added to an excess of methylmagnesium iodide over a period of five minutes with rapid stirring. The mixture was hydrolyzed by adding ice and dilute hydrochloric acid. The organic layer was dried and evaporated on the steam-bath. The colorless oil which remained after evaporation was crystallized from petroleum ligroin. The melting point was 75–77°, yield 1.02 g. This compound was not further investigated.

Two *meso* Forms of 2,5-Diphenyl-4-methyl-4-tetrahydropyranol.—Eighty ml. of a benzene solution containing 1.1 g. of *cis*-2,6-diphenyl-4-tetrahydropyrone[7] was added over a period of 20 min. to a solution of an excess of methylmagnesium iodide in ether which was being stirred rapidly. The reaction was allowed to proceed for 20 min. and hydrolyzed with ice and diluted HCl. The organic layer was dried and evaporated on a steam-bath and the residue chromatographed on the Magnesol–Celite column. A fraction of 20 mg. melting at 67–69°, which we believe to be the *meso*-1-compound, was followed by a larger fraction of *meso*-2-compound weighing 140 mg. which melted from 142–145°. This latter fraction proved to be identical with the material from the *t*-butyl alcohol–benzaldehyde reaction by mixed melting point.

2,6-Diphenyl-3,5-dimethyl-4-ethyl-4-tetrahydropyranol.—An excess of ethylmagnesium bromide in 180 ml. of ether was added to 11 g. of 2,6-diphenyl-3,5-dimethyl-4-tetrahydropyrone[8] dissolved in ether. After reaction, the material was hydrolyzed with NH_4Cl solution and the product recovered and crystallized from petroleum ligroin; yield, 9 g., m.p. 177–178°. This substance is identical with that from the acid condensation; the mixed melting point showed no depression.

2,6-Diphenyl-3,4,5-trimethyl-4-tetrahydropyranol.—This compound was prepared as above, substituting methyl Grignard for the ethyl compound; yield 8.5 g., m.p. 158–159°. This compound is not identical with the compound prepared by acid condensation above. The mixed melting point was 138–143°.

Infrared spectra were obtained by using a Nujol mull spread on a salt plate. A Baird model B recording spectrophotometer was used.

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(8) F. R. Japp and W. Maitland, *J. Chem. Soc.*, **85**, 1484 (1904).

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The Synthesis of 6-Thioguanine

BY GERTRUDE B. ELION AND GEORGE H. HITCHINGS

RECEIVED OCTOBER 18, 1954

As part of a program devoted to the investigation of antimetabolites of the purine and pyrimidine bases,[1,2] 6-thioguanine (2-amino-6-mercaptopurine) was synthesized. This compound has been found to behave as a purine antagonist similar to 6-mercaptopurine in *Lactobacillus casei*[3–5] and to exhibit activity against a number of animal tumors.[6,7]

Thioguanine was prepared in the first instance by the reaction of a suspension of guanine in tetrahydronaphthalene with phosphorus pentasulfide, a reaction which earlier had given satisfactory results with pyrimidines and quinazolines[8] and later was found fruitful in the conversion of hypoxanthine to 6-mercaptopurine.[9] However, erratic results were obtained with guanine; in many instances only unreacted guanine was isolated from the reaction mixture. This was believed to be due to the extreme insolubility of both starting material and product in the solvent, with a resultant dependence on the physical state of the starting material. This interpretation finds support in the superior results obtainable through the use of solvents, such as pyridine, in which a greater solubility is demonstrable.

