Carbohydrate Research, 172 (1988) 160–163
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Electron-impact and chemical-ionization mass-spectral identification of methylated derivatives obtained from 2-acetamido-2,4-dideoxy- and 3-acetamido-3,4-dideoxy-DL-pentopyranoses

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(Received February 2nd, 1987; accepted for publication, May 20th, 1987)

Mass spectrometry has become one of the most powerful techniques for structural investigations of complex carbohydrates[1,2].

Interest in the synthesis and the structural characterization of novel amino sugars has provided a stimulus to maintain the need for systematic mass-spectral studies of series of amino sugars.

The unusual and novel amino sugars 2-amino-2,4-dideoxy-DL-pentose and 3-amino-3,4-dideoxy-DL-pentose constitute the glycosyl portion of various 2′- and 3′-aminodideoxyribonucleosides that have been shown to exhibit antiviral and antitumor properties[3,4].

In a continuation of our studies on the mass spectrometry of some acetylated derivatives of these novel aminodideoxypentoses[5], we now report the electron-impact and chemical-ionization mass-spectra of some permethylated glycosides of, and methylated alditol acetates from, 2-acetamido-2,4-dideoxy- and 3-acetamido-3,4-dideoxy-DL-pentoses.

The electron-impact mass spectra of methyl 2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**1**) and methyl 3,4-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**2**) are shown in Table I, and the proposed modes of formation of the specific ions formed during the break-

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0008-6215/88/$03.50

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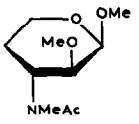
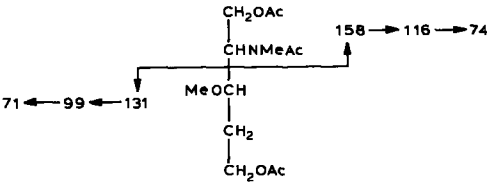


2



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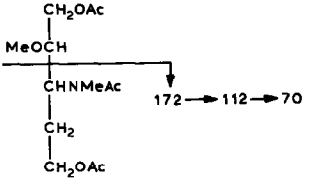

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down of the molecular radical-ion are identical to the ones obtained for the fully acetylated derivatives of **1** and **2** (see Ref. 5). The e.i.-mass spectra of *O*-(trideuteriomethyl)ated and *N*-(trideuterioacetyl)ated analogs of **1** and **2** support this conclusion.

The isobutane chemical-ionization mass spectrum of the methyl glycoside **1** gave, *inter alia*, peaks at the following *m/z* values: 144 (5.1), 154 (12.5), 186 (18.3) 218 (100), 219 (11.5), and 256 (2.8). The ion at *m/z* 256 is assigned to $[M + C_3H_3]^+$.

TABLE I

ELECTRON-IMPACT MASS SPECTRA AND RETENTION TIMES OF THE VARIOUS DERIVATIVES **1–4** OF 2-AMINO-2,4-DIDEOXY- AND 3-AMINO-3,4-DIDEOXY-DL-PENTOSE

Compound	m/z *(intensity, %)*	T_R[a]
Methyl 2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**1**)	217(3.1), 186(3.0), 185(2.2), 170(3.3), 154(18.5), 144(41.4), 129(28.0), 126(30.2), 125(38.1), 114(39.1), 112(12.1), 99(59.5), 98(39.2), 87(100.0), 84(29.9), 75(45.1), 72(63.1), 57(49.6), 43(60.3)	0.41
Methyl 3,4-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**2**)	218(4.8), 202(9.7), 187(3.2), 186(10.5), 170(5.1), 157(68.4), 144(13.6), 142(100.0), 129(29.7), 114(15.0), 113(34.4), 112(28.9), 100(10.1), 99(11.1), 88(71.2), 87(51.4), 86(36.4), 84(62.4), 43(57.1)	0.43
1,5-Di-*O*-acetyl-2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-DL-*threo*-pentitol (**3**)	158(20.0), 149(6.4), 142(2,8), 131(21.1), 116(100.0), 99(28.8), 74(38.5), 71(49.3), 57(18.5), 43(62.1)	0.62
1,5-Di-*O*-acetyl-3,4-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-DL-*threo*-pentitol (**4**)	216(3.1), 172(21.2), 112(100,0), 70(69.7), 43(23.2)	0.63

[a]Retention time relative to that of D-glucitol hexaacetate as unity.

The primary fragment-ion at *m/z* 186 is generated by the loss of a molecule of methanol from the protonated molecular ion $[M + H]^+$ at *m/z* 218 (base peak). The loss of a molecule of ketene from the ion at *m/z* 186 produces the ion at *m/z* 144.

