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Note

Electron impact mass spectra of per-*O*-acetyl derivatives of 4′-substituted glycopyranosylphenylamines

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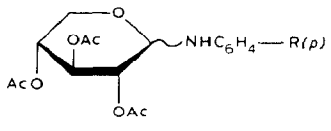
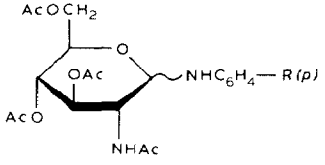
Some proteoglycans are covalently bound to proteins by a D-galactosyl-D-galactosyl-D-xylosyl linkage, with the D-xylosyl residue being attached to the hydroxy group of a seryl residue in the polypeptide[1]. Various alkyl β-D-xylopyranosides were shown to be artificial initiators in the biosynthesis[2–4], suggesting that they might modify the growth and metastatic potential of malignant neoplasms.

Koriya *et al.*[5] reported the inhibition of the growth of several malignant tumors[5] by *N*-phenoxycarbonyl-D-xylopyranosylamine in combination with a polysaccharide preparation, associated to the ability of this xyloside to serve as an artificial acceptor for proteoglycan chain initiation[6]. Wang *et al.*[7,8] recently reported the synthesis and ^{1}H-n.m.r. spectroscopy study of 4-substituted phenyl-D-xylopyranosylamines as the per-*O*-acetyl derivatives, as well as the inhibition of the replication of cultured B16 melanoma cells by these compounds, and their modifier capability in the synthesis of proteoglycans on the surface of these neoplastic cells.

In a previous study[9], we observed the structural parameters of some *N*-arylglycosylamines, where ^{1}H- and ^{13}C-n.m.r.-spectroscopy gave significant data for assessing the abundance of α and β anomers. Nelson and Lavin[10] have reported



1 R = NO_2
2 R = Cl
3 R = Me
4 R = MeO



5 R = NO_2
6 R = Cl
7 R = Me
8 R = MeO

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also ^{13}C-n.m.r. data for these derivatives obtained from D-glucose. However, the mass spectra of these compounds are lacking. The present report describes the mass spectral of several 4′-substituted per-*O*-acetyl derivatives of phenyl-D-xylopyranosyl- (**1–4**) and (2-acetamido-2-deoxy-*N*-phenyl-D-glucopyranosyl)-amine (**5–8**), and discuss the most significant fragmentation patterns.

EXPERIMENTAL

4′-Substituted phenyl-D-glycopyranosylamines[11]. — These compounds were synthesized by mixing equimolecular amounts of the reducing sugars and *p*-substituted anilines in water–ethanol, the ratio of which depended on the degree of solubility of the reactants. Glacial acetic acid (few drops) was added as catalyst. The mixture was stirred for 2–3 h <40° and extracted with diethyl ether. The reaction products were purified by t.l.c. in 2:2:1 benzene–acetone–methanol.

Per-O-acetyl derivatives (**1–8**)[12]. — The 4′-substituted phenyl-D-glycopyranosylamine (200 mg) was dissolved in 1:1 dry pyridine–acetic anhydride (4 mL) and heated to 100° in a sealed tube for 2 h. The reaction time was increased to 48 h when the reaction temperature was held at 0°. Methanol was added to the mixture after completion of the reaction, and all solvents were eliminated by evaporation under reduced pressure. Successive additions of toluene and evaporation were conducted until complete elimination of pyridine, and the end product was crystallized from chloroform.

Mass spectrometry. — Electron-impact mass spectra of **1–8** were obtained with a double-focusing JEOL, DX-300, mass spectrometer and a 3100 JEOL Data System with magnetic disks (4.56 Mw). The experimental conditions were: ionization energy, 15 and 70 eV; ionization current, 300 μA; accelerating voltage, 3 kV; and source temperature, 22°. The temperature of the direct inlet probe was programmed from 80 to 400° at $32° \cdot min^{-1}$. Mass spectra were recorded at 1 scan/s in a mass range of 30–500 amu.

