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Studies on the Smith Degradation

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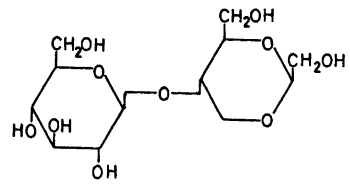
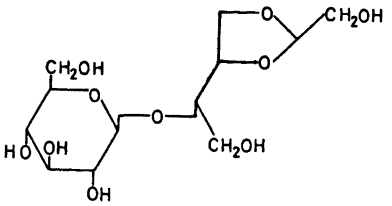
Two oligosaccharide derivatives, methyl 6-*O*-(β-D-glucopyranosyl)- 3-*O*-methyl-α-D- glucopyranoside (III) and *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-galactopyranosyl-(1→2)-glycerol (IV) have been subjected to the Smith degradation. Only the normal degradation products, methyl 3-*O*-methyl-α-D-glucopyranoside (IX) and 2-*O*-α-D-galactopyranosyl-glycerol (X), respectively, were obtained. The results indicate that acetal migration with the formation of cyclic acetals involving sugar moieties is insignificant in the Smith degradation.

Following Smith degradation of polysaccharides,[1] that is periodate oxidation, borohydride reduction, and mild acid treatment, glycosidic linkages of unoxidized sugar residues are not hydrolyzed while non-cyclic acetals in modified sugar residues are cleaved. Investigation of the resultant products, therefore, reveals structural features of the original polysaccharide.

One complication, observed by Smith and his coworkers,[1] is acetal migration during the mild acid treatment. Cyclic acetals formed by such migration are considerably more stable to acid hydrolysis than the non-cyclic acetals; consequently compounds containing cyclic acetal structures may be present in the product. Thus on Smith degradation of oat glycan,[1] not only 2-*O*-β-D-glucopyranosyl-D-erythritol but also 1,3-*O*-(2-hydroxyethylidene)-2-*O*-β-D-glucopyranosyl-D-erythritol (I) was obtained. Gorin and Spencer [2] observed that smaller amounts of analogous 3,4-*O*-(2-hydroxyethylidene)-2-glycopyranosyl derivatives of erythritol (*e.g.* II) were also formed from polysaccharides containing β-(1→4)-linked D-glucose or D-mannose residues. Such acetals became the main byproducts when polysaccharides containing



I



II

Fig. 1.

α-(1→4)-linked D-glucose or D-mannose residues were degraded.

It seemed possible that cyclic acetals, involving sugar moieties of the degraded product, could also be formed by acetal migration during the Smith degradation. This possibility has now been investigated, using suitable model substances.

RESULTS AND DISCUSSION

Two model substances were investigated. One, methyl 6-*O*-(β-D-glucopyranosyl)-3-*O*-methyl-α-D-glucopyranoside (III), was prepared by conventional methods from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and methyl 2,4-di-*O*-acetyl-3-*O*-methyl-α-D-glucopyranoside. Fully trideuteriomethylated III gave the ex-

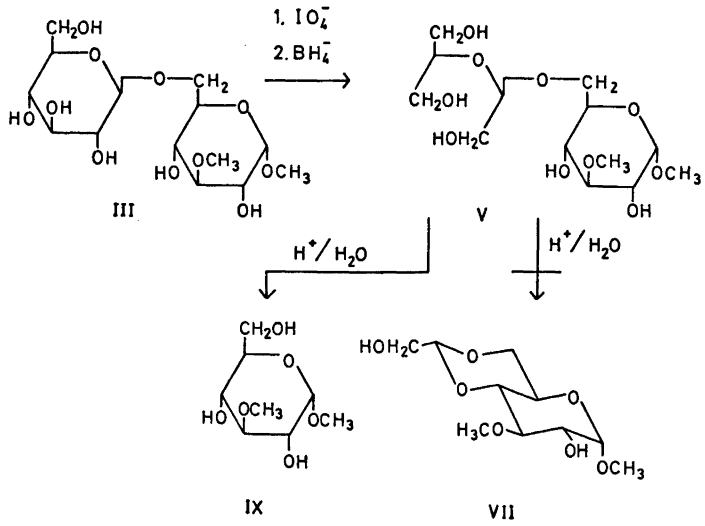


Fig. 2.

