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Note

Distribution of substituents in *O*-ethylcellulose

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O-Ethylcellulose (EC) as commercially produced, having a degree of substitution (d.s.) of 2.3–2.6, is soluble in organic solvents. Its properties vary with the molecular weight (degree of polymerization, d.p.), the d.s., and the distribution of the ethyl groups along the chain and in the residue. At lower d.s. values (0.8–1.7), it is soluble in water.

EC is prepared by treatment of cellulose fibers, obtained from cotton linters or wood pulp, with caustic solution, and ethylation of the resulting alkali-cellulose with ethyl chloride. Thus, the original homopolymer is transformed into a heteropolymer containing eight different residues randomly distributed along the chain. EC is thermoplastic, demonstrating strength and flexibility in a variety of compositions. Its uses include films, lacquers, adhesives, binders, and hot melts[1].

The relative reactivities of the three hydroxyl groups in cellulose are of great importance in the manufacture of cellulose derivatives. The distribution of substituents in EC has been analyzed, after hydrolysis, by quantitative separation of the D-glucose derivatives on a carbon column and further fractionation by paper chromatography and paper electrophoresis[2], or by use of an anion-exchange resin, with gradient elution[3]. All of the studies have found that the hydroxyl group on C-3 is the least reactive. Contradictory results have been reported as to whether the secondary hydroxyl group[2,3] on C-2 or the primary hydroxyl group[4,5] on C-6 is the more reactive. The discrepencies may be explained by use of less than satisfactory analytical methods.

This study now reports on the distribution of ethyl groups in five commercial ECs having d.s. values ranging from 2.32 to 2.64. The modified celluloses were completely hydrolyzed, and the partially ethylated monosaccharides resulting were reduced to alditols and these analyzed as acetates and trimethylsilyl ethers by combined g.l.c.–m.s. The ^{13}C-n.m.r. spectra of native and hydrolyzed EC, as well as those of the derived alditols, were also analyzed.

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RESULTS AND DISCUSSION

The ethoxyl content, percentage of C and H, and the degree of substitution of five commercial ethylcelluloses are shown in Table I.

For quantitative determination of D-glucose and D-glucose ethyl ethers, EC was hydrolyzed to a mixture of monomers. Fully or highly alkylated polysaccharides are often not soluble in hot water. For this reason, methanolysis, formolysis, or treatment with 72% H_2SO_4 is usually performed before hydrolysis with hot dilute acid. In this manner, degradation and dealkylation are kept to a minimum. The EC was treated with 90% formic acid for 2 h at 100°. After evaporation of the solution to dryness, the residue was hydrolyzed with different concentrations of CF_3CO_2H at 100° for various time-periods. The hydrolyzate was monitored by high-performance liquid chromatography (l.c.) in a Supelcosil LC-NH_2 column, using D-glucose and several oligosaccharides of D-glucose as standards. The result showed that complete hydrolysis had taken place with formolysis followed by 0.67M trifluoroacetic acid during 16 h at 100°, as no oligosaccharides were detected. Methylcellulose (d.s. 1.7) had also been shown to be completely hydrolyzed under these conditions[6]. The g.l.c. chart of the separation of the partially ethylated alditol acetates obtained by reduction and acetylation is shown in Fig. 1. All eight of the partially ethylated alditol acetates possible are completely separated, and easily quantified. Similar results were obtained when trimethylsilyl (Me_3Si) ethers of the alditols were analyzed. However, the resolution of the partially ethylated alditol trimethylsilyl ethers was less complete than that of the partially ethylated alditol acetates. As all of the ECs analyzed showed similar distribution of substituents, despite different degrees of substitution, only two of them are shown in Table II; the one with the lowest d.s. (A) and the one with the highest (B). The preponderant, partially ethylated alditol acetate was 2,3,6-tri-*O*-ethyl-D-glucose (fully *O*-ethylated cellulose residue). This is not surprising, because all of the *O*-ethylcelluloses analyzed have high d.s. values ranging from 2.3 to 2.6 (3.0 for fully ethylated cellulose). Among the disubstituted D-glucoses, 2,6-di-*O*-

