THE STRUCTURES OF THREE NEUTRAL OLIGOSACCHARIDES OBTAINED BY PARTIAL ACID HYDROLYSIS

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Preparation of degraded polysaccharides, obtained by stepwise hydrolysis with hydrochloric acid, is described. Three neutral oligosaccharides were isolated from a low-molecular-weight fraction, *viz.* 3-O-β-L-arabinopyranosyl-L-arabinopyranose, 6-O-β-D-galactopyranosyl-D-galactopyranose- and O-β-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl (1 → 6)-D-galactopyranose. Their structures were determined by mass spectrometry of their permethylated free or reduced forms.

In the previous papers[1,2] we described the structures of three neutral oligosaccharides isolated from the peach-tree gum polysaccharide (*Prunus persica* [L.] BATSCH), as constituents of the neutral side chain bound to the principal chain of the polysaccharide. The oligosaccharides were split off by partial hydrolysis of the polysaccharide studied (fraction a_1, Scheme 1). The present paper described another three oligosaccharides, obtained by further degradation of the polysaccharide (fraction a_2, Scheme 1). Similar oligosaccharides had been isolated from gum polysaccharides of the following trees: lemon[3], peach (*Prunus persica* [L]. BATSCH) and cherry (*Prunus avium* L. var. JULIANA L.)[4], *Acacio karroo*[5], *Anogeissus schimperi*[6], *Ghatti Ghatti* (Indian gum)[7], *Asafetide*[8], *Albizzia zygia* (mocbride)[9], *Virgilia oroboides*[10], Golden apple (*Spondias cytheria*)[11,12]. The purpose of our work was to ascertain the linkages between the individual saccharide units and to determine the ring of the reducing saccharide units in the fragments; the latter problem has been solved in no case yet.

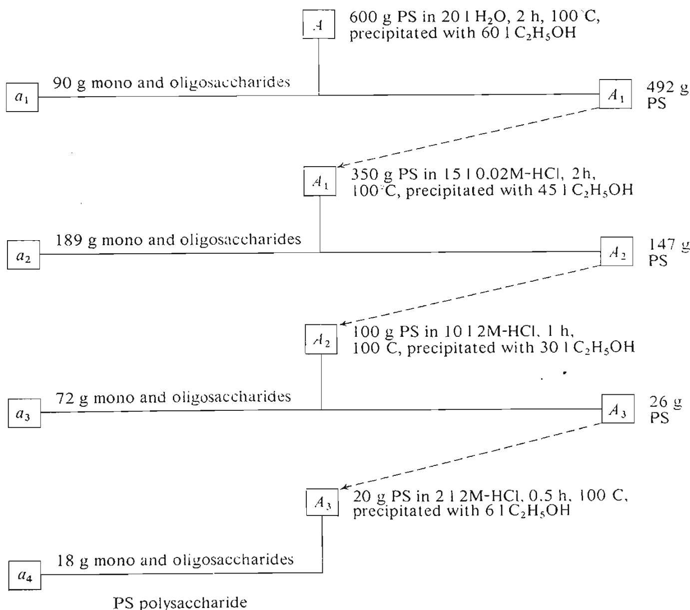
EXPERIMENTAL

Paper chromatography was carried out on paper Whatman No 1 in systems S_1, ethyl acetate–pyridine–water (8 : 2 : 1), and S_2, ethyl acetate–acetic acid–water (18 : 7 : 8). The saccharide spots were detected by aniline hydrogen phthalate[13] and an alkaline solution of silver nitrate[14]. The individual fractions eluted from a column packed with active carbon were detected by phenolsulphuric acid[15]. Infrared spectra were measured in a spectrophotometer Zeiss UR-10 using KBr pellets. Optical rotation was measured in an apparatus Perkin 141. Mass spectra of the per methylated disaccharides were measured in a mass spectrometer MCH 1306 at a sample temperature of 35°C and an ionization cell temperature of 120°C, using ionizing electrons of 70 eV.

The permethylated trisaccharide was measured in a mass spectrometer MS 902 S. Gas chromatography of persilylated oligosaccharides was performed in an apparatus Hewlett-Packard, Model 5711 A.; the column was packed with chromosorb W-AW-DMCS coated with SE-30 (3%), the programmed temperature was 120—200°C. Trimethylsilylation was carried out according to a described technique[16].

