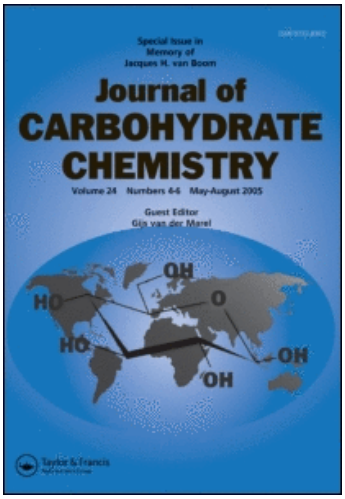

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Mass Spectrometry in the Structural Studies of Oligosaccharides

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MASS SPECTROMETRY IN THE STRUCTURAL STUDIES OF OLIGOSACCHARIDES#

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ABSTRACT

On the basis of the reported low-resolution electron impact (EI) mass spectral decomposition patterns of a few underivatised simple disaccharides[1-3] and the results of a high-resolution electron impact mass spectrum of a model disaccharide β-methyl pachybioside,[4] a common fragmentation pathway of such compounds could be worked out. Using the derived standard decomposition pathways, structure of a novel tetrasaccharide, orthenthose, has been elucidated as oleandrose tetrasaccharide.

INTRODUCTION

Although use of mass spectrometry is well established in the structural elucidation of natural products, its use in oligosaccharide structure elucidation has yet to find a suitable place. Because of the low volatility of such substances, very little is reported for the mass spectral interpretation of underivatised carbohydrates. In studies on the mass spectral analysis of underivatised oligosaccharides, it is apparent that the technique of field ionisation (FI) in conjunction with the usual electron-impact (EI) methods offers some appreciable

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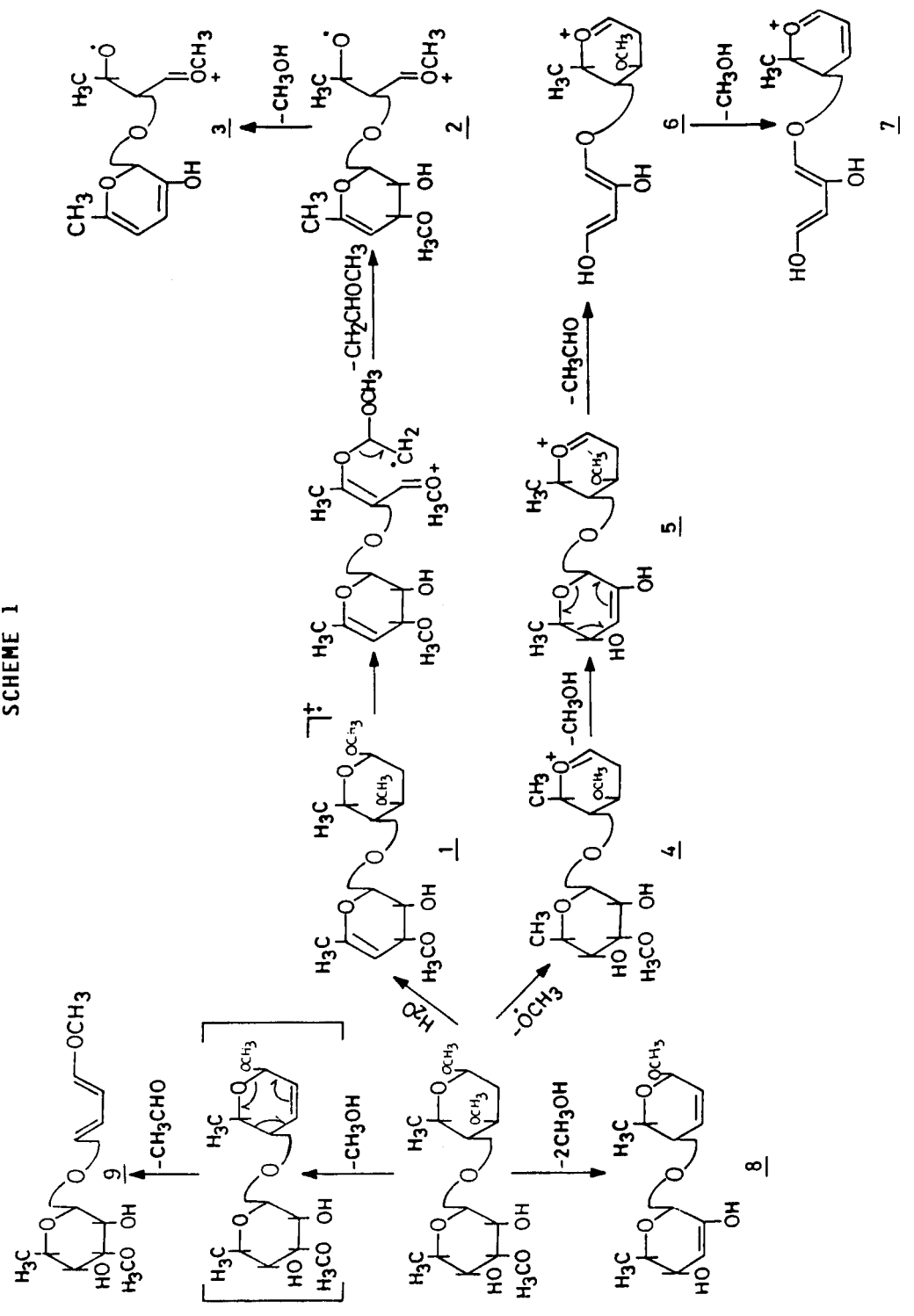
advantage. In particular, high-mass ion peaks such as molecular ions are more evident in FI spectra, but the ionisation does not give fragment ions as extensively as do EI mass spectra, which often provide more valuable structural information. Studies of EI-induced fragmentation of oligosaccharides thus potentially provide a very valuable analytical technique in this area. A limitation of this approach, however, is the inaccessibility of finer stereochemical details such as the configuration of glycosidic linkage. Oligosaccharides of O-methyl deoxyhexoses or dideoxyhexoses do not suffer from the drawback of low volatility, as shown by the underivatised mono- and oligosaccharides of normal sugars, which limits the observation of high-mass ion peaks.

