volatile phosphorus compounds isolated above. The mixture was refluxed for 0.5 hour and then distilled through an efficient column. The forerun boiling from 80–110° was discarded and the fraction boiling 110–112° accepted. A portion of this fraction was added to concentrated aqueous ammonia at 0° and the amide formed was recrystallized twice from water, m.p. 142–143° (lit. 141°), specific activity 3.0 cts./min./mg. $BaCO_3$ (corrected, 6.0).

Radioactivity Determinations.—All C^{14}-containing compounds were oxidized under reduced pressure with the oxidation mixture of Van Slyke and Folch,[8] the carbon dioxide collected in sodium hydroxide and precipitated in the usual manner.[9] The radioactivity was determined with thin uniform layers of barium carbonate, using a thin mica-windowed Geiger–Müller tube.

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(9) W. G. Dauben, J. C. Reid and P. E. Yankwich, *Anal. Chem.*, **19**, 826 (1947).

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The Acid-catalyzed Condensation of Tertiary Alcohols with Benzaldehyde[1]

BY FREDERICK R. DUKE AND MAX Q. FREELAND

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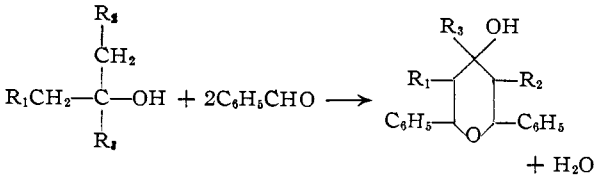
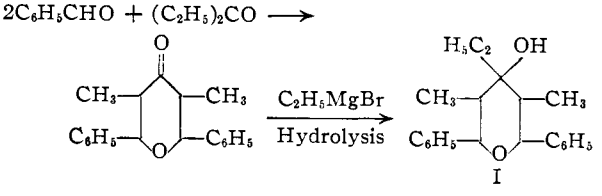
Primary and secondary alcohols have been shown to be oxidized by aromatic aldehydes.[2] Tertiary alcohols react with benzaldehyde to produce eventually colored products without oxidation. This evidence of reaction led us to try to isolate and independently synthesize some products of the reaction. It was found that the reaction follows the scheme



These tetrahydropyranols have not been reported previously. The reaction conditions suggest that the actual reactants are the aliphatic olefin with the conjugate acid of the aldehyde.[3] The condensation of triethylcarbinol with benzaldehyde yielded 2,6-diphenyl-3,5-dimethyl-4-ethyl-4-tetrahydropyranol (I) the structure of which was proved by the synthesis



The reaction of the ketone with ethylmagnesium bromide yielded a product identical in all respects with I. Since a large number of isomers is possible (4 *meso* forms and 6 racemates) it is noteworthy that both routes lead to the same compound.

2,6-Diphenyl-4-methyl-4-tetrahydropyranol was prepared by condensation of *t*-butyl alcohol and benzaldehyde. Reaction of the higher melting isomer of 2,6-diphenyl-4-tetrahydropyrone with methylmagnesium iodide gave the same product in low yield.

Dimethylethylcarbinol and methyldiethylcarbinol yielded crystalline products in the reaction, but both proved to be different from any compounds prepared by the other route.

Experimental

2,6-Diphenyl-4-methyl-4-tetrahydropyranol by Acid Condensation.—Twenty-five ml. each of benzaldehyde and *t*-butyl alcohol and 100 ml. of 20% (volume) sulfuric acid were added to a one-liter flask equipped with a sealed stirrer and condenser. The reaction mixture was maintained at a temperature of between 120 and 130° for one hour, with vigorous stirring. The unreacted benzaldehyde was then steam distilled out of the reaction mixture and the remaining viscous oily substance was dissolved in boiling petroleum ligroin. Upon cooling, clusters of colorless needles appeared which were recrystallized and washed with petroleum ligroin. The yield was 1.6 g. of crystals melting at 143–144°. The residue (200 mg.) remaining in solution in the petroleum ligroin was chromatographed on a Magnesol–Celite column,[4] using progressively richer mixtures of benzene in hexane.

