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 pH 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, pH 7. [c] Ref. 3. [d] Ref. 8. [e] Ref. 6, pH 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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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-