

on a Vigreux column, b.p.₁₂ 76–77°C, n_D^{20} = 1.5138.

A small amount of tetramethylsilane was used as internal reference and lock signal source. The neat sample was thoroughly degassed and sealed under vacuum.

The NMR spectra were run at ambient probe temperature (ca. 30°C) on a JEOL-C-60H spectrometer. The spectra used for the analyses were recorded at 54 Hz sweep width and calibrated every 5 Hz using a frequency counter. Line positions were obtained by averaging the results of four scans.

Computations were performed on an IBM/50H computer using the UEATR computer program.¹⁴ About 280 transitions were matched for each isomer. The root-mean-square deviations for the experimental and calculated lines were 0.042 Hz or less. The calculated probable errors for the parameters were 0.005 Hz or less.

The graphical output was obtained using a Calcomp Plotter.

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Formation of an Anhydride of 2-O-(α -D-Galactopyranosyluronic Acid)-L-rhamnose

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Studies by anion exchange chromatography of the uronic acids present in hydrolyzates from spruce¹ (*Picea abies*, Karst.) and in sulphite spent liquor² revealed that among other biouronic acids a previously unknown compound (I) of this type was present. Mild acid hydrolysis of the isolated compound gave rise to rhamnose and galacturonic acid as well as 2-O-(α -D-galactopyranosyluronic acid)-L-rhamnose (II) which is a constituent in hemicellulose from spruce and other sources.^{3,4} Moreover, the same compound (I) was produced upon mild acid treatment of II. It was not formed from rhamnose and galacturonic acid under similar conditions and therefore cannot be a reversion product.¹ Both compounds exhibited a similar behaviour when chromatographed in sodium acetate whereas I was held more strongly than II in 0.5 M acetic acid indicating a higher acidic strength (Table I). In 0.12 M sodium tetraborate I appeared earlier indicating that the unknown compound has a lower ability to give borate complexes (Table I). Moreover, this com-

Table I. Distribution coefficients (D_v) of the unknown compound I and of 2-O-(α -D-GalpA)-L-Rha (II) before and after reduction with sodium borohydride.

	D_v	D_v	D_v
	0.5 M HAc	0.08 M NaAc	0.12 M Na ₂ B ₄ O ₇
I	13.1	3.57	4.03
I:borohydride	13.2	3.59	
II	7.95	3.49	6.15
II:borohydride	8.23	2.63	

D_v = adjusted retention volume calculated in column volumes.¹¹

pond reacts more slowly with periodate than II.¹ A study of the products obtained after methylation according to Kuhn and subsequent reduction and methanolysis gave a very complicated pattern of products indicating incomplete substitution and for this reason the structure of the compound could not be established with certainty.

In the present investigation Hakomori's method⁵ was applied and found to give reliable information about the biouronic acids, their reduction products and fragmentation products obtained after hydrolysis or methanolysis. Gas-liquid chromatography on silicone gum, XE-60, at 200° after methylation of the unknown compound gave rise to a single peak (37.1 min), whereas two peaks (9.0 and 12.2 min), probably representing the α - and β -anomers, were obtained from II. The methylated products were treated with lithium aluminium hydride in tetrahydrofuran to reduce the esterified carboxylic acid group. The trimethylsilyl (TMS) derivative from methylated and reduced I gave rise to one gas chromatographic peak (8.2 min) whereas that of II gave two peaks (3.1 and 3.9 min). The values given within parenthesis refer to the adjusted retention times. The observation that these were much shorter after the reduction is explained by the decrease in polarity.

Methanolysis of methylated and reduced II gave, after trimethylsilylation, three new peaks besides those corresponding to the non-hydrolyzed sample. The positions of the methoxyl groups in the mixed derivatives were determined by mass spectrometry (*cf.* Refs. 6 and 7) using a gas chromatograph-mass spectrometer. The first peak contained the TMS ether of a methyl-3,4-di-*O*-methyl-6-deoxyhexopyranoside, whereas the second and the third peaks contained TMS ethers of methyl-2,3,4-tri-*O*-methylhexopyranosides. These results as well as the optical rotation values for different preparations of the acid, $[\alpha]_D^{25} = +118 - 123$ (water, *c* 0.5), and the agreement in the D_v values recorded with

an authentic sample kindly supplied by Dr. A. Roudier, Paris, confirm the identity of II.

When the methylated and reduced I was subjected to methanolysis the yield of monosaccharides was low and the substance was therefore subjected to acid hydrolysis instead. Gas chromatography of the TMS derivatives prepared from the reaction products showed that in addition to the non-hydrolyzed product four new peaks were produced. The first two peaks contained the TMS derivatives of 3,4-di-*O*-methyl-6-deoxyhexopyranoses and the two other peaks corresponded to fully TMS substituted 3,4-di-*O*-methylhexopyranoses.

