Mass spectrometric fragmentation of methyl 2-*O*-methyl-, 4-*O*-methyl-, and 2,4-di-*O*-methyl-α-D-glucopyranoside and its application to some synthetic problems

V. KOVÁČIK and P. KOVÁČ

Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava

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The study of the mass spectrometric fragmentation of methyl 2-*O*-methyl-, 4-*O*-methyl-, and 2,4-di-*O*-methyl-α-D-glucopyranoside revealed significant quantitative differences in the fragmentation of the two mono-*O*-methyl derivatives. The relative intensities of the peaks of the ions at *m/e* 74, 71, and 60 expressed in percent of the total ionization $\% \Sigma_{45}$ can be used for distinguishing between these two derivatives. Applying the obtained results it was possible to demonstrate that partial deacetylation of methyl 2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside gives the 4-*O*-monoacetate. It was proved also that by Purdie methylation of methyl 3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside almost exclusively the corresponding 2-*O*-methyl derivative is produced.

Interpretation of mass spectra of partially methylated sugars without derivatization [1, 2] is one of the latest approaches to the problem of identification of these substances. The fragmentation of methyl *O*-methyl-D-glucopyranosides [1] and methyl *O*-methyl-D-xylofuranosides [2] showed that mass spectra are important for determination of both the number and the location of the methyl groups present in the molecule of a partially methylated carbohydrate. In view of the fact that many of these compounds are directly amenable to and well separated by gas—liquid chromatography [1, 3, 4] the fragmentation of differently substituted partially methylated sugars can be used in identification of the products of methanolysis of methylated polysaccharides using the known GC-MS technique and also in solving some synthetic problems.

Fragmentation of a series of methyl ethers of methyl α-D-glucopyranoside was studied by *Heyns et al.* [1]. Although the quoted authors did not run the mass spectra of the 2-*O*-, 4-*O*-, and 2,4-di-*O*-methyl ethers they solved the fragmentation of all the compounds under their investigation and, based on the features of fragmentation, proposed a system of identification of all methyl *O*-methylhexopyranosides. Applying their assumptions to identification of the products of the reactions described in the Experimental Section we have found certain discrepancies between the proposed criteria [1] and our results. We have studied, therefore, the fragmentation of the title compounds, their deutero-analogs and confronted the obtained mass spectra with the features characteristic of the fragmentation of these substances as assumed by *Heyns et al.* [1]. Using the produced results it was possible to unambiguously identify the product of partial deacetylation of methyl 2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside and of partial methylation of methyl 3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside as 4-*O*-acetate and 2-*O*-methyl ether, respectively.

Experimental

Melting points were determined on a Kofler hot stage. Optical rotations were measured on a Bendix—Ericsson automatic polarimeter Model 143 A. I.r. spectrometry in KBr pellets was carried out with a Beckman IR Spectrometer Model 5 A. The n.m.r. spectra were taken at 80 MHz on a Tesla BS 487 B spectrometer. The mass spectra were obtained with MCh 1306 mass spectrometer at an ionizing potential 70 eV. The inlet temperature was 30—35°C and in the ionizing chamber 120°C. The peak intensities are given in percent of the total ionization (% Σ_{45}). The nomenclature of the ions ($A-K$) introduced by *Kochetkov et al.* [5] is used throughout. The *O*-deuterated compounds were prepared by evaporation of solutions of the substances on D_2O directly in the mass spectrometer.

Thin-layer chromatography (TLC) on Silica gel G and preparative chromatography on silica gel (0.05—0.1 mm) was performed using: *A*. hexane—ethyl acetate 4 1, and *B*. chloroform—methanol 6 : 1. The solvent ratios are based on volumes. The components were located by spraying with 5% sulfuric acid in ethanol and heating until permanent char spots were visible. Tetrahydrofuran and 1,2-dimethoxyethane were dried as described by *Perrin et al.* [6] and stored over sodium hydride.

Methyl 2-*O*-methyl-α-D-glucopyranoside *I* and methyl 2,4-di-*O*-methyl-α-D-glucopyranoside *III* were prepared as described previously [7, 8]. Methyl 4-*O*-methyl-α-D-glucopyranoside *II* was synthesized following the known sequence of reactions [9]. Complete methylation, in one step, at C_3—OH of methyl 2,3-di-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside was achieved by methylation with sodium hydride and methyl iodide in 1,2-dimethoxyethane [7, 8] and simultaneous removal of the blocking groups was done with sodium in liquid ammonia as described in [10]. Compound *II* had m.p. 96—97°C. Ref. [9] gives m.p. 98°C.

