chloride, each prepared in the absence of ether, gave in addition to benzoic acid very small quantities of *p*-phenylbenzoic acid, $C_6H_5C_6H_4COOH$. The formation of the latter acid is almost certainly due to free phenyl radicals initially developed in the preparation of the C_6H_5MgX.

Attention is directed to the theoretical, and in some cases practical, formation of a large and complex variety of Grignard reagents starting with a single and simple RX compound. Although the degree of these side reactions is fortunately very limited and in most cases insignificant, it emphasizes the difficulty or impossibility of preparing absolutely pure compounds. This difficulty is undoubtedly not confined to organomagnesium compounds.

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THE CHEMISTRY OF THE LIPOIDS OF TUBERCLE BACILLI. XXI. THE POLYSACCHARIDE OCCURRING IN THE PHOSPHATIDE FROM THE HUMAN TUBERCLE BACILLI[1]

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Introduction

Previous investigations in this Laboratory of the water-soluble constituents which are obtained on hydrolyzing the phosphatide A-3 from the human tubercle bacillus have shown that mannose,[3] inosite[4] and some other reducing sugar, probably invert sugar,[5] are present in about equal amounts in the aqueous solution. The phosphatide fractions isolated from the avian and bovine tubercle bacilli also yielded mannose and inosite on hydrolysis.[6]

In all of these cases prolonged boiling of the phosphatides with dilute acid was necessary before reducing sugars appeared in the solution. It seemed evident, therefore, that the various sugars which were liberated on complete hydrolysis existed in the original phosphatide molecules in firm chemical combination; possibly sugar complexes or polysaccharides

[1] The present report is a part of a coöperative investigation on tuberculosis and it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association. (An abstract of this paper was read before the Division of Medicinal Chemistry, at the meeting of the American Chemical Society, Cincinnati, Ohio, September, 1930.)

[2] Holder of a National Tuberculosis Association Fellowship at Yale University 1929–1930.

[3] Anderson and Renfrew, THIS JOURNAL, **52**, 1252 (1930).

[4] Anderson, *ibid.*, **52**, 1607 (1930).

[5] Anderson, Roberts, and Renfrew, *Proc. Soc. Expil. Biol. Med.*, **27**, 387 (1930).

[6] Unpublished data.

might be present with which the fatty acids were combined in the form of esters.

We were naturally interested in discovering some evidence of the existence of complex carbohydrates or polysaccharides in the bacillary phosphatides but for a long time we had to be content with the identification of the simple sugars which appeared as cleavage products after hydrolysis. It was difficult to discover a method by which a partial hydrolysis of the phosphatide could be effected without at the same time disrupting the carbohydrate groups.

In the analysis of the so-called purified tubercle bacilli wax[7] we found when this substance was saponified with dilute alcoholic potassium hydroxide that an alcohol-insoluble carbohydrate complex was obtained, while when the wax was hydrolyzed by boiling with acid–alcohol reducing sugars appeared in addition to fatty acids and so-called unsaponifiable wax. Further studies which have not yet been published on the water-soluble cleavage products obtained from the purified wax and from the bovine phosphatide made it seem probable that the polysaccharides or carbohydrate complexes that were present were very stable since only partial hydrolysis could be effected by means of acid–alcohol treatment.

A study has now been made of the cleavage products which are formed when the phosphatide A-3 derived from the human tubercle bacillus is boiled with dilute alcoholic potassium hydroxide. The results obtained were very interesting and entirely unexpected. The phosphatide is insoluble in alcohol but when it is boiled with dilute alcoholic potassium hydroxide the material gradually disintegrates, giving a soap solution of the fatty acids and leaving an amorphous mass of insoluble matter on the bottom of the flask. The latter is easily soluble in water and consists of two components: (a) the potassium salt of an organic phosphoric acid and (b) a neutral carbohydrate. The two products can be separated by dissolving the mixture in water and adding a solution of neutral lead acetate, when the organic phosphoric acid is precipitated as an insoluble lead salt, leaving the carbohydrate in solution. The carbohydrate is precipitated by alcohol as a gummy mass from the aqueous solution after the lead has been removed. The product can be converted into a snow white amorphous powder by pouring its aqueous solution with stirring into absolute alcohol. The substance does not reduce Fehling's solution but after it has been boiled with dilute acid reducing sugar is liberated and after complete hydrolysis it yields about equal parts of mannose and inosite.

