minutes and lyophilizing to dryness. The resulting salt was decarboxylated by heating the residue with phenol, and the acetone analyzed for O^{18}. Some of the pertinent data are shown in the table.

Experimental conditions[a]	% Exchange[b] at 20° Control (2.5 min.)	Control (15 min.)	Decarboxylation[c]
Acetone + buffer	15	65	
Acetone + 0.45 mg. enzyme	40	92	
Acetone + 0.90 mg. enzyme	57		
Acetoacetate + buffer[d]	25		
Acetoacetate + 0.45 mg. enzyme	45		98.5
Acetoacetate + 0.90 mg. enzyme	61		100

[a] All reactions were carried out in 2 ml. of 1 *M* phosphate buffer, *p*H 6.5, with 0.05 ml. of acetone or 0.06 to 0.15 g. of potassium acetoacetate. [b] Some of these values are averages. [c] The acetone was blown from the solution as soon as it was formed and was all collected within 2 min. after adding the acetoacetate. [d] An isolated experiment of this type gave a higher value (65%).

Inspection of these data shows that the exchange of O^{18} from the carbonyl group of acetoacetate is an obligatory part of the enzymatic decarboxylation process; control experiments establish that the direct exchange of O^{18} from acetone and acetoacetate, in the presence or the absence of enzyme, is incomplete. The results are consistent with the hypothesis that the reaction proceeds by way of Schiff-base formation[4] between the ketoacid and the enzyme, but do not of themselves demand this conclusion. Further tests of this hypothesis are in progress.

(4) K. J. Pedersen, *J. Phys. Chem.*, **38**, 559 (1934), proposed a similar mechanism for the non-enzymatic amine-catalyzed decarboxylation of acetoacetate.

(5) Holder, National Research Council of Canada Special Scholarship, 1957–1959.

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RECEIVED OCTOBER 15, 1959

STEREOCHEMISTRY OF THE FUMARASE AND ASPARTASE CATALYZED REACTIONS AND OF THE KREBS CYCLE FROM FUMARIC ACID TO *d*-ISOCITRIC ACID[1,2]

Sir:

The stereospecific synthetic approach used[3] to elucidate the stereochemistry of the *cis*-aconitase system has been applied to the fumarase and aspartase systems.

The racemate (I), m. 127–128.5°, neut. equiv. 67.0, of 3-monodeuterio-DL-malic acid, having the hydroxyl and deuterium in *trans* configuration, has been stereospecifically synthesized by the *trans* lithium aluminum deuteride opening[4] of the oxide ring of 3,4-epoxy-2,5-dimethoxy-tetrahydrofuran[4] and then acid hydrolysis[4] to the dialdehyde and nitric acid oxidation of the dialdehyde to 3-monodeuterio-DL-malic acid. The enantiomorphs may be referred[5] to as α-OH_{Ls}-β-H^2_{Ds}-malic acid and α-OH_{Ds}-β-H^2_{Ls}-malic acid. Nuclear magnetic resonance spectroscopic examination[6] of this 3-monodeuterio-DL-malic acid gave a coupling constant of 4.4 ± 0.2 c.p.s. It is thus identical in stereochemical configuration with the 3-monodeuterio-D-malic acid prepared[7] by inversion at the α-carbon atom of the 3-monodeuterio-L-malic acid obtained[7,8] by fumarase hydration of fumaric acid in deuterium oxide or by nitrous acid treatment,[7] with retention of configuration, of the 3-monodeuterio-L-aspartic acid obtained[7] by aspartase-catalyzed addition of ammonia to fumaric acid.

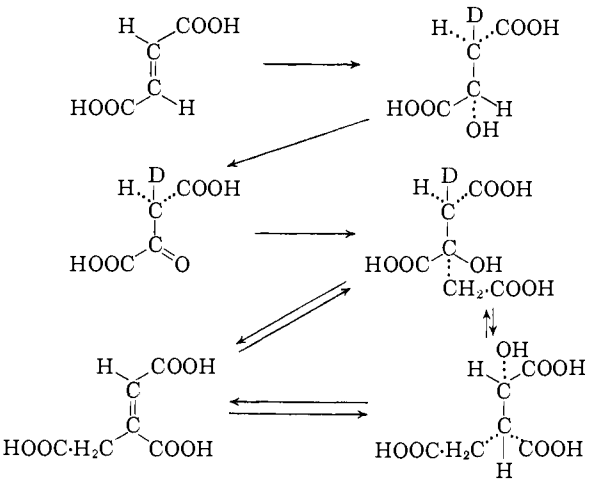

FIG. 1.—Stereochemistry of biochemical route from fumaric acid to *d*-isocitric acid.

