STRUCTURE OF THE CARBOHYDRATE CHAINS OF POLEMONIOSIDES B AND C

V. G. Bukharov, L. N. Karneeva, and V. A. Talan

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 6, pp. 511–515, 1969

UDC 547.597+547.918

In preceding papers we have described the properties of polemoniosides B and C isolated from Polemonium coeruleum [1] and have discussed the structure of their aglycone [2]. In the present paper we describe the determination of the structure of the sugar chains.

On hydrolysis with mineral acids, the two polemoniosides gave one and the same progenin which was then cleaved into the aglycone and a uronic acid by boiling with Kiliani's mixture. On paper chromatography, the uronic acid appeared in the form of two spots corresponding to the free acid and its lactone. When the methyl ether of the progenin was reduced with lithium aluminum hydride and the product obtained was hydrolyzed, D-glucose was identified in the hydrolysate.

Polemonioside B is hydrolyzed with mineral acids to the progenin and D-galactose.

Since the molecular weight of polemonioside B is 1130 and that of the progenin 660, the difference corresponds to three galactose molecules. To determine the structure of the carbohydrate chain, the full methyl ether of polemonioside B was obtained by its prolonged heating with methyl iodide in dimethylformamide. The completeness of the methylation was checked by IR spectroscopy from the absence of the absorption band of free hydroxyl groups. When the ether was subjected to hydrolytic cleavage, 2, 3, 4, 6-tetramethyl-D-galactose was identified. What has been said above permits the conclusion that in the sugar chain of polemonioside B all the hydroxyl groups of the uronic acid are bound to D-galactose residues.

As has been shown previously, polemonioside C is cleaved by alkalies into polemonioside B [1]. The difference in their molecular weights (~310) corresponds to two monosaccharide residues. Consequently, in the sugar chain of polemonioside C, in addition to the three molecules of galactose attached to the hydroxyls of the glucuronic acid, there is a disaccharide attached to its carboxyl group. It was impossible to isolate the disaccharide in the pure state, but it was found that it contains L-arabinose and D-galactose.

We have determined the ratio of the monosaccharides obtained in the acid hydrolysis of polemonioside C. Quantitative paper chromatography [3] showed that there are four molecules of D-galactose to one molecule of L-arabinose.

Of all the methods tested, methylation with dimethyl sulfate in dimethylformamide proved to be the most convenient for polemonioside C. The mixture of methylated sugars obtained on the hydrolysis of the ether was then separated preparatively on Schleicher und Schüll paper in system 2. In this way we isolated 2, 3, 4-trimethyl-L-arabinose, 2, 3, 6-trimethyl-D-galactose, and 2, 3, 4, 6-tetramethyl-D-galactose. The identification of the trimethyl-D-galactose was difficult, since 2, 3, 6- and 2, 4, 6-trimethyl-D-galactoses have similar R_f values. Consequently, the product obtained was reduced with sodium borohydride to 2, 3, 6-trimethyldulcitol, and the latter was oxidized with potassium periodate. Since 2, 4, 6-trimethyldulcitol cannot be oxidized by potassium periodate, it was established unambiguously that the initial sugar had the structure of 2, 3, 6-trimethyl-D-galactose.

The absence of a 1–3 bond between the sugars was also confirmed by the periodate oxidation of polemonioside C, which destroyed all the monosaccharide residues apart from the glucuronic acid.

When the methyl ether of polemonioside C was hydrolyzed, unmethylated D-glucuronic acid was detected. The appearance in the hydrolysate of not only 2, 3, 4, 6-tetramethyl-D-galactose but also 2, 3, 6-trimethyl-D-galactose and 2, 3, 4-trimethyl-L-arabinose shows that they are present in the disaccharide attached to the carboxyl group of the glucuronic acid, the galactose being attached directly to the carboxyl and the arabinose being terminal.

EXPERIMENTAL

Chromatography was carried out with type ASK silica gel and "Leningrad slow" paper in the following systems:

1) butan-1-ol–acetic acid–water (4 : 1 : 5), 2) butan-1-ol–ethanol–water (5 : 1 : 4), 3) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3), 4) toluene–butan-1-ol–water (1 : 1 : 1), and 5) toluene–butan-1-ol–water (1 : 2 : 1).

