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# IDENTIFICATION OF THE PRODUCTS OF HYDROLYSIS OF CARBOXYMETHYLCELLULOSE*†

KLAUS NIEMELÄ AND EERO SJÖSTRÖM
*Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Espoo (Finland)*
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## ABSTRACT

Hydrolysis of carboxymethylcelluloses, made from softwood and hardwood pulps, gave mainly glucose and its mono- and di-substituted derivatives, together with considerable amounts of products derived from hemicellulose constituents. Each hydrolysate contained 2- and 3-*O*-carboxymethylxylose; mono- and di-substituted mannose derivatives were also detected after hydrolysis of the carboxymethylated softwood pulp. 2,3,6-Tri-*O*-carboxymethyl derivatives of glucose and mannose were detected in hydrolysates of samples of higher degrees of substitution, and 2,3-di-*O*-carboxymethylxylose was found only after hydrolysis of hardwood-derived samples.

## INTRODUCTION

During the last 40 years, much attention has been paid[1–22] to the determination of the distribution of the substituents in carboxymethylcellulose (CMC). Recently, n.m.r. spectroscopy has attracted most interest[13–22], even though various chromatographic methods can be applied for the separation[7,9–12,18] of glucose and its carboxymethyl (CM) ethers after hydrolysis of CMC. In the g.l.c. methods, only packed columns have been used[9,10,12,18]. We now report on the application of capillary g.l.c. and mass spectrometry in identification of the products of hydrolysis of CMC.

## EXPERIMENTAL

*Materials.* — Samples of CMC, prepared either from spruce sulfite or birch sulfate pulps, were commercial products. The degrees of substitution (d.s.) varied from 0.5 to 1.

For g.l.c.–m.s., reference samples containing a mixture of CM ethers of

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glucose and xylose were prepared by carboxymethylation[23] of 100-mg samples of methyl α-D-glucopyranoside and methyl β-D-xylopyranoside, respectively, followed by hydrolysis with 2M hydrochloric acid for 30 min at 120°.

*Hydrolysis.* — For the hydrolysis of CMC (10-mg samples) with sulfuric acid, the procedure of Croon and Purves[7] was followed. Each hydrolysate was neutralised to pH 4 with barium hydroxide and then filtered. The difficulty in obtaining reproducible results, especially for di-*O*-CM-glucoses, suggested that some losses of carboxymethylated sugars occurred during the removal of sulfuric acid.

Hydrolysis with hydrochloric acid was preferred since the excess of acid could be evaporated and more reproducible results were obtained. A modification of a method used in the determination[24,25] of CMC in food was used. A solution of CMC in 6M HCl was stored for 2 h at room temperature, then diluted with water (to 2M), and heated for 30 min at 120°. An aliquot (containing 3 mg of sugars) of each hydrolysate was concentrated to dryness under reduced pressure, and the residue was shaken with pyridine (0.3 mL) and trifluorobis(trimethylsilyl)acetamide (0.3 mL) containing 5% of chlorotrimethylsilane[26] for 1 h at room temperature. The products were analysed by g.l.c. and g.l.c.–m.s.

G.l.c. was performed with a Hewlett–Packard 5890 A gas chromatograph equipped with a flame-ionisation detector and a fused-silica capillary column (OV-101 or SE-54, 25 m × 0.32 mm i.d.). The temperature programme was 2 min at 145°, 20°/min to 255°, and 10 min at 255°. The temperature of both the injection port and the detector was 260°. The carrier gas was hydrogen at 2 mL/min.

The e.i.-mass spectra were recorded[27] at 70 eV with a JEOL JMS-DX303 instrument in combination with a Hewlett–Packard 5790 A gas chromatograph and the same columns as above. Usually, the scanning range was 60 to 600, but, in order to detect the $M^+ - 15$ peaks from the di- and tri-*O*-CM-hexoses, a scanning range of 60 to 750 was used.