With the aim of using mass spectrometry in the structural elucidation of oligosaccharides, a high-resolution EI mass spectrum of a model 2-deoxyhexose disaccharide, β-methyl pachybioside, was taken to establish the principles which might govern fragmentation pathways for these compounds. The high-resolution mass spectral results, in addition to giving information about the elemental composition of the compound, also furnish equally well information on the composition of fragment ions and thus greatly assist in the correct interpretation of a mass spectrum. The verification of the elemental composition of an ion is quite important, whenever its genesis is discussed, for the purpose of determining a fragmentation mechanism for the given structure.

RESULTS AND DISCUSSION

The high-resolution mass spectrum of methyl pachybioside shows its highest-mass ion peak at m/z 318.1678 (1, M-H_2O, $C_{15}H_{26}O_7^+$). The first decomposition pathway (Scheme 1) shows the formation of those fragments which are formed by the usual loss of the elements of water, methanol, and CH_3CHO in different sequences. It also includes the retro-Diels-

SCHEME 1



Alder fragmentation initiated by the double bond created by the loss of water or methanol, yielding species having the following m/z values:

318.1678 (1, M-H_2O, $C_{15}H_{26}O_7$)

260.1243 (2, 318-CH_2CHOCH_3, $C_{12}H_{20}O_6$)

228.1000 (3, 260-CH_3OH, $C_{11}H_{16}O_5$)

305.1602 (4, M-OCH_3, $C_{14}H_{25}O_7$)

273.1326 (5, 305-CH_3OH, $C_{13}H_{21}O_6$)

229.1071 (6, 273-CH_3CHO, $C_{11}H_{17}O_5$)

197.0816 (7, 229-CH_3OH, $C_{10}H_{13}O_4$)

272.1257 (8, M-2CH_3OH, $C_{13}H_{20}O_6$)

260.1243 (9, M-CH_3OH-CH_3CHO, $C_{13}H_{20}O_6$)

Besides these, one can also visualize fragmentation pathway II (Scheme 2) wherein the formation of the radical ion at the oxygen atom of the 2-OH group of the normal sugar leads to a 2-3 bond cleavage and loss of a small fragment 10 ($C_3H_6O_2$) of mass m/z 74.0373. The same type of fragmentation can be anticipated in the 2-deoxy sugar unit by radical-ion formation at the C-3 oxygen atom resulting in the cleavage of the C3-C4 bond which again loses a small fragment 11 ($C_5H_9O_2$) of mass m/z 101.0603.