The major (88 mg.) crystalline substance separated was identical with that isolated above. Minor amounts of crystalline mixtures appeared both before and after this product in the chromatogram. *Anal.* Calcd. for $C_{18}H_{20}O_2$: C, 80.6; H, 7.51; mol. wt., 268. Found: C, 80.7; H, 7.62; mol. wt., 287 (Rast, using exaltone).

2,6-Diphenyl-3,5-dimethyl-4-ethyl-4-tetrahydropyranol by Acid Condensation. Method I.—Twenty-five ml. of benzaldehyde, 26 ml. of triethylcarbinol and 100 ml. of 20% (by volume) H_2SO_4 were treated as above, except that the temperature was 140° and the time three hours. The yield was 0.3 g. of crystalline substance melting at 177–178°.

Method II.—Forty-five grams of benzaldehyde, 20 g. of triethylcarbinol, 250 ml. of 95% alcohol and 320 ml. of 50% (by volume) sulfuric acid were mixed in a one liter flask. The flask was shaken at room temperature for 18 days. Before steam distillation, the mixture was partially neutralized by adding 50 g. of potassium hydroxide pellets with stirring and cooling. From here the procedure parallels that of method I. Chromatography of the residue on a Magnesol–Celite column yielded together with the original crop of crystals 3.7 g. of product melting 178–180°.

Anal. Calcd. for $C_{21}H_{26}O_2$: C, 81.3; H, 8.45; mol. wt., 310. Found: C, 81.2; H, 8.52; mol. wt. (Rast), 313.

Crystalline Substance from Dimethylethylcarbinol.—Sixty-six grams of benzaldehyde, 23 g. of *t*-amyl alcohol, 200 ml. of 95% ethanol and 100 ml. of distilled water were mixed in a two-liter round-bottom flask. With constant stirring and cooling, 90 ml. of concentrated H_2SO_4 was slowly added to the mixture. After stirring for two hours, 100 ml. of distilled water and 3 moles of KOH pellets were added slowly with cooling. The mixture was steam distilled, decanted and the oil dissolved in petroleum ligroin. The compound, separated by chromatography on the Magnesol–Celite column, melted at 142–143°, yield 0.5 g., is probably a 2,6-diphenyl-3,4-dimethyl-4-tetrahydropyranol.

Anal. Calcd. for $C_{19}H_{22}O_2$: C, 80.8; H, 7.85. Found: C, 80.8; H, 7.98.

Crystalline Substance from Methyldiethylcarbinol.—The procedure was essentially the same as that for *t*-amyl alcohol, except that the ethanol was not used, and the sulfuric acid and water were mixed and cooled before adding to the organic mixture. The whole was shaken for 50 hours. The yield was one gram of material melting from 161 to 162°. *Anal.* Calcd. for $C_{20}H_{24}O_2$: C, 81.0; H, 8.16. Found: C, 81.0; H, 8.37. The compound is probably a 2,6-diphenyl-3,4,5-trimethyl-4-tetrahydropyranol.

Racemic 2,6-Diphenyl-4-methyl-4-tetrahydropyranol.—Seventy ml. of a benzene solution containing 1.2 g. (0.0048 mole) of *trans*-2,6-diphenyl-4-tetrahydropyrone[5,6] were

(1) Work was performed in the Ames Laboratory of the Atomic Energy Commission.
(2) F. R. Duke, *Anal. Chem.*, **19**, 661 (1947).
(3) E. Arundale and L. A. Mikeska, *Chem. Revs.*, **51**, 505 (1952).
(4) G. S. Hammond and A. Raave, THIS JOURNAL, **73**, 1891 (1951).
(5) P. Petrenko-Kritschenko and D. Plotnikoff, *Ber.*, **30**, 2802 (1897).
(6) R. Cornubert and J. Viroit, *Compt. rend.*, **224**, 1114 (1947).

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.

(7) P. Cornubert and P. Robinet, *Bull. soc. chim. France*, [5] **1**, 90 (1934).

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The Synthesis of 6-Thioguanine

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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.

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

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

BY J. DE FLINES

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).