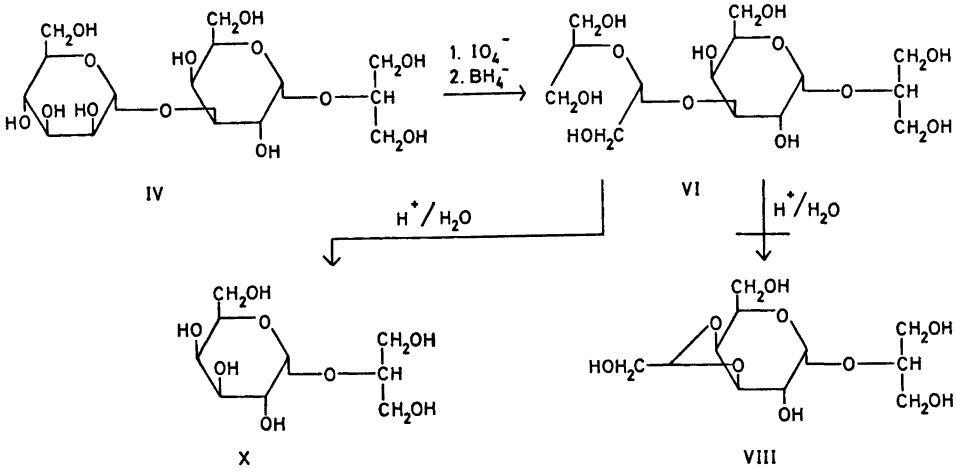


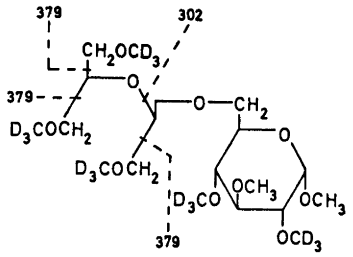
Fig. 3.

pected mass spectrum.[3] The other substance *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-galactopyranosyl-(1→2)-glycerol (IV) was previously isolated from the marine red alga *Furcellaria fastigiata*.[4] It seemed possible that the polyalcohols (V, VI) obtained from these should yield some 4,6- and 3,4-(2-hydroxyethylidene)-acetal (VII and VIII), respectively, on mild acid treatment.

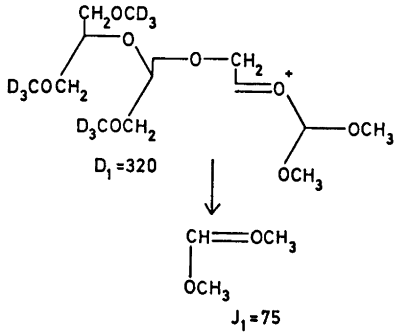
Each disaccharide derivative (III and IV) was oxidized with sodium metaperiodate in aqueous methanol and reduced with sodium borohydride. The product was isolated by chromatography on Sephadex G25 and its purity checked by TLC and, after permethylation, by GLC. From III a single product was obtained. The MS of its pertrideuteriomethylated derivative (XI) was in agreement with the postulated structure. The origins of some major fragments are indicated in the formula.

Two components were obtained from IV, probably due to preferential cleavage between

C-2 and C-3 and formation of a cyclic hemiacetal in equilibrium with the open structure, whereby further oxidation is slowed down. By subjecting the product to borohydride reduction and a second oxidation reduction, a single component was obtained. The MS of the permethylated derivative (XII) of polyalcohol VI indicated

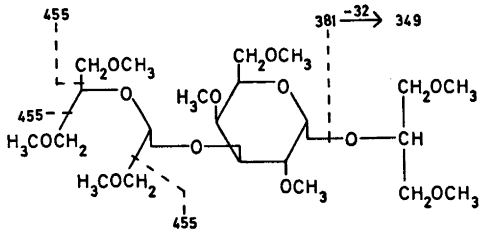


XI Mw 427



that it was the expected product. The origins of some major fragments are indicated in the formula.

