LV. A NOTE ON THE STRUCTURE AND CHEMISTRY OF GLUCURONIC ACID MONOBENZOATE.

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ALTHOUGH glucuronic acid was discovered over 50 years ago, its position in physiology remained until recently undefined. Now, however, it seems fairly certain that this compound is intimately related to carbohydrate metabolism. The depancreatized dog can still produce glucuronic acid from the glycogenetic fraction of the protein molecule, which would otherwise go to glucose and be excreted [Quick, 1926, 2]; furthermore, insulin can markedly increase while acetoacetic acid as well as lactic acid decidedly suppress its output [Quick, 1932]. The importance of glucuronic acid depends not only on its relationship to carbohydrates, but also on the fact that it represents a type of conjugation, which is commonly referred to as a detoxication mechanism. In the opinion of the writer these synthetic processes are constantly active in normal metabolism, but they often only become manifest when the organism applies them to abnormal substances. It is probable that the uric acid-carbohydrate compound normally present in the blood is produced by a conjugating mechanism and may represent an important step in the excretion of uric acid.

The desirability of studying the molecular structure and chemistry of the various conjugated glucuronic acids is evident. Recently Pryde and Williams [1933] reported studies on the structure of bornyl-*D*-glucuronide, glucurone, and the glucuronic acid-benzoic acid compound. This last compound was prepared and studied by Quick [1926, 1]. From the data then obtained, he concluded that the compound was not benzoylglucuronic acid as Magnus-Levy [1907] had postulated (I) but glucuronic acid monobenzoate (II).

I

II

Pryde and Williams, however, question the ester structure and believe that the formula originally proposed by Magnus-Levy is the correct one. The writer's contention that the compound has formula II is based in part on the finding that sodium cyanide reacts directly with glucuronic acid monobenzoate, thus indicating that the aldehyde group is free and is not combined with benzoic acid. Although Pryde and Williams obtained the same polariscopic changes as the author, they claim that the alkalinity of the sodium cyanide first caused a hydrolysis, and that it was the liberated glucuronic acid which reacted. They base their conclusions on the qualitative detection of benzoic acid, which obviously can be misleading, and was unfortunate in view of the fact that satisfactory quantitative methods were available, which would have prevented a hasty and erroneous conclusion.

In order to redemonstrate that sodium cyanide reacts with glucuronic acid monobenzoate directly without previous hydrolysis, the following experiment was performed.

0.4 g. of glucuronic acid monobenzoate was dissolved in 20 ml. of water and mixed with a solution containing 0.09 g. sodium cyanide in 20 ml. The resulting mixture was faintly alkaline to phenolphthalein and became progressively more so as the reaction progressed. At the end of 21 hours, the solution was acidified with hydrochloric acid until acid to Congo red, then extracted with 4 portions (25, 25, 15, 10 ml.) of neutral toluene and the benzoic acid determined in the usual manner by titration with 0.1 *N* sodium ethoxide. The amount of free benzoic acid found was 0.0145 g. while the total amount contained in 0.4 g. of glucuronic acid monobenzoate is 0.1635 g. Therefore only 8.9 % of the compound was hydrolysed in 21 hours. At the same time, a determination of the reducing action made on an aliquot by the method of Shaffer and Hartmann [1920-21] showed that only 3.9 % of the original reducing power remained, indicating that 96.1 % of the aldehyde had reacted to form the cyanohydrin derivative. It must be concluded that the benzoic acid is not attached to the aldehyde group, for, if it were, an amount of benzoic acid equivalent to the quantity of cyanohydrin produced should be formed. Therefore the benzoic acid must be linked to one of the hydroxy-groups of glucuronic acid.

Although the experiment presented answers the criticism of Pryde and Williams there are sufficient data in the writer's original paper to support adequately the claim that the compound under discussion is not benzoylglucuronic acid but a glucuronic acid monobenzoate.

In the first place, when 0.2 g. of glucuronic acid monobenzoate (which requires 0.033 g. of sodium cyanide) was treated with a slight excess, *i.e.* 0.045 g. of sodium cyanide, the solution was alkaline to phenolphthalein and became more alkaline as the reaction progressed. If hydrolysis had occurred, the solution should have become acid, since the amount of benzoic acid liberated would have greatly exceeded the amount of base available for neutralisation.

In the second place, glucuronic acid monobenzoate showed mutarotation in an alkaline solution. As the writer expressly stated, this change of rotation occurred with scarcely any hydrolysis.

Further study is required to determine by which hydroxy-group the benzoic acid is linked to glucuronic acid. Probably the most promising approach to the problem is the preparation of the methyl ester methylglucoside, as described by the writer, before attempting complete methylation.

SUMMARY.

The compound resulting from the conjugation of glucuronic acid with benzoic acid is a glucuronic acid monobenzoate, and not benzoylglucuronic acid, for it acts directly with sodium cyanide to form the cyanohydrin, and it exhibits mutarotation.

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