DL-I

Thus the stereochemical configuration of the 3-monodeuterio-D-malic acid is α-OH_{Ds}-β-H^2_{Ls}, the structure of the product of the fumarase reaction in deuterium oxide is α-OH_{Ls}-β-H^2_{Ls} and the product of the aspartase reaction is α-NH_{2Ls}-β-H^2_{Ls}. With these configurations in mind, it is now seen that both the fumarase-catalyzed hydration of fumaric acid and the aspartase-catalyzed addition of ammonia to fumaric acid proceed via a *trans* addition.[9] The *trans* nature of these additions is in

(1) Support of this work by grant RG-6245, from the National Institutes of Health is gratefully acknowledged.

(2) With the technical assistance of David Belitskus.

(3) O. Gawron, A. J. Glaid, III, A. LoMonte and S. Gary, THIS JOURNAL, **80**, 5856 (1958).

(4) J. C. Sheehan and B. M. Bloom, *ibid.*, **74**, 3825 (1952).

(5) Using the nomenclature of Ref. 3.

(6) In 2 *M* D_2O solution after replacement of exchangeable protons with deuterium. We are indebted to Dr. Paul Lauderbur for this determination.

(7) A. I. Krasna, *J. Biol. Chem.*, **233**, 1010 (1958). A coupling constant of 4 c.p.s. is reported for this 3-monodeuterio-D-malic acid. A coupling constant of 6 c.p.s. was found for the 3-monodeuterio-L-malic acid.

(8) R. A. Alberty and P. Bender, THIS JOURNAL, **81**, 542 (1959). A coupling constant of 7.1 c.p.s. was found for the 3-monodeuterio-L-malic acid. A sample of enzymatically prepared 3-monodeuterio-L-malic acid, kindly supplied us by Dr. Alberty, was found by us to have a coupling constant of 7.3 c.p.s.

(9) Previously, Ref. 10, 8 and 7, these additions have been considered to proceed *via cis* additions.

(10) T. C. Farrar, H. S. Gutowsky, R. A. Alberty and W. G. Miller, THIS JOURNAL, **79**, 3978 (1957). Stereochemical assignments were based on nuclear magnetic resonance conclusions and the assumption that the carboxyl groups of malic acid in the solid state are in a *trans* relationship. Doubts of the correctness of this assumption have been raised, ref. 8, and personal communication, R. Alberty.

keeping with the *trans* nature[3] of the *cis*-aconitase system. The stereochemistry of the Krebs cycle from fumaric acid to isocitric acid may now be traced (Fig. 1) utilizing the finding[11,12] that the 3-monodeuterio-L-malic acid (from the fumarase reaction) gives, biochemically, isocitric acid lacking deuterium and utilizing our previously suggested[3] scheme for the *cis*-aconitase system.

(11) (a) S. Englard, *Fed. Proc.*, **18**, 222 (1959). (b) S. Englard, personal communication. We wish to thank Dr. Englard for sending us his manuscript prior to publication.

(12) The other results, Ref. 11, are also consistent with the scheme presented in Fig. 1.

(13) National Science Foundation Cooperative Graduate Fellow.

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RECEIVED SEPTEMBER 25, 1959

THE ROLE OF A TRIPLET STATE IN THE PHOTOREDUCTION OF BENZOPHENONE

Sir:

Irradiation of benzene solutions of benzophenone and benzhydrol with near ultraviolet light produces benzpinacol stoichiometrically according to equation (1).

$$(C_6H_5)_2CO + (C_6H_5)_2CHOH \longrightarrow (C_6H_5)_2C(OH)-C(OH)(C_6H_5)_2 \quad (1)$$

Experiments were carried out using a collimated beam from a Westinghouse SAH800-C Mercury arc filtered to give a band having a maximum at 3660 Å. and a band pass of 500 Å. Photolysis rates were measured by spectrophotometric determination of residual benzophenone and by titration of the pinacol with lead tetraacetate.[1] Quantum yields were based upon uranyl oxalate actinometry although the reaction of benzophenone with 2-propanol[2] was used as a secondary standard. Solutions were degassed to 10^{-3} mm. A series of experiments was run using 0.1 *M* benzophenone and varying concentrations of benzhydrol.[3] Graphical analysis showed the $1/\Phi$ was a linear function of $1/[BH_2]$. This relationship indicates a simple competition between deactivation of the chemically active state and its reaction with benzhydrol. Since the intercept of the plot is one, physical quenching by benzhydrol must be negligible. The mechanism shown accounts for these facts.

