium nitroacetate as determined spectrophotometrically. Hydrolysis of the solution with ice and hydrochloric acid led to the isolation of nitroacetic acid, m.p. 89–92° (reported 87–89°,[2] 91.5–92°[4]) in 63% yield. This result demonstrates that the well-known decarboxylation of nitroacetic acid is a reversible reaction, the position of equilibrium being completely shifted by chelation.

Other primary nitroparaffins react similarly. Nitroethane is converted to **2-nitropropionic acid** in 49% yield under conditions found best for nitroacetic acid, although these conditions may not be optimum in this and other cases. The following amino acids were prepared by hydrogenating the crude nitro acids, prepared as described above, in acetic acid at room temperature with 10% Pd/C, in the specified over-all yield based on nitroparaffin: **DL-alanine,**[8] dec. 292–294° (46%) from nitroethane; **DL-α-aminobutyric acid,** dec. 283–285° (34%) from 1-nitropropane; and **DL-norvaline,** dec. 290–292° (42%) from 1-nitrobutane.

2-Nitropropane, which could not give rise to a species such as II, fails to undergo the reaction.

The use of magnesium methyl carbonate as a carboxylating agent for other active hydrogen compounds is being investigated.

(1) K. J. Pedersen, *Trans. Faraday Soc.*, **23**, 316 (1927).

(2) W. Steinkopf, *Ber.*, **42**, 3925 (1909).

(3) D. J. Suter, F. J. Llewellyn and H. S. Maslen, *Acta Cryst.*, **7**, 145 (1954).

(4) K. J. Pedersen, *Acta Chem. Scand.*, **3**, 676 (1949).

(5) The atomic ratio of nitrogen to aluminum was 1.43 and 1.42 for two samples prepared by mixing alkoxide and acid in molar ratios of 1.4 and 0.5, respectively. The elemental analyses [Found: C, 33.5; H, 6.0; N, 8.2; Al, 11.1 (former sample) and C, 29.5; H, 4.6; N, 7.7; Al, 10.4 (latter sample)] suggest that the identity and number of solvent molecules in the salt depend upon the details of preparation. Two molecules of ether or isopropyl alcohol are probably involved. (Calcd. for $C_{12}H_{19}O_{14}N_3Al_2$: C, 29.8; H, 4.0; N, 8.7; Al, 11.2; for $C_{18}H_{23}O_{14}N_3Al_2$: C, 32.9; H, 4.5; N, 8.2; Al, 10.6).

(6) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, 1952, p. 29.

(7) E. Szarvasy, *Ber.*, **30**, 1836 (1897).

(8) The amino acids were identified by comparison of their decomposition temperatures and infrared spectra (Nujol) with those of commercial samples.

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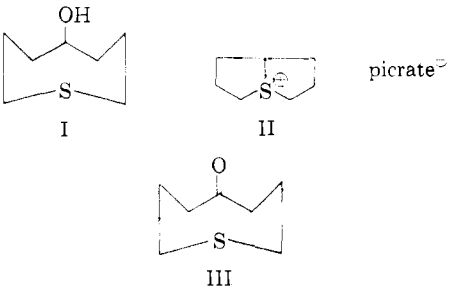
RECEIVED DECEMBER 4, 1958

A TRANSANNULAR REACTION IN AN EIGHT-MEMBERED RING SULFIDE

Sir:

We wish to report a novel transannular reaction in a cyclic 8-membered ring sulfide. The carbinol (I), 5-hydroxythiacycloöctane, on treatment with phosphoric anhydride and then treatment of the purified solution (ion exchange) with picric acid, gave the bicyclic sulfonium salt II, 57%, bicyclo-[3,3,0]octane-1-thianium picrate, m.p. 262–264° (microblock, corr.) C, 43.55; H, 4.23; N, 11.75.



This sulfonium picrate was identical (infrared spectrum) with the same compound prepared by R. H. Eastman[1] and G. Kritschevsky, m.p. 261–263°, mixed m.p. 261–264 (micro-block, corr.)

The eight-membered ring ketone (III),[2] 5-oxothiacycloöctane, b.p. 120–123 (19 mm.), m.p. 50–52°; C, 58.26; H, 8.39; (2,4-dinitrophenylhydrazone, m.p. 194–195°, N, 17.34) was prepared by the Dieckmann cyclization of ethyl thio-di-*n*-butyrate with subsequent hydrolysis and decarboxylation (31%). The infrared spectrum (0.01 *M* in CCl_4) showed a strong normal carbonyl absorption for this compound at 1707 cm.$^{-1}$, with a shoulder at 1690 cm.$^{-1}$. We had reported previously a single absorption for the 7-membered

(1) We are indebted to Dr. R. Eastman for a sample of this picrate; Gene Kritschevsky, Ph.D. Thesis, Stanford University, September 1955; prepared from 3-(2-tetrahydrofuryl)-propyl chloride (from the corresponding alcohol by reaction with thionyl chloride) by conversion of the latter into the isothiuronium salt and then by treatment with ammonium hydroxide to yield the mercaptan, which then was treated with concd. hydrochloric acid at 100° (74% yield) to give the bicyclo[3,3,0]octane-1-thianium chloride—isolation was then carried out by the formation of the picrate.

(2) This product was identical with a sample of the same material kindly forwarded to us by Dr. N. J. Leonard of the University of Illinois and described in the accompanying communication by N. J. Leonard, T. L. Brown and T. W. Milligan.