TABLE I

PERCENTAGE OF ETHOXYL, C AND H, AND DEGREE OF SUBSTITUTION OF *O*-ETHYLCELLULOSES ANALYZED

O-Ethylcellulose	*Ethoxyl (%)*		*Analysis (%)*		*D.s.*[b]	
	Found	*Range*[a]	*C*	*H*	*Found*	*Range*
No. 2354(A)	46.0	45.5–46.8	55.07	8.31	2.32	2.28–2.38
No. 5429	48.0	45.5–49.0	56.75	8.60	2.46	2.43–2.54
No. 5430	49.8	49.0 +	56.93	8.67	2.60	2.54 +
K-type	46.9	46.1–47.2	56.68	8.62	2.38	2.33–2.41
T-type (B)	50.2	49.6 +	55.92	8.52	2.64	2.58 +

[a]Suggested by supplier. [b]Calculated from ethoxyl content.

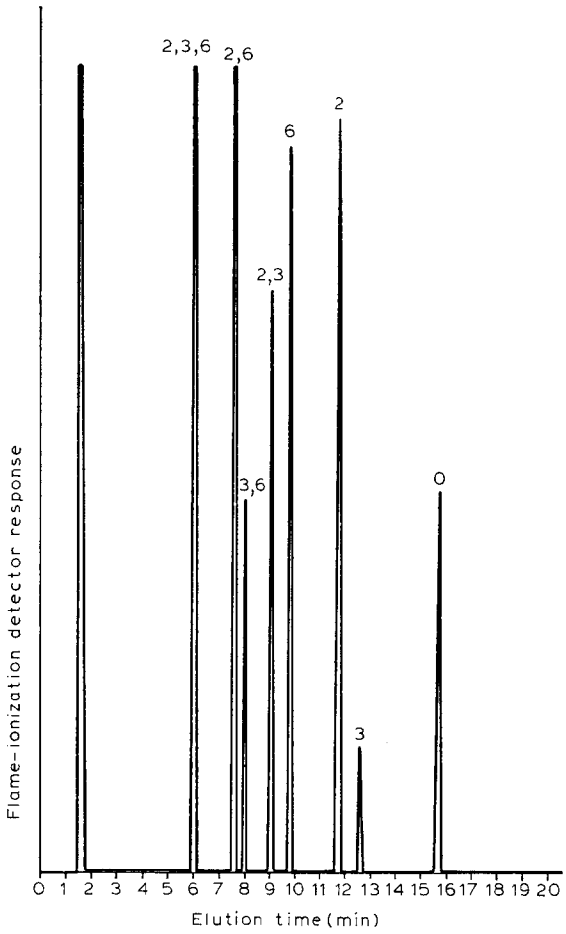


Fig. 1. G.l.c. chart of separation of partial ethylated alditol acetates obtained from *O*-ethylcellulose by hydrolysis, reduction, and acetylation. The numbers indicate the positions of the ethyl groups.

ethyl-D-glucose was found to be by far the major isomer, showing that O-2 and O-6 are the easiest to ethylate. Comparison between the two ECs of different d.s. values showed a large increase in the molar percentage of 2,3,6-tri-*O*-ethyl-D-glucose and a decrease of 2,6-di-*O*-ethyl-D-glucose from the highest-substituted EC. This suggests that the hydrogen atoms of the hydroxyl groups on C-3 atoms are only replaced when the majority of the O-2 and O-6 atoms have been ethylated. The data in Table II agree with the ethoxyl content (see Table I), suggesting that the hydrolytic conditions used in this study resulted in complete hydrolysis and that no degradation of the sugar residues or dealkylation had taken place.

The percentage of ethyl group on the individual oxygen atoms was calculated from the values in Table II, and are presented in Table III. High values of ethyl

TABLE II

ETHYL ETHERS OF D-GLUCOSE OBTAINED ON HYDROLYSIS OF PARTIALLY ETHYLATED CELLULOSE OF D.S. 2.30 (A) AND 2.65 (B)

Glucose ethyl ether[a]	*T*[b]	*Molar percentage*			
		A	*Calculated*	*B*	*Calculated*
2,3,6	**1.00**	43.7	43.0	65.7	66.2
2,6	1.13	31.7	31.7	23.3	22.7
3,6	1.17	6.3	6.8	5.3	4.8
2,3	1.24	6.8	6.8	3.3	3.3
6	1.28	5.3	4.9	1.0	1.6
2	1.41	4.9	4.9	1.0	1.6
3	1.46	0.8	1.0	0.2	0.2
0	1.61	1.2	0.8	0.1	0.1