Identification of the ring isomers of L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose was based on the use of an automatic library search (NIH/EPA, 31,000 spectra), and was confirmed by reference g.l.c. retention times, obtained after per(trimethylsilylation) of pure sugars. Identification of carboxymethylated compounds was based on the interpretation of the mass spectra.

## RESULTS AND DISCUSSION

The gas chromatograms from the CMC hydrolysates contained 70–80 peaks, of which only some minor compounds remained unidentified (Fig. 1). Mutarotation during hydrolysis explains the formation of the α-pyranose forms, and it is known[28] that their trimethylsilyl derivatives are eluted before those of the corresponding β-anomers. In addition, several furanose compounds were detected in small amounts.

The hydrolysates from all samples contained the α- and β-D-pyranose forms of 2- (**1**) and 3-*O*-CM-xylose (**3**), 2- (**5**), 6- (**7**), and 3-*O*-CM-glucose (**9**), and 2,3-

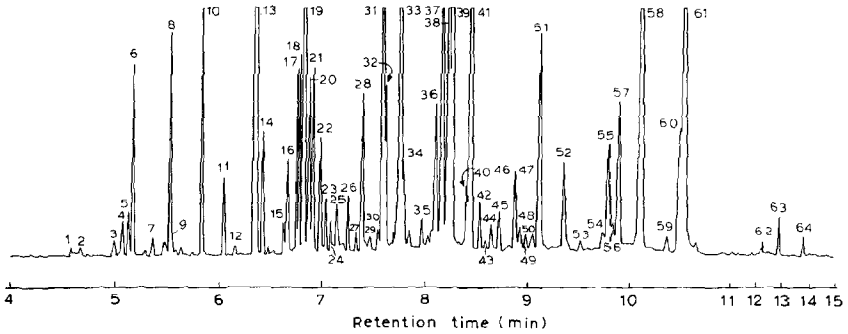
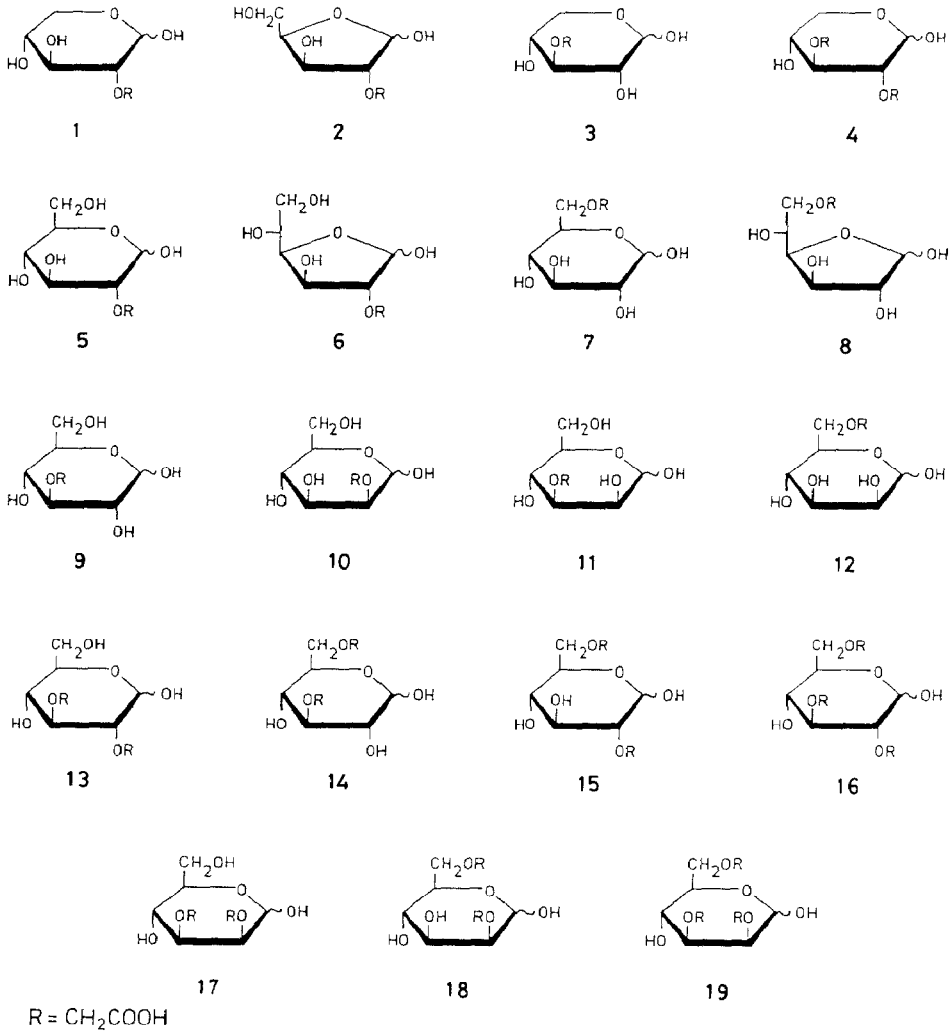