The nature of the new compound has not been established definitely but the results so far obtained indicate clearly that the phosphatide contains a new type of polysaccharide. So far as we are aware this is the

[7] Anderson, *J. Biol. Chem.*, **83**, 505 (1929).

first time that a compound of the nature of a polysaccharide or possibly a glucoside composed of mannose and inosite has been found in nature and we propose to name this substance maninositose.

It is an interesting fact that the action of dilute acid and dilute alcoholic alkali upon the phosphatide produces such different cleavage products. When the phosphatide is boiled with dilute acid the molecule is completely disrupted with the formation of fatty acids and glycerophosphoric acid together with reducing sugar and inosite. By the action of hot dilute alcoholic alkali only the fatty acids are split off; the polysaccharide binding is not disturbed and no reducing sugar is liberated. It seems probable, therefore, that the fatty acids are combined as esters with hydroxyl groups in the polysaccharide and the fact that no free glycerophosphoric acid can be isolated from the saponification mixture makes it probable that this substance is also linked in ester combination with the sugar molecules.

The organic phosphoric acid referred to above was converted into a barium salt. The barium salt is very soluble in water but it is insoluble in alcohol and up to the present time it has been obtained only as a snow white amorphous powder. The substance does not reduce Fehling's solution but after it has been boiled for some time with dilute acid some reducing sugar is liberated. The composition of the substance does not agree with any ordinary hexosemonophosphate since the relation of carbon to phosphorus is approximately C : P = 9 : 2. It is not impossible that it represents a compound of a hexosephosphate with glycerophosphoric acid.

The discovery of maninositose, a bacterial polysaccharide or glucoside of mannose and inosite will open up a new chapter in the study of the function of inosite in cell metabolism. It should also lead to interesting experiments in the synthesis of similar combinations between hexoses such as glucose or mannose and inosite. Biological values or reactions of maninositose are still unknown but various experiments are planned and will be carried out as soon as possible.

Experimental Part

The general properties of the cleavage products obtained on saponification together with methods of separating the organic phosphoric acid and the neutral polysaccharide were worked out in preliminary experiments. Three separate saponifications were conducted in the course of which the various cleavage products were isolated. In all of these experiments very similar results were obtained both qualitatively and quantitatively. The experimental procedure outlined below can, therefore, be recommended as the shortest and most satisfactory.

Saponification of the Phosphatide.—The phosphatide had been prepared by the H. K. Mulford Company from the human tubercle bacillus, strain H-37, according to our

original method.[8] The product was a white amorphous powder and its properties were identical with those that we described for the phosphatide A-3. We desire to express our thanks to the H. K. Mulford Company who generously supplied this expensive material.

The saponification[9] was carried out by refluxing a mixture of 18 g. of the phosphatide and 600 cc. of 1% alcoholic potassium hydroxide on the steam-bath for twenty-four hours. The phosphatide agglutinated to a solid mass on the bottom and sides of the flask and the alcoholic solution became slightly straw colored. After the mixture had cooled the insoluble product was a hard brittle mass which could be easily rubbed to a powder. The supernatant liquid was decanted, the flask was rinsed several times with hot alcohol and the solution was reserved for the isolation of the fatty acids.

Examination of the Alcohol-Insoluble Residue.—The material which was insoluble in alcohol was dissolved in 100 cc. of water and the solution was acidified with acetic acid, when a slight scummy precipitate was obtained which consisted mainly of fatty acids which had been occluded in the amorphous mass. The precipitate was filtered off, washed with ether and the extract was combined with the fatty acids obtained from the alcoholic solution.