Preparation of Oligosaccharides

Oligosaccharides were prepared by degradation according to Scheme 1. The low-molecular-weight fraction a_2 was neutralized by silver carbonate and the silver ions were removed on Dowex 50 W X 2 in the H^+ form. Neutral saccharides were separated from acidic ones on Dowex 1X8 in the acetate form. The neutral portion was eluted by water (fraction a_2N). It was crystallized from methanol to be freed from L-arabinose and D-xylose and the oligosaccharides were separated on a column of active carbon Darco G 60 (100 × 3 cm). Gradient elution by ethanol was effected



SCHEME 1

in a concentration range 5—10% at a flow rate of 0·5 ml/min. The saccharides were eluted in the order *I*, *II*, *III*.

Reactions of Oligosaccharides

Partial and total hydrolysis. The individual oligosaccharides (20 mg) were heated in 2 ml of 0·5M sulphuric acid in a sealed tube at 100°C. After 1 h 1 ml of the solution was withdrawn and the rest was heated for 7 h. The samples were neutralized with barium carbonate and deionized on Dowex 50WX2 in the H^+ form.

Reduction. To a 100-mg sample was added 100 mg of sodium borodeuteride in 5 ml of water and the solution was stirred for 12 h at 5°C. The excess of sodium borodeuteride was decomposed by an addition of acetic acid. Sodium ions were removed on Dowex 50WX2 in the H^+ form. Boric acid was removed by evaporation with methanol (five times) in a vacuum rotary evaporator at 40°C.

Methylation. A freeze-dried oligosaccharide (300 mg) was dissolved in 1 ml of dimethyl sulphoxide. Five ml of methylsulphinylmethyl sodium was added, then gradually under stirring 10 ml of methyl iodide with the exclusion of aerial moisture. The mixture was stirred for 20 h. The methylated oligosaccharides were extracted into chloroform and purified with active carbon[17].

RESULTS

The neutral oligosaccharides (a_2N), were resolved into fractions *I*, *II*, *III*. The fraction (a_2N) *I*, $(\alpha)_D^{21}$ +232° (*c* 1·2, water), was chromatographed on paper in the system S_2 and proved to be a pure compound, R_{Ara} 0·70. Its purity (individuality) was corroborated by gas chromatography of the silyl derivatives. After total hydrolysis only L-arabinose was left. We measured the mass spectrum of the permethylated saccharide or permethylated alditol after reduction by sodium borodeuteride. The peaks of ions characteristic of pyranose units were interpreted as described[18]. Fragmentation of the mass spectrum of the permethylated, reduced saccharide was also described previously[19]. In addition to peaks of other ions the mass spectrum of the permethylated disaccharide exhibited the following ones: *ba* B_1 *m/e* 336, *ab* J_1 *m/e* 235, *b* A_1 *m/e* 175, *b* A_2 *m/e* 143. This proves that either saccharide unit is in the pyranose form. The spectrum lacked the ion peak *m/e* 161, which suggests that the disaccharide under study does not contain a 1→4 glycoside linkage. The possible linkages are 1 → 3 or 1 → 2; the spectrum did not allow of distinguishing between the two. After reduction with sodium borodeuteride and permethylation the mass spectrum of the disaccharide contained ion peaks characteristic of 3-O-pentapyranosylpentitol with a deuterium atom on the carbon $C_{(1)}$ (Fig. 1). The presence of peaks *m/e* 89 and *m/e* 90, absence of peaks *m/e* 133 and low intensities of the peak *m/e* 46 established *a* 1 → 3 glycoside linkage[19]. The molecular weight (366) was calculated from the equation[18] $M = a\ A_1 + b\ A_1 + 16$.

Judging by specific optical rotation and a strong absorption band at 890 cm^{-1} in the IR spectrum we believe that the glycoside linkage is β, the structure being 3-O-β-L-arabinopyranosyl-L-arabinopyranose.

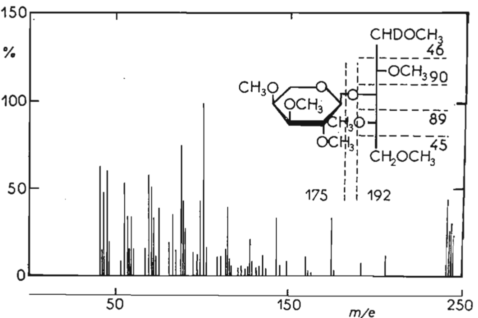


FIG. 1

Mass Spectrum of Permethyl 3-O-β-L-Arabinopyranosyl-L-arabitol-1-*d*

FIG. 2

Mass Spectrum of Permethyl-O-hexapyranosyl-(1 → 6)-O-hexapyranosyl-(1 → 6)-hexapyranoside

