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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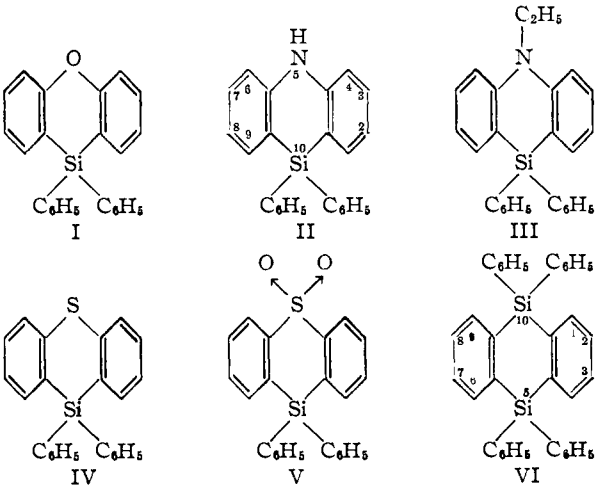
RECEIVED OCTOBER 10, 1957

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).

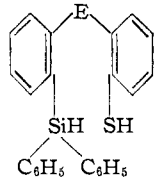
manner to give 10,10-diphenylphenazasiline[2] (II) in a 1.2% yield, m.p. 197–198°; *Anal.* Calcd. for $C_{24}H_{19}NSi$: C, 82.49; H, 5.48; Si, 8.03. Found: C, 82.08, 82.27; H, 5.28, 5.23; Si, 8.10. On metalation of II with *n*-butyllithium, followed by treatment with ethyl sulfate, 5-ethyl-10,10-diphenylphenazasiline (III) was formed in an almost quantitative yield, m.p. 122–123°. *Anal.* Calcd. for $C_{26}H_{23}NSi$: C, 82.74; H, 6.14; N, 3.71; Si, 7.41. Found: C, 82.35; H, 6.03; N, 4.39, 4.12; Si, 7.48. The same compound III was obtained in a 6.6% yield from the reaction of diphenylsilane with 10-ethylphenothiazine after heating for 6 days at reflux temperature.

From the reaction of diphenylsilane and thianthrene (1:1 ratio), after heating for 3.5 days at 250–260° and working up in the manner described above, 10,10-diphenylphenothiasilin[2] (IV) was obtained in a 4.7% yield, m.p. 157–158°. *Anal.* Calcd. for $C_{24}H_{18}SSi$: Si, 7.66. Found: Si, 7.72. The compound was oxidized with hydrogen peroxide in acetic acid to form 10,10-diphenylphenothiasilin-5-dioxide (V), m.p. 208–209°, which was shown to be identical with an authentic sample obtained from the reaction of 2,2′-dilithiodiphenyl sulfone and diphenyldichlorosilane.[3] An attempt to isolate 5,5,10,10-tetraphenylsilanthrene[2] (VI) from the reaction of diphenylsilane and thianthrene in a 2:1 ratio has so far been unsuccessful; only 4.05% of IV was obtained. Thianthrene-5-dioxide[4] also evolved hydrogen sulfide on heating with diphenylsilane, but attempts to isolate V from the reaction mixture were unsuccessful.

Whereas in all reactions essentially no diphenylsilane was recovered, the sulfur heterocycle was recovered in yields ranging from 20 to 50%. Triphenylsilane[5] was obtained in 5–16% yields as a by-product in all reactions, together with traces of tetraphenylsilane.[5]

Since hydrogen sulfide was evolved on exposure of the crude reaction mixtures to moisture and on chromatography of the distillation fractions on alumina, the formation of Si–S–H or Si–S–Si type by-products is postulated. Such products might have resulted as a consequence of reaction between hydrogen sulfide and diphenylsilane or triphenylsilane.