The aqueous solution and washings, after they had been concentrated under reduced pressure to a volume of about 30 cc., were mixed with 400 cc. of alcohol, when a sticky mass was precipitated. The mixture was allowed to stand until the supernatant liquid was clear; the solution was decanted, and the precipitate was washed with alcohol and dried. The material was dissolved in 10 cc. of water, precipitated by adding alcohol and the supernatant liquid was decanted. After the precipitate had been washed with alcohol and dried, it was dissolved in 60 cc. of water and a slight excess of neutral lead acetate solution was added. The dense amorphous precipitate was filtered off and washed thoroughly with water. The filtrate and washings were saved for the isolation of maninositose.

Isolation of the Organic Phosphoric Acid.—The lead salt was suspended in water, decomposed with hydrogen sulfide and the lead sulfide was filtered off and washed with water. The filtrate, after it had been concentrated under reduced pressure to a volume of about 50 cc., was neutralized with barium hydroxide. A slight amount of an amorphous precipitate separated which was filtered off and washed with water.

This precipitate, which gave a strong reaction for inorganic phosphoric acid, evidently consisted of barium phosphate and it was discarded. The colorless filtrate was mixed with an equal volume of 95% alcohol, when a white amorphous precipitate seprated. The latter was filtered off, washed with 60% alcohol and with alcohol. After the substance had been dried *in vacuo* it weighed 3.15 g. The barium salt was dissolved in a few cc. of water and the perfectly clear solution, after it had been diluted to 40 cc. with water, was precipitated by adding 40 cc. of alcohol. The snow white product after it had been filtered, washed and dried, weighed 3.1 g.

The substance did not directly reduce Fehling's solution but after it had been boiled with an acid for some time it did reduce Fehling's solution.

For analysis the barium salt was dried at 105° *in vacuo* over dehydrite.

Anal. Subs., 0.2223: $BaSO_4$, 0.1481; $Mg_2P_2O_7$, 0.0715. Subs., 0.1722: H_2O, 0.0479; CO_2, 0.0997. Found: C, 15.79; H, 3.11; P, 8.96; Ba, 39.20.

The analytical values agree approximately with the formula $C_9H_{20}O_{14}P_2Ba_2$, which might represent a mixture of equal parts of a hexosemonophosphate and glycerophos-

[8] Anderson, *J. Biol. Chem.*, **74**, 525 (1927).

[9] Throughout the various operations air was displaced by nitrogen or carbon dioxide until the fatty acids had been isolated. Freshly distilled solvents were used and the alcohol had been distilled over potassium hydroxide.

phate. It is evident, however, that the substance does not contain any ordinary hexose-monophosphate, because it does not reduce Fehling's solution until after it has been boiled for some time with an acid. It appears most reasonable, at present, to assume that the compound contains a complex binding which is broken on boiling with acid, yielding a reducing sugar. Further work on this subject will be necessary before we can determine the definite formula of the acid as well as of its cleavage products.

Isolation of Maninositose.—The filtrate from the lead acetate precipitation was freed of lead by means of hydrogen sulfide and the lead sulfide was filtered off and washed with water. The clear colorless filtrate was concentrated to a sirup under reduced pressure and the sirup was washed into a beaker with 30 cc. of water. The solution was acidified with 2 cc. of glacial acetic acid and poured with constant stirring into 400 cc. of absolute alcohol, when an amorphous precipitate was obtained. The product was collected on a Büchner funnel, washed thoroughly with absolute alcohol and finally dried in a vacuum desiccator. The snow white amorphous powder weighed 4.4 g. For further purification the product can be reprecipitated a number of times in the same manner but it is recovered practically quantitatively.

The substance is optically active and on moist litmus paper it shows a neutral reaction. It has no definite melting point. When heated it begins to sinter at 100°; it begins to swell or froth at 150° without showing any noteworthy change on further heating, but above 250° it slowly darkens. When the substance is ignited it swells up and burns, leaving a very light voluminous white ash which on further heating forms a fused mass. The ash consists principally of potassium phosphate.

