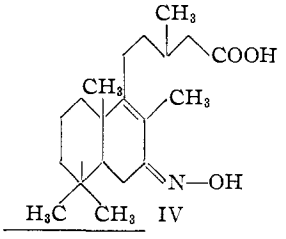
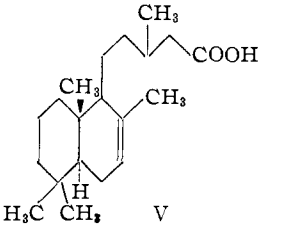
subjected to a two-step Barbier–Wieland degradation from whence a methyl ketone was derived, whose **semicarbazone** (III), m.p. 201.5–202°, $[\alpha]^{31}D$ +66.6° ($CHCl_3$), was found to have the same constants and infrared spectrum as that derivative of the methyl ketone (II) arising from the degradation of manoöl.[6] Repetition of this degradation yielded a semicarbazone which melted undepressed with III and had a specific rotation of +65.4°. Stereochemically and structurally, therefore, cativic acid is related to the dicyclic diterpenes.

Cativic acid contains one element of unsaturation (perbenzoic acid titration) which must lie in the ring system, for no carbon atoms were lost when the acid was ozonized. Furthermore, the ozonolysis product gave a positive iodoform reaction, from which test the inference was drawn that C-6 was one of the double bond terminals. Although manoöl and agathic acid have exocyclic unsaturation, the ozonization and infrared data on cativic acid allowed the elimination of this possibility. Methyl cativate (from diazomethane esterification of the acid), $n^{25}D$ 1.4954, $[\alpha]^{30}D$ −7.51°, when reacted with amyl nitrite and concentrated hydrochloric acid in chloroform at −30° and then warmed to room temperature over a total time of about 10 minutes, was converted directly to the α,β-unsaturated oxime (IV),[7] m.p. 121.5–122°, λ_{max} 246 mμ (log ε 4.01); λ (—C=C—C=N—OH) 6.17 μ. *Anal.* Calcd. for $C_{21}H_{35}O_3N$: C, 72.16; H, 10.09; N, 4.01. Found: C, 71.97; H, 10.31; N, 4.24. This evidence permits the complete structure of cativic acid to be written as V.



IV



V

(6) J. R. Hosking and C. W. Brandt, *Ber.*, **68**, 1311 (1935).

(7) Another example of this reaction is the conversion of α-pinene to nitrosopinene by boiling pinene nitrosochloride in carbon tetrachloride for two minutes: J. C. Earl and J. Kenner, *J. Chem. Soc.*, 1269 (1927).

CONTRIBUTION NO. 1245 FROM
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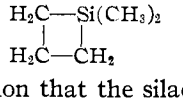
RECEIVED AUGUST 16, 1954

A SILICON-CONTAINING 4-RING

Sir:

We wish to record the synthesis of a highly strained silicon heterocycle with silicon as the hetero atom, 1,1-dimethyl-1-silacyclobutane, compound I.



I

On the assumption that the silacyclobutane ring is planar and that the C–C–C angle is tetrahedral (at least to a first approximation) calculation using 1.94 Å. for C–Si and 1.54 Å. for C–C bond lengths 80°48′ for the C–Si–C bond angle and 84°52′ for each of the two C–C–Si bond angles.[1]

The reaction sequence used for the synthesis of compound I was

$$Me_3Si(CH_2)_3Br \xrightarrow[(2)\ H_2O]{(1)\ H_2SO_4} [Br(CH_2)_3SiMe_2]_2O \xrightarrow[H_2SO_4]{NH_4Cl}$$

$$Br(CH_2)_3SiMe_2Cl \xrightarrow[Et_2O]{Mg} (CH_2)_3SiMe_2$$

Ring closure in dilute ether solution gave a 66% yield of the silacyclobutane, b. p. 81° (730 mm.,) $n^{20}D$ 1.4270, d^{20} 0.7746, MR_D 33.2 (calcd., 32.8); *Anal.* Calcd. for $C_5H_{12}Si$: Si, 28.0; C, 60.0; mol. wt., 100.2. Found: Si, 28.0; C, 60.3; mol. wt., 98. An infrared spectrum showe l no Si—H band and no maximum for C=C.

Chemical effects of the strain at the silicon atom are quite interesting. Preliminary experiments showed that compound I gives a highly exothermic reaction with 1 *N* potassium hydroxide in ethyl alcohol merely on mixing at room temperature. In further contrast to ordinary tetraalkylsilanes the silacyclobutane reacts violently with concentrated sulfuric acid at room temperature despite the heterogeneous nature of the reaction. The above reactions proceed without gas evolution and thus involve ring-opening.

