Streptomycin Semicarbazone Hydrochloride.—A mixture of 401 mg. of streptomycin hydrochloride, 60.1 mg. of semicarbazide hydrochloride, and 55 mg. of pyridine dissolved in 10 ml. of water was allowed to stand overnight. The amorphous semicarbazone was isolated as described above for the oxime. The product had a rotation of $[\alpha]^{25}D$ −70° (*c*, 1.08 in water).

Anal. Calcd. for $C_{22}H_{42}N_{10}O_{12}\cdot 3HCl$: C, 35.32; H, 6.06; N, 18.73. Found: C, 35.66; H, 6.14; N, 18.16.

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Summary

Streptomycin hydrochloride has been degraded by methanol containing hydrogen chloride to streptidine and methyl streptobiosaminide dimethyl acetal hydrochloride, a derivative of the disaccharide-like molecule streptobiosamine, $C_{13}H_{23}NO_9$. The disaccharide derivative was further characterized by conversion to crystalline methyl tetraacetylstreptobiosaminide dimethyl acetal. In like manner, degradation of dihydrostreptomycin gave two isomeric methyl glycosides, α- and β-methyl dihydrostreptobiosaminide, which were separated and characterized as the crystalline pentaacetyl derivatives. The preparation of the oxime and semicarbazone of streptomycin hydrochloride has been described.

It has been shown that the streptobiosamine portion of streptomycin contains a reactive carbonyl group, a C-methyl group, a methylamino group, three acetylatable hydroxyl groups, and one hydroxyl group which is resistant to acetylation.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

A Microbiological Synthesis of 2-Thiophenecarbinol

BY FLOYD W. DUNN[1] AND KARL DITTMER

Erlenmeyer[2] first pointed out the isosteric relationship between the vinylene group and a divalent organic sulfur atom. Many biologically important compounds have since been prepared wherein the vinylene group and the sulfide group have been interchanged. One group of the isosters resulting from such an exchange produces inhibition of the normal biological processes[3,4,5]; whereas the other group retains some of the natural biological activity.[6,7,8,9] It therefore seemed desirable to investigate whether the substitution of a thiophene ring for a benzene ring would alter the synthetic abilities of a fermenting yeast system. Neuberg and co-workers[10,11] demonstrated that yeast could synthesize benzyl alcohol and acetylphenylcarbinol from benzaldehyde; Lintner and Liebig[12] showed that 2-furfuryl alcohol was obtained when yeast acted on 2-furaldehyde. In this report are presented the results of studies of the effect of fermenting yeast on 2-thiophenealdehyde.

For the microbiological synthesis herein reported, a suspension of fermenting yeast was prepared in a manner similar to that employed by Neuberg, *et al.*[10,11] With the Budweiser strain of yeast and under the conditions used in this investigation a large part of the 2-thiophenealdehyde was converted to thiophenecarbinol. In this respect 2-thiophenaldehyde is attacked by the yeast system in a manner analogous to the reaction with benzaldehyde and furaldehyde. The thiophenecarbinol was isolated by precipitation of the 5-chloromercuri-2-thiophenecarb nol derivative. The mercury could be removed with hydrogen sulfide, liberating the thiophenecarbinol.

To establish the identity of the carbinol produced microbiologically, thiophenecarbinol was synthesized from 2-thiophenealdehyde by the crossed Cannizzaro reaction with formaldehyde This procedure, developed by Davidson and Bogert[13] as a general one for aromatic alcohols, was found to apply equally as well to the thiophene compound. The carbinols prepared synthetically and microbiologically were compared by mixed melting points of the phenylurethan and α-naphthylurethan derivatives and by elementary analysis. The thiophenecarbinol produced by the fermenting yeast was found to be identical in every respect with the thiophenecarbinol prepared by chemical synthesis.

Experimental[14]

5-Chloromercuri-2-thiophenecarbinol.—The microbiological synthesis was carried out with a mixture of 50 g. of Budweiser baker's yeast, 50 g. of sucrose, 1250 cc. of water and 5 g. of thiophenealdehyde. At the end of four days the yeast was removed by filtration, and 500 cc. of 5% mercuric chloride was added to the aqueous solution. At the end of several days, when precipitation was complete, the supernatant liquid was decanted and the pre-

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cipitate washed with cold water. The yield was 5.27 g. (33.4% of the theoretical) of crude 5-chloromercuri-2-thiophenecarbinol. Repeated recrystallization from hot water produced a white powder melting at 183–185°, with decomposition.

Anal. Calcd. for $C_5H_5SOHgCl$: C, 17.18; H, 1.44; Hg, 57.4. Found: C, 16.80; H, 1.42; Hg, 56.31.