$$B + h\nu \xrightarrow{n \rightarrow \pi} B^1 \text{ (singlet)} \quad (2)$$

$$B^1 \longrightarrow B^3 \text{ (triplet)} \quad (3)$$

$$B^3 \xrightarrow{k_d} B \text{ (all deactivation steps)} \quad (4)$$

$$B^3 + BH_2 \xrightarrow{k_r} 2(C_6H_5)_2\dot{C}OH \longrightarrow \text{Benzpinacol} \quad (5)$$

Application of steady state kinetics to the concentrations of excited states gives the rate law.

$$\frac{1}{\Phi} = 1 + \frac{k_d}{k_r[BH]_2}$$

From the slope of the plot of $1/\Phi$ *vs.* $1/[BH_2]$, the value of k_d/k_r is found to be 0.050. *Consideration of this number compels the conclusion that the triplet state is responsible for the chemical reaction.* The largest value that k_r can be imagined to have is 10^9 liter mole^{-1} sec.$^{-1}$, the diffusion controlled rate.[4] This gives an upper limit of 5×10^7 sec.$^{-1}$ for k_d. Fluorescence rate constants for singlet states[6] are believed to be of the order of 10^8 sec.$^{-1}$ Since benzophenone solutions have no visible fluorescence, the actual rate of non-radiative quenching of the lowest singlet state must be at least 10^{10} sec.$^{-1}$. It is clear that some longer lived state must be involved in the measured competition.

Reaction (5) actually must be much slower than diffusion-controlled since a substantial isotope effect is observed when α-deuteriobenzhydrol is used as the hydrogen donor. The value of $k_d/k_{r(D)}$ is 0.133, which indicates that $k_{r(H)}/k_{r(D)}$ is 2.7.

In summary, the results indicate that intersystem crossing to a triplet state must be complete, and that the triplet is responsible for chemical reaction.

Acknowledgments.—This work was supported by grants from the Film Department of the du Pont Company and from the National Science Foundation.

(1) R. Criegee, *Ber.*, **64B**, 264 (1931).

(2) J. N. Pitts, R. L. Letsinger, R. P. Taylor, J. M. Patterson, G. Rechtenwald and R. B. Martin, THIS JOURNAL, **81**, 1068 (1959).

(3) Light absorption was essentially complete. Other experiments show that the quantum yields are independent of both light intensity and ketone concentration.

(4) Calculated by the method of Schultz[5] using a diffusion coefficient of 10^{-5} cm.^{2} sec.$^{-1}$ for benzophenone and benzhydrol in benzene.

(5) G. V. Schultz, *Z. physik. Chem.*, **8**, 284 (1956).

(6) M. Kasha, *Disc. Faraday Soc.*, **9**, 14 (1950). However, see J. W. Sidman, *Chem. Revs.*, **58**, 689 (1958), for a possible lower estimate.

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RECEIVED SEPTEMBER 8, 1959

THE ADDITION OF NITRONES TO OLEFINS. A NEW ROUTE TO ISOXAZOLIDINES

Sir:

The isolation of *cis*-N-methyl-3-oxa-2-azabicyclo[3.3.0]octane (III) from the pyrolysis of a mixture of the isomeric N-methyl-α-pipecoline oxides[1] suggests that the unsaturated nitrone I may be an intermediate. We have therefore investigated the cyclization of I and a homolog.

Monofunctional aliphatic nitrones have not been reported, since their preparations generally lead to aldol-type dimers.[2] Nevertheless, the reactive nitrone linkage of I, generated *in situ*, might well undergo intramolecular addition to the terminal olefin group.

Oxidation of an ether solution of N-methyl-N-5-hexenylhydroxylamine (V)[1] with excess mercuric oxide afforded III, characterized as its hydrogen oxalate (m.p. and m.m.p. 82–82.5°) and by comparison of the infrared spectra,[1] in 24% yield.

(1) A. C. Cope and N. A. LeBel, Abstracts of Papers, 133rd Meeting, American Chemical Society, San Francisco, California, April 13–18, 1958, p. 62-N; THIS JOURNAL, in press.

(2) R. Bonnett, R. F. C. Brown, V. M. Clark, I. O. Sutherland and A. Todd, *J. Chem. Soc.*, 2094 (1959), present a summary of the various dimeric structures for nitrones including aldolization structures, and report the syntheses of several monomeric alicyclic nitrones. *Cf.* also R. F. C. Brown, V. M. Clark, I. O. Sutherland and A. Todd, *ibid.*, 2109 (1959); R. F. C. Brown, V. M. Clark, M. Lamchen, B. Sklarz and A. Todd, *Proceedings of the Chemical Society*, 169 (1959).