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The Synthesis of Salicyl Glucuronide Derivatives*

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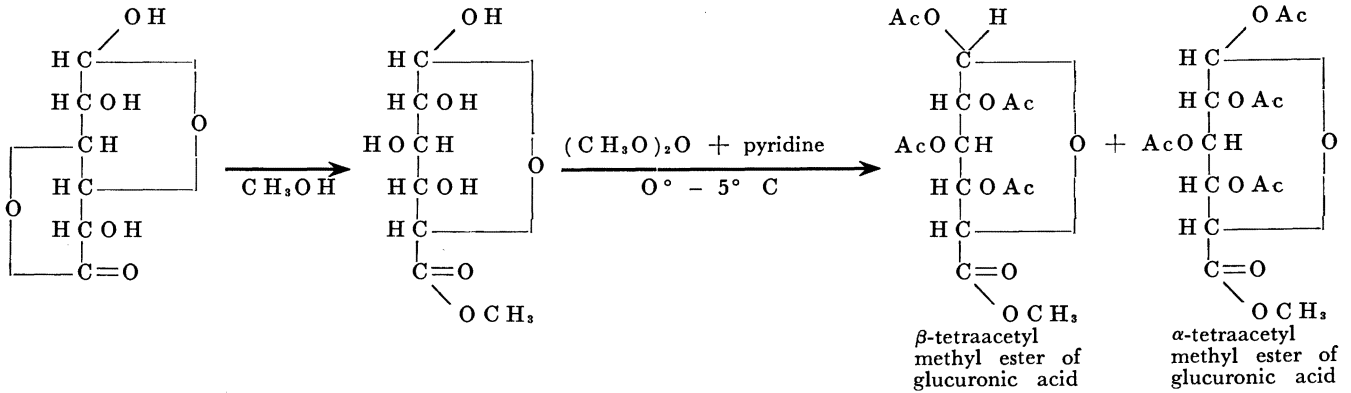
The formation of glucuronides of salicylic acid as conjugated products in the body has been demonstrated for many years. Quick (1) in 1932 found salicyl diglucuronates in the urine of dogs fed salicylic acid, whereas, Kapp and Coburn (2) demonstrated the presence of both mono- and di-glucuronides of salicylic acid in human urine. Bray and coworkers (3) observed small amounts of ester linked glucuronides and ether linked glucuronides in the urine of rabbits fed salicylic acid. Recently, Smith *et al.* (4) using C^{14} carboxyl labeled salicyclic acid obtained evidence for the excretion of the two types of mono-glucuronides in human urine. One had an ester linkage between the carboxyl group of salicylic acid and a hydroxyl group of glucuronic acid. The other had an ether linkage between the hydoxyl groups of salicylic and glucuronic acids. To our knowledge, none of the salicyl glucuronides have been isolated in crystalline form. The present investigation was an attempt to synthesize both forms of salicyl-β-d-glucuronide.

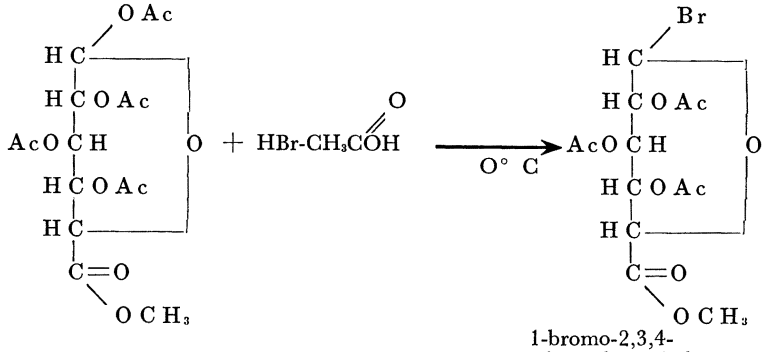
Glucurone lactone was converted to the methyl ester (Goebel and Babers (5) which was then acetylated to the α- and β-tetra-acetyl methyl esters of glucuronic acid (6).

Equation on following page.