Hydrolysis of the progenin. A solution of 0.5 g of the progenin in 7 ml of acetic acid, 11 ml of ethanol, and 2 ml of conc HCl was heated for 5 hr. After the ethanol had been distilled off, the precipitate of aglycone that had deposited was filtered off. Then the resulting hydrolysate, glucuronic acid (R_{Rha} 0.53) and its lactone (R_{Rha} 0.91) were identified by paper chromatography in system 1 in comparison with authentic samples.

Reduction of the progenin. A solution of 0.29 g of the substance in 10 ml of absolute ether was treated with an excess of an ethereal solution of diazomethane. The reaction mixture was evaporated to dryness, the residue was dissolved in 20 ml of absolute ethyl ether, and this solution was added dropwise to a suspension of 0.6 g of lithium aluminum hydride in 30 ml of absolute ethyl ether. The solution was heated with vigorous stirring for 6 hr. The complex was decomposed with a 1% aqueous solution of acetic acid, and the ethereal layer was separated off, washed with water, the evaporated to dryness. The residue was heated in solution in 5 ml of methanol containing 0.3 ml of HCl. In the hydrolyzate D-glucose was identified by paper chromatography in systems 2 and 4 in comparison with an authentic sample.

Full methyl ether of polemonioside B. A mixture of 0.1 g of polemonioside B, 4 ml of dimethylformamide, 6 g of barium oxide, and 6 ml of methyl iodide was heated in the boiling water bath in a sealed tube for 14 hr. After the addition of a large volume of water, the product was extracted with chloroform (100 ml × 3). This gave 0.1 g of the full methyl ether of polemonioside B with decomp. p. 108–113° C; $[\alpha]_D^{20}$ + 39° (c 1.5; ethanol). A mixture of this product (0.05 g) and 5 ml of methanol containing 0.4 ml of HCl was heated for 1 hr.

Found, %: C 61.33, 61.39; H 8.41, 8.71. Calculated for $C_{69}H_{116}O_{28}$, %: C 61.0; H 8.61.

The methanol was distilled off in vacuum and the precipitate of the progenin that had deposited was filtered off. 2,3,4,6-Tetramethyl-D-galactose was identified in the hydrolysate by paper chromatography in system 2.

Alkaline hydrolysis of polemonioside C. A mixture of 4 g of polemonioside C and 50 ml of 10% aqueous caustic soda was heated in the water bath for 3 hr. Then 50 ml of ethanol was added and the mixture was neutralized with KU-2 cation-exchange resin, after which the resin was filtered off, the filtrate was evaporated to small volume, and the precipitate of polemonioside B that had deposited was filtered off. After hydrolysis of the filtrate with 2% HCl, L-arabinose and D-galactose were identified in the hydrolysate by paper chromatography in systems 2 and 3 in comparison with authentic samples.

Determination of the ratio of the monosaccharide residues in polemonioside C. A mixture of 0.12 g of polemonioside C and 6 ml of 5% HCl was boiled for 1 hr. The precipitate that had deposited was filtered off and the filtrate was diluted with ethanol to 25 ml. Amounts of 0.025, 0.05, and 0.075 ml of hydrolysate were deposited on chromatograms. After chromatography had been carried out in system 3 for 48 hr and the spots had been revealed with aniline phthalate, the zones were cut out and extracted with glacial acetic acid, and the optical densities D of the extracts were measured on a FEK-M instrument in a 0.5-cm cell with a blue filter. The figures are given below:

	D	mg	D	mg	D	mg
arabinose	90	31	108	40	172	62
galactose	179	110	288	160	455	232

Full methyl ether of polemonioside C. A mixture of 5 g of polemonioside C, 13 g of barium oxide, 13 g of barium hydroxide, 7 ml of dimethyl sulfate and 160 ml of dimethylformamide was heated with stirring for 2 hr. Then 10 g of BaO and 18 ml of dimethyl sulfate were added and stirring and heating were continued for 16 hr with the gradual addition of another 10 g of BaO and 15 ml of dimethyl sulfate. The reaction mixture was diluted with 300 ml of water, and the precipitate that had deposited was filtered off and washed with chloroform (220 ml × 2). The chloroform extracts were combined with the filtrate, washed with water (100 ml × 3), and evaporated in vacuum. The residue was remethylated by the method described above twice. The final product was transferred to a column containing 20 g of silica gel and was eluted with 750 ml of benzene–chloroform (1 : 1). This gave 0.5 g of the ether, with decomp. p. 102–105° C, $[\alpha]_D^{20}$ + 30° (c 1; ethanol).