The isobutane chemical-ionization mass spectrum of the methyl glycoside **2** gave, *inter alia*, peaks of the following *m/z* values: 256 (2.7), 219 (10.9), 218 (100), 217 (4.2), 187 (5.0), 186 (48.5), and 185 (4.0). The ions at *m/z* 256 and *m/z* 186 are assigned as before. It should be noted that the ion at *m/z* 186 does not lose a molecule of ketene, contrary to its corresponding ion in the chemical-ionization mass spectrum of the isomeric methyl glycoside **1**.

The simple and well established behavior of partially methylated alditol acetates upon electron impact[2,6] makes these derivatives suitable for the identification of these novel aminodideoxypentoses.

The electron-impact mass spectra of 1,5-di-*O*-acetyl-2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-DL-*threo*-pentitol (**3**) and 1,5-di-*O*-acetyl-3,4-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-DL-*threo*-pentitol (**4**), given in Table I, were found to obey the same fragmentation pattern as other partially methylated alditol acetates of amino sugars[6]. As expected, the fragmentation pattern of alditol derivatives **3** and **4** was governed by fission between C-2 and C-3 of the alditol chains, to afford the primary fragment-ions at *m/z* 158 and 172, respectively. The breakdown processes leading to the production of the aformentioned fragment-ions were investigated by *N*-(trideuterioacetyl)ation. In effect, the corresponding *N*-[methyl(trideuterioacetyl]-ated derivatives of **3** and **4** gave a fragmentation pattern analogous to that of their precursors, and consequently, the ions at *m/z* 158 and 172 shifted to three a.m.u. higher respectively.

From Table I, it may be seen that the relative retention time cannot be depended on as a diagnostic tool for the identification of this series of reported derivatives. This makes it essential that the final identification be made by electron-impact mass spectrometry.

In conclusion, the relevant data obtained from the electron-impact and chemical-ionization mass spectra provide valuable information for the clear identification of this novel series of reported derivatives of aminodideoxypentoses.

EXPERIMENTAL

Reagents. — All of the reagents and solvents were of analytical grade, were glass-distilled before use, and were stored over molecular sieves 4A.

Synthesis of the methyl glycosides. — The methyl 2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**1**) and methyl 3,4-dideoxy-2-*O*-methyl-3-(*N*-methylacetamido)-β-DL-*threo*-pentopyranoside (**2**) were obtained from the fully acetylated precursor derivatives[5] by methylation of the corresponding acetylated methyl glycosides by the Hakomori method[7] and were purified by passage through a column of Sephadex LH-20.

Synthesis of the alditol acetates. — The methyl glycosides **1** and **2** were hydro-

lyzed with 0.5M trifluoroacetic acid for 1 h at 100°, and the solutions were evaporated to dryness. The free dideoxyamino sugars resulting were reduced with sodium borohydride in water for 1 h at room temperature, the base neutralized with dilute acetic acid, the solution evaporated to dryness, and traces of solvents co-distilled with methanol–acetic acid. Each alditol was acetylated with 1:1 acetic anhydride–pyridine for 1 h at 100°, and the solution evaporated to afford, respectively, 1,5-di-*O*-acetyl-2,4-dideoxy-3-*O*-methyl-2-(*N*-methylacetamido)-DL-*threo*-pentitol (**3**) and 1,5-di-*O*-acetyl-3,4-dideoxy-2,*O*-methyl-3-(*N*-methylacetamido)-DL-*threo*-pentitol (**4**).

Gas–liquid chromatography. — Gas–liquid chromatography was performed on a fused-silica capillary column (0.25 × 0.23 mm, i.d., film thickness 0.15 μm) of WCOT CP-Sil 5CB (Chrompack), at 150°, mounted in a Perkin–Elmer Model 8310 gas chromatograph equipped with a flame-ionization detector.

Gas–liquid chromatography–mass spectrometry. — Combined gas–liquid chromatography–electron-impact mass spectrometry was performed in a Hewlett Packard Model 5985 A GC/MS/DS instrument equipped with a dual e.i./c.i. source. E.i. spectra were recorded at a source temperature of 160° and an ionizing voltage of 70 eV. C.i. spectra were recorded at a source pressure of 120 Pa (using isobutane as the reagent gas and carrier), a source temperature of 150°, and an ionization voltage of 230 eV. The temperature program for the e.i. and c.i. spectra started at 120° and was increased to 270° at 10°/min, using a packed glass column of 2% of OV-17 on Chromosorb W (H.P.) (80–100 mesh).

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