THE CHEMISTRY OF THE LIPIDES OF TUBERCLE BACILLI

LXXVI. CONCERNING INOSITOL GLYCEROL DIPHOSPHORIC ACID, A COMPONENT OF THE PHOSPHATIDE OF HUMAN TUBERCLE BACILLI*

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In studies conducted in this laboratory dealing with the composition of the phosphatide isolated from the human tubercle bacillus, strain H-37, it was recognized early that an organic phosphoric acid of the formula \( \text{C}_9\text{H}_{20}\text{O}_{14}\text{P}_2 \) could be separated in the form of the barium salt from the water-soluble cleavage products after the phosphatide had been saponified with alcoholic potassium hydroxide (I). This substance was regarded as a complex composed of a hexose monophosphoric acid and glycerophosphoric acid. The acid gave no reduction with Fehling’s solution until it had been refluxed for some time with dilute sulfuric acid. The presence of a reducing sugar was thus demonstrated, but the sugar was not identified at that time.

A compound of similar composition was also isolated from the cleavage products of the phosphatide of the leprosy bacillus (2). In a later investigation (3) of the organic phosphorus compounds contained in the phosphatide of the human tubercle bacillus, strain H-37, a barium salt was isolated which corresponded in composition to the formula \( \text{C}_9\text{H}_{16}\text{O}_{16}\text{P}_2\text{Ba}_2 \). Analysis of this substance indicated that it was mannose glycerol diphosphoric acid. The reducing sugar contained in this compound was identified as mannose.

In addition to the acid of the formula \( \text{C}_9\text{H}_{20}\text{O}_{14}\text{P}_2 \), the isolation of glycerophosphoric acid (4), inositol monophosphoric acid (5, 6), and the phosphorus-containing glycoside manninositose (1, 3) has been described.

The isolation of an acid of the formula \( \text{C}_9\text{H}_{20}\text{O}_{14}\text{P}_2 \) in the form of the barium salt was described in the preceding paper (6) and it was suggested that the substance was inositol glycerol diphosphoric acid. The substance

* The data are taken from the dissertation submitted by G. I. de Sütő-Nagy to the Faculty of the Graduate School, Yale University, 1946, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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was obtained after alkaline saponification of the phosphatide prepared from Lot 5 of cell residues which remained after the preparation of the purified protein derivative, PPD. Since such a compound has not been isolated or described previously, we present in this paper a brief account of the properties and cleavage products of this unusual acid.

**EXPERIMENTAL**

The barium salt of the acid had been isolated as described in the preceding paper (6) and it was a white amorphous powder which weighed 2.313 gm. It gave no Molisch reaction, but a positive Scherer reaction, and when heated with acid potassium sulfate the evolution of acrolein was evident. In the Zeisel determination a volatile iodide was obtained which when calculated as isopropyl iodide corresponded to 48.3 per cent of barium glycerophosphate.

For analysis the substance was dried over phosphorus pentoxide in vacuo at 110°.

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C_9H_{16}O_{11}P_2Ba_2 (684.8). \quad \text{Calculated.} \quad \text{P } 9.05, \text{ Ba } 40.12
\]

\[
\text{Found.} \quad 9.32, 9.39, \text{Ba } 40.05, 39.99
\]

The results of this preliminary examination indicated that the substance contained both inositol and glycerol combined with 2 molecules of phosphoric acid.

*Complete Hydrolysis of the Organic Phosphoric Acid*—In order to determine the cleavage products of the above organic phosphoric acid a portion of the barium salt weighing 1 gm. was heated in a sealed tube with 11 cc. of 10 per cent sulfuric acid for 3 hours and 15 minutes at 160°. There was no pressure on opening the tube and its contents were washed into a beaker. The sulfuric acid and phosphoric acid were removed quantitatively by means of barium hydroxide. The filtrate was evaporated to dryness in vacuo, and the residue was triturated with absolute alcohol, which left a white insoluble substance, and the latter was filtered off and washed with alcohol.

*Identification of Inositol*—The alcohol-insoluble substance, after it had been dried in vacuo weighed 280 mg. It was dissolved in water, decolorized with norit, and the clear solution was mixed with alcohol to faint turbidity. The solution was warmed until it was clear. On cooling and scratching, colorless needle-shaped crystals separated, which were filtered off after a few hours and twice recrystallized from water by the addition of alcohol. The crystal form was characteristic of inositol and the crystals gave the Scherer reaction. The crystals melted at 222° and there was no depression when mixed with inactive inositol. The properties of the substance identify it as inactive inositol.
Examination of Alcohol-Soluble Portion of Cleavage Products. Identification of Glycerol—The alcoholic filtrate and washings were combined and evaporated to dryness in vacuo, when a syrupy residue was obtained which weighed 110 mg. The substance, when heated with acid potassium sulfate, gave a strong indication of acrolein, thus suggesting that it was crude glycerol. In order to confirm the presence of glycerol the tribenzoyl derivative was prepared in the usual manner and crystallized from methyl alcohol. The derivative was obtained in the form of colorless needles which melted at 74°, and there was no depression when mixed with an authentic sample of glycerol tribenzoate.

