[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

THE CHEMISTRY OF THE LIPOIDS OF TUBERCLE BACILLI. XIII. THE OCCURRENCE OF MANNOSE IN THE PHOSPHATIDE FROM HUMAN TUBERCLE BACILLI[1]

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Introduction

In a study of the cleavage products of the phosphatide fraction[3] isolated from the human type of tubercle bacilli, strain H-37, it was found that the water-soluble constituents amounted to about 33%. The aqueous solution contained reducing sugars, glycerophosphoric acid and some substance which formed a crystalline derivative with phenylhydrazine. These products were separated by first precipitating the glycerophosphoric acid as the barium salt with alcohol. After removal of the excess of barium with sulfuric acid, the concentrated solution was mixed with phenylhydrazine, when a crystalline derivative separated and was filtered off. The filtrate on treatment with phenylhydrazine hydrochloride and sodium acetate yielded typical glucosazone crystals. In addition to the three components mentioned above, a small quantity of another crystalline compound was isolated from the residual sirup but this substance has not yet been identified.

The crystalline phenylhydrazine compound was regarded as a salt of some sugar acid but owing to stress of other work it was impossible at that time to analyze or identify the substance. Recently we have had an opportunity to resume the investigation of the compound and we have come to the conclusion that it is not a salt of a sugar acid but that it is mannose phenylhydrazone.

The identification was accomplished by comparing the properties of the purified substance with those of pure mannose phenylhydrazone. Both substances formed colorless crystals that melted with decomposition at 195° and a mixed melting point showed no depression. Examination of the optical properties of the two crystals also showed that they were identical.

Since a considerable amount of glucosazone could be isolated from the original hydrolysis mixture after removing the insoluble mannose phenylhydrazone, it is evident that the carbohydrate complex contained in the phosphatide yields on hydrolysis at least two reducing sugars, *viz.*, mannose and probably glucose, and in addition some other as yet unidentified sub-

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[1] The present report is a part of a coöperative investigation on tuberculosis; it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association.

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[3] R. J. Anderson, *J. Biol. Chem.*, **74**, 537 (1927).

stance is present which crystallizes rather easily from a concentrated aqueous solution.

Experimental Part

The crude phenylhydrazone had been obtained as already described.[3] The individual crystals appeared to be colorless but in bulk they exhibited a yellowish color. The crystals were almost insoluble in alcohol and very slightly soluble in hot water. The substance dissolved slowly in boiling water but the color became deep yellow and on cooling large brownish-yellow crystals separated. The crude crystals melted with decomposition at 194–195°.

For purification, 0.6 g. of the crystals was dissolved in 60 cc. of hot 60% alcohol, giving a slightly yellowish solution. Dense colorless plate-shaped crystals separated slowly after the solution had cooled. The crystals were filtered off and washed with alcohol, acetone and ether. After the crystals had been dried *in vacuo* over sulfuric acid, they weighed 0.5 g.

A sample of mannose phenylhydrazone was prepared as follows: 4 g. of crude mannose[4] prepared from the ivory nut was dissolved in 25 cc. of water, treated with norite and filtered. The clear filtrate was mixed with a slight excess of phenylhydrazine dissolved in a little alcohol. The hydrazone soon began to crystallize and after the mixture had stood overnight the crystals were filtered off and washed with water and with alcohol. The product was recrystallized from 350 cc. of hot 60% alcohol, yielding about 3 g. of large colorless plate-shaped crystals.

In crystal form and in solubilities the two products mentioned above appeared to be identical. Both substances melted with decomposition at the same temperature but the melting point varied considerably depending upon the rate of heating. When heated with moderate rapidity the melting point was 195–196°. When the rate of heating was more rapid, the melting point was 197–198° and in another case 204–205°. A mixture of the two products melted at the same temperature; the capillary tubes were attached to the same thermometer and heated simultaneously.

The nitrogen content of the crystals isolated from the phosphatide corresponded to that of a hexose phenylhydrazone.

Anal. Subs., 0.1837: 16.90 cc. of N_2 at 22° and 763 mm. Calcd. for $C_{12}H_{18}O_5N_2$ (270): N, 10.37. Found: N, 10.64.

Mannose phenylhydrazone according to Hoffmann[5] shows in pyridine solution a specific optical rotation of about +27°, while Levene and Mori[6] report a specific rotation of +32.5° for a solution of the substance in 50% pyridine and water. We were unable to determine the optical rotation with any satisfactory degree of accuracy because both preparations were so slightly soluble in pyridine or in a 50% mixture of pyridine and water that prolonged warming was necessary before 0.2 or 0.1 g. of substance could be dissolved in 25 cc. of the solvent. The solutions which were obtained turned decidedly yellow in color during the warming and we were unable to make any accurate readings.

The optical properties of the crystals obtained from the phosphatide

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[4] We are indebted to Dr. R. H. F. Manske of this Laboratory for kindly supplying the mannose.

[5] A. Hoffmann, *Ann.*, **366**, 277 (1909).

[6] P. A. Levene and T. Mori, *J. Biol. Chem.*, **84**, 49 (1929).

were compared with those of pure mannose phenylhydrazone by Dr. E. J. Roberts of this Laboratory and the two crystals were found to be identical. The results of the examination are stated by Dr. Roberts as follows. "Mannose phenylhydrazone crystallizes in the form of rhombic plates which are biaxial positive with $2V \doteqdot 75^\circ$; $\beta = 1.65$, $\alpha \doteqdot 1.60$, $\gamma \doteqdot 1.74$. Most of the plates show an *almost* perpendicular emergence of an optic axis, have an acute angle of 75° and an extinction angle of 23° in the acute angle. The trace of the axial plane passes through the acute angle of the rhomb. The crystals obtained from the phosphatide have identical properties."

Summary

One of the reducing sugars formed on hydrolyzing the phosphatide isolated from the human tubercle bacillus has been identified as mannose.

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THE THERMAL DECOMPOSITION OF SODIUM AND POTASSIUM METHIDES

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In a previous paper[1] the results of a study of the thermal decomposition of sodium methide were reported.

The thermal decompositions of sodium and potassium methides have now been studied and found to proceed in accordance with Equation 1.

$$8MCH_3 \longrightarrow 6CH_4 + M_2C_2 + 6M \quad (M{=}Na,\ K) \tag{1}$$

Pure methane is liberated in the calculated amount and, on treatment of the residue with water, the rest of the carbon is set free as acetylene mixed with ethylene and ethane.[2]

Sodium and potassium methides are primary salts of the very weak volatile polybasic acid, methane. When salts of this general class are heated the usual result is the liberation of free acid and the formation of a new salt in which all the available hydrogens of the residual acid have been replaced. This process consists in the displacement of a prototropic equilibrium[3] by the constant removal of one of the participating entities. In accordance with this general behavior is the liberation of methane from sodium or potassium methide

$$4CH_3^- \longrightarrow 3CH_4 + C^{----} \tag{2}$$

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[1] Carothers and Coffman, THIS JOURNAL, **51**, 588 (1929).

[2] The presence of ethylene and ethane may be ascribed to the fact that the acetylene is formed under powerfully reducing conditions (action of water on excess sodium or potassium).

[3] Lowry, *Institute International de Chemie Solvay*, **2**, 150 (1925).