Methyl 4-O-acetyl-3-O-benzyl-6-O-triphenylmethyl-α-D-glucopyranoside (IV)

A suspension of methyl 2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside [8] (2 g) in dry methanol (60 ml) containing a catalytic amount of sodium methoxide was magnetically stirred with the exclusion of moisture at 35°C. A few minutes after the starting material had dissolved the solution was cooled and deionized with Dowex 50W (H^+ form), filtered rapidly through a fine sintered glass funnel and evaporated to dryness. The solid residue was crystallized from methanol or ether—hexane to give 1.3 g (70.5%) of *IV*, m.p. 142—143°C, $[\alpha]_D^{24}$ +67.4° (c 1, chloroform). I.r. spectrum showed a strong absorption at $\tilde{\nu}_{max}$ 3300 (OH), 1740 (acetyl), and 1225 (ester) cm^{-1}. The integrated n.m.r. spectrum (in $CDCl_3$) showed a total of 36 protons, 20 of which were aromatic (multiplet at τ 2.5—2.9). The other definite signals were at τ 7.01 (singlet, CH_3O), and τ 5.65 (singlet, $COCH_3$).

For $C_{35}H_{36}O_7$ (568.64) calculated: 73.92% C 6.38% H, 5.46% CH_3O; found: 73.93% C, 6.32% H, 5.81% CH_3O.

Methyl 4-O-acetyl-3-O-benzyl-2-O-methyl-6-O-triphenylmethyl-α-D-glucopyranoside (V)

A mixture of monoacetate *IV* (1.7 g), methyl iodide (20 ml) containing a few drops of methyl sulfide, and silver oxide (3 g) was shaken in the dark for 6 hours at which time a fresh portion of silver oxide (1.5 g) was added. The shaking was continued over-

night and then TLC in the system *A* showed that the reaction was complete. The product, isolated in the usual manner, spontaneously crystallized upon evaporation of the solvents and on recrystallization from ether—hexane had m.p. 125.5—126.5°C. I.r. spectrum showed an intense peak at $\tilde{\nu}_{max}$ 1740 cm^{-1} (acetyl) and no hydroxyl absorption could be detected.

For $C_{36}H_{38}O_7$ (582.66) calculated: 74.20% C, 6.57% H, 10.65% CH_3O; found: 74.47% C, 6.59% H, 11.11% CH_3O.

Methyl 3-O-benzyl-2-O-methyl-6-O-triphenylmethyl-α-D-glucopyranoside (VI)

Methyl 3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside [8] (0.8 g), methyl iodide (10 ml), and silver oxide (1.5 g) were shaken in the dark for 72 hours during which time fresh portions of methyl iodide (2 ml) and silver oxide (5 × 0.25 g) were added. TLC in the system *A* showed then that the starting material disappeared from the reaction mixture almost completely. One major product, slightly contaminated with the di-*O*-methyl derivative [8], (R_F 0.3, *cf.* 0.1 for the starting material) was detected. The reaction mixture was worked up in the usual manner and the product *VI* was isolated in a chromatographically pure state by elution from a silica gel column (1.5 × 15 cm). Compound *VI*, obtained as a white foam (0.7 g, 85%), had $[\alpha]_D^{24}$ +41.6° (*c* 1.03, chloroform).

For $C_{31}H_{36}O_6$ (540.63) calculated: 75.53% C, 6.71% H, 11.48% CH_3O; found: 75.56% C, 6.68% H, 11.15% CH_3O.

Methyl 2-O-methyl-α-D-glucopyranoside (VII)

A. A solution of *VI* (0.4 g) in tetrahydrofuran (4 ml) was slowly added with stirring into a mixture of liquid ammonia (40 ml) and tetrahydrofuran (4 ml). Sodium (0.15 g) was added portionwise and with continued stirring and when permanent blue colour developed, a small amount of ammonium chloride was added and ammonia was allowed to evaporate. The solid was filtered off, washed with acetone and the filtrate combined with the washings was evaporated to dryness. The residue was partitioned between chloroform and water and the water layer containing a single charring component (R_F 0.4, solvent *B*) was concentrated. The syrupy residue crystallized immediately when seeded with authentic methyl 2-*O*-methyl-α-D-glucopyranoside [7], m.p. 146—148°C. Ref. [7, 11] give m.p. 147—148°C.

B. Hydrogenolysis of a small amount of *V* carried out in the above described manner using 1,2-dimethoxyethane as the inert solvent yielded a substance melting at 147—148°C.

