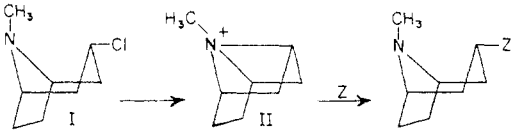
benzylamine to give 3α-benzylaminotropane isolated as the dihydrochloride, m.p. 272° dec., undepressed when mixed with the above samples. This result confirms the belief that chloride belonged in the alpha series.

The previous assignment[3] of configuration to the isomeric 3-aminotropanes was based on analogy. Direct and independent chemical evidence was obtained by transforming authentic methyl tropane-3β-carboxylate[4] (our oxalate melted 149–151°, undepressed with an authentic sample[4]) to be the corresponding carboxamide (m.p. 151–152°; *Anal.* Calcd. for $C_9H_{16}N_2O$: N, 16.66. Found: N, 16.34). The latter was converted to 3β-aminotropane (phenylthioureide, m.p. 174.5–175°, undepressed when admixed with an authentic sample[3]) by the Hofmann rearrangement, a reaction known to proceed with retention of configuration.[5]

Sodium azide and 3α-chlorotropane gave a liquid azide (b.p. 58–60° (0.2 mm.) *Anal.* Calcd. for $C_8H_{14}N_4$: N_{AP}, 8.47. Found: N_{AP}, 8.53.[6] Hydrochloride, m.p. 167–169°, *Anal.* Calcd. for $C_8H_{13}ClN$: C, 47.42; H, 7.41; N, 27.66; Cl, 17.50. Found: C, 46.55; H, 7.66; N, 27.54; Cl, 17.35) which on catalytic hydrogenation afforded 3α-aminotropane isolated as the phenylthioureide, m.p. 156–158° (no depression when mixed with other samples).

Thus the over-all result of the reaction of 3α-chlorotropane with nucleophilic agents is retention of configuration. Undoubtedly this apparent retention is the result of two inversions, one of which involves participation of the nitrogen. Accordingly it is suggested that the reaction of the chloride I with a nucleophile Z proceeds *via* the ion II



This scheme is supported further by the facts that (1) conversion of tropine or pseudotropine to the corresponding chlorides proceeds with inversion and (2) the benzyl bromide quaternary salt of I (m.p. 213°; *Anal.* Calcd. for $C_{15}H_{21}BrClN$: C, 54.47; H, 6.40; Br, 24.17. Found: C, 54.46; H, 6.12; Br, 24.28) does not appear to react with potassium cyanide under conditions sufficient to permit the chloride I, to produce a crystalline nitrile (m.p. 64–66°; *Anal.* Calcd. for $C_9H_{14}N_2$: N_{AP}, 9.33. Found: N_{AP}, 9.28[6]). In both instances the nitrogen is positively charged, a circumstance which precludes participation with C-3 of the tropane nucleus.

The crystalline nitrile furnished a benzoyl ketone[7] (b.p. 130–134° (0.2 mm.), n^{25} 1.5540. *Anal.* Calcd. for $C_{15}H_{23}NO$: N, 6.11. Found: N, 6.05) on treatment with phenylmagnesium bromide. Methanolysis of the nitrile gave a methyl ester, whose oxalate melted at 149–151° and thus must be the β-ester.[4] Since it is not known whether inversion occurred at the alcoholysis stage[4] definite assignment of configuration to the nitrile awaits the preparation of authentic isomers, a project which is engaging our attention at the present time.

(4) C. Zirkle, *et al.*, "Abstracts XVI International Congress for Pure and Applied Chemistry," Paris, July, 1957, Vol. II, p. 153. We are deeply grateful to Dr. Zirkle for supplying us with directions for preparing the isomeric methyl tropane-3-carboxylates, for giving us samples of derivatives of each of the isomers and for informing us that the predominant hydrolysis product of methyl tropane 3α-carboxylate in either water or hydrochloric acid is tropane 3β-carboxylic acid; all by private communication.

(5) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 501.

(6) Perchloric acid titration for basic nitrogen.

(7) This compound is claimed but not described in a patent recently issued to Zirkle, U. S. Patent 2,800,480 (July 23, 1957). Treatment of the benzoyl ketone with phenyllithium gave the corresponding carbinol, m.p. 184–185°, which was reported by Zirkle (U. S. Patent 2,800,478, July 23, 1957) to melt at 185–186°.

