STUDY OF STABILITY OF PHENOL-GLUCOSIDE LINKAGE IN COMPOUNDS MODELING THE LIGNIN-CARBOHYDRATE LINKAGE

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The existence of lingnocarbohydrates has been firmly established at the present time, which support the hypothesis of lignin being linked to the hemicellulose portion of cholocellulose. However, up to now an unequivocal answer is lacking to the problem of the number and types of chemical linkages between lignin and carbohydrates. These linkages can arise via phenolic and alcoholic hydroxyl groups (and the CO groups of lignin are capable of enolization), on the one hand, and the OH, CO, and COOH groups of the carbohydrate components, on the other hand. As a result, the most probable types of linkages can be ether, ester, acetal and phenol-glycoside linkages.

The difficulties that face investigators when trying to elucidate the character of the chemical bond on natural complexes, make it expedient to study this problem on simple model compounds.

In this respect the studies on the acid and alkaline stability of the phenol-glycoside linkage in the β -D-glucosides of phenol and acetovanillone (I), and the β -D-xylosides of phenol, guaiacol, acetovanillone, α -hydroxypropiosyringone (III), are of interest [1]



It was shown that an acyl grouping in the p-position to the glycoside linkage does not affect its resistance toward acids, but it does make this linkage more resistant toward alkalis. The presence of o-methoxyl groups decreases the stability of the glycoside linkage with respect to both acids and alkalis. The great stability of the alkyl-glucoside linkage in the β -D-glucoside of α -hydroxypropioveratrone (IV) made it possible to postulate [1] that an aryl-glycoside linkage is more probable between lignin and carbohydrates, and not the alkyl-glycoside linkage.

The present paper represents an attempt to study the comparative stability of the chemical bonds in lignocarbohydrate complexes on model compounds. Most of the methods existing at the present time for the isolation of lignin from wood is associated with the use of acid catalysts and solvolytic agents, like water, alcohols, ketones and acetals. We studied the comparative acid stability of the phenol-glucoside linkage on the simpler compounds that model this type of linkage: the β -D-glucosides of phenol, guaiacol, 1-guaiacyl-propane (V) and the methyl ether of 1-guaiacyl-1-propanol (VI), and on coniferin (VII) and dihydroconiferin



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β-D-Glucopyranoside	pH 1,08				pH 2,02	
	80°		100°		100°	
	k·10³ (±5%)	τ _{1/2}	$(\pm 5\%)^{h \cdot 10^2}$	τ1/2	k · 10 ³ (±5%)	T1/2
Phenol Guaiacol 1-Guaiacylpropane	$2,15 \\ 5,05 \\ 4,6$	320 137 150	1,5 5,1 4,6	46 14 15	1,2 5,7 5,0	577 120 139
Methyl ether of 1-guaia-	4 ,93	140	4,2	16,5	5,2	133
1-propanol Coniferyl alcohol (coniferin)	12,4	56	11,5	6	13,2	53
Dihydroconiferyl alcohol (dihydroconiferin)	5 ,05	137	5,1	14	5,2	133

TABLE 1. Hydrolysis Rate Constants and Half-Decomposition Periods of Phenol β -D-Glucopyranosides (k, min⁻¹, $\tau_{1/2}$, min)

We were the first to obtain compounds (V) and (VI). The synthesis of phenol glycosides containing labile aglycons, which most natural aglycons are, presents definite difficulties. A large excess of the phenol, quite drastic reaction conditions, and low yields are the main disadvantages of these methods. In the present paper the main attention was devoted not to the yields, but to the purity of the glucosides. The glucosides of phenol, guaiacol, 1-guaiacylpropane and the methyl ether of 1-guaiacyl-1-propanol were synthesized by a modification of the Königs-Knorr method, by the condensation of acetobromoglucose with the appropriate phenol, with subsequent removal of the acetate protection using sodium methylate. Coniferin (VII) was isolated from the cambial sap of freshly cut pine, while the hydrogenation of (VII) in the presence of Pd on $BaSO_4$ gave dihydroconiferin.

