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Note

E.i.- and c.i.-mass spectrometry of peracetylated methyl glycosides of and peracetylated alditols from 2-amino-2,4-dideoxy- and 3-amino-3,4-dideoxy-DL-pentopyranoses

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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[1–3]. These aminodideoxypentoses could also be possible constituents of bacterial lipopolysaccharides, which are a unique class of macromolecules and constitute a characteristic attribute of Gram-negative bacteria[4,5].

In a continuation of our studies on the mass spectrometry of carbohydrates[6–9], we now report the electron-impact and chemical-ionization mass spectra, and proposed fragmentation-patterns, of some peracetylated methyl glycosides of, and alditols from, these novel 2-amino-2,4-dideoxy- and 3-amino-3,4-dideoxy-DL-pentoses.

The mass spectra of peracetylated methyl glycosides have been extensively studied[10], and it has been established that the fragmentation routes are similar to those established for the fully methylated glycosides[11,12]. In the following rationale, the nomenclature adopted by Kochetkov and Chizhov[11] will be used, but only when fragment-ions having similar structures, and produced by analogous sequences of fragmentation, are recognized.

In the c.i.-mass spectrum of methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-β-DL-*threo*-pentopyranoside (**1**), the molecular radical-ion $M^{+\cdot}$, formed by expulsion of one electron upon impact and subsequent ionization of the ring-oxygen atom, is

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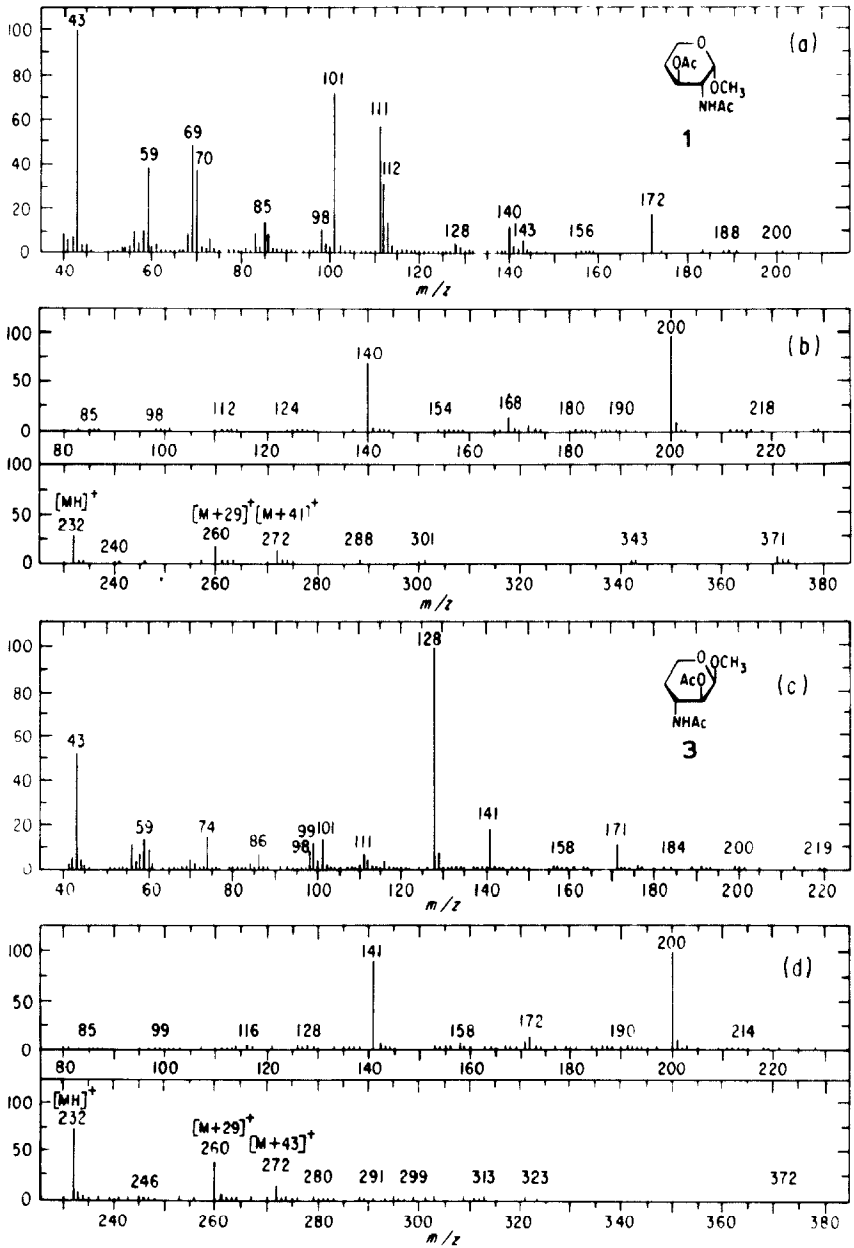

