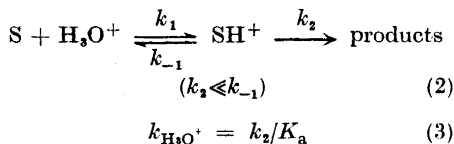


methyl-substituted derivatives but the substituent effect is much smaller than in the corresponding acyclic acetals. Thus we can conclude that the protonation of the oxygen atoms is less sensitive to inductive polar effects of the substituents in 1,3-dioxolanes than in the acyclic acetals.

The overall rate coefficient $k_{\text{H}_3\text{O}^+}$ for the hydrolysis of a 1,3-dioxolane by the A-1 mechanism of acetals (2) can be expressed by eqn. (3), where k_2 is the rate coefficient



of the rate-determining heterolysis and K_a the equilibrium constant for the protonation pre-equilibrium. Because only small differences in the equilibrium constants K_a are found in alkyl-1,3-dioxolanes, the differences in the observed rate coefficients are mainly due to the partial reactions in which the 1,3-dioxolane ring is cleaved. This observation confirms the previous conclusions that the relatively low rates of the hydrolysis of a number of 1,3-dioxolanes is due to low rates of ring cleavage and that the retardation must be steric in character.

The rate coefficients k_2 can be evaluated from the experimental data using eqn. (3). As recorded above, the values of K_a are of the order of 10^4 M for most of the studied 1,3-dioxolanes. Using the second-order rate coefficients measured in a previous work,² the following rate coefficients are obtained for the heterolysis reactions: $10^{-2} - 10^{-3} \text{ s}^{-1}$ for the 1,3-dioxolanes derived from formaldehyde, $10 - 10^2 \text{ s}^{-1}$ for the acetaldehyde derivatives, and $1 - 10^3 \text{ s}^{-1}$ for the acetone derivatives. When these rate coefficients are compared with the estimated maximum value, $10^6 - 10^8 \text{ s}^{-1}$,⁴ for the rate coefficient k_{-1} of a proton transfer from the protonated acetal to water, it is found that also in the case of acetone derivatives, in which the heterolysis is greatly accelerated owing to the high stability of the intermediate ion, k_2 is much smaller than k_{-1} . Thus additional and independent evidence has been obtained for the A-1 hydrolysis of 5-membered cyclic acetals.

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Synthesis of Sambubiose

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Sambubiose, 2-*O*- β -D-xylopyranosyl-D-glucose, was prepared by condensation of benzyl 3,5,6-tri-*O*-benzyl- α -D-glucoside and 2,3,4-tri-*O*-acetyl- α -D-xylopyranosyl bromide followed by removal of blocking groups.

The disaccharide moiety of a cyanidine glycoside isolated from *Sambucus nigra* was first believed to be primverose, 6-*O*- β -D-xylopyranosyl-D-glucose, but was later shown to be 2-*O*- β -D-xylopyranosyl-D-glucose, and was given the name sambubiose.¹ It is an uncommon constituent of flavanoids,² and has never been isolated as a free sugar. In the present communication, the synthesis of sambubiose is reported.

Benzyl 3,5,6-tri-*O*-benzyl- α -D-glucoside was prepared from 1,2-*O*-isopropylidene-D-glucoside and purified *via* its crystalline acetate.³ It was then condensed with 2,3,4-tri-*O*-acetyl- α -D-xylopyranosyl bromide, using mercuric cyanide-mercuric bromide in acetonitrile, as devised by Helferich.⁴ The condensation product was isolated by column chromatography on silicic acid, deacetylated and, after a further chromatographic purification, debenzylated by catalytic hydrogenation. Crystallisation of the resulting syrup from ethanol yielded sambubiose, m.p. 202–203°. The optical rotation of the disaccharide, in water, decreased from $[\alpha]_{578} +32^\circ$ (5 min) to $+17^\circ$ (48 h), demonstrating that it had crystallised as the α -form.