The acid-catalyzed hydrolysis of glycosides has been extensively studied, but comparatively little information exists on the hydrolysis of the glucosides of substituted phenols. The glucosides synthesized by us were investigated for their resistance to acids at a definite acidity of the medium. The hydrolysis was run inacetate — hydrochloric acid buffer solution at pH 1 and 2, and 80 and 100°. As it proved, D-glucose under these conditions remains unchanged and is quantitatively isolated from the hydrolyzate as the chromatographically pure material. The half-decomposition period was taken as a measure of the stability of the glycoside linkage. In order to estimate the degree of decomposition we used a colorimetric method that was based on measuring the optical density of the orange-yellow solution that was obtained by treating the sugar with phenol and conc. H_2SO_4 . This method makes it possible to determine microamounts of the sugars (10-80 μ g) with an accuracy of $\pm 5\%$, which has great importance when working with difficultly accessible glycosides [3].

Aliquot samples of the hydrolyzate were deposited on chromatographic paper, the chromatogram was developed with a mixture of ethanol-n-butanol-water (5:5:1), and the glucose was extracted with water and determined quantitatively from the calibration curve. Using the equation for first order reactions we calculated the rate constants and the half-decomposition periods. In Table 1 are given the average values of the half-decomposition periods and the hydrolysis rate constants of 0.1 M solutions of the β -D-glucosides investigated by us. The obtained data show that the stability of the phenol-glucoside linkage depends on the pH of the medium and the reaction temperature. The hydrolysis practically does not go at room temperature, while its rate increases 10 times when the temperature is changed from 80 to 100°.

As can be seen from Table 1, the $o-OCH_3$ group exerts a much greater effect on the stability of the phenol-glucoside linkage than do alkyl substituents, in which connection the presence and position of an OH group in the p-substituent are practially without effect on the stability of this linkage, since the double bond, conjugated with the aromatic ring of the aglycon, weakens it greatly.

EXPERIMENTAL METHOD

The cambial sap of freshly cut pine was carefully heated up to the boil, the liberated plant proteins were filtered, while the clear sap was evaporated to 1/3 of the original volume. Crystalline coniferin (VII) deposited on cooling, mp 173-176° (water); $[\alpha]_D - 71.2°$ (C 1.5; methanol).

Dihydroconiferin was obtained in 87% yield by the hydrogenation of coniferin in alcohol in the presence of Pd/C, mp 139-140.5° (acetone), see [4]; $[\alpha]_D - 57.5°$ (C 2; methanol). Found: C 54.38; H 7.08%. C₁₆H₂₄O₈.¹/₂ H₂O. Calculated: C 54.37; H 7.17%.

<u>Tetraacetate of the β -D-Glucopyranoside of 1-Guaiacylpropane.</u> 1-Guaiacylpropane was obtained by the hydrogenation of freshly distilled eugenol in ethanol over 10% Pd/BaSO₄ [5], bp 113-114° (5 mm), see [6].

Acetobromoglucose was synthesized as described in [7], mp 86-88°. To a solution of the guaiacylpropane in aqueous alkaline solution (0.63 g of NaOH in 25 ml of water) was added 3.57 g of acetobromoglucose in 35 ml of acetone, and the reaction mixture was allowed to stand overnight in the dark at ~20°. Then the mixture was extracted with benzene, and the benzene solution was washed in succession with 2 N NaOH solution and water, and then dried over anhydrous CaCl₂. Removal of the benzene gave 2.04 g (12%) of the crystalline glucoside, mp 120-122° (from ethanol); $[\alpha]_{\rm D}$ -40.6° (C 1.5; methanol). Found: C 58.24; H 6.43%. C₂₄H₃₂O₁₁. Calculated: C 58.06; H 6.45%.

<u> β -D-Glucopyranoside of 1-Guaiacylpropane</u>. To a solution of 3.5 g of 1-guaiacylpropane β -D-glucopyranoside tetraacetate in 25 ml of methanol was added 7 ml of 0.1 N CH₃ONa solution, and the mixture was kept overnight at ~20°. After removal of the sodium ions with a slight excess of cationite KU-2, and distilling off the methanol, we obtained 3.1 g (90%) of substance, mp 138-140° (from water), after drying at 108° (1.5 \cdot 10⁻² mm); [α]_D -62.3° (C 2; methanol). Found: C 57.91; H 7.54%. C₁₆H₂₄O₇. Calculated: C 58.54; H 7.31%.