RESULTS AND DISCUSSION

Per-O-acetyl derivatives of 1-N-aryl-D-xylopyranosylamines (**1–4**). — The main characteristic of the mass spectra (15 eV) of **1–4** was the occurrence of the molecular ion either at 15 or 70 eV, which is related to the molecule stabilization owing to the presence of a substituted aromatic ring. A similar behavior has been observed for the per-*O*-methyl ethers of phenyl glycosides[13], although the relative intensity of the molecular ion is comparatively smaller for the latter compounds. The molecular ions for the most commonly studied derivatives such as per-*O*-methyl[13,14], per-*O*-acetyl[15–17], per-*O*-benzoyl[18], per-*O*-trimethylsilyl[19] aldopyranoses, and per-*O*-benzoylated aldopyranosylamines[20] have not been observed. Another characteristic was the occurrence of the fragment type (M − NHPhR) with subsequent elimination of acetic acid (60 amu) and ketene (42 amu), leading

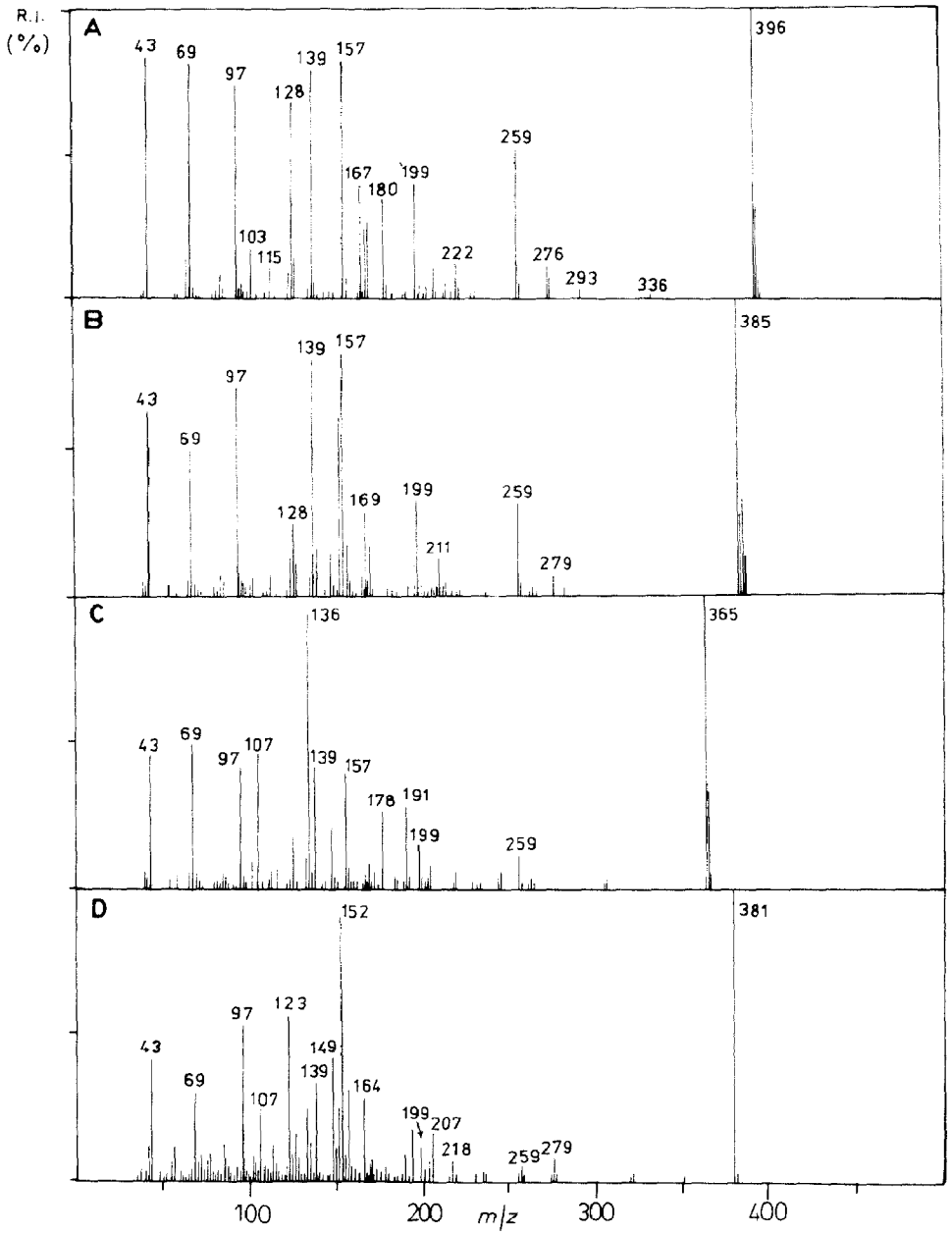

Fig. 1. Mass spectra (15 eV) of compounds **1**(A), **2**(B), **3**(C), and **4**(D).