Fig. 1. Separation on an SE-54 fused-silica capillary column of the trimethylsilylated compounds obtained after hydrolysis with hydrochloric acid of a carboxymethylcellulose (prepared from spruce sulfite pulp): 1, α-D-xylofuranose; 2, β-D-xylofuranose; 3, α-glucoisosaccharinolactone; 4, β-glucoisosaccharinolactone; 5, a pentopyranose; 6, α-D-xylopyranose; 7, an *O*-CM-D-xyloselactone; 8, β-D-xylopyranose; 9, α-D-glucofuranose; 10, α-D-mannopyranose; 11, β-D-glucofuranose; 12, α-D-galactopyranose; 13, α-D-glucopyranose; 14, β-D-mannopyranose; 15, an *O*-CM-D-glucoselactone; 16, 3-*O*-CM-α-D-xylopyranose; 17, an *O*-CM-D-glucoselactone; 18, 2-*O*-CM-α-D-xylopyranose; 19, β-D-glucopyranose; 20, 3-*O*-CM-β-D-xylopyranose; 21, 2-*O*-CM-β-D-xylopyranose; 22–25, *O*-CM-D-glucoselactones; 26, 3-*O*-CM-D-mannopyranose; 27, 2-*O*-CM-D-glucofuranose; 28, 2-*O*-CM-α-D-mannopyranose; 29, 6-*O*-CM-D-glucofuranose; 30, an *O*-CM-D-glucoselactone; 31, 3-*O*-CM-α-D-glucopyranose; 32, 6-*O*-CM-α-D-mannopyranose; 33, 2-*O*-CM-α-D-glucopyranose; 34, 2-*O*-CM-β-D-mannopyranose; 35, an *O*-CM-hexose; 36, 6-*O*-CM-β-D-mannopyranose; 37, 3-*O*-CM-β-D-glucopyranose; 38, 2-*O*-CM-β-D-glucopyranose; 39, 6-*O*-CM-α-D-glucopyranose; 40, an *O*-CM-hexose; 41, 6-*O*-CM-β-D-glucopyranose; 42, an *O*-CM-hexose; 43–46, di-*O*-CM-D-glucoselactones; 47, 2,3-di-*O*-CM-α-D-mannopyranose; 48–50, di-*O*-CM-D-glucoselactones; 51, 2,3-di-*O*-CM-α-D-glucopyranose; 52, 2,6-di-*O*-CM-D-mannopyranose; 53, a di-*O*-CM-hexofuranose; 54, 2,3-di-*O*-CM-β-D-mannopyranose; 55, 2,3-di-*O*-CM-β-D-glucopyranose; 56, a di-*O*-CM-hexose; 57, 3,6-di-*O*-CM-α-D-glucopyranose; 58, 2,6-di-*O*-CM-α-D-glucopyranose; 59, a di-*O*-CM-hexose; 60, 3,6-di-*O*-CM-β-D-glucopyranose; 61, 2,6-di-*O*-CM-β-D-glucopyranose; 62, 2,3,6-tri-*O*-CM-D-mannopyranose; 63, 2,3,6-tri-*O*-CM-α-D-glucopyranose; 64, 2,3,6-tri-*O*-CM-β-D-glucopyranose.