Fragmentation route III is presented in Scheme 3 in which H-transfers break the disaccharide into monosaccharide units which undergo further fragmentation yielding species having the following m/z values:

145.8815 (12, 176-OCH_3, $C_7H_{13}O_3$)

113.0601 (13, 145-CH_3OH, $C_6H_9O_2$)

95.0498 (14, 113-H_2O, C_6H_7O)

144.0753 (15, 176-CH_3OH, $C_7H_{12}O_3$)

100.0519 (16, 144-CH_3CHO, $C_5H_8O_2$)

118.0616 (17, 176-$CH_3CH{=}CHOH$, $C_5H_{10}O_3$)

87.0450 (18, 118-OCH_3, $C_4H_7O_2$)

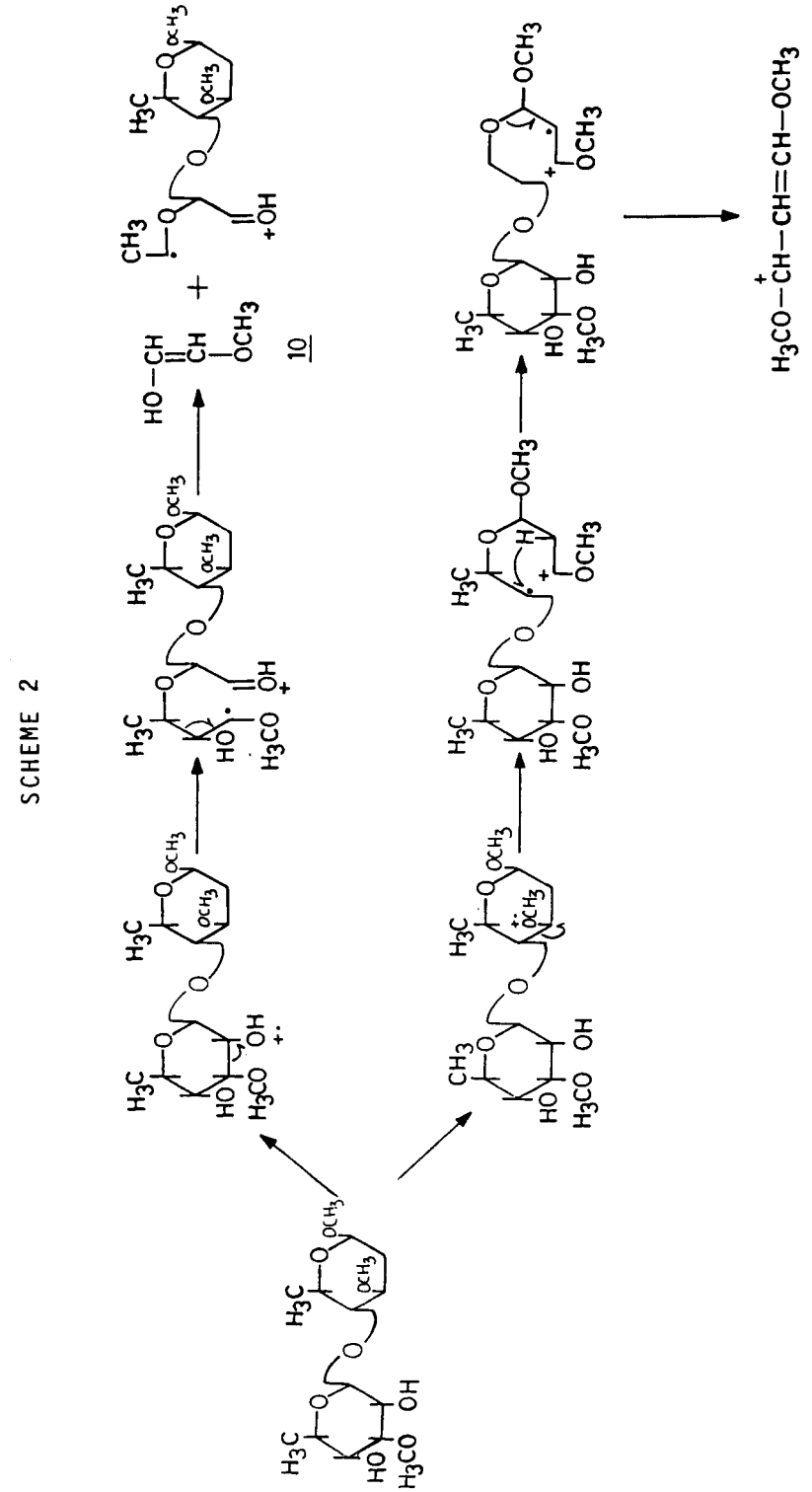
117.0551 (19, 118-H, $C_5H_9O_3$)