to fragments of *m/z* 259, 199, 139, and 97 for nitro, chloro, methyl, and methoxy substituents, respectively, but which considerable higher intensities for the nitro and chloro derivatives. The origin of the fragments at *m/z* 157 and 115 in all derivatives could be deduced (a) by successive loss of ketene and acetic acid from the parent ion (*m/z* 199), and (b) by ring breakdown of the monosaccharide unit leading to the fragment from C-2, -3, and -4 with acetyl groups at O-2 and -4, which explains the high intensity for these fragments, even at 70 eV. The intensities of fragments formed from the molecular ion having the benzene ring substituent were of less intensity. However, the breakdown of the C-1–N bond gave relatively stable fragments, the intensity of which increased in the order methoxy, methyl, chloro, and nitro owing to charge localization on the arylamine group.

The occurrence of fragments derived from acetylation of the amine group was not observed for the nitro- (**1**), chloro- (**2**), and methyl-substituted compounds (**3**). I.r. and n.m.r. spectra of the acetyl derivatives showed that the amino group was not acetylated when the reaction was performed either at 0 or 100°. Therefore, fragments of type A (AcNHPhR) would not appear in the mass spectra of these compounds (*m/z* 180, 169, and 149 for nitro, chloro, and methyl derivatives, respectively). Nevertheless, fragments at *m/z* 222, 211, and 191, respectively, were observed which could be considered as fragments of type A + 42. If the simultaneous breakdown of the hemiacetal and C-2–C-3 bonds is considered, it would explain the occurrence of A + 42 fragments, by the subsequent loss of ketene. This assumption was confirmed by the study of the mass spectrum at 15 eV of the per-*O*-methyl derivative of **1**, the most intense fragments of which were at *m/z* 326, 295, 208, 193, 115, and 88.

The acetylation of the amine group in the methoxy-substituted compound **4** could be confirmed by repetitive scans of its reconstructed ion chromatogram. Both molecular ions of the unsubstituted and amine-substituted derivatives were observed at *m/z* 381 and 423, respectively, and a considerable increase in the relative intensity for the fragment at *m/z* 165 upon substitution. This increase is explained by the formation, from the acetylaryl group, of a fragment having the same *m/z* ratio, which led to fragments of *m/z* 108 (base peak) by loss of the AcNH group.

Per-O-*acetyl derivatives of 4'-substituted 2-acetamido-2-deoxy-1*-N-*phenyl*-D-*glucopyranosylamines* (**5**–**8**). — The presence of the nitrogen atom at C-2 in the glucose ring imparted some differences to the fragmentation pattern of 4'-substituted 1-*N*-aryl-2-acetamido-2-deoxy-D-glucopyranosylamines, although many fragmentation pathways are similar to those discussed above for *N*-arylxylosylamines (Fig. 2). Fragments having a higher relative intensity were those formed by loss of the acetamido group (M − 59), and subsequent elimination of acetic acid and ketene. The relative intensities of the fragments characteristic for acetyl derivatives of hexopyranoses were considerably reduced, as well as those formed from the arylamino group. However, the fragments formed from the simultaneous breakdown of the hemiacetal and C-2–C-3 bonds, as discussed above for *N*-aryl-