(**13**), 3,6- (**14**), and 2,6-di-*O*-CM-glucose (**15**). Most samples contained also 2- (**6**) and 6-*O*-CM-glucofuranose (**8**). All samples from softwood CMC contained 2- (**10**), 3- (**11**), and 6-*O*-CM-mannose (**12**), and 2,3- (**17**) and 2,6-di-*O*-CM-mannose (**18**). In addition, those obtained from CMC with d.s. ≥0.7 contained tri-*O*-CM-glucose (**16**) and -mannose (**19**). 2-*O*-CM-xylofuranose (**2**) and 2,3-di-*O*-CM-xylopyranose (**4**) were found only after the hydrolysis of hardwood-derived CMC.

During hydrolysis or concentration, some lactonisation occurred because peaks indicating the presence of one *O*-CM-xyloselactone, 7 *O*-CM-glucoselactones, and 7 di-*O*-CM-glucoselactones were detected (Fig. 1). No attempts were made to determine the structures of these lactones. The formation of some *O*-CM-glucoselactones has been reported[18,21] previously.

In general, the OV-101 and SE-54 columns gave quite similar separations and orders of elution (Table I). However, 2-*O*-CM-β-D-xylopyranose and β-D-glucopyranose were not resolved on the OV-101 column, which is therefore less suitable for the analysis of samples containing carboxymethylated xylan. Another




drawback of OV-101 is the difficulty in separating some di-*O*-CM-D-glucopyranoses.

Tachibana and Sumimoto[12] found some different orders of elution for an SE-30 column (which is similar to OV-101). In contrast to our results, they reported that the 3-*O*-CM-D-glucopyranoses appear after other *O*-CM-D-glucopyranoses and that the 2,6-di-*O*-CM-D-glucopyranoses are eluted before the 3,6-di-*O*-CM-D-glucopyranoses. Confusion is also caused by the extremely small peaks corresponding to $\alpha$-D-glucopyranose and 6-*O*-CM-$\beta$-D-glucopyranose.

*Mass spectra of O-CM-xyloses.* — Trimethylsilyl derivatives are useful in the determination of the structure of partially substituted aldoses, as exemplified by the e.i.-mass spectra of partially *O*-methylated pentoses[29], *O*-methylated hexoses[30,31],

TABLE I

RELATIVE RETENTION TIMES[a] OF THE PER(TRIMETHYLSILYL) DERIVATIVES OF THE PYRANOSE FORMS OF D-XYLOSE, D-GLUCOSE, D-MANNOSE, AND THEIR CARBOXYMETHYL (CM) ETHERS