Fig. 1. Mass spectra of methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-β-DL-*threo*-pentopyranoside (**1**) and methyl 3-acetamido-2-*O*-acetyl-3,4-dideoxy-β-DL-*threo*-pentopyranoside (**3**) (a) and (c), electron-impact ionization; (b) and (d), chemical-ionization.

not observed, as it undergoes rapid transformation into stable ions (see Fig. 1a).

A summary of the proposed modes of formation, and suggested structures, of the specific ions formed during the breakdown of the molecular radical ion are given in Fig. 2.

It may be seen that one of the important, initial cleavages is dominated by elimination of the 2-acetamido group, by a route which involves loss of one molecule of acetamide to afford the primary fragment-ion at *m/z* 172, to which we tentatively assign structure **b**. Elimination of a molecule of acetic acid from ion **b** produces the secondary fragment-ion* at *m/z* 112, to which is assigned structure **c**.

A conjugated electronic shift in the sugar molecule following the well known, H sequence of fragmentation[11], affords the primary fragment-ion $\mathbf{H}_1^2$ at *m/z* 143; this loses a molecule of ketene, to yield the intense, secondary fragment-ion at *m/z* 101, which, in turn, loses a molecule of ketene, to generate the ion at *m/z* 59.

The primary fragment-ion $\mathbf{A}_1$ at *m/z* 200 is produced by the loss of the methoxyl radical from the molecular radical-ion[11]. Elimination of one molecule of acetic acid from the ion $\mathbf{A}_1$ generates the secondary fragment-ion $\mathbf{A}_2$ at *m/z* 140, which loses a molecule of ketene to form the ion* at *m/z* 98.

Elimination of the 3-acetoxyl group, by a route which involves loss of a molecule of acetic acid, presumably produces an unstable, primary fragment-ion, which breaks down by electronic shifts, to yield the intense, secondary fragment-ion at *m/z* 111, to which we assign structure **d**, and it appears to be mainly composed of C-2, C-3, C-4, and C-5. Elimination of one molecule of ketene from ion **d** gives the ion at *m/z* 69, assigned structure **e**.

The alternative, heterolytic cleavage of the C-1–C-2 bond of the sugar molecule logically leads, by two different routes, to either of the primary fragment-ions at *m/z* 98 or 112, respectively assigned structures **f** and **g**. The ion **f** appears to be mainly composed of C-2, C-3, and C-4, and it loses a molecule of ketene to afford the ion at *m/z* 56. Similarly, the ion **g** seems to be mainly composed of C-2, C-3, C-4, and C-5, and it loses a molecule of ketene to afford the ion at *m/z* 70.

The breakdown processes leading to the production of the aforementioned fragment-ions were investigated by using a deuterium-labeling procedure. In effect, when the methyl glycoside **1** was *O*-deacetylated, and the product (trideuterioacetyl)ated, the corresponding methyl 2-acetamido-2,4-dideoxy-3-*O*-(trideuterioacetyl)-β-DL-*threo*-pentopyranoside (**2**) was obtained. The e.i.-mass spectrum of the (trideuterioacetyl)ated methyl glycoside **2** gave, *inter alia*, peaks at the following *m/z* values: 43 (53.1), 46 (54.5), 56 (12.4), 58 (11.6), 60 (50.8), 68 (14.7), 69 (92.5), 70 (66.4), 85 (24.9), 98 (17.3), 100 (7.5), 102 (100), 111 (92.5), 112 (49.1), 113 (22.7), 128 (6.4), 140 (22.6), 146 (7.7), 175 (32.5), and 203 (1.3). These peaks are in agreement with the fragmentation pattern expected for this (trideuterioacetyl)ated glycoside.