[a]Numerals refer to position of ethoxyl groups. [b]Retention time of the corresponding alditol acetate, relative to that of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-ethyl-D-glucitol as unity.

groups were found in almost equal proportions on O-2 and O-6, compared to a low value on O-3, regardless of the degree of substitution. Partial methylation of cellulose (d.s. 1.7) had shown[6] that O-2 is the most reactive, followed by O-6, with much less substitution on O-3. (EC of a lower degree of substitution will probably show the same distribution of ethyl groups as the methyl groups in *O*-methylcellulose, as similarities between the distribution of ethyl and methyl groups have been observed[7].) The difference in the reactivities between the three hydroxyl groups has been explained by steric hindrance resulting in the O-2 atom's being the most accessible in cellulose for this reaction. This hydroxyl group has also been shown to be the most acidic of the hydroxyl groups, due to its proximity to the glucosidic and ring-atom oxygens[8]. The 3-hydroxyl group in cellulose seems to be the least reactive in both methylation and ethylation reactions. This fact has been explained by the 3-hydroxyl group's hydrogen bonding intramolecularly with the ring-oxygen atom in adjacent D-glucosyl units[9].

Assuming that (*a*) there is no influence of end groups, (*b*) the substitution reaction is of first order with respect to the hydroxyl groups, and (*c*) there is no influence of substitution on the relative reaction-constant, it should be possible to

TABLE III

PERCENTAGE OF *O*-ETHYLATION OF O-2, O-3, AND O-6 IN THE D-GLUCOSYL RESIDUES OF THE *O*-ETHYLCELLULOSE, AND ITS D.S. CALCULATED FROM TABLE II

Sample	*% O-Ethyl group in position*			*D.s.*
	2	*3*	*6*	
A	86.5	57.6	86.4	2.31
B	93.3	74.5	95.3	2.63

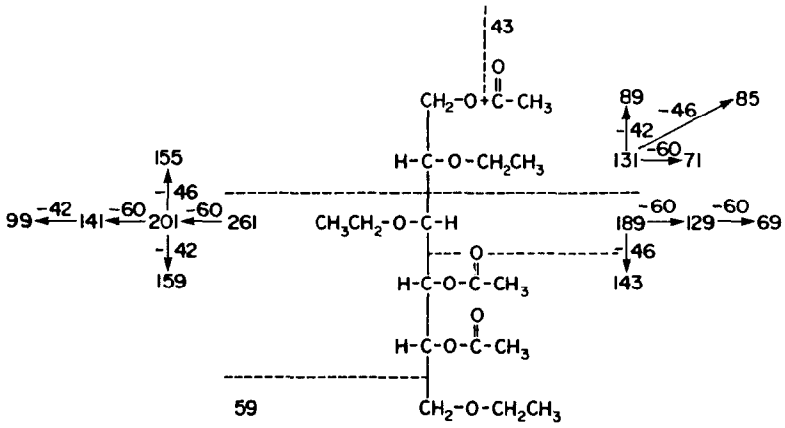
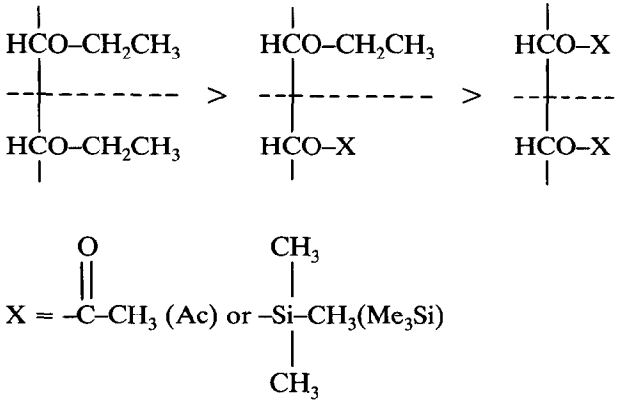


Fig. 2. The origin of the major fragment-ions obtained from e.i.-mass spectrometry of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-ethyl-D-glucitol.

calculate the percentages of D-glucosyl residues ethylated at different positions from the percentage of ethylation in each position[10]. Such a calculation has been performed by using the values in Table I, and the good agreement between observed and calculated values (see Table II), indicates that the aforementioned assumptions are justified. This suggests that the substitution is of first order, and that the relative rate-constant of hydroxyl groups is not affected by earlier substitution. This result had been found for *O*-(carboxymethyl)cellulose[10], cellulose acetate[11], and *O*-methylcellulose[6].