| *Derivative of* | *OV-101* | *SE-54* |
|---|---|---|
| α-Xylose | 0.713 | 0.759 |
| β-Xylose | 0.779 | 0.810 |
| 2-*O*-CM-α-xylose | 0.973 | 0.994 |
| 2-*O*-CM-β-xylose | 0.998 | 1.016 |
| 3-*O*-CM-α-xylose | 0.957 | 0.979 |
| 3-*O*-CM-β-xylose | 0.995 | 1.010 |
| 2,3-Di-*O*-CM-α-xylose | 1.174 | 1.183 |
| 2,3-Di-*O*-CM-β-xylose | 1.205 | 1.210 |
| α-Glucose | 0.914 | 0.931 |
| β-Glucose | 1.000 | 1.000 |
| 2-*O*-CM-α-glucose | 1.138 | 1.138 |
| 2-*O*-CM-β-glucose | 1.209 | 1.209 |
| 3-*O*-CM-α-glucose | 1.113 | 1.115 |
| 3-*O*-CM-β-glucose | 1.201 | 1.200 |
| 6-*O*-CM-α-glucose | 1.204 | 1.213 |
| 6-*O*-CM-β-glucose | 1.237 | 1.243 |
| 2,3-Di-*O*-CM-α-glucose | 1.305 | 1.343 |
| 2,3-Di-*O*-CM-β-glucose | 1.383 | 1.444 |
| 3,6-Di-*O*-CM-α-glucose | 1.383 | 1.459 |
| 3,6-Di-*O*-CM-β-glucose | 1.450 | 1.551 |
| 2,6-Di-*O*-CM-α-glucose | 1.403 | 1.491 |
| 2,6-Di-*O*-CM-β-glucose | 1.453 | 1.556 |
| 2,3,6-Tri-*O*-CM-α-glucose | 1.652 | 1.906 |
| 2,3,6-Tri-*O*-CM-β-glucose | 1.740 | 2.041 |
| α-Mannose | 0.839 | 0.857 |
| β-Mannose | 0.934 | 0.946 |
| 2-*O*-CM-α-mannose | 1.083 | 1.087 |
| 2-*O*-CM-β-mannose | 1.138 | 1.144 |
| 3-*O*-CM-mannose | 1.065 | 1.068 |
| 6-*O*-CM-α-mannose | 1.113 | 1.121 |
| 6-*O*-CM-β-mannose | 1.187 | 1.193 |
| 2,3-Di-*O*-CM-α-mannose | 1.280 | 1.310 |
| 2,3-Di-*O*-CM-β-mannose | 1.370 | 1.438 |
| 2,6-Di-*O*-CM-mannose | 1.330 | 1.381 |
| 2,3,6-Tri-*O*-CM-mannose | 1.599 | 1.810 |

[a]Relative to the β-glucopyranose derivative; 6 min for OV-101 and 6.8 min for SE-54.

*O*-ethylated glucoses[32], *O*-(2-hydroxyethylated) glucoses[33,34], *O*-acetylated methyl glucopyranosides[35], and *O*-palmitoylated methyl glucopyranosides[36]. General schemes in the interpretation of the spectra as well as the structures of the main fragments are well described in these papers, and will not be repeated here in detail.

Fig. 2 shows partial mass spectra of the trimethylsilyl derivatives of 2- (**1**) and

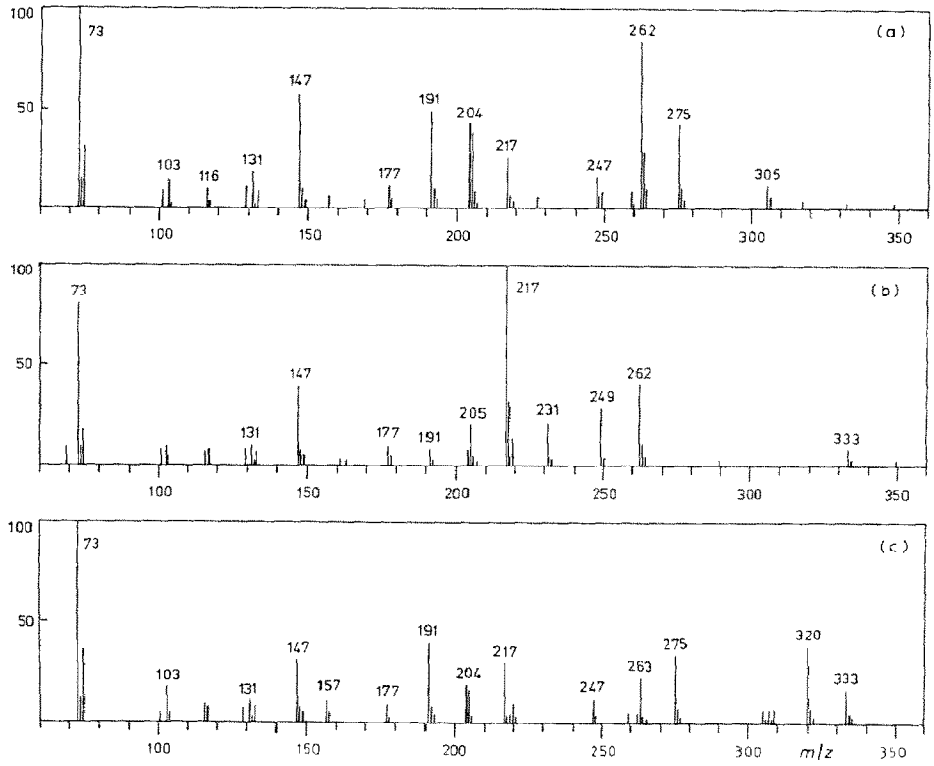



Fig. 2. Partial e.i.-mass spectra at 70 eV of the trimethylsilylated derivatives of 2- (*a*) and 3-*O*-CM-D-xylopyranose (*b*), and 2,3-di-*O*-CM-D-xylopyranose (*c*).