Individuality of the fraction $(a_2N)II$, $(\alpha)_D^{21}$ +28° (*c* 1·5, water), was confirmed by gas chromatography after conversion into the corresponding silyl derivative and by paper chromatography in the system S_2 (R_{Gal} 0·54). Following total hydrolysis only D-galactose was left. The saccharide $(a_2N)II$ was permethylated and investigated by mass spectrometry. Apart from other ions the peaks were characteristic of the following ones: $a\ A_1$ m/e 219, $a\ A_2$ m/e 187, $a\ A_3$ m/e 155, $ab\ J_1$ m/e 279, $b\ A_1$ m/e 219, $b\ A_2$ m/e 187, $ba\ D_1$ m/e 353. These confirm safely that the saccharide was permethyl 6-O-hexapyranosylhexapyranoside[18], molecular weight $M = a\ A_1 + b\ A_1 + 16 = 454$. The IR spectrum (a strong absorption band at 890 cm^{-1}) and a low specific optical rotation testify to a β-glycoside linkage between the two saccharide units. The disaccharide *II* was 6-O-β-D-galactopyranosyl-D-galactopyranose.

Individuality of the fraction $(a_2N)III$, $(\alpha)_D^{21}$ +16° (*c* 1·5, water) was confirmed after silylation by gas chromatography and paper chromatography in the system S_2 (R_{Gal} 0·32). Following total hydrolysis only D-galactose was demonstrated by paper chromatography in the systems S_1 and S_2. After a partial hydrolysis we detected D-galactose and oligosaccharides (R_{Gal} 0·54 and R_{Gal} 0·32), respectively. After exhaustive methylation the mass spectrum of this trisaccharide, contained, among others, the following ion peaks: $a\ A_2$, $c\ A_2$ m/e 187, $a\ A_1$, $c\ A_1$ m/e 219, $bc\ J_1$ m/e 279, $ba\ A_1$ $bc\ A_1$ m/e 423, $abc\ J_1$ m/e 483, $cba\ D_1$ m/e 557; these are characteristic[20] of permethyl-O-hexapyranosyl-(1 → 6)-O-hexapyranosyl-(1 → 6)-hexapyranoside. Fragmentation of the trisaccharide is depicted in Fig. 2.

A low value of the specific optical rotation and the presence of an absorption band at 890 cm^{-1} in the IR spectrum suggest that the three saccharide units are bound by two β-glycoside linkages. Consequently, the trisaccharide *III* appears to be a homologue of the disaccharide *II*, *viz.* O-β-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 6)-D-galactopyranose, molecular weight $M = ba\ A_1 + c\ A_1 + 16 = 658$.

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REFERENCES

1. Rosík J., Kubala J., Kardošová A., Kováčik V.: Chem. Zvesti *27*, 688 (1973).
2. Rosík J., Kubala J., Kardošová A.: Chem. Zvesti *28*, 128 (1974).
3. Andrews P., Hough L., Powell D. B.: Chem. Ind. (London) *1956*, 658.
4. Andrews P., Hall D. H., Jones J. K. N.: J. Chem. Soc. *1953*, 4090.
5. Charlson A. J., Nunn J. R., Stephen A. M.: J. Chem. Soc. *1955*, 1428.
6. Aspinall G. O., Christensen T. B.: J. Chem. Soc. *1961*, 3461.
7. Aspinall G. O., Auret B. J., Hirst E. L.: J. Chem. Soc. *1958*, 4408.
8. Jones J. K. N., Thomas G. H. S.: Can. J. Chem. *39*, 192 (1961).
9. Drummond D. W., Percival E.: J. Chem. Soc. *1961*, 3908.
10. Smith F., Stephen A. M.: J. Chem. Soc. *1961*, 4892.

11. Andrews P., Jones J. K. N.: J. Chem. Soc. *1954*, 4134; 1955, 583.
12. Lindgren B. O.: Acta Chem. Scand. *11*, 1365 (1957).
13. Patridge S. M.: Nature *164*, 443 (1949).
14. Trevelyan W. E., Proctor D. P., Harrison J. S.: Nature *166*, 444 (1950).
15. Montgomery R.: Biochim. Biophys. Acta *48*, 491 (1961).
16. Sweeley C. C., Bentley R., Makyta M., Wells W. W.: J. Amer. Chem. Soc. *85*, 2497 (1963).
17. Hakamori S.: J. Biochem. (Tokyo) *55*, 205 (1964).
18. Kováčik V., Bauer Š., Rosík J., Kováč P.: Carbohyd. Res. *8*, 282 (1968).
19. Kärkkäinen J.: Carbohyd. Res. *14*, 27 (1970).
20. Kärkkäinen J.: Carbohyd. Res. *17*, 1 (1971).

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