Results and discussion

The mass spectra of methyl 2-*O*-methyl- and 4-*O*-methyl-α-D-glucopyranoside *I* and *II* (Table 1) are qualitatively identical. The ions $A_1 + E_1$ are at *m/e* 177 (after deuteration A_1 = *m/e* 180, E_1 = *m/e* 179). Elimination of the molecules of water or methanol gives the ions at *m/e* 159 and 145 (m^* = 142.8 for 177 → 159; m^* = 118.7 for 177 → → 145), which disintegrate to give the ions at *m/e* 127 (m^* = 111.2 for 145 → 127). The peaks at *m/e* 158 in the upper, less intense, part of the spectra, which could not originate in the series *B* and *C* by analogy with the fragmentation of permethylated methylhexopyranosides [5, 12], are probably those of the ions $(M-CH_3OH-H_2O)^+$ and the ones at *m/e* 130 are given rise to by dehydration of the ions B_1 at *m/e* 148 through the

Table 1

The mass spectra of *I—III*

m/e	Rel. int.			m/e	Rel. int.		
	2*	4*	2,4*		2*	4*	2,4*
191			0.075	101	0.734	0.477	14.684
177	0.264	0.141		99	0.998	0.913	1.095
173			0.279	98	0.352	0.296	
162			0.086	97	0.470	0.403	
159	0.558	0.558	0.397	89	1.262	0.907	1.374
158	0.382	0.159		88	4.403	9.215	7.353
148	0.022	0.033		87	14.967	14.869	5.195
147	0.066	0.144		85	2.788	1.460	1.073
145	0.910	0.507	0.075	75	2.348	4.138	4.208
144	0.205	0.101		74	36.836	19.233	19.300
141			0.097	73	3.375	2.481	2.394
131			0.193	71	3.375	13.028	18.516
130	0.851	0.388		69	0.881	1.653	
129	0.117	0.100	0.129	61	3.816	3.043	1.310
127	0.235	0.318	0.118	60	1.174	5.517	1.116
117	0.235	0.337	0.365	59	3.816	3.373	3.456
115	0.264	0.200	2.225	58	0.881	2.452	2.244
113	0.323	0.192	0.247	57	3.229	3.265	2.329
112	0.558	0.211		56	1.174	1.531	
111	0.352	0.318	0.397	55	1.027	0.817	
103	0.499	0.699		45	5.870	6.220	7.203
102	0.382	0.296	4.465				

The numbers refer to the position of the methoxyl groups on methyl α-D-glucopyranoside (*I—III*).

interaction of the two hydroxyl groups (the value 130 remains unchanged after deuteration). According to the earlier workers [1] the relative intesities of the ions H_1 (K_1) at *m/e* 88, 74, 60 and F_1 (G_1) at *m/e* 87 determine the position of the methoxyl group in the case of methyl 2-*O*-methyl and methyl 4-*O*-methyl derivatives. The ions at *m/e* 74 found in the spectrum of the 2-*O*-methyl derivative are almost twice as intense as the ions of *m/e* 74 found in the spectrum of the 4-*O*-methyl ether. This, together with the fact that the intensity of the ions at *m/e* 87 is the same in the spectra of both derivatives, is in accordance with the postulated data [1]. The intensity of the ions at *m/e* 88, however, disagrees with the previous prediction *i.e.* these ions are more intense in the case of 4-*O*-methyl derivative than in the case of 2-*O*-methyl analog.

This surprising finding was clarified by deuteration analysis which proved that the ions at *m/e* 88 in the spectrum of methyl 4-*O*-methyl-α-D-glucopyranoside belong to the series K_1 ($\overset{+}{C}H_3O{=}\overset{4}{C}H{-}\overset{5}{\underset{\bullet}{C}}H{-}CH_2OH$) whereas in the case of methyl 2-*O*-methyl-α-D-glucopyranoside the ions at *m/e* 88 are of the series H_1 ($\overset{+}{C}H_3O{=}\overset{2}{C}H{-}\overset{1}{\underset{\bullet}{C}}H{-}OCH_3$). In addition, much more intense are also the ions K_2 at *m/e* 71 and H_1 at *m/e* 60 in the case of 4-*O*-methyl derivative. We suggest, therefore, the different

intensities of the ions at *m/e* 74, 71, and 60 as a criterion for distinguishing between these two mono-*O*-methyl methylhexopyranosides. Taking into account the differences in the fragmentation of 3-*O*-methyl and 6-*O*-methyl methylhexopyranosides [1] it can be concluded that the location of the methoxyl group in any methyl mono-*O*-methyl hexopyranoside can be unambiguously assigned by interpreting their mass spectra taken without prior derivatization.