3-*O*-CM-D-xylopyranose (**3**), and 2,3-di-*O*-CM-D-xylopyranose (**4**). In the upper mass range, weak (<2%) $M^+ - 15$ and $M^+ - 15 - 90$ peaks confirmed the molecular weights, namely, 496 for **1** and **3**, and 554 for **4**.

In the mass spectra of the trimethylsilyl derivatives of pentopyranoses having no 2- or 3-substituent, a prominent peak at *m/z* 204 was observed[29]. The presence of a 2- or 3-CM group resulted in the formation of a prominent peak at *m/z* 262 (204 + 58), which was more intense for **1**. A prominent peak at *m/z* 249 (191 + 58) was possible for **3** only (*cf.* ref. 37). The base peak in the mass spectra of the trimethylsilyl derivatives of **3** and 3-*O*-methylxylopyranose[29] was at *m/z* 217.

The mass spectrum of the trimethylsilyl derivative of 2-*O*-CM-D-xylofuranose (**2**) differed from that of **1**, mainly by the following structure-specific[29] intensities: *m/z* 275 (55%), 262 (33), 217 (27), 204 (19), and 191 (46). The presence of the two CM-substituents at positions 2 and 3 in **4** is clearly shown by the intense peak at *m/z* 320 (262 + 58) in the spectrum of its trimethylsilyl derivative.

*Mass spectra of mono-O-CM-glucoses.* — Fig. 3 shows the partial mass spectra of the trimethylsilyl derivatives of 2- (**5**), 3- (**9**), and 6-*O*-CM-D-glucopyranose (**7**). Again, weak (<2%) peaks for $M^+ - 15$ and $M^+ - 15 - 90$ were used to confirm