The mother liquors, which on paper-chromatography exhibited a single spot indistinguishable from that of the crystalline disaccharide, showed $[\alpha]_{578} + 65^\circ$. This optical rotation value suggests that the solution contains a mixture of the α - and β -linked disaccharides. The crystalline disaccharide after reduction and methylation, gave a single peak on GLC. The syrup from the mother liquors on similar treatment, gave the same peak on GLC plus another peak of about the same magnitude. These two peaks probably correspond to the α - and β -linked disaccharides. This is to be expected since both α - and β -glycosides are often produced when mercuric cyanide-mercuric bromide is used in the Koenigs-Knorr reaction.

Sambubiose was also obtained, but in lower yields, when the condensation was performed in the presence of silver oxide or silver carbonate.

The crystalline disaccharide on acid hydrolysis yielded equimolar amounts of D-glucose and D-xylose. It showed no mobility on paper electrophoresis in germanate buffer⁵ of pH 10, suggesting that the glycosidic linkage in the disaccharide is 1 \rightarrow 2. NMR of the reduced and trimethylsilylated disaccharide, in carbon tetrachloride, showed *inter alia* a doublet (τ 5.71, J_{12} 6.5 Hz) attributed to the anomeric proton of the D-xylose residue. The high coupling constant indicates a β -pyranose configuration for the xylose residue.

The disaccharide was methylated by the Hakomori procedure,⁶ hydrolysed and the methyl ethers in the hydrolysate analysed, as their alditol acetates by GLC⁷ (ECNSS-column)-mass spectrometry.⁸ Two peaks with *T*-values (retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol), 0.65 and 1.90, were obtained. The former was identified by its *T*-value and mass spectrum, as being derived from 2,3,4-tri-*O*-methyl-D-xylose. The latter, from its mass spectrum, was considered derived from a 3,4,6-tri-*O*-methyl hexose. Although no material of 3,4,6-tri-*O*-methyl-D-glucose was available as reference, it is clear that the component in this last peak is derived from the D-glucose residue. The absence of the primary fragment of *m/e* 117, always found in mass spectra of alditol acetates having an acetoxyl at C-1 and a methoxyl at C-2, conclusively demonstrates that the disaccharide does not have a free hydroxyl at C-2 in the D-glucose residue.

Experimental. General methods. Paper chromatography was performed on Whatman No. 1 paper, using the solvent systems ethyl acetate-pyridine-water, 8:2:1, and ethyl acetate-acetic acid-water, 3:1:1. *p*-Anisidine hydrochloride in butanol was used as spraying reagent. TLC was on silica gel G and G₂₅₄ (E. Merck AG) and column chromatography on silica gel G (<0.08 mm). GLC was performed on ECNSS columns, with a Perkin-Elmer 881 instrument. A Perkin-Elmer 270 instrument was used for the combined GLC-mass spectrometry. Paper electrophoresis was performed on Whatman 3MM paper in 0.05 M germanate buffer⁵ of pH 10. Melting points are corrected. Solutions were concentrated under reduced pressure, at a bath temperature not exceeding 40°.

Benzyl 3,5,6-tri-O-benzyl- α -D-glucofuranoside was prepared essentially as described by Finan and Warren.³ It was purified by conversion into its acetate and crystallisation from ethanol. The m.p. 55–56°, and rotation $[\alpha]_{\text{D}}^{23} + 67^\circ$ (c 1.0 chloroform) were higher than the values previously reported for this substance (m.p. 53–54°, $[\alpha]_{\text{D}} + 57^\circ$ (chloroform)). Deacetylation and concentration yielded the title compound as a syrup.