The presence of the peaks of ions $(M\text{-}31)^+$ at *m/e* 191 in the spectrum of methyl 2,4-di-*O*-methyl-α-D-glucopyranoside *III* which after deuteration give a pair of peaks at *m/e* 192 and 193, as well as the absence of the ions $(M\text{-}45)^+$ is in accordance with the fragmentation of di-*O*-methyl methylhexopyranosides having the C_6-OH group unsubstituted. The ions at *m/e* 191 (A_1, E_1) give after elimination of water or methanol the ions at *m/e* 173 and 159 (A_2, E_2) ($m^* = 156.7$ for $191 \rightarrow 173$; $m^* = 132.1$ for $191 \rightarrow 159$) and at *m/e* 141 (A_3, E_3). The relative intensities of the ions F_1 (G_1), given rise to by the disintegration of the pyranoid ring, at *m/e* 101 and 87, H_1 at *m/e* 88 and 74 (together with the ions K_1) and J_1 at *m/e* 75 are, essentially, in agreement with the assumed values [1]. The intensities of the ions at *m/e* 87 (5.195%) and 75 (4.208%) are only slightly different from the presumed values (1—5% and 5—10%, respectively).

Methyl 2,4-di-*O*-acetyl-3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside shows noticeable resistance to complete deacetylation with a catalytic amount of sodium methoxide. While this can be achieved by prolonged treatment under the conditions of catalytical deacetylation [13] or, alternatively, by boiling in methanolic potassium hydroxide [8], a brief treatment with a catalytic amount of sodium methoxide in methanol yielded 70.5% of crystalline mono-*O*-acetate *IV*

Methylation of *IV* with methyl iodide and silver oxide gave methyl *O*-acetyl-3-*O*-benzyl-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranoside *V* in which the location of the methoxyl group was unequivocally proved by interpreting the mass spectrum of *VII* obtained from *V* by removal of the protecting (acetyl, benzyl, triphenylmethyl) groups. The mass spectrum of *VII* was identical with that of methyl 2-*O*-methyl-α-D-glucopyranoside *I*.

The determination of the position of the methoxyl group at C-2 in *VII* establishes, in addition, the location of the *O*-acetyl group at C-4 in the monoacetate *IV* The formation of *V* from the 2-*O*-acetate of methyl 3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside might have occurred by methylation with Purdie's reagent only if the *O*-acetyl group originally present at C-2 had migrated to the C-4 position during the methylation process. This can hardly be assumed in the case of an acetyl group stable under the conditions of deacetylation.

It is known that a carbohydrate bearing the bulky triphenylmethyl group is difficult to convert to the fully substituted derivative by conventional methods of methylation. The previous authors [14] despite repeated treatment of 3-*O*-benzyl-6-*O*-triphenylmethyl-D-glucose with methyl iodide and silver oxide obtained only low yield of the desired fully methylated product and explained the fact by severe undermethylation. That this explanation was correct was proved by methylation of methyl 3-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside with the same methylation agent which, in our hands, gave a high yield of methyl 3-*O*-benzyl-2-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranoside. The assigned structure *VI* was proved by the mass spectrum of *VII* obtained from *VI* by debenzylation and detritylation.

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References

1. Heyns, K., Sperling, K. R., and Grützmacher, H. F., *Carbohyd. Res.* ***9***, 79 (1969).
2. Kováčik, V. and Kováč, P., *Carbohyd. Res.* ***24***, 23 (1972).
3. Anderle, D., Petríková, M., and Kováč, P., *J. Chromatogr.* ***58***, 209 (1971).
4. Anderle, D., Kováč, P., and Anderlová, H., *J. Chromatogr.* ***64***, 368 (1972).
5. Kochetkov, N. K. and Chizhov, O. S., *Advan. Carbohyd. Chem.* ***21***, 39 (1966).
6. Perrin, D. D., Armarego, W. L. F., and Perrin, D. R., *Purification of Laboratory Chemicals*. Pergamon Press, Oxford, 1966.
7. Kováč, P. and Longauerová, Ž., *Chem. Zvesti* ***27***, 415 (1973).
8. Kováč, P. and Longauerová, Ž., *Carbohyd. Res.* ***25***, 253 (1972).
9. Whistler, R. L., Linke, E. G., and Kazeniac, S., *J. Amer. Chem. Soc.* ***78***, 4704 (1956).
10. Kováč, P. and Bauer, Š., *Tetrahedron Lett.* ***1972***, 2349.
11. Haworth, W. N., Hirst, E. L., and Teece, E. G., *J. Chem. Soc.* ***1931***, 2858.
12. Heyns, K., Grützmacher, H. F., Scharmann, H., and Müller, D., *Fortschr. Chem. Forsch.* ***5***, 488 (1966).
13. Kováč, P., unpublished results.
14. Adams, M. H., Reeves, R. F., and Goebel, W. F., *J. Biol. Chem.* ***140***, 653 (1941).

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