Separation of the methylated sugars. A mixture of 1 g of the methyl ether of polemonioside C, 9 ml of methanol and 1 ml of HCl was heated for 1 hr. Then 15 ml of water was added, the methanol was distilled off in vacuum, and the residue was filtered. The filtrate was passed through Dowex-2 anion-exchange resin and evaporated to dryness.

The residue (0.9 g) was transferred to a column containing 50 g of cellulose and was eluted successively with systems 4 and 5 and with butan-1-ol saturated with water, 100-ml fractions being collected. The separation of the fractions was monitored by chromatography in system 2.

Sixteen fractions were collected. Nos. 1–6 (0.7 g) were combined and distilled from a rotating flask, bp 120–130° C (4 mm). Amounts of 0.025 g of the product in ethanolic solution were deposited in a band on a sheet of Schleicher und Schüll paper (29 × 58 cm) and a chromatogram was run in system 2 for 24 hr. To detect the zones of the sugars, narrow strips were cut out from the edges and from the center of the sheet and were sprayed with aniline phthalate. The corresponding sections of the main chromatograms were extracted with ethanol. This yielded 2, 3, 6-trimethyl-D-galactose with $[\alpha]_D^{20}$ + 100° (c 1; ethanol), R_f 0.695. Literature data: R_f 0.71 in system 2 [4].

The sample obtained (0.005 g) was heated with 0.5 ml of 48% HBr in the boiling water bath for 5 min. Then it was diluted with 5 ml of water and was evaporated to dryness in vacuum at 50° C, after which the residue was dissolved in the minimum amount of water and extracted with benzene. The aqueous solution was shown by paper chromatography in systems 2 and 4 to contain D-galactose.

2, 3, 6-Trimethyl-D-galactose (0.1 g) was dissolved in 2 ml of ethanol, and 0.025 g of $NaBH_4$, two small crystals of boric acid, and 1 g of KU-2 ion-exchanger were added and the mixture was stirred at room temperature for 5 hr. Then the resin was filtered off, and the solution was evaporated to minimum volume and transferred to a chromatogram. Subsequently the chromatogram was sprayed with a saturated solution of sodium metaperiodate and a 0.1 M solution of benzidine in methanol–water–acetone (2 : 2 : 1). 2, 3, 6-Trimethyldulcitol appeared in the form of a white spot on a blue background.

The 2, 3, 4, 6-tetramethylgalactose had $[\alpha]_D^{20}$ + 100° (c 6.3; ethanol), R_f 0.86. Literature data: $[\alpha]_D$ + 117.8° (water); R_f 0.88 in system 2 [4]. On demethylation by the method described above, D-galactose was obtained.

The 2, 3, 5-trimethyl-L-arabinose had $[\alpha]_D^{20}$ + 117.5° (C 0.23; ethanol), R_f 0.95. Literature data: $[\alpha]_D$ + 133.4° (water) [6]; R_f 0.95 in system 2 [4].

D-Glucuronic acid and its lactone. The Dowex-2 ion-exchange resin through which the methylated sugars had been passed was treated with 200 ml of 20% acetic acid. The filtrate was evaporated to small volume in vacuum. D-Glucuronic acid and its lactone were identified by paper chromatography in systems 2 and 3.

Periodate oxidation of polemonioside C. A solution of 1 g of polemonioside C in 50 ml of acetate buffer (pH 4.5) was treated with 0.92 g of potassium periodate. The mixture was left in the dark for 3 days. Then 10 drops of ethylene glycol were added, the precipitate was filtered off, the solution was evaporated in vacuum to dryness, and the residue was extracted with 50 ml of ethanol. The evaporation of the ethanol yielded a viscous oily liquid which was hydrolyzed by being heated with 50 ml of aqueous ethanol and 0.8 ml of conc HCl for 4 hr. The residue was distilled off, and the precipitate was filtered off and washed with water, giving 0.4 g of progenin with mp 211–215° C. No monosaccharides were detected in the filtrate by paper chromatography in systems 2 and 3.

CONCLUSIONS

The structure of the carbohydrate chains of polemoniosides B and C have been established.

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2 December 1968

Arbuzov Institute of Organic and Physical Chemistry, AS USSR