*The ions at *m/z* 112 and 98 are also represented by two other, diastereomeric fragments, assigned structures **q** and **f**, respectively, as they account for the ions at *m/z* 70 and 56.

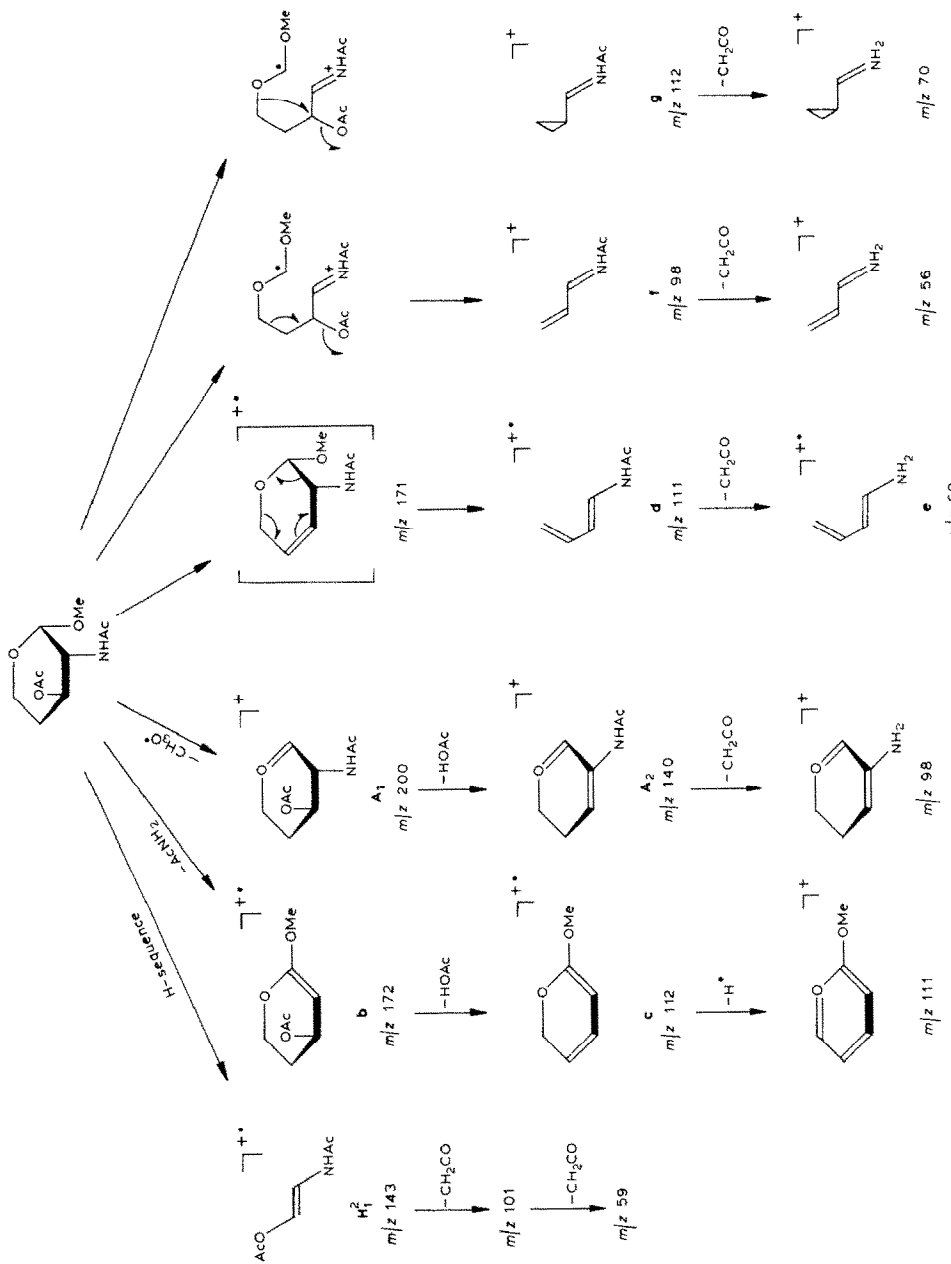

Fig. 2. Proposed fragmentation pattern of methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-β-D-*threo*-pentopyranoside (**1**).

Thus, the primary fragment-ions $\mathbf{A}_1$, **b**, and $\mathbf{H}_1^2$, at *m/z* 203, 175, and 146, respectively, have, indeed, shifted to three a.m.u. higher than the corresponding ions obtained in the e.i.-mass spectrum of the methyl glycoside **1**. Similarly, the secondary fragment-ions, at *m/z* 102 and 60, respectively, have shifted to one a.m.u. higher. The aforementioned shifts observed in the e.i.-mass spectrum of the deuterium-labeled isomer **2** reinforce the proposed fragmentation-pattern of methyl glycoside **1**.

The (methane) c.i.-mass spectra of the methyl glycoside **1** showed the protonated molecular-ion $[MH]^+$ at *m/z* 232. The ions $[M + 29]^+$ and $[M + 41]^+$, are at *m/z* 260 and 272, respectively. The primary fragment-ion at *m/z* 200 (base peak) is generated by the loss of a molecule of methanol from the protonated molecular-ion. The loss of a molecule of acetic acid from the ion at *m/z* 200 produces the ion at *m/z* 140 (see Fig. 1b).

The salient features of the e.i.-mass spectrum of methyl 3-acetamido-2-*O*-acetyl-3,4-dideoxy-β-DL-*threo*-pentopyranoside (**3**) (see Fig. 1c) are the absence of the molecular radical-ion $M^{\ddagger}$ and the high abundance of the fragment-ion at *m/z* 128 (base peak). A summary of the tentative modes of formation, and fragmentation routes, of the specific ions is proposed in Fig. 3.