Rotation. 0.5998 g. of substance dried at 60° *in vacuo* over dehydrite was dissolved in water at 23° and made up to 10 cc. In a 1-dm. tube $\alpha = +3.42°$; hence $[\alpha]_D^{23} +57°$.

For analysis the substance was dried at 105° *in vacuo* over dehydrite. The loss in weight was 3.5%.

Anal. Found: ash, 16.27; P, 2.33; K, 3.92.

The phosphorus is present in organic combination but it is impossible to state definitely, at present, whether the phosphorus content is due to incomplete removal of the organic phosphoric acid compound or forms an integral part of the carbohydrate molecule.

Hydrolysis of Maninositose.—A sample of the neutral carbohydrate which had been repeatedly precipitated from dilute acetic acid with absolute alcohol was hydrolyzed. The snow white amorphous powder, which weighed 1.1 g., was refluxed with 100 cc. of 5% sulfuric acid for four hours. The faintly straw-colored solution was freed of sulfuric acid quantitatively with barium hydroxide and the barium sulfate was removed. The filtrate was concentrated under reduced pressure to 50 cc. and the solution, which showed a slight acid reaction, was neutralized with barium hydroxide. The addition of two volumes of alcohol caused a small amount of an amorphous precipitate to separate. The latter was filtered off, washed with alcohol and dried. The white powder weighed only 0.15 g. and contained organic phosphorus. The properties of the substance resembled those of barium glycerophosphate but the small quantity of the material prevented its definite identification.

Isolation of Mannose Phenylhydrazone.—The filtrate from the above-mentioned barium salt was freed of barium quantitatively with sulfuric acid and, after removing the barium sulfate, the filtrate was concentrated to 30 cc. On adding 1 g. of phenylhydrazine dissolved in 2 cc. of alcohol a crystalline precipitate began to separate immediately. After the mixture had stood overnight the crystals were filtered off, washed with water, alcohol and ether. The dried crystals were slightly straw-colored and they weighed 0.55 g. The substance was recrystallized from 50 cc. of hot 60% alcohol and as the solution cooled there separated slowly large colorless rhombic plates which were

filtered off, washed and dried. The crystals weighed 0.35 g. and the optical properties were identical with those of mannose phenylhydrazone. The substance melted with decomposition when rapidly heated at 195–196° and there was no depression of the melting point when some of the substance was mixed with a sample of pure mannose phenylhydrazone. The crystal form and melting point definitely identify the substance as mannose phenylhydrazone.

Isolation of Inosite.—The filtrate from the crude mannose phenylhydrazone was shaken with an excess of benzaldehyde for several hours and the precipitated hydrazone was removed by filtration. The solution, after it had been extracted several times with ether, was concentrated under reduced pressure to a sirup and the latter was dried in a vacuum desiccator. A snow-white crystalline residue, free from any sirupy admixture, was obtained that weighed 0.25 g. It was dissolved in a little dilute acetic acid and brought to crystallization by the addition of alcohol. Colorless prismatic needles were obtained that weighed 0.2 g. The crystals gave the Scherer reaction and melted at 225°. There was no depression of the melting point when the substance was mixed with pure inactive inosite prepared from phytin.

It is evident from the results of this analysis that the only products obtained on hydrolyzing the neutral carbohydrate were: (a) a small quantity of a barium salt of an organic phosphoric acid which resembled barium glycerophosphate, (b) mannose, isolated as mannose phenylhydrazone, and (c) inactive inosite. We are forced to the conclusion, therefore, that the carbohydrate consists of a combination of mannose and inosite in the form of a polysaccharide or a glucoside. For want of a better name we designate the substance by the name maninositose.

Isolation of the Fatty Acids.—The alcoholic soap solution, obtained on saponifying the phosphatide, was concentrated to a volume of about 200 cc., diluted with water, acidified with hydrochloric acid, and the fatty acids were extracted with ether. The aqueous solution was saved and examined for glycerol. The ether extract was washed with water until the washings were neutral to litmus and the ether was distilled off. The residue, consisting of the mixed fatty acids, weighed 10.8 g. after it had been dried *in vacuo*. The fatty acids were not separated at this time but they were preserved for future use.