<u>Tetraacetate of β -D-Glucoside of Methyl Ether of 1-Guaiacyl-1-propanol.</u> 1-Guaiacyl-1-propanol was obtained by the Grignard reaction from vanillin in 80% yield, mp 84-85.5° (from benzene), see [8]. The alcohol group was methylated by treatment with methanol, containing 0.5% HCl, at ~20° for a day. After neutralization of the reaction mixture with alcoholic KOH solution, and removal of the methanol, the residue was extracted with ether. From the ether was isolated the methyl ether of 1-guaiacyl-1-propanol with bp 122.5-123.5° (5 mm).

To 0.58 g of the methyl ether of 1-guaiacyl-1-propanol in caustic solution (0.15 g of NaOH in 4 ml of water) was added 0.8 g of acetobromoglucose in 6 ml of acetone. The mixture was allowed to stand overnight (~20°), after which it was extracted with benzene. The benzene solution was washed in succession with 2 N NaOH solution and water, and then dried over anhydrous Na₂SO₄. After removal of the benzene we obtained a crystalline product in 63% yield, mp 107-110° (from alcohol); $[\alpha]_{D} - 37.4^{\circ}$ (C 2; methanol). Found: C 56.68; H 6.66%. C₂₅H₃₄O₁₂. Calculated: C 57.02; H 6.46%.

<u> β -D-Glucopyranoside of Methyl Ether of 1-Guaiacyl-1-propanol.</u> The compound was obtained by the saponification of its tetraacetate with a 0.1 N solution of CH₃ONa in methanol at ~20°, yield 100%, mp 152-154° (from ethanol, precipitated with ether), after drying at 110° (10⁻² mm); $[\alpha]_D - 53.4°$ (C 2; methanol). Found: C 56.66; H 7.34%. C₁₇H₂₆O₈. Calculated: C 56.92; H 7.26%.

<u>Phenyl- β -D-glucopyranoside</u>. Obtained as described above from phenol and acetobromoglucose, mp 175-177° (from water); $[\alpha]_D - 73.7°$ (C 0.93; water), see [9].

<u>Guaiacyl- β -D-glucopyranoside</u>. Obtained in a similar manner via the tetraacetate of the glucoside starting with guaiacol and acetobromoglucose, mp 151-152° (from ethanol), after drying at 100° (10⁻⁴ mm); $[\alpha]_{\rm D}$ -76.03° (C 1; methanol).

Hydrolysis of Phenol β -D-Glucopyranosides. The hydrolysis was run in 3 ml glass ampuls, containing 1 ml of a 0.1 M solution of the glucoside in acetate-hydrochloric acid buffer solution (50 ml of 1 N CH₃COONa solution with the appropriate amount of 0.1 N HCl solution was diluted up to 250 ml), at pH 1.08 and 2.02, and temperatures of 80 and 100° (in a thermostat). The pH was controlled potentiometrically. The ampul was cooled at definite time periods, opened, and 0.2 ml aliquots of the hydrolyzate were removed, which were deposited on Whatman No.1 chromatographic paper (12×30 cm, with a control band of 3 cm) that had been previously washed with water. The chromatogram was developed with the system: n-butanol -ethanol-water (5:5:1) for 4 h; the control band was treated with a 3% solution of p-anisidine in butanol. The band on the paper, corresponding to the position of the glucose ($R_f 0.12$), was cut out and extracted with 20 ml of water, and the solution was filtered through a glass filter. To 2 ml of the glucose solution were added 3 drops of 80% phenol and 5 ml of conc. H₂SO₅. After 10 min the solution was shaken and kept at 30-35° for 20 min. The optical density was measured on an FEK-56 photoelectric colorimeter at 490 nm. The amount of the sugar was determined from the calibration curve, and the rate constant was calculated using the equation of a first order reaction. The results were verified by repeating the experiments, after which the average values of the reaction rate constants were taken and the half-decomposition period of the substance was calculated. The obtained results are summarized in Table 1; the accuracy of the determination was $\pm 5\%$.

CONCLUSIONS

1. A study was made of the comparative stability of the synthesized β -D-glucopyranosides of phenol, guaiacol, 1-guaiacylpropane and the methyl ether of 1-guaiacyl-1-propanol, and also of coniferin and dihy-droconiferin, at pH 1 and 2.

2. The stability of the glucoside linkage in these compounds depends on the temperature, the acidity of the medium, and the structure of the aglycon.

3. A methoxyl group in the o-position of the aromatic ring and an α,β -double bond in the side chain of the aglycon weaken the glucoside linkage. The presence and position of an alcohol group in the side chain do not affect the stability of the linkage.

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