Further proof of structure was afforded by treatment of the silacyclobutane with concentrated sulfuric acid at 0°. Ring-opening followed by hydrolysis of the reaction product gave as the expected product di-*n*-propyltetramethyldisiloxane, identical with an authentic sample prepared from hydrolysis of the product obtained from dimethyldiethoxysilane and *n*-propylmagnesium bromide, b. p. 182° (730 mm.), $n^{20}D$ 1.4088, MR_D 67.4 (calcd., 67.4). *Anal.* Calcd. for $C_{10}H_{26}SiO_2$: Si, 25.8. Found: Si, 25.9.

(1) Given a nearly planar structure for the silacyclobutane ring, it follows that the C–Si–C angle cannot be greatly increased without expansion of the C–C–C angle beyond the tetrahedral value. For small deviation from planarity in perfluorocyclobutane see H. P. Lenaire and R. L. Livingston, THIS JOURNAL, **74**, 5732 (1952).

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RECEIVED JULY 22, 1954

ISOCITRITASE: A NEW TRICARBOXYLIC ACID CLEAVAGE SYSTEM

Sir:

Campbell, Smith and Eagles[1] reported the formation of glyoxylic acid from citric and *cis*-aconitic acids by crude extracts of *Pseudomonas aeruginosa*. The pertinence of this observation arises from its indication of a new enzyme system for the cleavage of tricarboxylic acids, and in the formation of glyoxylic acid, a biosynthetic precursor of glycine[2] and of active C_1.[3]

By the use of sonic extracts of this pseudomonad, fractionated to remove aconitase, we have shown

(1) J. J. R. Campbell, R. A. Smith and B. A. Eagles, *Biochim. et Biophys. Acta*, **11**, 594 (1953).

(2) S. Weinhouse and B. Friedmann, *J. Biol. Chem.*, **191**, 707 (1951).

(3) S. Weinhouse and B. Friedmann, *ibid.*, **197**, 733 (1952).

that a new enzyme, "isocitritase," catalyzes the reaction

Isocitric acid ⟶ glyoxylic acid + succinic acid (1)

The conditions for growing the cells, preparing the extracts, and separating aconitase from isocitritase are shown in Table I. Isocitrate is the substrate

TABLE I

SEPARATION OF ISOCITRITASE FROM ACONITASE

Reaction 10 minutes, 30° under nitrogen in 3-ml. containing: 200 μM. tris (tris-(hydroxymethyl)-aminomethane) buffer *p*H 7.6; 10 μM. $MgCl_2$; 10 μM. glutathione; 20 μM. DL-isocitrate; reaction started with enzyme (0.5 to 5 units), stopped with 0.3 ml. 100% TCA.

	Fraction	Absorption ratio 280/260	Protein g.	Isocitritase[b] units × 10^3	recov. %	Aconitase[c] units × 10^5
1	Extract[a]	0.57	2.3	6.46	100	12.4
2	$AmSO_4$-1 0.25–0.88 satd.	0.58	1.8	3.8	59	
3	2 + protamine	0.93	1.6	2.9	44	
4	$AmSO_4$-2 0.43–0.62 satd.	1.49	0.46	2.7	42	nil

[a] *P. aeruginosa*, ATCC 9027, was grown with aeration in an acetate mineral salts medium; 22 g. of cell paste, suspended in 200 ml. of *M*/50 phosphate buffer *p*H 7.0 containing 50 mg. of glutathione, was oscillated 15 min. in a 10 KC. Raytheon, and centrifuged 1 hour at 16,000 × *g* to obtain an extract. [b] 1 unit = 1 μM. glyoxylate formed per 10 min. in protocol above. [c] 1 unit = 0.001 OD increase/min. at 240 mμ; ≈ 0.05 μM. aconitate accumulated/hour/3 ml. cuvette: Racker, *Biochim. et Biophys. Acta*, **4**, 211 (1950).

of this enzyme, as shown by the data in Table II; *i.e.*, with crude preparations all three tricarboxylic acids yield glyoxylate, whereas after the removal of aconitase only isocitrate serves as substrate. The stoichiometry of the reaction with both crude and fractionated extracts is also indicated in Table II.

TABLE II

STOICHIOMETRY AND SUBSTRATE SPECIFICITY OF ISOCITRITASE

Reaction as in Table I except run 20 minutes.