It may be observed that one of the important, initial cleavages is dominated by elimination of the 2-acetoxyl group by two distinct routes, which involve loss of a molecule of acetic acid from either C-2 and C-1 or C-2 and C-3, to afford the primary fragment at *m/z* 171, to which we tentatively assign the two structures **h** and **h'**. The ion **h** loses a molecule of acetamide, to form the secondary fragment-ion at *m/z* 112, assigned structure **i**, which, in turn, loses a hydrogen radical to afford the ion at *m/z* 111. Also, the ion **h'** breaks down by electronic shifts, to give the secondary fragment-ion at *m/z* 141, to which is assigned structure **j**. Elimination of one molecule of ketene from ion **j** produces the ion at *m/z* 99, assigned structure **k**.

It is important to note that elimination of a molecule of acetamide from the primary fragment $\mathbf{A}_1$ (ref. 11) at *m/z* 200, also produces another ion, at *m/z* 141, assigned structure $\mathbf{A}_2'$. Similarly, elimination of a molecule of ketene from ion $\mathbf{A}_2'$ produces the ion at *m/z* 99 assigned structure **k'**.

The primary fragment-ion $\mathbf{H}_1^2$ at *m/z* 143, produced by fragmentation of the sugar molecule by the H sequence[11], fragments further, by elimination of a molecule of ketene, to yield the ion at *m/z* 101, which, in turn, loses a molecule of ketene to afford the ion at *m/z* 59.

The alternative, heterolytic cleavage of the C-3–C-4 bond of the sugar molecule logically leads to the prominent, primary fragment-ion at *m/z* 128 (base peak), assigned structure **1**. The latter ion is stabilized by resonance, and seems to be mainly composed of C-1, C-2, and C-3.