The polyalcohols V and VI were hydrolysed at different temperatures. The rate constants



XII Mw 500

Table 1. Rate constants and activation energies for the acid hydrolysis of the mixed acetals V, VI, and XIII.[5]

Compound	Temp. °C	$k \times 10^4$ s^{-1}	E kJ/mol
	20	0.25	
V	30	0.99	114.0
	40	4.9	
	20	0.47	
VI	30	2.2	108.5
	40	8.1	
	20	0.89	
XIII	30	3.62	98.5
	40	11.30	

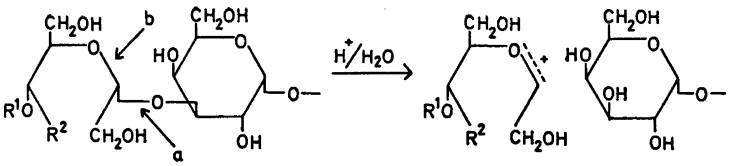
were of the same order of magnitude as those for XIII,[5] obtained on oxidation-reduction of methyl α-D-glucopyranoside (Table 1).

During the hydrolyses of V and VI, samples were withdrawn, borohydride reduced, and analysed by TLC and, after permethylation, by GLC. Only unchanged starting material and the expected degradation product, methyl 3-*O*-methyl-α-D-glucopyranoside (IX) and 2-*O*-α-D-galactopyranosyl-glycerol (X), respectively, were observed and not even traces of cyclic acetals, such as VII and VIII. The results of the present study therefore indicate that the formation of cyclic acetals involving unoxidized sugar residues is insignificant during Smith degradation. One explanation for the findings that cyclic acetals involving erythritol occur, but those involving sugar residues do not, is that the first linkage cleaved when the non-cyclic acetal (*e.g.* XIV) is hydrolysed is the one to the sugar residue (a) and not the other linkage to the alditol residue (b).

Although an explanation is not obvious, perhaps there is strain due to crowding of substituents in the solvated ion formed by protonation of the oxygen at *b*, which should precede the fission, and less strain in the corresponding ion formed by protonation at *a*, and that this is responsible for the observed effect.

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed at reduced pressure at a bath temperature not exceeding 40 °C. Optical rotations were determined using a Perkin-Elmer 141 instrument. GLC was per-



XIV

R^1 = H, sugar residue
R^2 = H, CH_2OH

Fig. 4.

formed using a column (0.15 × 180 cm) of 3 % OV 1 on Gaschrom Q at 210 °C and a Perkin-Elmer 900 instrument. Permethylated melibiitol ($T_M = 1$) was used as standard. GLC-MS was performed using Perkin-Elmer 270 and Varian Mat 311 instruments. TLC was performed on precoated plates with Silica Gel 60 F_{254} (Merck), 0.25 mm.

Methyl 3-O-methyl-α-D-glycopyranoside was obtained together with the 2-*O*-methyl derivative by partial methylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (7.6 g), fractionation of the product by silicic acid chromatography and catalytic hydrogenation, as previously described.[5] Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucoside (2.1 g) was eluted as the second component and showed, after crystallization from ethanol, m.p. 149–150 °C and $[\alpha]_{578}^{20}$ +116° (*c* 1.0, $CHCl_3$), in good agreement with published values. The title compound was obtained as a chromatographically pure syrup (1.25 g). The MS of the permethylated (CD_3I) product agreed with the postulated structure.[3]

Methyl 2,4-di-O-acetyl-3-O-methyl-6-trityl-α-D-glucopyranoside. Triphenylmethyl chloride (3.9 g) was added to a solution of methyl 3-*O*-methyl-α-D-glucopyranoside (3.0 g) in dry pyridine (100 ml). The solution was heated on a steam bath for 20 min, acetic anhydride (80 ml) was added and the heating continued for further 20 min. The solution was cooled and poured into a mixture of ice (300 g) and water (100 ml), which was then extracted with chloroform (4 × 100 ml). The chloroform solution was washed with 8 % aqueous sodium hydrogen carbonate (4 × 100 ml) and water (3 × 100 ml), dried (Na_2SO_4) and concentrated. The product (3.5 g) crystallized from ethanol and showed m.p. 158–159 °C and $[\alpha]_{578}^{20}$ +90° (*c* 1.9, $CHCl_3$). (Found: C 69.8; H 6.23. $C_{31}H_{34}O_8$ requires: C 69.7; H 6.41).

Methyl 2,4-di-O-acetyl-3-O-methyl-α-D-glucopyranoside. A saturated (at 0 °C) solution (1.5 g) of hydrogen bromide in acetic acid was added to a solution of the above trityl compound (3.0 g) in acetic acid (12 ml), kept at 10 °C. The mixture was shaken for 60 s and then rapidly filtered into ice and water (50 g). The trityl bromide was washed with acetic acid (10 ml) and the combined filtrate and washings extracted with chloroform (4 × 20 ml). The chloroform solution was washed with ice-water (4 × 20 ml) and dried (Na_2SO_4). Concentration of the solution yielded a syrup (1.4 g), used without further purification in the following step.