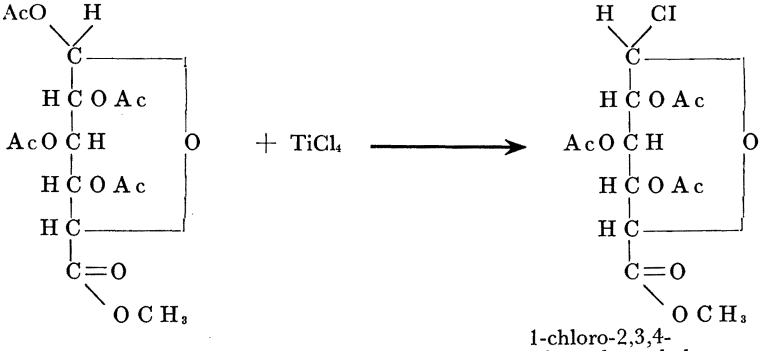
*This investigation was aided by a grant from the Institute for the Study of Analgesic and Sedative Drugs.


CH3OH
(CH3O)2O + pyridine
0° – 5° C
β-tetraacetyl methyl ester of glucuronic acid
α-tetraacetyl methyl ester of glucuronic acid

The α-compound was converted into the 1-bromo-2,3,4-triacetyl methyl ester (5), while the β-compound was converted into the α<-1chloro compound (5).

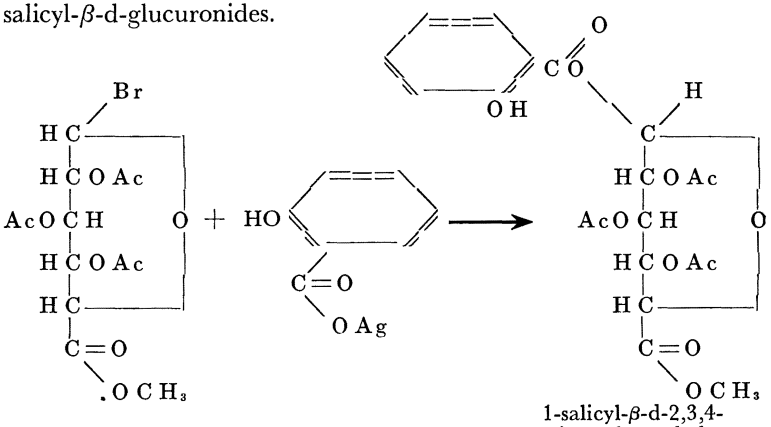


1-bromo-2,3,4-triacetyl methyl ester of glucuronic acid



1-chloro-2,3,4-triacetyl methyl ester of glucuronic acid

The halogen derivatives were used for the preparation of the salicyl-β-d-glucuronides.



1-salicyl-β-d-2,3,4-triacetyl methyl ester of glucuronic acid

The ester-type linkage compound was prepared following Goebel's (7) procedure, while the ether-type linkage glucuronide was prepared according to the procedures of Robertson and Waters (8), and of Helferich and Smitz-Hillebrecht (9).

The criteria for purity were melting points, optical rotation, the reduction of Benedict's solution, the color reaction with $FeCl_3$, and the determination of the number of acetyl and methyl groups.

Laboratory Procedure*

Methyl ester of glucuronic acid—33 g. of glucurone lactone were refluxed for 72 hours with 400 cc. of absolute methanol; decolorized with charcoal, and the alcohol evaporated in vacuo. The residue was taken up in absolute ethanol, allowed to stand 48 hours, and the unreacted glucurone removed by filtration; 9 g. were recovered. The filtrate was evaporated in vacuo to a constant weight. No boiling point or optical rotation of the syrup are available.

1,2,3,4-tetraacetyl methyl esters of glucuronic acid—A cold mixture (0°) of 92 cc. dry pyridine and 62 cc. of acetic anhydride was added to the flask containing the methyl ester of glucuronic acid. The temperature was kept between 0° - 5° C., while the reaction mixture was constantly stirred to insure solution of the ester. The flask was allowed to stand overnight at 5° C. The precipitate was filtered off, and the filtrate poured over ice-water, stirred vigorously for 30 minutes; then filtered and washed several times with distilled water.