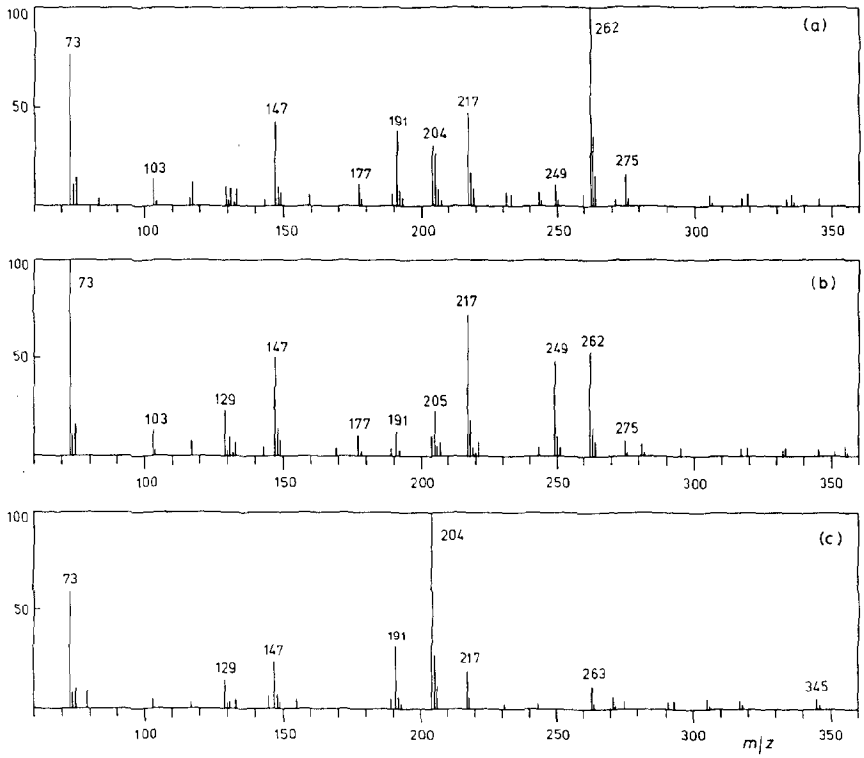



Fig. 3. Partial e.i.-mass spectra at 70 eV of the trimethylsilylated derivatives of (*a*) 2-, (*b*) 3-, and (*c*) 6-*O*-CM-D-glucopyranose.

the molecular weight of 598. The identification of **5** and **9** was analogous to that of the corresponding xylopyranoses and needs no further discussion.

The base peak in the mass spectrum of the trimethylsilyl derivative of **7** (no 2- or 3-substituent) was at *m/z* 204. Analogous mass spectra have been reported for the trimethylsilyl derivatives of many other 6-substituted hexopyranoses[30–36], including also 6-*O*-benzyl-β-D-glucopyranose[38], 6-*O*-(*N*-acetyl-α-D-neuraminyl)-D-galactose[39], D-hexopyranose 6-phosphates[40,41], and β-D-glucopyranose 6-malate[42]. The mass spectra reported by Bach Tuyet *et al.*[18] for the trimethylsilyl derivatives of **5, 7,** and **9** are confusing, and that of **7** is erroneous.

The trimethylsilyl derivatives of hexofuranoses are readily recognised[30,43] from the intense peaks (often base peaks) at *m/z* 217. This ion remains intense also after introduction of another 6-substituent, as demonstrated by the spectrum of the trimethylsilyl derivative of α-D-galactofuranose 6-phosphate[40]. Thus, the abundance of this ion allowed identification of peak 29 (Fig. 1) as 6-*O*-CM-D-glucofuranose (**8**): *m/z* 493 (1%), 305 (4), 275 (7), 232 (11), 217 (78), 205 (4), and 204 (3). Analogously, 2-*O*-CM-D-glucofuranose (**6**) showed an intense peak at *m/z* 275

