CONTROLLED FORMATION OF *cis*- AND *trans*-DECALIN-9-CARBOXYLIC ACIDS BY CARBONYLATION

Sir:

Koch and Haaf[1] have described the preparation of decalin-9-carboxylic acid (80% *cis*) from β-decalol, sulfuric and formic acids. In a recent use of this preparation, we have found that products all the way from 84% *cis* (V) to 90% *trans* (IV) can be obtained, the only variable being the amount of fuming sulfuric acid included in the reaction medium (see Table I).

TABLE I

COMPOSITION OF DECALIN-9-CARBOXYLIC ACID IN PREPARATIONS BY UNIFORM PROCEDURE

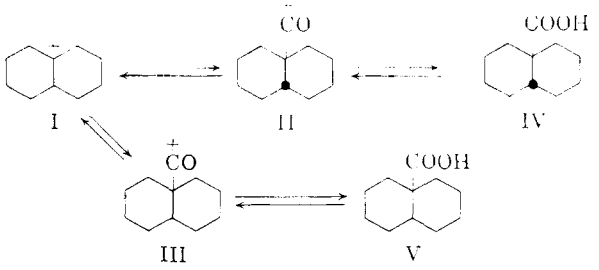
41.5 g. 98% H_2SO_4; 4.6 g. 88% formic acid; 0–5°, 1–1.5 hr.

30% oleum, g.	% *trans* (10.29 μ)	% *cis* (11.26 μ)	100-(% *cis*) − (% *trans*)
None	90	10	0
5	74	22.5	3.5
10	56	41	3
15	19	62.5	18.5
20	5	84	11
40	7.5	79	13.5
(Isomerization product)	9	87.5	3.5

For the rapid assay of the total acid fraction calibration curves were prepared by the infrared examination of six synthetic mixtures of the purified isomers (*cis*, m.p. 122°; *trans*, m.p. 135°) at total concentrations of 25 mg. of acid per cc. of carbon disulfide. Estimates made separately from the "*trans*" peak at 10.29 μ and the "*cis*" peak at 11.26 μ agreed within 3% on isomer composition of the products made with 0–10 g. of fuming sulfuric acid present, but indicated the presence of 11–18% of a further isomer from the more strongly acid media.

A sample of the acid assaying 90% *trans* was introduced into a mixture of 41.5 g. of 98% sulfuric acid and 20 g. of fuming sulfuric acid (30%), along with 5 g. of formic acid, over a period of 10 minutes. After 1.5 hours at 5° decalin-9-carboxylic acid was recovered in 90% yield, having the composition 87.5 *cis*, 9% *trans*. Thus kinetic control of carbonylation leads to *trans*-decalin-9-carboxylic acid, eventual equilibrium favoring the *cis* isomer.

Models show that the *trans*-acylium ion II,



with the C—C≡O⁺ atoms in a straight line, has little axial-axial interaction and should be rapidly formed without strain. On the other hand, in the *trans* acid IV, or its protonation product, the axial carboxyl appears more crowded than a methyl group, which in analogous cases is known to reverse the usual order of stability and to favor the *cis*-decalin system over the *trans*.[2,3,4,5]

In strong enough acid to make all the steps reversible, equilibrium is approached; K = (V)/(IV) = 4–11 at 5°, depending upon the composition of the unisolated 10% of the material. This is consistent with the value 1.5 at 250° obtained for 9-methyl-1-decalone.[3]

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(1) H. Koch and W. Haaf, *Angew. Chem.*, **70**, 311 (1958); *Ann.*, **618**, 251 (1958).

(2) W. E. Bachmann, A. Ross, A. S. Dreiding and P. A. S. Smith, *J. Org. Chem.*, **19**, 222 (1954).

(3) A. Ross, P. A. S. Smith and A. S. Dreiding, *ibid.*, **20**, 905 (1955).

(4) N. L. Allinger, *ibid.*, **21**, 915 (1956).

(5) W. G. Dauben and K. S. Pitzer in Newman, "Steric Effects in Organic Chemistry," John Wiley and Sons, New York, N. Y., 1956, pp. 30, 31.

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ON THE MECHANISM OF THE ENZYMATIC DECARBOXYLATION OF ACETOACETATE[1]

Sir:

In order to investigate the mechanism of the enzymatic decarboxylation of β-ketoacids, we have examined the oxygen exchange which accompanies the decarboxylation of acetoacetic acid, labeled in the carbonyl group with O^{18}, in the presence of the crystalline[2] decarboxylase from *Clostridium acetobutylicum*.

Acetone was labeled with O^{18} by allowing it to stand with enriched water (Stuart Oxygen Co., 1.4% O^{18}) and a trace of sodium hydroxide. Ethyl acetoacetate was saponified in 2 *M* potassium hydroxide in enriched water, and the potassium acetoacetate, labeled in the carbonyl and carboxyl groups, was obtained by evaporation *in vacuo* and purified by precipitation of an alcoholic solution with ether. The O^{18} content of the acetone was determined mass spectrometrically by measuring the ratio of the 58 and 60 peaks, using a Consolidated model 21-103C mass spectrometer; the cracking pattern of the acetone established its purity. Acetone from the enzymatic decarboxylation and from control experiments was swept from the reaction mixture with a stream of air through an ice-water condenser, and trapped at −78°. A sample of acetone vapor then was prepared for mass spectrometry by equilibrating liquid and vapor at 0°. Some water undoubtedly was present in the liquid sample, but earlier work[3] has shown that, in the absence of buffers, exchange between acetone and water at low temperatures is very slow. The internal consistency of the results further supports this conclusion. Control experiments with potassium acetoacetate were conducted by freezing the solution after 2.5

(1) This research was supported in part by a grant from the National Institutes of Health.

(2) G. A. Hamilton and F. H. Westheimer, THIS JOURNAL, **81**, 2277 (1959).

(3) M. Cohn and H. C. Urey, *ibid.*, **60**, 679 (1938).

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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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 con-

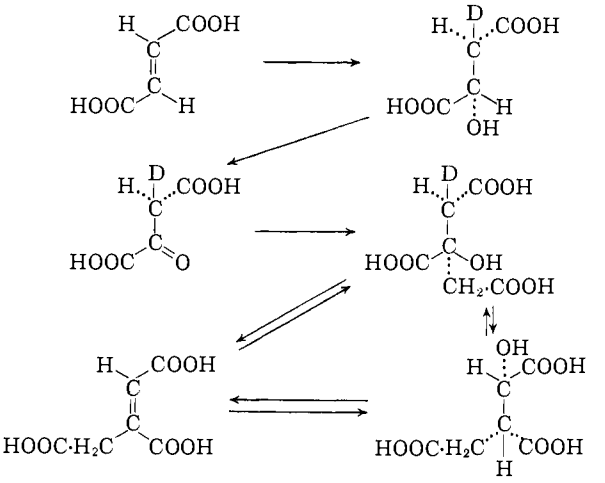

FIG. 1.—Stereochemistry of biochemical route from fumaric acid to *d*-isocitric acid.

DL-I

stant 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.

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.