161.0814 (20, $C_7H_{17}O_4$)

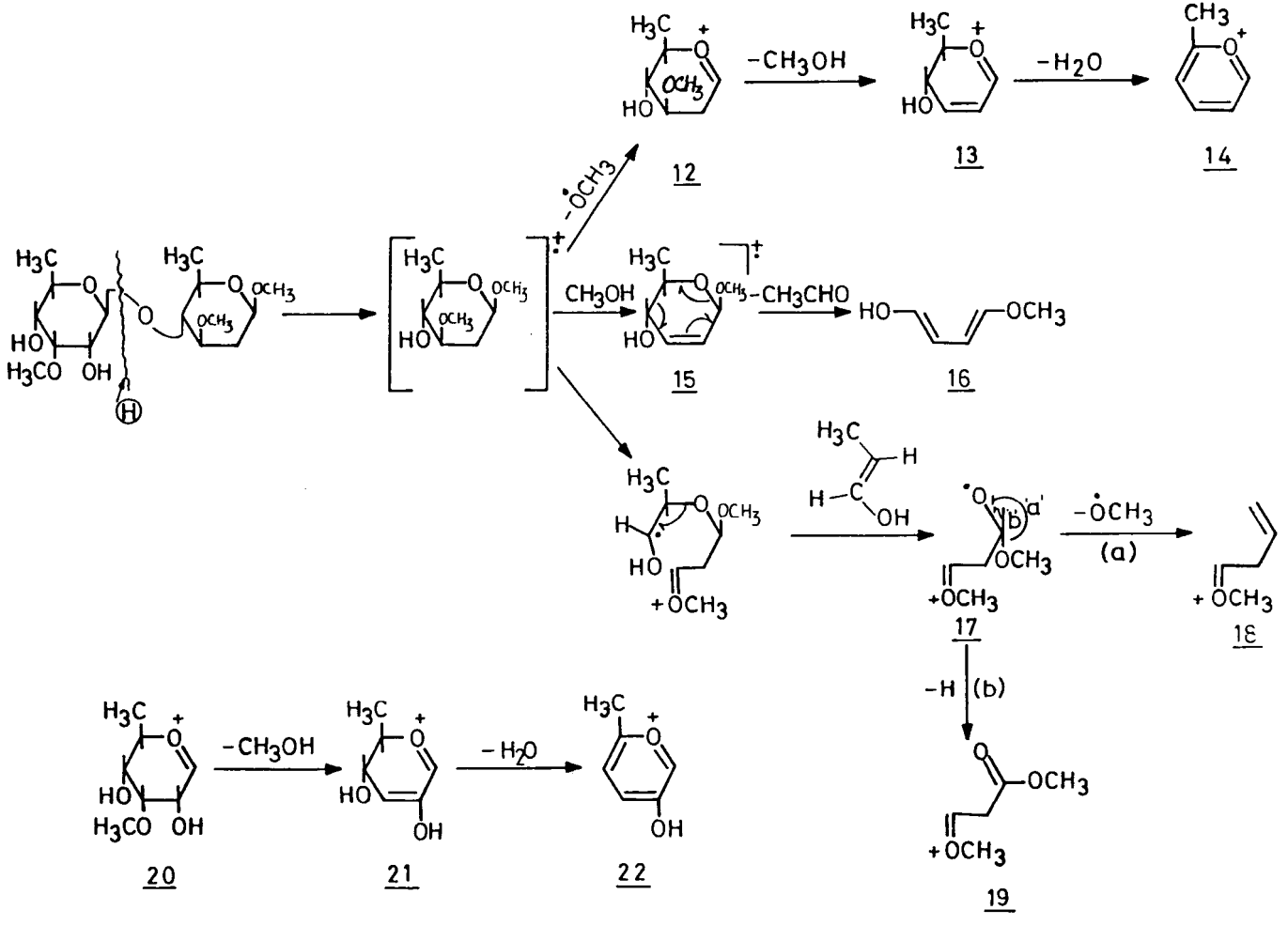
129.0549 (21, 161-CH_3OH, $C_6H_9O_3$)