Examination of the Aqueous Solution for Glycerol and Glycerophosphoric Acid.—The acid aqueous solution, which remained after the fatty acids had been extracted, was concentrated to dryness under reduced pressure and the residue was extracted with absolute alcohol. The insoluble potassium salts were removed by filtration, the alcohol was evaporated and the residue was again extracted with absolute alcohol, filtered and diluted with an equal volume of water. The mixture was made faintly alkaline with barium hydroxide when a very slight amorphous precipitate was obtained. The precipitate was filtered off, washed with alcohol and dried. The amount of the substance was very small and since it gave a reaction for inorganic phosphoric acid it evidently consisted of barium phosphate.

The filtrate from the barium precipitate was concentrated to dryness *in vacuo*. The residue was extracted with absolute alcohol, the extract was filtered and the alcohol was distilled off when a slight sirupy residue was obtained. The sirup gave no reduction on boiling with Fehling's solution but when it was heated with acid potassium sulfate a strong odor of acrolein was noted, thus indicating the presence of glycerol.

The alcohol-insoluble potassium salts, which were mentioned above, were dissolved in water and the solution was made faintly alkaline with barium hydroxide. A slight amount of an amorphous precipitate was obtained but there was no further precipitate on adding alcohol. The precipitate gave a reaction for inorganic phosphate and it evidently consisted of barium phosphate. The results of the examination indicate that the

aqueous solution contained some free glycerol and some free phosphoric acid but no glycerophosphoric acid could be isolated.

Summary

1. A study has been made of the action of hot dilute alcoholic potassium hydroxide on the phosphatide A-3 from the human tubercle bacillus. When treated in this manner the phosphatide is saponified, yielding an alcoholic soap solution of the fatty acids together with small amounts of free glycerol and phosphoric acid, while an alcohol-insoluble residue remains which consists of a mixture containing a complex organic phosphoric acid and a neutral carbohydrate.

2. The organic phosphoric acid has not been studied fully but when it is boiled with dilute acid it is hydrolyzed with the formation of a reducing sugar which has not yet been identified.

3. The neutral carbohydrate, called maninositose, represents a new type of polysaccharide or a glucoside which on hydrolysis with dilute acid yields mannose and inosite.

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THE SYNTHESIS OF INDOLYL-BUTYRIC ACID AND SOME OF ITS DERIVATIVES

BY RICHARD W. JACKSON AND RICHARD H. MANSKE

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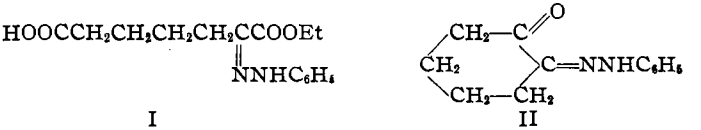
The interest which has of late centered in the metabolism of certain indole derivatives[1,2,3] has made it desirable to prepare new members, particularly some of the higher homologs, of an already known series. For example, to make available a sequence of 3-indolyl acids among which indolyl-propionic acid has heretofore been the highest known homolog, it became desirable to construct the corresponding butyric, valeric and caproic acids. At the same time, the chemical aspects of the syntheses presented several interesting features.

The recently much exploited[4] Japp and Klingemann reaction, when applied to ethyl cyclohexane-1-one-2-carboxylate, produces an excellent yield of the half ester of the phenylhydrazone of α-ketopimelic acid (I)



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[1] Jackson, *J. Biol. Chem.*, **73**, 523 (1927); **84**, 1 (1929).

[2] Berg, Rose and Marvel, *ibid.*, **85**, 207, 219 (1929).

[3] Jackson, *ibid.*, **87**, XIV (1930).

[4] Manske, Perkin and Robinson, *J. Chem. Soc.*, 1 (1927); Manske and Robinson, *ibid.*, 240 (1927); and others.