The breakdown processes leading to the production of the aforementioned fragment-ions were investigated by (trideuterioacetyl)ation. The e.i.-mass spectrum of methyl 3-acetamido-3,4-dideoxy-2-*O*-(trideuterioacetyl)-β-DL-*threo*-pento-

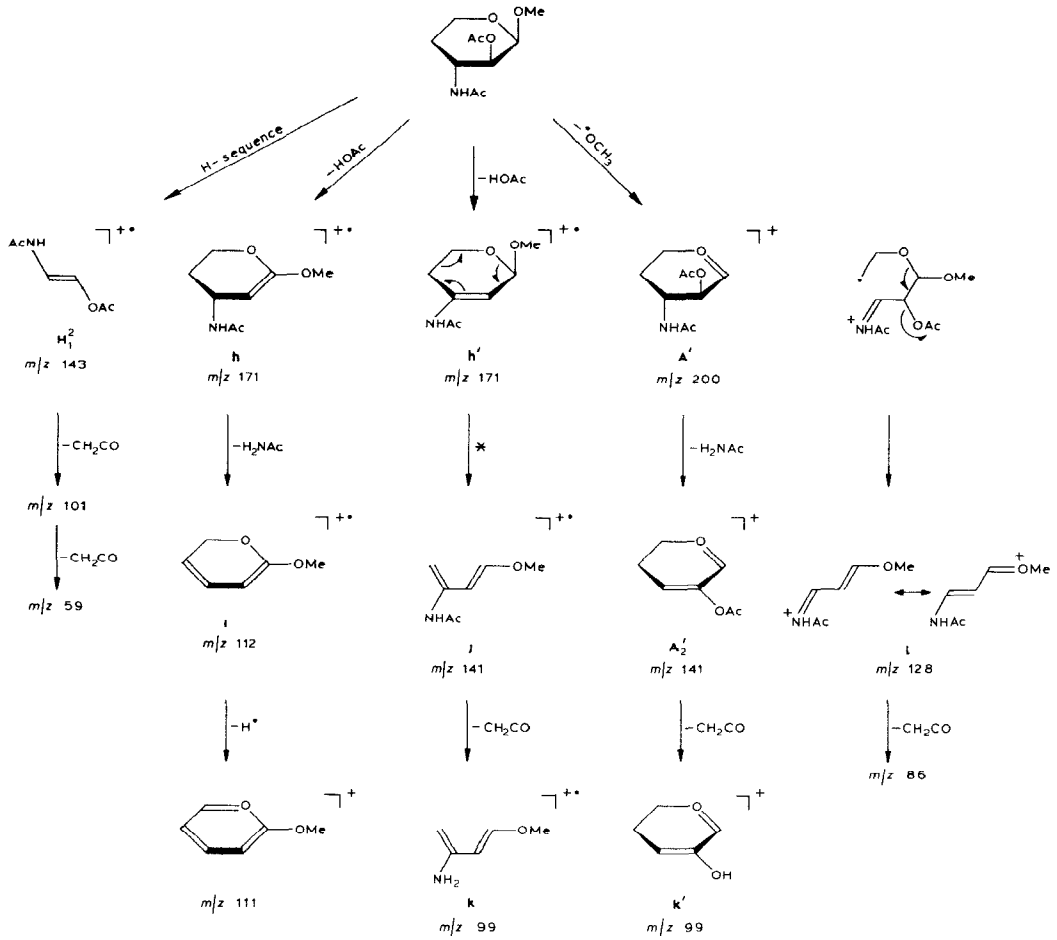

Fig. 3 Proposed fragmentation pattern of methyl 3-acetamido-2-*O*-acetyl-3,4-dideoxy-β-DL-*threo*-pentopyranoside (**3**). *, Fragmentation route proven by *O*-trideuterioacetylation not to occur.