The first precipitate was washed with a cold mixture of ether-ethanol, and recrystallized from ethanol, m.p. 178°, $(\alpha)_D^{20} = +8.70$. This was the β-form. The second precipitate was recrystallized from ethanol and gave the same melting point and optical rotation.

The filtrates were combined and evaporated in vacuo. The residue was washed with chilled ether and recrystallized from ethanol, m.p. 118°, $(\alpha)_D^{20} = +98°$. This was the α-form.

1-chloro-2,3,4-triacetyl methyl ester of glucuronic acid—50 g. of $TiCl_4$ were added to 10 g. of β-tetraacetyl methyl ester of glucuronic acid dissolved in 80 cc. of anhydrous chloroform. The solution was kept at 45-50° for three hours, cooled and poured into ice-water. The water layer was decanted and the chloroform layer washed repeatedly with ice-water, dried over $CaCl_2$, and evaporated in vacuo. The residue was dissolved in 20 cc. of ether and

*We wish to thank Dr. R. L. Shriner for suggestions which greatly facilitated portions of the experimental work.

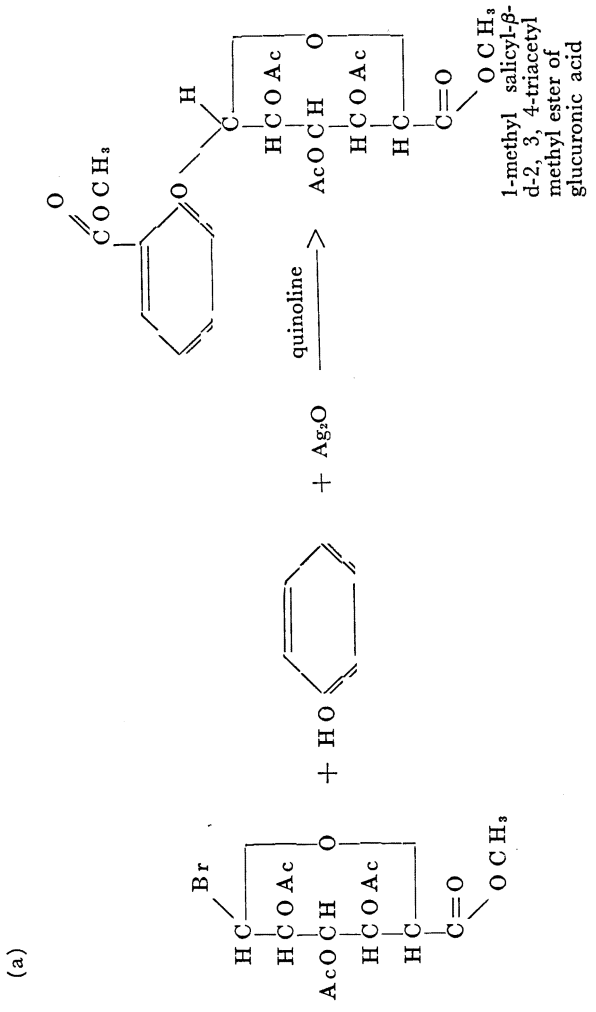
kept in the refrigerator. The precipitate was filtered and recrystallized from ether, m.p. 150.5 - 1.5°, $(\alpha)_D^{20} = + 167.2°$.

1-bromo-2,3,4-triacetyl methyl ester of glucuronic acid—5 g. of α-tetraacetyl methyl ester of glucuronic acid were dissolved in 20 cc. of acetic acid previously saturated with HBr at 0°, allowed to stand for 2 hours; 40 cc. of chloroform added, and the mixture poured over ice-water with stirring. The chloroform layer was separated and the aqueous layer extracted repeatedly with chloroform. The chloroform extracts were combined, dried over $CaCl_2$, and evaporated in vacuo. The precipitate was filtered and recrystallized from ether, m.p. 107 - 8°, $(\alpha)_D^{20} = + 198°$.