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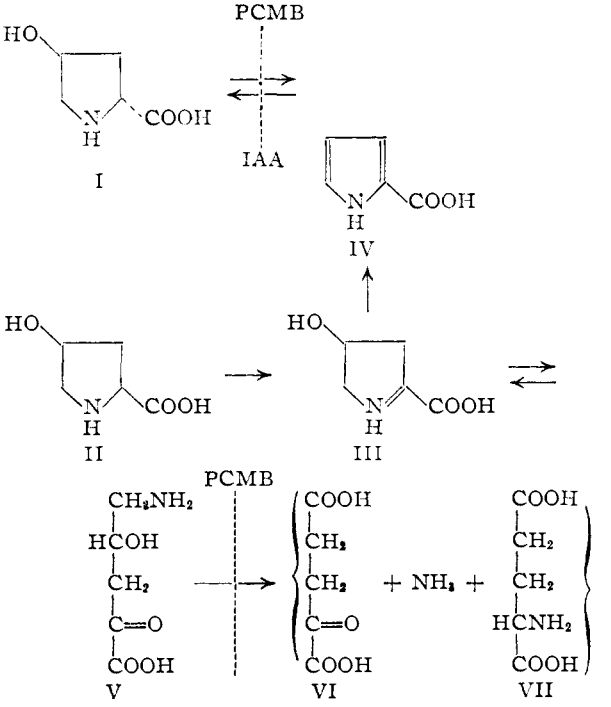
ENZYMATIC CONVERSION OF D-ALLOHYDROXYPROLINE TO L-GLUTAMATE

Sir:

As reported earlier,[1,2] extracts of hydroxyproline-adapted soil bacteria catalyze the conversion of hydroxyproline to glutamic acid. An inducible epimerase,[2] catalyzing rapid interconversion of the two hydroxyproline epimers, permits equally efficient formation of L-glutamate (VII) from either L-hydroxyproline (I) or D-allohydroxyproline (II). The simplest reaction sequence would indicate conversion of L-hydroxyproline to L-glutamate with retention of configuration at the α-carbon. Recent evidence, however, indicates D-allohydroxyproline as the more direct glutamate precursor, according to the tentative reaction sequence



Supernatants of bacterial sonicates centrifuged at 25,000 × *g* catalyze the over-all reaction: L

(1) E. Adams, *Federation Proc.*, **15**, 209 (1956).

(2) E. Adams, *ibid.*, **16**, 142 (1957).

hydroxyproline (or D-allohydroxyproline) → α-ketoglutarate (VI) + L-glutamate. Further centrifugation at 105,000 × *g* yields particles which cannot utilize I but oxidize II to Δ^1-pyrroline-4-hydroxy-2-carboxylic acid (III). Accumulation of this unstable product is recognized by certain of the criteria cited by Radhakrishnan and Meister,[3] particularly condensation with *o*-aminobenzaldehyde to form a characteristic yellow compound, and rapid conversion in acid to pyrrole-2-carboxylic acid (IV). In analogy with the corresponding oxidation product of D-proline,[4] III is considered to be in equilibrium with its hydrolyzed form, α-keto-γ-hydroxy-δ-aminovaleric acid (V).

While the ultracentrifugal pellet catalyzes only the conversion of II to III (and secondarily to IV), the supernatant contains hydroxyproline-2-epimerase (I ⇄ II) and enzymes for steps beyond V. Thus the ultra-centrifugal supernatant alone cannot utilize either hydroxyproline epimer, but after recombination with the pellet again catalyzes L-glutamate formation from either epimer. As further evidence for the step II → III, kidney D-amino acid oxidase can replace the ultracentrifugal pellet in restoring the over-all reaction.

Sulfhydryl reagents acting as selective inhibitors of hydroxyproline epimerization provide additional support for the postulated reaction sequence. Thus 10^{-4} *M* *p*-chloromercuribenzoate (PCMB) in unfractionated sonicates prevents any reaction with L-hydroxyproline but permits accumulation of III and IV from D-allohydroxyproline. Iodoacetate (IAA) at 10^{-2} *M* is more selective, permitting no reaction with L-hydroxyproline but almost stoichiometric accumulation of L-glutamate plus α-ketoglutarate from D-allohydroxyproline. These two inhibitors are therefore believed to act in the reaction sequence as shown above.[5]

TABLE I

SELECTIVE CONVERSION OF D-ALLOHYDROXYPROLINE TO L-GLUTAMATE

Incubation mixtures contained per ml.: 6 mg. of enzyme protein; 50 μmoles of *p*H 7.9 tris-(hydroxymethyl)-aminomethane, 10 μmoles of hydroxyproline, 10 μmoles of iodoacetic acid. Aliquots taken before and after 2-hour incubation in O_2 (1 atm.) at 25° were brought to 0.5 *N* HCl and assayed for hydroxyproline,[6] L-glutamate[7] (enzymatic decarboxylation) and α-ketoglutarate.[8] All values are in μmoles/ml.