Experimental

A mixture of 10 g. of finely powdered guanine and 50 g. of powdered phosphorus pentasulfide in 250 ml. of dry pyridine was heated under reflux conditions for 2.5 hours. The pyridine was removed by distillation under reduced pressure and the residue was heated with 200 ml. of water for about ten minutes. After cooling, 100 ml. of concentrated ammonium hydroxide was added and the mixture thoroughly chilled. The insoluble residue and the precipitate of ammonium phosphate was filtered off. The orange filtrate was acidified to *p*H 4 with hydrochloric acid and kept at 4° overnight. The precipitate of crude thioguanine was collected and treated with 200 ml. of 6 *N* ammonium hydroxide. The insoluble residue consisting mainly of guanine was removed by filtration. After removal of most of the excess ammonium hydroxide from the filtrate under reduced pressure, the solution was adjusted to *ca.* *p*H 4 with hydrochloric acid and chilled. Pale yellow needles of thioguanine were collected, washed with water and dried at 110°. This product (3.5 g.) was 93% pure on the basis of its ultraviolet absorption spectrum. A sample was purified for analysis by recrystallization from 1000 parts of hot water. The colorless needles thus obtained did not melt below 360°. Ultraviolet absorption spectrum: at *p*H 1, λ_{max} 258, 347 mμ (E_m 8,100, 20,900); at *p*H 11, λ_{max} 242, 270, 322 mμ (E_m 8,700, 7,200, 16,000).

Anal. Calcd. for $C_5H_5N_5S$: C, 35.9; H, 3.0; N, 41.9. Found: C, 36.0; H, 3.3; N, 41.8.

(8) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2138 (1947).

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The Occurrence of a Sulfuric Acid Ester of Choline in the Mycelium of a Strain of *Penicillium chrysogenum*

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RECEIVED OCTOBER 29, 1954

In a recent publication Gordon, *et al.*,[1] stated, that they had found a relatively high quantity of rather loosely bound methionine in a hot water extract of the mycelium of *P. chrysogenum* (Wis 49-133). We too are investigating the sulfur metabolism of this particular strain of *P. chrysogenum* and we wish to report the occurrence of the sulfuric acid ester of choline, $(CH_3)_3N^+$-CH_2-CH_2-O-SO_3^-, in mycelial extracts of this mould. The culture filtrates did not contain this ester.

The presence of ethereal sulfates in culture filtrates of Penicillia has been reported,[2] but so far as we are aware this particular ester has only been found by Woolley and Peterson[3] in the mycelium of *Aspergillus sydowi*.

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(3) D. W. Woolley and W. H. Peterson, *J. Biol. Chem.* **122**, 213 (1937).

(1) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950).

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(6) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock and G. H. Hitchings, *Proc. Am. Assoc. Cancer Res.*, **1**, 9 (1954).

(7) L. W. Law, *Proc. Soc. Exper. Biol. Med.*, **84**, 109 (1953).

In a typical experiment the mould was cultivated in 20 ml. of synthetic medium[4] containing 300 microcuries of radioactive sulfur as sulfate.[5] Vegetative inoculum was used. After three days incubation at 26° on a rotatory shaker (300 r.p.m., stroke 1.5 cm.) the mycelium was filtered off, washed thrice with 5 ml. of cold water and homogenized in cold dilute hydrochloric acid (*p*H 2). The insoluble cell material was centrifuged off and the supernatant deproteinized according to Sevag, *et al.*[6]

One-dimensional paper chromatograms were made of this extract using as solvents propanol–water (70/30, v./v.),[7] *t*-butyl alcohol–formic acid–water (70/15/15, v./v.),[8] and propanol–water–diethylamine (85/15/3, v./v.).[9] Autoradiographs revealed besides a strong spot of sulfate a second one with R_f value 0.52, 0.45 and 0.23, respectively, on Whatman paper no. 1 in the above mentioned solvents. Weaker spots due to oxidized glutathione, methionine and unidentified compounds were observed.

The substance having R_f 0.45 in *t*-butyl alcohol–formic acid–water was eluted from chromatograms on paper cylinders and again chromatographed using as solvent propanol–water. This gave one radioactive zone with R_f 0.52. After elution the sulfur compound was characterized as follows: it passed columns of Dowex-50 (H^+-form) and Amberlite IRA-400 (OH^--form) and did not move on paper electrophoresis at *p*H 5.6 or 8.5 (except for electroendosmosis). Hydrolysis with 1 *N* HCl for 15 minutes at 100° gave radioactive sulfate, which was identified by paper chromatography and paper electrophoresis.

Radioactive choline sulfate synthesized from $H_2S^{35}O_4$ and choline chloride[10] behaved exactly in the same manner. Chromatography of the compound from the mycelial extract and the synthetic choline sulfate together gave only one radioactive spot in the three solvents used.