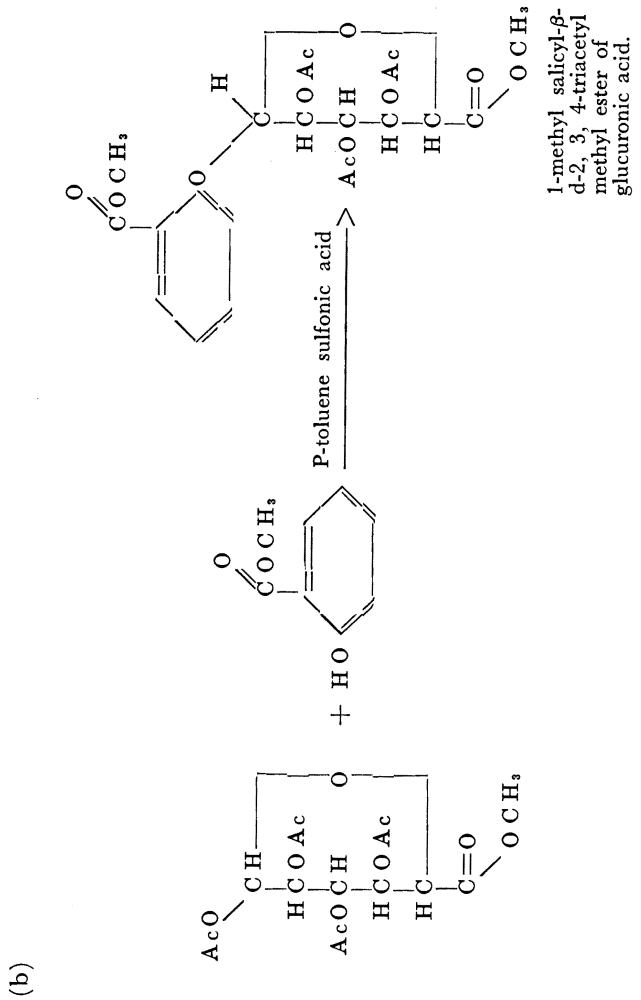
1-salicyl-β-d-2,3,4-triacetyl methyl ester of glucuronic acid—4 g. of 1-bromo-2,3,4-triacetyl methyl ester of glucuronic acid and 8.23 g. of silver salicylate were refluxed in 100 cc. of anhydrous alcohol-free chloroform for 3½ hours. The solution was filtered, and the chloroform evaporated in vacuo. The oily residue was recrystallized from methyl alcohol, m.p. 166 - 70°, $(\alpha)_D^{20} = + 32.5°$.

1-methylsalicyl-β-d-2,3,4-triacetyl methyl ester of glucuronic acid —Two procedures have been attempted:

Equation on following page.



According to Robertson and Waters (8): 2 g. of the halogen acetylated compound and 1 cc. of methyl salicylate were dissolved in 4 cc. pure quinoline and 1.39 g. of dry active Ag_2O were added and stirred keeping the reaction cool. The mixture turned into a stiff mass, and was kept in a dessicator for 30 minutes, taken up in 15 cc. of acetic acid, stirred and the salts filtered off. The filtrate was poured into 250 cc. of H_2O, and the oily precipitate was washed with water. No crystals could be produced from this oil, even after evaporation under vacuum.



(b)

5 g. of β-tetraacetyl compound, 4 cc. of methyl salicylate, and 0.079 g. of p-toluene sulfonic acid were stirred for 90 minutes on the steam bath. The mixture liquified, turned brown and the production of acetic acid was noted by its odor. After cooling, it was taken up in either benzene or chloroform and washed with water. The solvent layer was then dried over $CaCl_2$, evaporated in vacuo, and the residue was dissolved in hot ethanol. On cooling, crystals precipitated, which were filtered and identified as unreacted glucuronic acid compound (3 g. recovered). The alcohol from the filtrate was evaporated and gave a brown thick syrup from which

the unreacted methyl salicylate was removed in vacuo. The resultant syrup was dissolved in 5 cc. of methanol and allowed to stand at 5° C for 24 hours. The precipitate was filtered and recrystallized from methanol, m. p. 176-78°, $(\alpha)_D^{20} = +35.7°$.

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