The free 2-thiophenecarbinol was isolated by treating a warm aqueous solution of the mercury derivative with hydrogen sulfide, removal of the mercuric sulfide and extraction of the carbinol with ether. The α-naphthylurethan derivative of this biologically synthesized thiophenecarbinol was prepared as described below. The melting point (148°) of this derivative and the corresponding derivative from synthetic thiophenecarbinol showed no depression when the two were mixed. The analysis of the α-naphthylurethan derivative further established the identity of the isolated thiophenecarbinol with the synthetic product.

Anal. Calcd. for $C_{16}H_{13}O_2SN$: C, 67.78; H, 4.62; N, 4.94; S, 11.31. Found: C, 67.78; H, 4.67; N, 4.98; S, 11.20.

Synthesis of 2-Thiophenecarbinol.—Into a three-necked flask, equipped with stirrer, dropping funnel and thermometer, were placed 3.5 g. of 2-thiophenealdehyde, 10 cc. of absolute methanol and 5 cc. of formalin. With stirring the mixture was heated on the water-bath to 65°, at which time a solution of 6 g. sodium hydroxide in 6 cc. of water was added. Heating was continued at 65° for thirty minutes, and then the solution was refluxed for a short time. The dark-colored reaction mixture was extracted with benzene. Distillation yielded 2.1 g. (59% of the theoretical) of the carbinol boiling at 102–105° (20 mm.).

Phenylurethan Derivative of 2-Thiophenecarbinol.—A mixture of a few drops of phenyl isocyanate with an equal volume of 2-thiophenecarbinol was heated on the water-bath for thirty minutes. Recrystallization of the product from petroleum ether produced colorless, monoclinic crystals melting at 72.3–74°.

Anal. Calcd. for $C_{12}H_{11}O_2SN$: C, 61.77; H, 4.75; N, 6.00; S, 13.74. Found: C, 61.69; H, 4.80; N, 6.13; S, 13.72.

α-Naphthylurethan Derivative of 2-Thiophenecarbinol.—A mixture of equal amounts of the carbinol and α-naphthyl isocyanate was heated for thirty minutes on the water-bath. After recrystallization from a mixture of chloroform and petroleum ether the colorless, monoclinic crystals melted at 148°.

Anal. Calcd. for $C_{16}H_{13}O_2SN$: C, 67.78; H, 4.62; N, 4.94; S, 11.31. Found: C, 67.81; H, 4.60; N, 4.97; S, 11.30.

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Summary

2-Thiophenecarbinol was synthesized from 2-thiophenealdehyde by fermenting yeast in a manner similar to the microbial synthesis of benzyl and furfuryl alcohols from the corresponding aldehydes. The carbinol obtained from the fermenting yeast was identical with 2-thiophenecarbinol synthesized from 2-thiophenealdehyde by a crossed Cannizzaro reaction with formaldehyde. Three derivatives of 2-thiophenecarbnol were prepared and described.

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Δ^6-*i*-Cholestadiene

BY BYRON RIEGEL, GEORGE P. HAGER AND BERNARD L. ZENITZ

An investigation was made of the Barbier–Wieland degradation[1] of 3-hydroxy-5-cholenic acid where the 3-hydroxyl group and the 5–6 carbon–carbon double bond were protected by the *i*-ether structure. The 3-hydroxyl group and the double bond can be easily regenerated from the *i*-steroid structure. When the carbinol was dehydrated with activated alumina in boiling xylene, there was a simultaneous loss of methyl alcohol with the introduction of an ethylenic bond in ring B. An attempt to apply this reaction to the carbinol of the bis*nor*-cholenic acid[2] resulted in some tar formation but chiefly in recovery of the starting material.

To further clarify this reaction and the structure of the resulting hydrocarbon, *i*-cholesteryl methyl ether (I) was treated with alumina in boiling xylene. There was obtained a white, crystalline hydrocarbon that melted at 73°, with a specific rotation of −47°, for which formula (II) has been assigned as a result of the physical and chemical evidence.

There was also isolated a small quantity of cholesteryl methyl ether (V). In order to determine whether this was a reaction product or an inpurity in the starting material, the *i*-cholesteryl methyl ether was extensively purified by chromatographic technique. The normal ether, however, was isolated from the reaction using the purified *i*-ether and was considered to be a reaction product, probably formed by the addition of methanol to the *i*-diene.

The physical evidence for the cyclopropane ring conjugate to the ethylenic bond was the ultraviolet absorption spectrum of the hydrocarbon.[3] The *i*-diene showed a maximum absorption near 2100 Å., which is between the absorption peaks of a conjugated diene and an ethylenic double bond. This was analogous to the shift of the absorption maxima of *i*-cholestenone and carone compared to those of 4-cholestene-3-one and 5-cholestene-3-one.

The hydrocarbon was found to display unusual addition reactions. When refluxed with glacial acetic acid, cholesteryl acetate (IV) was obtained.

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