111.0443 (22, 129-H_2O, $C_6H_7O_2$)

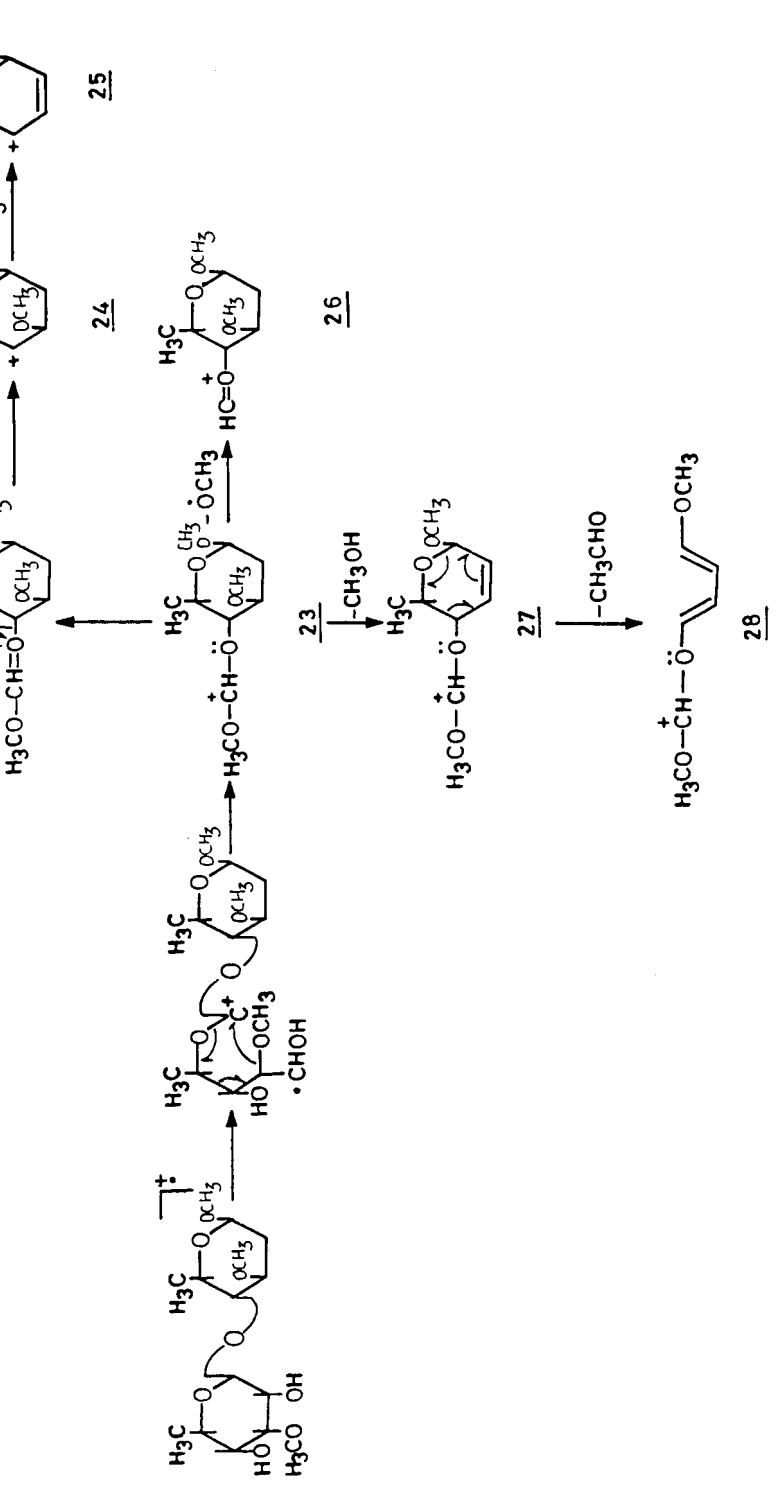
SCHEME 2



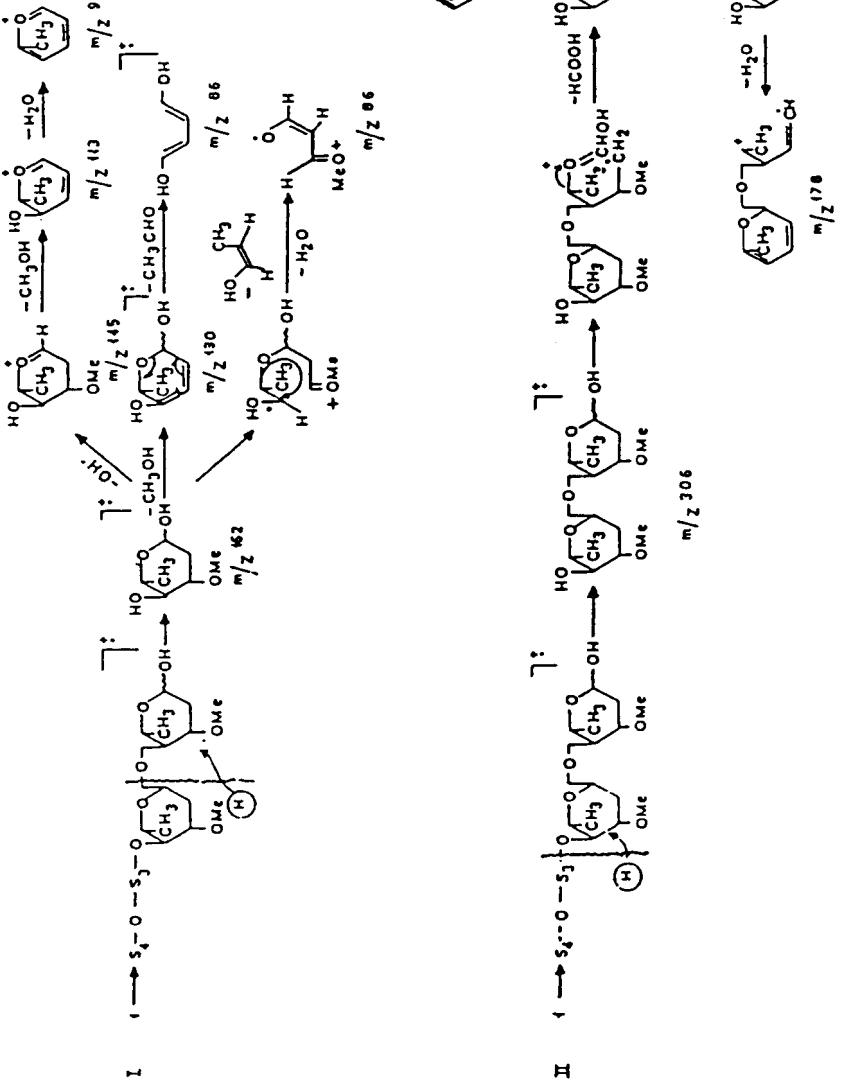
SCHEME 3

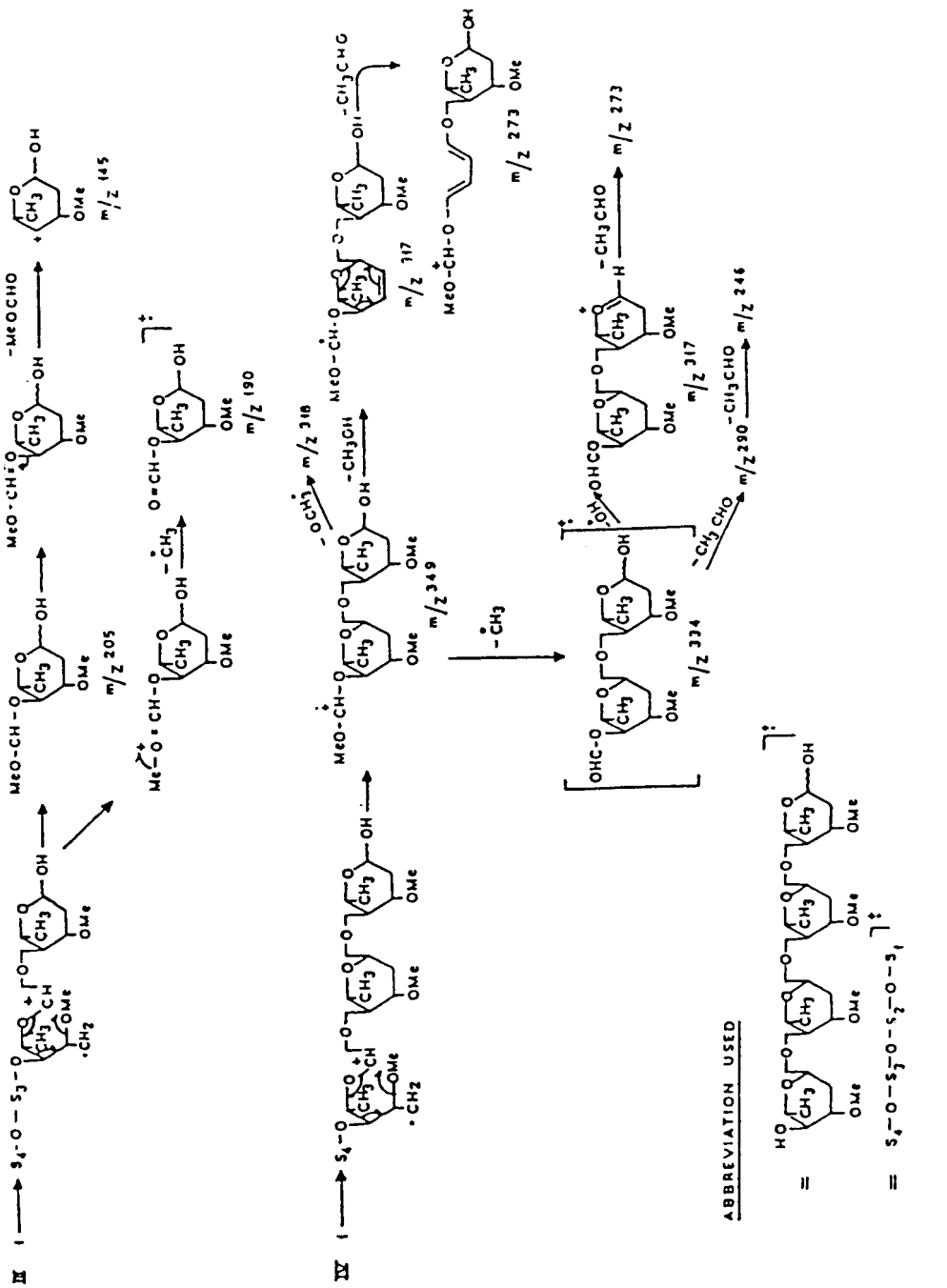


SCHEME 4



SCHEME 5





Fragmentation route IV (Scheme 4) defines the genesis of ions to be found in the rearrangement involving migration of the methoxyl group[5] after radical-ion cleavage of the C_1-C_2 bond, followed by the migration of the C-3 methoxyl group to C-1, resulting in the cleavage of a normal sugar unit. Further fragmentation of the monosaccharide unit is presumed to be arising from the characteristic fragmentation pattern of 2,6-dideoxyhexoses reported by Brown et al.[6] This route gives species with the following m/z values:

219.1231 (23, $C_{10}H_{19}O_5$)
159.1021 (24, 219-MeOCHO, $C_8H_{15}O_3$)
127.0758 (25, 159-CH_3OH, $C_7H_{11}O_2$)
188.1059 (26, 219-OCH_3, $C_9H_{16}O_4$)
187.0951 (27, 219-CH_3OH, $C_9H_{15}O_4$)
143.0708 (28, 187-CH_3CHO, $C_7H_{11}O_3$)

Based on the major fragmentation pathways that are operative in the mass spectra of underivatised disaccharides studied so far, almost all the prominent fragment ions of the tetrasaccharide orthenthose[7] could be interpreted in the context of the proposed structure. The mass spectrum of orthenthose fails to display its molecular ion, as it contains mass peaks of only smaller fragments comprised of monosaccharide and dissacharide units. A structurally significant ion peak is recorded at m/z 306 (6%), which corresponds to a disaccharide fragment. The relatively intense peak in the higher-mass region at m/z 290 (100%) corresponds to a fragment formed from the disaccharide fragment resulting from the rearrangement-cleavage of 1. Fragmentation routes I and II (Scheme 5) represent repeated H-transfers in the oligosaccharide, accompanied by the elimination of terminal sugars less water, giving rise to an ion of the same minimal mass as the molecular ion of the corresponding oligosaccharide with one less monosaccharide residue, and so on until only the monosaccharide remains.

