with 0.01 molar ketone.[9] In general, a concentrated solution (2.25 molar and upwards) of phenylmagnesium bromide appears to be best suited for the tests described in this paper.

Summary

The sensitive color test for reactive organometallic compounds can be used as a delicate test for Michler's and related ketones. It is less satisfactory for phosgene and dialkyl anilines.

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THE CHEMISTRY OF THE LIPOIDS OF TUBERCLE BACILLI. XIV. THE OCCURRENCE OF INOSITE IN THE PHOSPHATIDE FROM HUMAN TUBERCLE BACILLI[1]

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Introduction

The phosphatide fraction, A-3, isolated from the human type of tubercle bacilli, strain H-37, after it had been hydrolyzed with dilute sulfuric acid, was found to yield about 67% of fatty acids and about 33% of water-soluble material.[2] On concentrating the aqueous solution, a thick sirup was obtained from which we isolated glycerophosphoric acid, a small quantity of a colorless crystalline substance, crude mannose phenylhydrazone[3] and finally obtained a good yield of glucosazone.

We have recently examined the crystalline substance which separated from the concentrated sirup and it has been identified as ordinary inactive inosite. That inosite should be present in tubercle bacilli is not surprising, since it appears to be universally distributed in all living cells, but the occurrence of inosite in an ether-soluble lipoid was unexpected. The nature of the inosite linkage in the phosphatide molecule is still unknown, but it must have been combined in some manner either with the carbohydrate complex or with fatty acids.

Experimental Part

The inosite crystals had been obtained in the following manner. After the phosphatide, A-3, had been hydrolyzed by boiling with dilute sulfuric acid, the fatty acids

[9] This is in agreement with earlier work by Gilman and Heck (see Ref. 1b of this paper), who showed that the color was more pronounced with more concentrated solutions of Michler's ketone.

[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.

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

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

were removed by extraction with ether and the aqueous solution was freed of sulfuric and phosphoric acids by adding a slight excess of barium hydroxide. After the solution had been filtered, the excess of barium was removed quantitatively with sulfuric acid and the filtered solution was concentrated under reduced pressure to a thick sirup. The sirup was extracted with alcohol in order to remove the glycerophosphoric acid and on neutralizing the alcoholic extract with barium hydroxide, the barium glycerophosphate separated as a white amorphous precipitate which was filtered off. The filtrate was concentrated to dryness, the residue was dissolved in water and the excess of barium was removed quantitatively with sulfuric acid. The barium sulfate was filtered off and the filtrate was concentrated and combined with the main portion of the sirup which was insoluble in alcohol. The sirup was then concentrated in a vacuum desiccator, when crystals formed; these were isolated by washing off the adhering sirup with cold dilute alcohol. The crude crystals after combining the material from several hydrolyses weighed about 0.7 g. On combustion a small amount of ash was left which apparently consisted of magnesium carbonate.

For purification the substance was twice recrystallized by dissolving it in a little hot dilute acetic acid and adding alcohol until crystals began to form in the hot solution. On standing for a few hours or overnight at room temperature, the substance separated almost completely. It was recrystallized twice in the same manner from hot water by adding alcohol. The purified crystals weighed 0.55 g. and were practically free from ash. Large, colorless prismatic crystals were obtained which did not contain any water of crystallization.

The substance gave the Scherer reaction and when heated in a capillary tube it melted at 224–225°. There was no depression of the melting point when the substance was mixed with some purified inactive inosite, prepared from phytin, which melted at 224–225°.

For analysis the powdered crystals were dried at 61° in vacuo over dehydrite but there was no loss in weight.

Anal. Subs., 0.1387: CO_2, 0.2033; H_2O, 0.0860. Calcd. for $C_6H_{12}O_6$ (180): C, 40.00; H, 6.66. Found: C, 39.97; H, 6.93.

Summary

One of the water-soluble cleavage products of the phosphatide obtained from the human type of tubercle bacilli has been identified as inactive inosite.

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