Methyl 6-O-β-D-glucopyranosyl-3-O-methyl-α-D-glucopyranoside (III). The above syrup (0.8 g), silver oxide (0.7 g), Drierite (2.5 g) and dry ethanol-free chloroform (10 ml) were stirred in the dark for 1 h and iodine (0.2 g) was added. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.0 g) in chloroform (10 ml) was added in 1 ml portions over 2 h and stirring continued for further 24 h. The mixture was filtered, concentrated and fractionated on a silica gel column (5 × 40 cm), using chloroform – acetone (5:1) as irrigant. The fractions were investigated polarimetrically and by TLC. The main component was obtained as a syrup (1.0 g) that crystallized from ethanol, m.p. 126–128 °C, $[\alpha]_{578}^{20}$ +49° (*c* 0.4, $CHCl_3$). (Found: C 50.1; H 6.09. $C_{26}H_{38}O_{17}$ requires: C 50.2; H 6.15). This product (0.9 g) was dissolved in methanol (10 ml) and methanol saturated (room temperature) with ammonia (50 ml) was added. The solution was kept overnight at room temperature, concentrated and the resulting syrup (0.5 g) purified by chromatography on a Sephadex G25 column to give the title compound as a chromatographically pure syrup.

α-D-Mannopyranosyl-(1→3)-α-D-galactopyranosyl-(1→2)-glycerol. The nonaacetate of the title compound was deacetylated and the product purified as above, yielding a chromatographically pure syrup.

The mixed acetal V. To the disaccharide glycoside III (0.2 g) in methanol (20 ml) was added 0.23 g sodium metaperiodate in water (5 ml). The mixture was stirred for 2 h at room temperature in the dark, ethylene glycol (0.1 g) was added and stirring continued for 15 min. Methanol (30 ml) was then added and the mixture filtered, concentrated and dissolved in water (10 ml). Sodium borohydride (0.2 g) was added, the solution kept at room temperature

for 12 h, excess borohydride was decomposed by addition of acetic acid and the solution was concentrated. Boric acid was removed by codistillations with methanol and the product purified by chromatography on Sephadex G25, $[\alpha]_{578} \simeq +117°$. TLC (ethanol—chloroform—water, 15:5:1) and GLC after permethylation ($T_M = 0.54$) showed that the product (0.15 g) was pure. The MS of the permethylated (CD_3I) product showed, *inter alia*, the following peaks (relative intensities in brackets): 48(100), 74(39), 75(21), 76(10), 91(51), 92(12), 104(9), 107(14), 109(12), 134(8), 151(2), 176(1.5), 179(1.6), 193(2.4), 211(1.2), 225(1.8), 301(0.6), 302(0.1), 320(0.2), 321(0.1), 357(0.1), 379(0.6).

The mixed acetal VI, $[\alpha]_{578} \simeq +94°$ was prepared as described above for V, except that the oxidation-reduction had to be repeated. The permethylated product showed $T_M = 1.23$, compared to $T_M = 1.89$ for the component that disappeared after the second oxidation-reduction. MS of the former showed, *inter alia*, the following peaks: 45(100), 71(41), 73(10), 101(12), 103(14), 127(6.1), 145(2.9), 159(2.0), 163(1.3), 177(3.0), 187(1.2), 207(0.4), 215(0.4), 233(0.4), 247(0.2), 275(0.2), 349(0.1), 380(0.1), 381(0.2), 455(0.1).

Acid hydrolysis studies. The mixed acetal (V or VI) (about 10 mg) in 0.125 M sulfuric acid (1 ml) was transferred to a jacketed polarimeter tub (10 mm) maintained at the required temperature. The optical rotation, at 365 nm, was determined at intervals and the reaction constant calculated, assuming first order kinetics. Samples from a parallel experiment were withdrawn at intervals, borohydride reduced and analysed by TLC and, after permethylation, by GLC and GLC-MS. In addition to unchanged starting material, methyl 3-*O*-methyl-α-D-glucopyranoside (T_M too low to be determined accurately) and 2-*O*-α-D-galactopyranosyl-glycerol ($T_M = 0.24$), respectively, were observed but no components indicative of acetal migration.

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