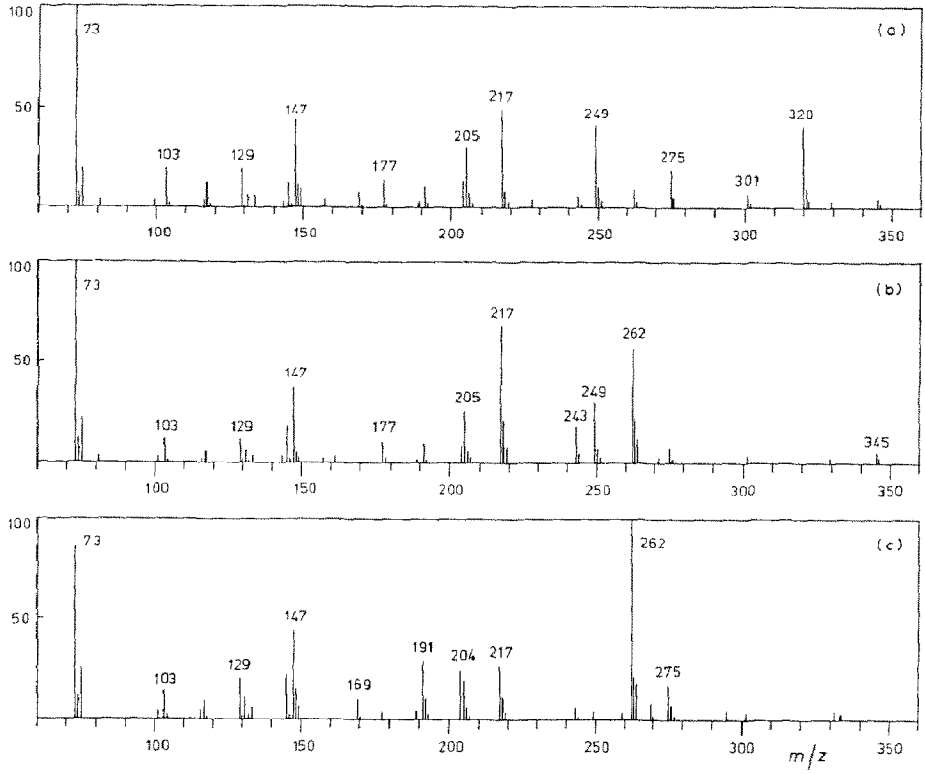



Fig. 4. Partial e.i.-mass spectra at 70 eV of the trimethylsilylated derivatives of (*a*) 2,3-, (*b*) 3,6-, and (*c*) 2,6-di-*O*-CM-D-glucopyranose.

(217 + 58): *m/z* 493 (1%), 315 (4), 275 (55), 262 (2), 217 (10), 205 (4), 204 (4), and 191 (6).

*Mass spectra of di- and tri-O-CM-glucopyranoses.* — Fig. 4 shows the partial mass spectra of the trimethylsilyl derivatives of 2,3- (**13**), 3,6- (**14**), and 2,6-di-*O*-CM-D-glucopyranose (**15**). Weak (≤1%) but detectable peaks for $M^+ - 15$ and $M^+ - 15 - 90$ confirmed their molecular weights as 656.

Identification of the mono-*O*-CM-D-glucopyranoses **5, 7,** and **9** facilitates the interpretation of the spectra of disubstituted compounds. A prominent ion at *m/z* 262 is possible for **14** and **15**, but only **14** can have abundant ions at *m/z* 217 and 249. The prominent peak at *m/z* 320 in the spectrum of **13** clearly indicates the presence of the substituents at positions 2 and 3. The previous mass spectrum of **13**, interpreted by Bach Tuyet *et al.*[18] with the help of n.m.r. spectroscopy, is misleading.

As expected, the partial mass spectrum of the trimethylsilyl derivative of 2,3,6-tri-*O*-CM-D-glucopyranose (**16**) resembled that of **13** (Fig. 5), even though the relative intensities of many important ions differ. However, longer retention

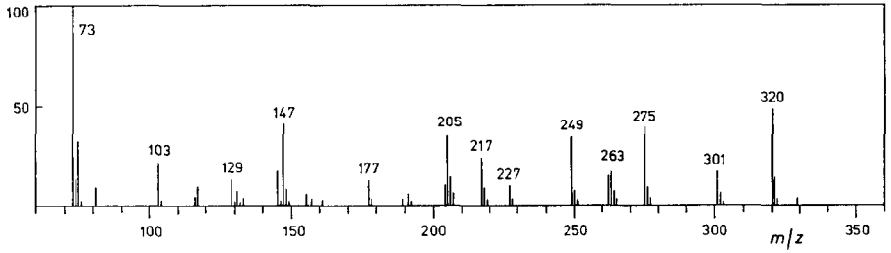



Fig. 5. Partial e.i.-mass spectrum at 70 eV of the trimethylsilylated derivative of 2,3,6-tri-*O*-CM-D-glucopyranose.

times and the weak (<1%) peak for the $M^+ - 15 - 90$ ion at *m/z* 609 confirm the presence of the three substituents.

*Detection of lactones.* — The presence of 15 lactones was recognised from the prominent peaks for $M^+ - 15$ at *m/z* 319 (7%) for an *O*-CM-D-xyloselactone, 421 (2–5%) for *O*-CM-D-glucoselactones, and 479 (2–13%) for di-*O*-CM-D-glucoselactones. The structures of these lactones were not determined.

## CONCLUSIONS

Although many neutral and carboxymethylated monosaccharides are obtained by acid hydrolysis of technical carboxymethylcelluloses, the mixtures can be analysed by capillary g.l.c. and thereby provide a detailed characterization including the contents of (carboxymethyl)xylan and (carboxymethyl)mannan, the d.s., and the distribution of the substituents in the cellulose and hemicellulose constituents.

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