The e.i.-mass spectra of the partially ethylated alditol acetates showed the main peaks listed in the Experimental section, with the intensities in parentheses. Only peaks having intensities of 10% above, and 20% below, *m/z* 120 are shown.

1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-ethyl-D-glucitol and the origins of its major fragment-ions are illustrated in Fig. 2. The fragmentation of the partially ethylated alditol acetates follow as for the methylated derivatives[12], with the most stable ions (highest intensity) presumed to derive from fission in the following order.



The secondary fragments observed may be derived from the primary fragments by single or consecutive eliminations of acetic acid (60), ketene (42), ethanol (46), or acetaldehyde (44).

The mass fragmentation of partially ethylated alditol trimethylsilyl ethers followed a similar pattern. This is not surprising, because close fragmentation analogies have been reported for methyl and Me_3Si derivatives of other species, including some carbohydrates[13,14].

The ^{13}C-n.m.r. spectrum of EC (d.s. 2.30) is shown in Fig. 3. *O*-Alkylation of polysaccharides promotes strong deshielding of the substituted carbinol group, resulting[15,16] in a downfield shift of ~9 p.p.m. The signal for the anomeric carbon atom at 103 p.p.m., is indicative of polymeric β-D-glucosyl residues. The downfield resonances between 81 and 83 p.p.m. belong to the substituted C-2 and C-3. The three sharp peaks of equal proportions, at 76.7, 77.0, and 77.3 p.p.m., are due to the resonances of the solvent chloroform. The resonances of unsubstituted C-2 and C-3, as well as of C-4 and C-5, are in the region of 72–78 p.p.m. The peak at 67 p.p.m. derives from the substituted C-6 atom. The peaks in the region of 69 p.p.m. belong to the methylene carbon atoms in the three ethoxyl groups. Unsubstituted primary carbon atoms (C-6) would resonate at ~60 p.p.m., and it is obvious that almost all of the C-6 atoms are substituted with ethoxyl groups, as no resonance could be detected in this region. The three resonances at 14.9, 15.1, and 15.4 p.p.m.

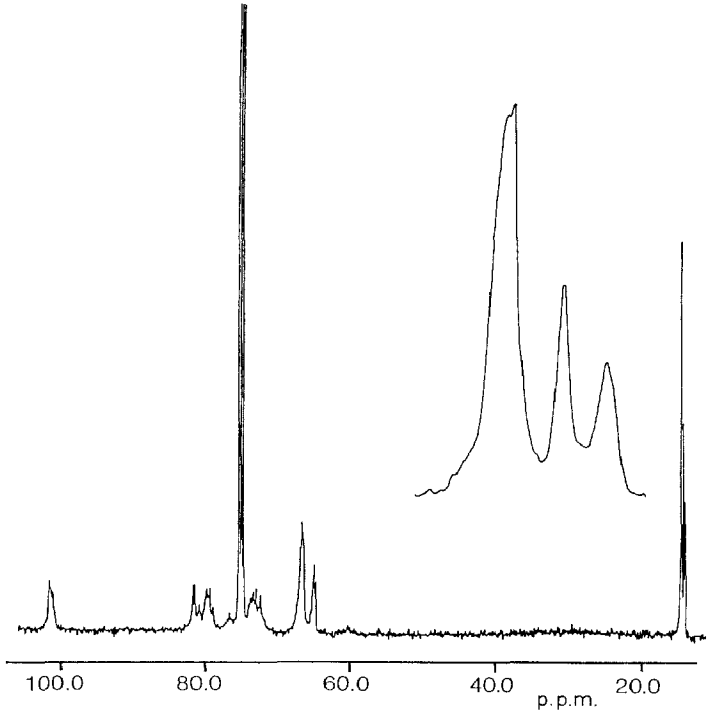


Fig. 3. 400-MHz, ^{13}C-n.m.r. spectrum of *O*-ethylcellulose (d.s. 2.3) in $CDCl_3$, recorded at 21°. Chemical-shift values are relative to internal acetone (δ 31.4).