From the results obtained it is evident that the products liberated on complete hydrolysis of the organic phosphoric acid consisted of phosphoric acid, together with inositol and glycerol. By calculation, 1 gm. of a barium salt of the composition C₉H₁₆O₁₄P₂Ba₂ should yield on complete hydrolysis 0.3971 gm. of organic compounds, and if inositol and glycerol were present in equimolecular proportions there should be obtained 0.2628 gm. of inositol and 0.1343 gm. of glycerol. The amounts of inositol and glycerol actually isolated correspond approximately to the theoretical values. The experimental results reported indicate that the organic phosphoric acid was inositol glycerol diphosphoric acid.

Partial Hydrolysis of the Organic Phosphoric Acid—The balance of the barium salt was dissolved in water and the barium was removed quantitatively with sulfuric acid. The solution on evaporation to dryness in vacuo left a solid residue which was insoluble in alcohol. Attempts to crystallize the acid were unsuccessful.

For partial hydrolysis the acid was dissolved in 10 per cent sulfuric acid and the solution was heated at 45° for about 6 hours. The sulfuric acid was removed with barium hydroxide and the barium sulfate was filtered off. The filtrate was concentrated in vacuo to a thick syrup and the latter was triturated with absolute alcohol until a white powder was produced which was filtered off and washed with absolute alcohol.

Isolation of Barium Inositol Monophosphate—The alcohol-insoluble substance mentioned above gave a positive Scherer reaction. It was dissolved in a little water and the solution which showed a strong acid reaction was neutralized with barium hydroxide. The clear solution was mixed with 2 volumes of alcohol and the white amorphous precipitate that separated was filtered off and washed with alcohol. After the substance had been reprecipitated from aqueous solution with alcohol, it was collected, washed with alcohol, and dried in vacuo. For analysis the substance was dried to constant weight at 78° in vacuo over phosphorus pentoxide.

Analysis—C₉H₁₆O₁₄PBa (395.4). Calculated. P 7.84, Ba 34.75

Found. " 7.38, 7.60, Ba 34.55, 34.14
The properties of the substance and the composition of the barium salt indicate that it was barium inositol monophosphate.

Isolation of Barium Glycerophosphate—The alcoholic filtrate from the above alcohol-insoluble inositol monophosphoric acid showed an acid reaction to litmus. The solution was neutralized with barium hydroxide and the precipitate that separated was filtered off and washed with alcohol. The substance, after it had been reprecipitated from aqueous solution by the addition of alcohol, was filtered off, washed with alcohol, and dried in vacuo. The product was a snow-white amorphous powder that gave no Scherer reaction. For analysis it was dried at 110° in vacuo over phosphorus pentoxide.

Analysis—C_{9}H_{20}O_{14}P_{2}Ba (307.4). Calculated. P 10.08, Ba 44.70

Found. " 9.93, 10.23, Ba 44.27, 44.62

The analytical values found for the barium salt are in close agreement with the calculated composition of barium glycerophosphate.

DISCUSSION

Since it was possible to isolate both inositol monophosphoric acid and glycerophosphoric acid after mild hydrolysis of the acid of the formula C_{9}H_{20}O_{14}P_{2}, it seems evident that the molecules of inositol and glycerol must have been combined either as esters of phosphoric acid or by means of an easily hydrolyzable oxygen bridge. Unfortunately, lack of material prevented a complete investigation of the constitution of the acid, but the results illustrate the diversity of organic phosphoric acids that can be produced by the tubercle bacillus. It would appear also that almost every lot of bacilli that is grown on an artificial medium elaborates different organic phosphoric acid compounds. In future investigations of the organic phosphoric acids isolated from the phosphatide of the human tubercle bacillus it is suggested that special attention should be given to distinguish between mannose glycerol diphasphoric acid and inositol glycerol diphasphoric acid as well as other phosphoric acid compounds. The great variety of metabolic products produced by the organism renders it impossible to predict what compounds may be found.

SUMMARY

An organic phosphoric acid of the formula C_{9}H_{20}O_{14}P_{2} was isolated from the phosphatide of a special lot of tubercle bacilli residues from the preparation of tuberculin.

Complete hydrolysis of the acid by heating in a sealed type to 160° yielded phosphoric acid and approximately equimolecular quantities of inositol and glycerol.
When the acid was subjected to mild hydrolysis, it yielded inositol monophosphoric acid and glycerophosphoric acid.

It is concluded, therefore, that the acid is inositol glycerol diphosphoric acid, but the chemical constitution of the acid is as yet unknown.

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