In order to throw light on the possible intermediate formation of a triphenylsilane-thiophenol type VII, which in a further step splits off hydrogen sulfide to form the silicon heterocycle



VII

$(C_6H_5)_3Si—S—C_6H_5—CH_3$-*p*

VIII

the reaction between triphenylsilane and *p*-thiocresol was investigated. On heating an equimolecular mixture for 5 days at 220–230°, however, none of the expected triphenyl-*p*-tolylsilane was formed, but triphenyl-*p*-thiocresoxysilane (VIII) was obtained in a 69% yield, m.p. 79–80°. *Anal.* Calcd. for $C_{25}H_{22}SSi$: Si, 7.33. Found: Si, 7.27. The structure of VIII was established by hydrolysis with alkali, which gave *p*-thiocresol, triphenylsilanol and hexaphenyldisiloxane. From the reaction of VIII with triphenylsilyllithium in tetrahydrofuran, hexaphenyldisilane was isolated in an 82% yield and *p*-thiocresol in a 57% yield.

Acknowledgment.—This research was supported by the United States Air Force under Contract AF 33(616)-3510 monitored by Materials Laboratory, Directorate of Laboratories, Wright Air Development Center, Wright-Patterson AFB, Ohio. The authors are grateful to the Materials Laboratory at Dayton, Ohio, for very helpful suggestions. Infrared analyses were obtained through the courtesy of the Institute for Atomic Research, Iowa State College, and special acknowledgment is made to E. Miller Layton and Miss M. Powers for the spectra.

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RECEIVED OCTOBER 5, 1957

(2) The names and the numbering systems used herein were recommended by the editorial staff of *Chemical Abstracts*.

(3) K. Oita and H. Gilman, *J. Org. Chem.*, **22**, 336 (1957).

(4) H. Gilman and D. R. Swayampati, THIS JOURNAL, **77**, 5946 (1955).

(5) These compounds may have been formed by disproportionation of diphenylsilane. See H. Gilman and D. Miles, *ibid.*, in press.

BIOSYNTHESIS OF GLYCOGEN FROM URIDINE DIPHOSPHATE GLUCOSE[1]

Sir:

Previous work has shown that UDPG[2] acts as glucose donor in the synthesis of trehalose phosphate,[3] sucrose,[4] sucrose phosphate[5] and cellulose.[6]

TABLE I

ANALYTICAL CHANGES

The complete system contained: 0.5 μmole of UDPG, 0.33 μmole of glycogen, tris-(hydroxymethyl)-aminomethane buffer of *p*H 7.4, 0.01 *M* ethylenediaminetetraacetate and 0.02 ml. of enzyme. Total volume 0.07 ml. Incubation: 45 min. at 37°. The enzyme was prepared from an aqueous extract of rat liver by acidification to *p*H 5. The precipitate was washed four times with acetate buffer of *p*H 5 and redissolved in buffer. Results in μmoles.

	Δ UDP[a]	Δ Glycogen[b]
Complete system	0.22	0.27
No UDPG	0	−0.03

[a] Estimated with pyruvate kinase.[7] [b] Measured with a phenol–sulfuric acid reagent after precipitation with ethanol[8] and expressed as glucose.

When UDPG is incubated with a liver enzyme and a small amount of glycogen the chemical changes shown in Table I were found to take place. Approximately equal amounts of UDP and of glycogen were formed. Such an increase in glycogen could only be detected with liver preparations freed from amylase. Other preparations obtained

(1) This investigation was supported in part by a research grant (No. G-3442) from the National Institutes of Health, U. S. Public Health Service, and from Laboratorios de Investigación de E. R. Squibb & Sons Argentina, S. A.

(2) UDPG = uridine diphosphate glucose; UDP = uridine diphosphate.

(3) L. F. Leloir and E. Cabib, THIS JOURNAL, **75**, 5445 (1953).

(4) L. F. Leloir and C. E. Cardini, *ibid.*, **75**, 6084 (1953).

(5) L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **214**, 157 (1955).

(6) L. Glaser, *Biochim. et Biophys. Acta*, **25**, 436 (1957).

(7) E. Cabib and L. F. Leloir, *J. Biol. Chem.*, in press.

(8) H. Montgomery, *Arch. Biochem. Biophys.*, **67**, 378 (1957).