Fraction and substrate	μM.	Used μM.	Products formed Glyoxylate[b] μM	Succinate[c] μM
Extract, 1.1 mg. P				
DL-isocitrate,	20	4.14[a]	3.64	4.18
cis-aconitrate,	12.5		2.24	
citrate	12.5		0.72	
Isocitritase (Fr. 4, Tbl. I, 0.75 mg P)				
DL-isocitrate,	20	4.54	4.81	4.58
cis-aconitate,	12.5		nil	
citrate	12.5		nil	

[a] D-Isocitric acid determined by method of Ochoa in J. B. Sumner and K. Myrback, "The Enzymes," Academic Press, Inc., New York, N. Y., 1952, Vol. II, p. 1017. [b] Glyoxylate determined by the method of Friedemann and Haugen, *J. Biol. Chem.*, **147**, 415 (1943), using crystalline glyoxylic acid 2,4-dinitrophenylhydrazone as standard. [c] Succinate measured with succinoxidase according to Dietrich, *et al.*, *Arch. Biochem.*, **41**, 118 (1952).

Early fractionations revealed requirements for a divalent metal and for sulfhydryl as activators. Dialysis of Fraction 4, Table I, against *M*/50 Versene *p*H 7.4 for 20 hours at 4°, followed by 14 hours dialysis *vs.* *M*/50 KCl at the same temperature, rendered the isocitritase completely dependent on magnesium ion and a sulfhydryl compound, as indicated by the saturation curves in Fig. 1. Glutathione and cysteine were equally effective activators for isocitritase at all stages of enzyme purity so far studied. Ferrous and cobaltous ions were 40% as active, and manganous about 20% as active, as magnesium.

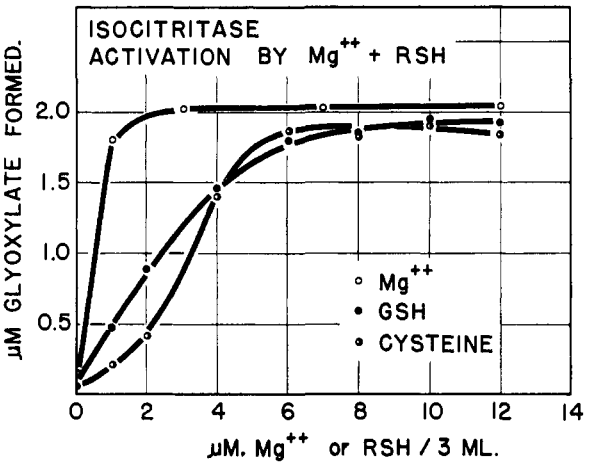

Fig. 1.—Magnesium and sulfhydryl activation of isocitritase: reaction 10 minutes, 30° under nitrogen in 3 ml. containing: 200 μM. tris buffer, *p*H 7.6; 3 μM. $MgCl_2$ or as indicated; 10 μM. RSH or as indicated; enzyme Fr. 4, table I ≈ .38 mg. protein; reaction started with 20 μM. DL-isocitrate; stopped with 0.3 ml. 100% TCA.

Isocitritase does not require Coenzyme A, as indicated by retention of full activity after Dowex treatment,[4] which completely removed the Coenzyme A from the extracts, as indicated by transacetylase assay.[5]

Although the reaction does not proceed to completion, and succinate inhibits the forward reaction, our experiments have not so far demonstrated a reversibility either with crude or fractionated preparations unsupplemented, or upon the addition of ATP or other activators. Further data will be required to clarify this point.

The isocitritase reaction (1) constitutes an aldolase cleavage of isocitric acid analogous to the inducible citritase, or citridesmolase, which cleaves citrate to oxalacetate and acetate, as previously described in *Streptococcus faecalis*[6] and *Escherichia coli*.[7]

(4) H. Chantrenne and F. Lipmann, *J. Biol. Chem.*, **187**, 757 (1950).
(5) E. R. Stadtman, G. D. Novelli and F. Lipmann, *ibid.*, **191**, 365 (1951).
(6) D. C. Gillespie and I. C. Gunsalus, *Bact. Proc.*, **80**, (1953).
(7) M. Grunberg-Manago and I. C. Gunsalus, *ibid.*, **73** (1953), and *J. Bact.*, 1954, in press.

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RECEIVED AUGUST 16, 1954

THE DEGREE OF POLYMERIZATION OF THE CELLULOSE COMPONENT OF BALSAM FIR

Sir:

Many attempts have been made to establish the degree of polymerization (D.P.) of native wood cel-