These results show that not only is a free hydroxyl group absent at C-2 in the rhamnose moiety but also in the galacturonic acid moiety in I. Free hydroxyl groups which could be TMS substituted were obtained in both C-2 positions after mild acid hydrolysis.

These results clearly indicate that I contains two glycosidic linkages namely the 1,2'-bond present in II and an additional 2,1'-bond formed during the acid treatment. Moreover, the results show that the pyranose structure is retained during the acid treatment and that the rhamnose moiety in I is in the pyranoside form. Since the compound is formed from II under very mild conditions the α -configuration of the 1,2'-glycosidic bond is most likely retained which means that the substance has the structure given in Fig. 1 and should be named α -D-(galactopyranuronic acid)-1-rhamnopyranose 1,2':2,1'-dianhydride.

No decomposition was observed when the original compound I was kept at pH 8 for 20 h at room temperature whereas, as mentioned above, it was reconverted in part to II after acid treatment. Additional evidence for the presence of two glycosidic linkages in I was obtained by a study of its reaction with sodium borohydride and subsequent ion exchange chromatography in sodium acetate and acetic acid (Table 1). For II the D_v value was changed markedly

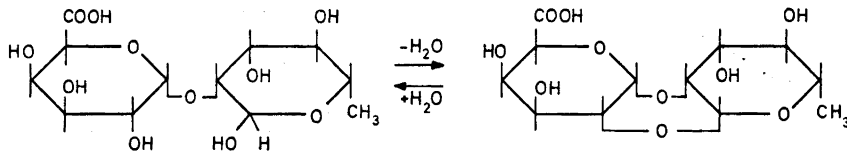


Fig. 1. Anhydridization and hydrolysis reactions.

after the reduction, whereas this treatment had no influence upon the D_v value of I. Moreover, I was hardly affected by treatment with cyanoferrate(III), whereas a rapid oxidation was obtained with II. These results lend additional support to the structure given above.

The mass spectrum of the methylated and reduced (but not methanolized) compound I recorded after trimethylsilylation exhibited several interesting features. The spectrum was quite different from those recorded for disaccharides. Several peaks with high m/e values were obtained and even the molecular ion (m/e 436) was clearly distinguished. The presence of a prominent peak at $M-15$ is explained by the loss of a methyl group from the TMS group. Peaks recorded at $M-32$ and $M-2 \times 32$ as well as the expected peaks at $M-15-32$ and $M-15-2 \times 32$ can be attributed to the loss of methanol.⁶ The results confirm the structure given above and show that a stabilization of the structure against fragmentation is obtained due to the presence of a third ring. A prominent peak at $M-103$ is explained by a loss of $\text{CH}_2\text{O-TMS}$ at C-5 in the galactose moiety.⁶ The base peak was recorded at m/e 88 which corresponds to the well-known radical ion produced from methylated sugars containing *O*-methyl groups at two adjacent carbon atoms.⁸

Theoretically, two different disaccharides could be obtained by acid hydrolysis of one of the glycosidic linkages in I. Only II was present in detectable amounts, which is explained by the well-known stabilization of the glycosidic bond due to the carboxylic acid group in aldobiouronic acids of this type. The fact that no detectable amounts of a hydrolysis product with a 2,1'-linkage were observed is explained by the instability of the pseudo-aldobiouronic acids.⁹

One question which remains to be answered is whether the 2,1'-linkage is in the α - or β -position. The high positive optical rotation value, $[\alpha]_D^{25} + 105$ (water, c 0.5), of I might suggest a β -linkage. Moreover, the formation of an α -linkage would require that the rhamnose moiety be transformed from the preferred C_1^4 conformation to C_1^1 conformation. In the β -form the rhamnose moiety retains its preferred conformation. It is therefore concluded that the 2,1'-linkage is in the β -position.

Experimental. 2-*O*-(α -D-Galactopyranosyluronic acid)-L-rhamnose was prepared from 20 g of Karaya gum by acid hydrolysis¹⁰ and isolated by anion exchange chromatography¹ as a syrup crystallized after drying over phosphorus pentoxide (1.2 g). Compound I (0.2 g) was obtained in the same procedure. Methanolysis of the methylated and reduced compounds in 0.65 M methanolic HCl for 18 h under reflux gave a reasonable yield of methylated monosaccharides from II, whereas a low yield was obtained from I. For this reason acid hydrolysis in 0.5 M H_2SO_4 at 100° for 16 h was applied to prepare the corresponding products from I.

The TMS derivatives were prepared in pyridine solution by adding an excess of bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (2:1). The mass spectra were recorded using a LKB 9000 gas chromatograph-mass spectrometer. The column packing was 3% DC QF-1 on Gas Chrom Q. The column temperature was 140° for the monosaccharides and 185° for the dianhydride and the ionizing voltage 70 eV.

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