Fragmentation routes III and IV (Scheme 5) show the genesis of ions formed in the rearrangement involving migration of the methoxyl group

after radical-ion cleavage of the C1-C2 bond, followed by the migration of the C-3 methoxyl group to C-1, resulting in the cleavage of the oligosaccharide. Further fragmentation of the smaller monosaccharide units is likely via processes characteristic of the fragmentation pattern of 2,6-dideoxyhexoses reported by Brown et al.[6] These account for most of the major peaks in the spectrum that fully support the structure for oleandrotetrose.

EXPERIMENTAL

General. Mass spectra were determined on AEI-MS-30 and JEOL-300 mass spectrometers.

β-Methyl pachybioside: Chromatographically pure crystalline cubes of this glycoside were obtained by extraction[8] of the dried aerial part of the plant Sarcostemma brevistigma: mp 135-36 °C, $[\alpha]_D^{25}$ -38° (MeOH) (identical with an authentic sample). Mass spectrum (m/z) of β-methyl pachybioside:

318.1678 (1, $C_{15}H_{26}O_7$)
305.1602 (4, $C_{14}H_{25}O_7$)
273.1326 (5, $C_{13}H_{21}O_6$)
272.1257 (8, $C_{13}H_{20}O_6$)
260.1243 (2 & 9, $C_{12}H_{20}O_6$)
229.1071 (6, $C_{11}H_{17}O_5$)
228.1000 (3, $C_{11}H_{16}O_5$)
219.1231 (23, $C_{10}H_{19}O_5$)
197.0816 (7, $C_{16}H_{13}O_4$)
188.1059 (26, $C_9H_{16}O_4$)
187.0951 (27, $C_9H_{15}O_4$)
161.0814 (20, $C_7H_{17}O_4$)
159.1021 (24, $C_8H_{15}O_3$)
145.0865 (12, $C_7H_{13}O_3$)
144.0753 (15, $C_7H_{12}O_3$)
143.0708 (28, $C_7H_{11}O_3$)

129.0549 (21, $C_6H_9O_3$)
127.0758 (25, $C_7H_{11}O_2$)
118.0616 (17, $C_5H_{10}O_3$)
117.0551 (19, $C_5H_9O_3$)
113.0601 (13, $C_6H_9O_2$)
111.0443 (22, $C_6H_7O_2$)
101.0603 (11, $C_5H_9O_2$)
100.0519 (16, $C_5H_8O_2$)
95.0498 (14, C_6H_7O)
87.0450 (18, $C_4H_7O_2$)
74.0373 (10, $C_3H_6O_2$)

Orthenthose. Chromatographically pure amorphous orthenthose (41 mg) was isolated from the acid hydrolysate of the extract obtained by a method reported earlier[8] from the shade twigs of Orthenthera viminea: $[\alpha]_D^{25}$ +47.5° (MeOH). The compound gave a blue coloration (for a 2-deoxy sugar) with vanillin-perchloric acid spray reagent,[9] gave positive test in both the xanthydrol[10] and Keller-Kiliani reactions,[11] and reduced Fehling's solution. Mass spectrum (m/z) of orthenthose: 594 (M^+ not observed), 420 (6%), 366 (2), 349 (9), 324 (2), 318 (2), 317 (2), 308 (28), 306 (6), 290 (100), 283 (2), 276 (38), 273 (17), 260 (3), 254 (6), 246 (4), 242 (3), 222 (3), 211 (2), 205 (12), 196 (3), 190 (2), 178 (16), 162 (51), 148 (12), 145 (9), 130 (21), 113 (31), 101 (29), 97 (42), 95 (54), 86 (13) and 78 (5).

Very mild acid hydrolysis of orthenthose. To a solution of orthenthose (2 mg) in methanol (0.5 mL) was added 0.01 M HCL (0.5 mL) in 99.5% aqueous methanol, and the solution was maintained at room temperature. After 7 days, it showed four spots on paper chromatography, two zones having mobilities identical to those of oleandrose (R_{Ole} 1.0) and orthenthose (R_{Ole} 0.29), respectively; the third spot (R_{Ole} 0.77) and the fourth spot (R_{Ole} 0.38) were presumably the partially hydrolysed products, i.e., the disaccharide and trisaccharide, respectively. After 15 days, the hydrolysate showed only

one spot which had the same mobility as oleandrose. Concentration of the solution afforded a colorless syrup (2 mg) having $[\alpha]_D^{25}$ +13.4° (MeOH), a specific rotation comparable to that of a sample authentic L-oleandrose.[12]

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