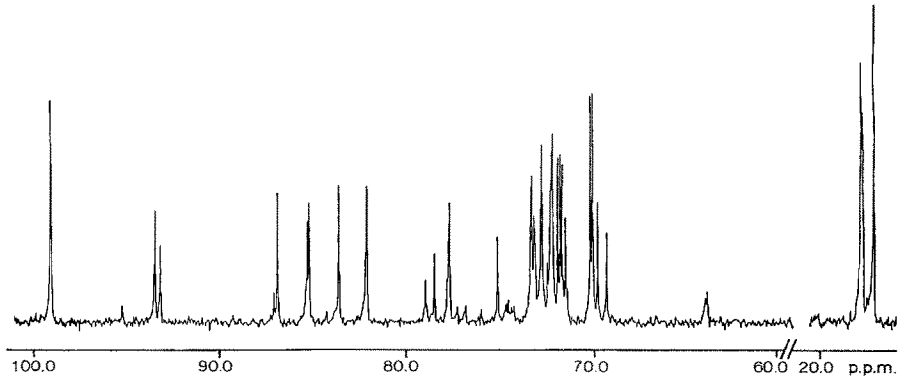


Fig. 4. 400-MHz, ^{13}C-n.m.r. spectrum of hydrolyzed *O*-ethylcellulose (d.s. 2.3) in D_2O, recorded at 21°. Chemical-shift values are relative to internal acetone (δ 31.4).

belong to the methyl carbon atoms in the ethoxyl groups. The sharpness of these peaks is probably due to long T_2 relaxation times, resulting from internal rotation of the methyl groups.

The proportions of the 3 peaks were not those expected from the results of the determination of the distribution of ethoxyl groups in *O*-ethylcellulose by analysis, using g.l.c.–m.s., of the monosaccharide derivatives obtained by complete hydrolysis. Further examination revealed that the downfield peak represented, indeed, two resonances, which did not separate completely (see Fig. 3). The partial assignments of the chemical shifts are based on data reported earlier on fully ethylated cellulose[17]. The broad peaks in the spectrum are caused by the high viscosity of the chloroform solution. This could be improved by partial acid hydrolysis of the polysaccharide to lower the molecular weight. The determination of the distribution of ethyl groups in *O*-ethylcellulose by ^{13}C-n.m.r. spectroscopy is shown to be difficult due to the poor resolution.

To improve the resolution of the spectrum, the polysaccharide was completely hydrolyzed with 90% formic acid followed by 0.67M trifluoroacetic acid. The ^{13}C-n.m.r. spectrum of the partially ethylated monosaccharides resulting is depicted in Fig. 4. The spectrum shows 40 resonance peaks, which indicates that at least 14 additional resonance peaks are not separated, and therefore cannot be quantified. No attempt will be made to assign all of the resonances, but with the assumption that *O*-methyl- and *O*-ethyl-substituted cellulose should have similar carbon-13 chemical shifts for the cellulose carbon atoms, the following tentative assignments were made based on chemical shifts obtained from hydrolysis of *O*-methylcellulose[18] and methyl glycosides from *O*-ethylcellulose[19]. The resonance peaks at 99.0, 93.4, and 93.1 p.p.m. belong to C-1β, C-1α unsubstituted at O-2, and C-1α substituted at O-2, respectively. The resonances between 82 and 87 p.p.m. belong to substituted C-2 and substituted C-3. The area between 63 and 75 p.p.m. contains the rest of the ring resonances, including those of the methylene