It is worth noting that choline sulfate is not retained by a strong acid or basic ion exchanger although it has a negative and a positive charge. Apparently one group prevents the other one from attachment to the ion exchanger.

The insoluble cell material left after the extraction with dilute hydrochloric acid was treated with hot 5% trichloroacetic acid (TCA). In this extract we found as sulfur compound sulfate only and no trace of methionine. This latter substance was of course detected in hydrolysates of the insoluble residue left after the TCA extraction.

Although we did find some methionine in our first mycelial extract, the quantities are much less than those reported by Gordon, *et al.*[1] This might be due to the different method used in making the extracts.

(4) F. G. Jarvis and M. J. Johnson, THIS JOURNAL, **69**, 3010 (1947).

(5) Sometimes the sulfur content was lowered, but this makes not much qualitative difference in the sulfur compounds produced.

(6) M. G. Sevag, D. B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).

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(8) R. J. Block, *Anal. Chem.*, **22**, 1327 (1950).

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Redistribution of Et_4Si and Pr_4Si[1]

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RECEIVED OCTOBER 13, 1954

The random redistribution of organic groups in certain metal alkyls upon heating with anhydrous aluminum chloride has been well established, particularly in the case of lead alkyls.[3] The redistribution of Et_4Si and Pr_4Si has also qualitatively been demonstrated[4]; however a number of the products were not identified, and it was not possible to conclude whether isomerization of propyl groups had occurred.

We undertook to examine this redistribution reaction in greater detail because of its possible utility in synthesizing mixed tetraalkylsilanes and because of the growing importance of redistribution reactions in silicone technology.[5]

It has been found that, just as with lead alkyls,[3b] the reaction does indeed proceed without appreciable isomerization. This was established by carefully separating the redistribution products and independently synthesizing the expected products for a direct comparison of refractive indexes and infrared spectra.

$$Et_4Si + Pr_4Si \xrightarrow{AlCl_3} Et_4Si + PrSiEt_3 + Pr_2SiEt_2 + Pr_3SiEt + Pr_4Si$$

Fractional distillation of the redistribution product from equimolar amounts of tetraethylsilane and tetra-*n*-propylsilane gave three major boiling point plateaus and two less well-defined plateaus corresponding to the five tetraalkylsilanes expected. This was of little significance with regard to possible isomerization because the literature showed that *n*-propyl- and isopropylsilane isomers boil within 1° of each other. Refractive index proved to be a satisfactory criterion; isopropylsilanes have refractive indexes 0.003 unit per isopropyl group higher than their *n*-propyl isomers. Infrared spectra provided a more sensitive criterion; it was found that *n*-propylsilanes show a characteristic absorption at 7.50 μ which is not exhibited by isopropyl compounds while the latter show a characteristic absorption at 11.37 μ which is not exhibited by *n*-propyl compounds. The refractive indexes and the infrared spectra of the redistribution products were the same as those of the authentic compounds prepared by conventional methods. No evidence for isopropyl derivatives was found even in the interfractions. These results show that no appreciable, *i.e.*, less than 3–5%, isomerization occurred.

Experimental

Di-*n*-propyldiethylsilane.—The intermediate di-*n*-propyldichlorosilane,[6] b.p. 170° at 730 mm., was prepared conventionally in 35% yield from silicon tetrachloride and *n*-propylmagnesium bromide in ether. Addition of an ether solution of 0.94 mole of the dichlorosilane to 3.5 moles of ethylmagnesium bromide was followed by distillation of the ether, heating of the residue at 100° for 16 hours, hydrolysis of the residue, and distillation of the liberated ether. The crude product was washed twice with cold concentrated sulfuric acid, then with water, dilute ammonium hydroxide and finally water again. After being dried with anhydrous sodium sulfate, the crude product was fractionally distilled, and there was obtained 110 g. (0.64 mole, 68% yield) of di-*n*-propyldiethylsilane, b.p. 185–186° at atmospheric pressure, $n^{20}D$ 1.4338–41. The product was freed of an aromatic odor with activated alumina, and the following

(1) Paper 46 in a series on organosilicon compounds. For paper 45, W. D. English and L. H. Sommer, THIS JOURNAL, **77**, 170 (1955).

(2) Deceased.

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