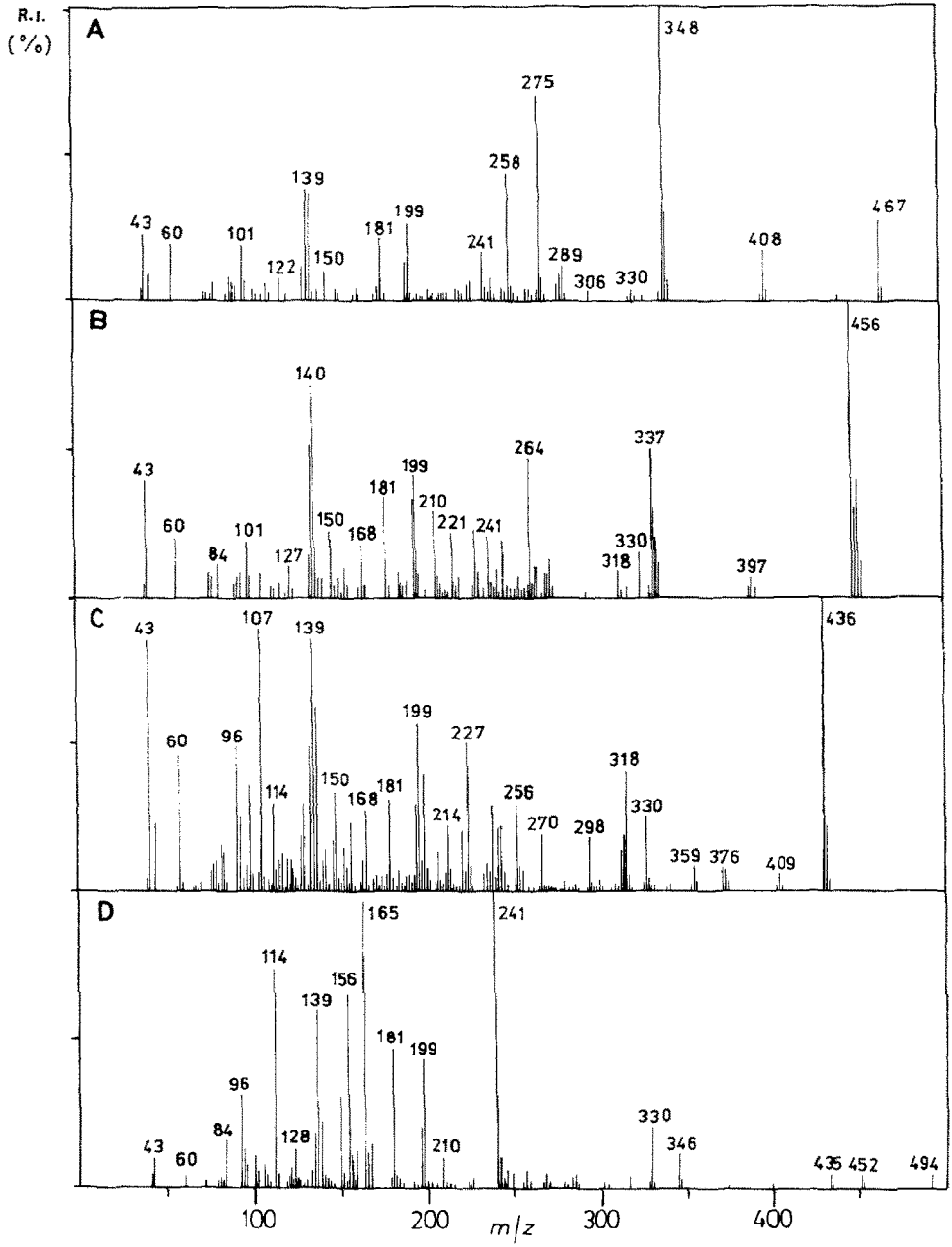

Fig. 2. Mass spectra (15 eV) of compounds **5**(A), **6**(B), **7**(C), and **8**(D).

xylosylamines, were not observed (*m/z* 222, 211, 191, 180, 169, and 149). Thus, the nitrogen atom at C-2 influenced markedly the fragmentation pattern of 4′-substituted 2-acetamido-2-deoxy-1-*N*-phenyl-D-glucopyranosylamines.

Some fragments (*m/z* 199 and 139) were observed in the mass spectra of acetyl derivatives of both xylosyl- and glucosyl-amine, but the fragmentation pathways considered for the former compounds could not be applied to the latter compounds. If the simultaneous breakdown of the hemiacetal and C-1–C-2 bonds is considered in a similar way as for the *N*-arylxylosylamines, it would explain the origin of fragments at *m/z* 199 and 140. The different mass spectra for *p*-substituted *N*-aryl-D-xylopyranosylamines and 4′-substituted 2-acetamido-2-deoxy-1-*N*-phenyl-D-glucopyranosylamines led to consider this technique for the analytical differentiation between 2-amino-2-deoxy and neutral monosaccharides. Low-voltage- and chemical-ionization spectroscopy were similarly applied to these compounds[21] and, therefore, they may be used alternatively.

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