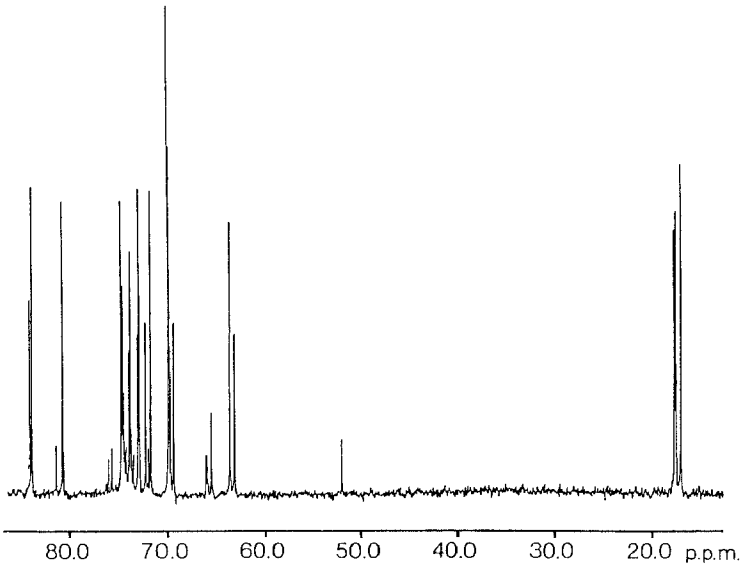


Fig. 5. 400-MHz, ^{13}C-n.m.r. spectrum of hydrolyzed and reduced *O*-ethylcellulose (d.s. 2.3) in D_2O, recorded at 21°. Chemical-shift values are relative to internal acetone (δ 31.4).

carbon atoms in the ethyl groups. The methyl carbon atoms resonate in the region of 17.0–17.7 p.p.m. Despite the improved resolution in this spectrum compared to that of the native one, difficulties in determining an accurate distribution of the ethyl groups remained.

In order to avoid complexities associated with α/β mutarotational equilibria of the partially ethylated sugars in solution, the resulting partially ethylated monosaccharides obtained by hydrolysis were reduced to alditols by sodium borohydride, and these analyzed by carbon-13 n.m.r. spectroscopy and by g.l.c.-m.s. (see Table II). The spectrum is shown in Fig. 5. Thirty-two resonance peaks, ranging from 16.99 to 84.00 p.p.m., were well separated. This indicates that at least 16 additional resonance peaks are not separated, and therefore cannot be quantified. No attempt will be made to assign the resonances in the spectrum. Despite fewer resonance peaks in this spectrum compared to the one of the mixture containing monosaccharides, it still remains very difficult to determine the location of the ethyl groups.

From the results presented herein, it seems probable that complete hydrolysis, and characterization by g.l.c.–m.s. of the D-glucose ethers obtained, will give a more accurate, quantitative and qualitative estimation of the distribution of substituents compared to that provided by ^{13}C-n.m.r. spectroscopy.

EXPERIMENTAL

Materials. — ECs were obtained from Polysciences, Inc., Warrington, PA,

U.S.A. (Nos. 2354, 5429, and 5430) and Hercules Inc., Wilmington, DE, U.S.A. (K- and T-type). The ethoxyl content was determined as described by Hodges *et al.*[20]. The C and H analyses were performed by Canadian Microanalytical Service, Ltd., Vancouver, B.C.

Evaporations were conducted under diminished pressure at a bath temperature not exceeding 40°.

Hydrolysis and analysis of the ethyl ethers. — The partially ethylated cellulose (5 mg) was treated with 90% HCO_2H (2 mL) for 2 h at 100°. After evaporation to dryness, and codistillation with MeOH (3 mL), the residue was hydrolyzed with CF_3CO_2H (2 mL), using different concentrations and time periods, in an ampoule at 100°. The hydrolyzate was evaporated to dryness under a stream of N_2. The residue was dissolved in water (5 mL) and $NaBH_4$ (20 mg) was added. After 2 h at room temperature, an excess of Dowex 50 (H^+) resin was added. The resin was removed by filtration, and the filtrate was evaporated to dryness. Boric acid was removed by repeated codistillation with MeOH (3 × 5 mL). Part of this residue, dissolved in 3:1 MeCN–H_2O, was analyzed on a Supelcosil LC-NH_2 column (25 cm × 4.6 mm), using a Hewlett–Packard 1084A Liquid Chromatograph connected to a Hewlett–Packard 79850 LC Terminal. The eluant was 3:1 MeCN–H_2O and the eluate was monitored with a refractive index detector.

Gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.). — The partially ethylated alditols obtained after complete hydrolysis were derivatized in two ways. (*a*) Part of the residue was acetylated by treatment with 1:1 Ac_2O–C_5H_5N (2 mL) for 15 min at 100°. The solution was evaporated to dryness, and the resulting, partially ethylated alditol acetates were dissolved in a small volume of methanol and analyzed by g.l.c.–m.s. (*b*) Another part was treated with TRI-SIL Z reagent (Pierce Chemical Company, Rockford, IL, U.S.A.) for 1 h at room temperature, and the resulting partially ethylated alditol trimethylsilyl ethers were analyzed by g.l.c.–m.s.

G.l.c. was conducted in a Hewlett–Packard 5880 A gas–liquid chromatography, equipped with a flame ionization detector and connected to an electronic integrator. The separations were performed at 200° (isothermal) on a fused-silica, capillary column (DB 1, 15 m × 0.2 mm). Combined g.l.c.–m.s. was achieved in a Hewlett–Packard 5985 B GC/MS/DS using the same column and an ionization potential of 70 eV.

Electron-impact mass spectra of alditols. — *2,3,6-Tri-*O*-ethyl-*D*-glucose: m/z* 261(31.9), 201(12.9), 189(20.5), 171(13.9), 159(73.5), 156(14.8), 155(15.7), 143(81.3), 142(30.0), 141(86.8), 132(36.0), 131(100.0), 130(14.0), 129(82.3), 127(12.9), 103(58.9), 101(86.4), 99(45.4), 97(20.7), 89(35.0), 88(33.5), 85(97.6), 73(79.3), 72(24.3), 71(30.7), 69(35.1), 61(29.8), 60(34.6), 59(81.3), 57(24.6), and 43(73.5).

*2,6-Di-*O*-ethyl-*D*-glucose: m/z* 159(17.2), 143(21.8), 139(10.0), 129(82.8), 118(23.7), 117(100.0), 115(24.3), 97(20.0), 87(70.3), 58(37), and 43(71.7).

*3,6-Di-*O*-ethyl-*D*-glucose: m/z* 189(20.2), 131(13.9), 129(100.0), 113(44.3), 99(30.5), 87(69.9), and 43(29.3).

2,3-Di-O-ethyl-D-glucose: m/z 131(100.0), 129(13.1), 127(16.7), 113(24.7), 101(22.0), 85(25.7), and 43(27.0).

6-O-Ethyl-D-glucose: m/z 198(10.4), 173(13.6), 157(54.0), 156(20.5), 145(27.5), 143(61.1), 140(18.5), 139(41.4), 131(19.0), 128(19.7), 127(13.9), 128(19.7), 127(13.9), 115(90.4), 103(28.5), 101(100.0), 98(35.4), 97(26.6), 85(27.1), 73(25.9), 59(93.9), and 43(88.0).

2-O-Ethyl-D-glucose: m/z 139(17.1), 131(100.0), 97(14.4), and 43(30.9).

3-O-Ethyl-D-glucose: m/z 271(15.1), 270(23.7), 203(10.0), 157(10.6), 155(10.5), 143(100.0), 140(12.0), 131(18.7), 129(14.9), 127(30.8), 113(73.0), 103(21.7), 101(41.0), 85(75.7), 83(57.4), 69(20.8), 60(26.4), 59(24.0), 44(43.6), and 43(96.1).

D-Glucose: m/z 346(14.0), 310(14.3), 271(10.6), 270(35.1), 253(12.3), 217(13.3), 207(14.4), 207(14.4), 187(40.5), 183(11.6), 170(33.8), 169(14.3), 157(33.5), 153(10.3), 147(17.3), 145(40.6), 139(37.0), 129(14.2), 128(62.6), 127(29.1), 117(24.9), 116(23.8), 115(94.2), 103(54.5), 98(28.5), 97(44.0), 86(22.5), 73(21.4), 69(25.3), 45(21.4), 44(25.0), and 43(100.0).

Nuclear magnetic resonance (n.m.r.) spectroscopy. — ^{13}C-N.m.r. spectra were recorded at a probe temperature of 21° with a Bruker WM-400 instrument operated in the pulsed, Fourier-transform mode, with complete proton-decoupling. The chemical shifts are reported in p.p.m., and are related to internal acetone (δ 31.4). The samples were dissolved in $CDCl_3$ or D_2O.

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