Substrate	Hydroxyproline[a] consumed	L-Glutamate formed	α-Ketoglutarate formed
L-Hydroxyproline	0.6	Trace[b]	0.3
D-Allohydroxyproline	9.2	7.1	1.3
None	...	0.0	0.1

[a] No formation of pyrrole-2-carboxylate (which would mask hydroxyproline disappearance) was found by direct assay with *p*-dimethylaminobenzaldehyde in acid. [b] Detected on paper chromatograms but insufficient for assay.

(3) III was first described (A. N. Radhakrishnan and A. Meister, *J. Biol. Chem.* **226**, 559 (1957)) as the product of D-allohydroxyproline with kidney D-amino acid oxidase. The specificity of the bacterial D-allohydroxyproline oxidase is shown by failure to oxidize other D-amino acids including D-hydroxyproline.

(4) A. Meister, *ibid.*, **206**, 577 (1954).

(5) Direct assays of hydroxyproline-2-epimerase indicate virtually complete inhibition by these two reagents at the concentrations cited.

(6) R. E. Neuman and M. A. Logan, *J. Biol. Chem.*, **184**, 299 (1950).

(7) V. A. Najjar and J. Fisher, *ibid.*, **206**, 215 (1954).

(8) T. E. Friedemann and G. E. Haugen, *ibid.*, **147**, 415 (1943).

Direct demonstration of efficient glutamate formation from the postulated intermediate III[9] has been equivocal owing to the latter's rapid conversion to pyrrole-2-carboxylic acid. However small amounts of glutamate were consistently formed both in crude sonicates and ultracentrifugal supernatants.

(9) A sample of this compound, partly purified and free of hydroxyproline, was kindly provided by Dr. A. N. Radhakrishnan and Dr. A. Meister.

(10) Research supported by funds from the National Science Foundation.

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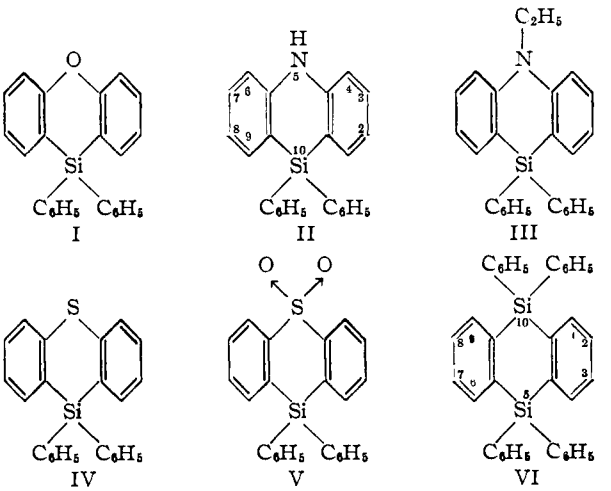
THE REPLACEMENT OF SULFUR IN SOME HETEROCYCLES BY THE DIPHENYLSILYLENE GROUP

Sir:

We are reporting a novel type of reaction for the formation of some cyclic compounds containing silicon as a hetero atom. On heating diphenylsilane with sulfur-containing heterocycles such as phenoxathiin, phenothiazine, 10-ethylphenothiazine and thianthrene, hydrogen sulfide was evolved slowly, and from the crude reaction mixtures compounds were isolated in which the sulfur atom is replaced by the diphenylsilylene group.

An equimolecular mixture of diphenylsilane and phenoxathiin was refluxed for 6 days, at which time the evolution of hydrogen sulfide had essentially ceased. The reaction mixture was worked up by distillation under reduced pressure, followed by chromatography of the high-boiling fractions on alumina with petroleum ether (b.p. 60–70°), and recrystallization of the resulting product from ethanol. There was obtained 10,10-diphenylphenoxasilin (I), m.p. 178–179°, in a 2.0% yield. The compound was shown to be identical by mixed melting point and infrared spectra with an authentic sample obtained from the reaction of *o,o'*-dilithiodiphenyl ether and diphenyldichlorosilane.[1]



A mixture of diphenylsilane and phenothiazine was refluxed for 3 days and worked up in the same

(1) K. Oita and H. Gilman, THIS JOURNAL, **79**, 339 (1957); C. H. S. Hitchcock, F. G. Mann and A. Vanterpool, *J. Chem., Soc.*, 4537 (1957).