2-O- β -D-Xylopyranosyl-D-glucose. Benzyl 3,5,6-tri-*O*-benzyl- α -D-glucofuranoside (4.0 g), mercuric bromide (1.3 g) and mercuric cyanide (0.9 g) were dissolved in dry acetonitrile (50 ml), and 2,3,4-tri-*O*-acetyl- α -D-xylopyranosyl bromide (3.1 g) was added in portions during a 2 h period. The solution was kept at room temperature for 2 h, concentrated to a small volume and diluted with chloroform (200 ml). The precipitated mercuric salts were filtered off and the chloroform solution was washed, first with 1 M potassium bromide (4 \times 100 ml) and then with water (2 \times 100 ml). The material was next dried over magnesium sulphate, concentrated to a syrup (7.0 g) and added to the top of a silica gel column (50 \times 8 cm), which was irrigated with light petroleum (60–71°)-ethyl acetate, 1:1. The effluent was analysed by TLC with the same solvent system, and the fractions containing the major component were combined and concentrated. The product was suspended in dry methanol (50 ml), sodium methoxide (from 5 mg sodium in 1 ml methanol) was added and the solution was kept at room temperature for 20 h. It was then neutralised (Dowex 50, H⁺), concentrated to a syrup, and fractionated as above, using ethyl acetate as irrigant, to yield the major component as a syrup (2.8 g). This product (0.5 g) in anhydrous ethanol (50 ml) was hydrogenated at room temperature and atmospheric pressure, using a catalyst of 10 % palladium on charcoal (0.1 g). When hydrogen

consumption had ceased (69 ml or 4 molar equivalents) the catalyst was filtered off and the solution kept at room temperature overnight. The crystals of sambubiose, thus obtained (0.1 g) showed: m.p. 202–203° and $[\alpha]_{578}^{27} +32^\circ$ (5 min) $\rightarrow +17^\circ$ (48 h) (c 1.0 water). (Found: C 42.31; H 6.54; C₁₁H₂₀O₁₀ requires: C 42.30; H 6.47).

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Nuclear Quadrupole Relaxation of ⁸⁵Rb in an Aqueous Solution of a Humic Acid

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The ion-exchange properties of humic acids are of agronomic importance as well as of theoretical interest. The total possible exchange capacity can be experimentally determined by potentiometric titrations between two arbitrarily selected pH values but it would be of great interest to have a more direct method to study the

actual attachment of the metal ions to the humic acid molecules. It seemed possible that the study of the nuclear relaxation of ⁸⁵Rb in humic acid solutions would offer such a possibility. In the present paper results are reported which show that the method actually is very promising.

The principles of the method used have been described in a number of papers.¹ The present measurements were made at $28 \pm 1^\circ\text{C}$ on a Varian V-4200 NMR spectrometer. For experimental details, see Ref. 2. The humic acid was obtained by pyrophosphate extraction at pH 7 from a peat soil. The fractionation and the soil has been described elsewhere.³ The humic acid was precipitated in acid solution and dissolved by RbOH in a solution containing 0.5 M RbCl.

For rubidium ions which are free to move in an aqueous solution of 0.5 M RbCl the line width of the ⁸⁵Rb magnetic resonance signal is 0.24 gauss (Fig. 1a). When the same measurements are carried out in a neutral humic acid solution, containing 5 g humic acid/l solution an appreciable line-

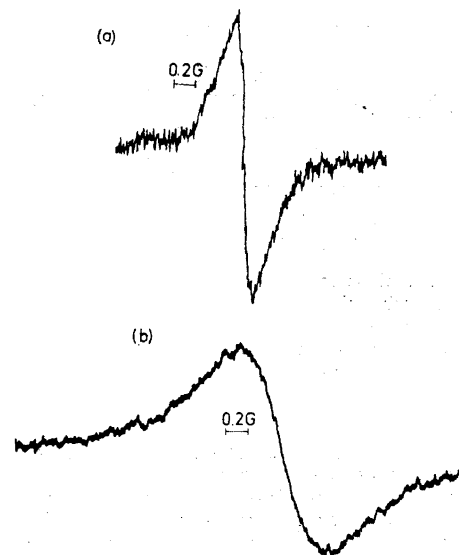


Fig. 1. The ⁸⁵Rb nuclear magnetic resonance signals (the figure shows the derivatives of the absorption signals) from (a) a 0.5 M RbCl solution and (b) a 0.5 M RbCl solution to which have been added 5 g humic acid/l solution and a small amount of RbOH in order to dissolve the acid. The spectra were recorded at a magnetic field strength of 14.0 kG.