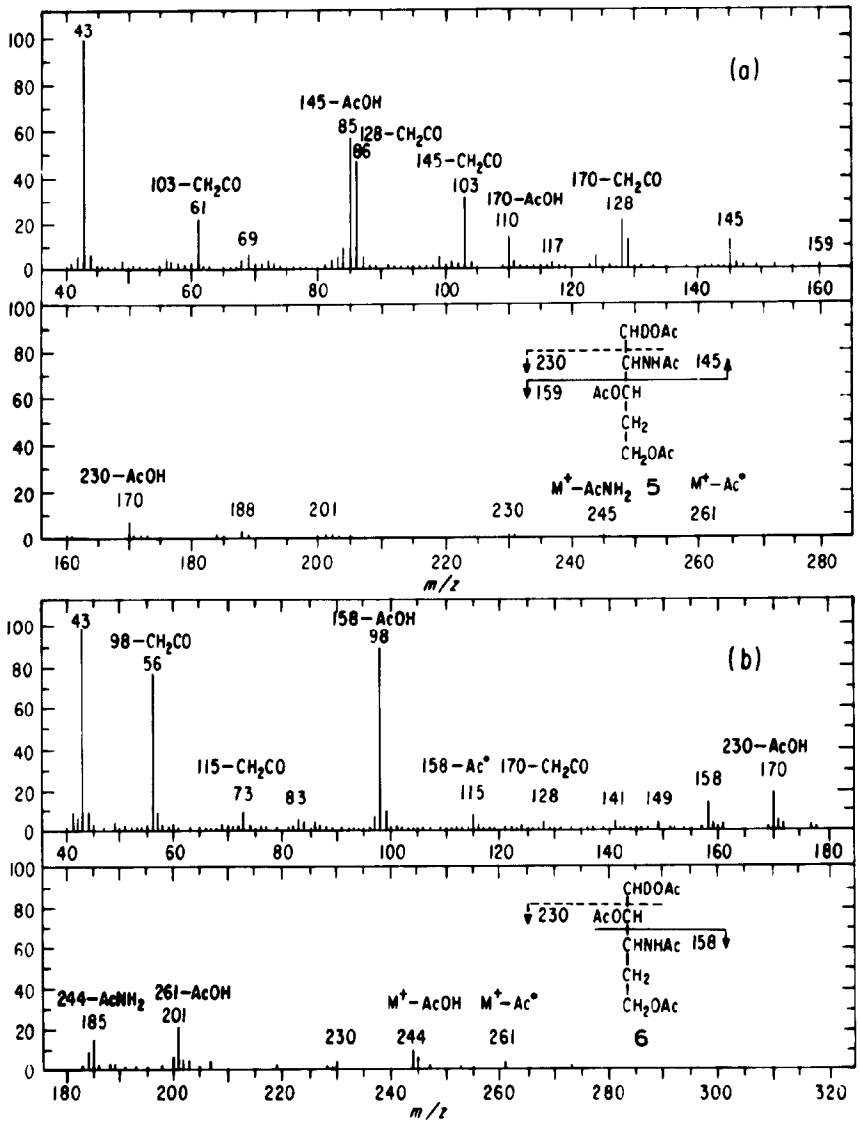

Fig. 4. E.i.-mass spectra of (a), 2-acetamido-1,3,5-tri-*O*-acetyl-1-deuterio-2,4-dideoxy-DL-*threo*-pentitol (**5**), and (b), 3-acetamido-1,2,4-tri-*O*-acetyl-1-deuterio-3,4-dideoxy-DL-*threo*-pentitol (**6**).

pyranoside (**4**) gave, *inter alia*, peaks at the following *m/z* values: 43 (55.9), 46 (64.7), 56 (15.9), 57 (11.6), 60 (21.7), 61 (15.1), 75 (22.4), 86 (7.3), 100 (13.5), 102 (14.3), 111 (8.1), 112 (6.3), 128 (100), 144 (18.3), 171 (4.8), and 203 (4.0).

These peaks are in agreement with the fragmentation pattern expected for this (trideuterioacetyl)ated glycoside. Thus, the fragment-ions $\mathbf{A}_1$ and $\mathbf{A}_2'$, at *m/z* 203 and 144, respectively, have indeed shifted to three a.m.u. higher than the

corresponding ions obtained in the e.i.-mass spectrum of the methyl glycoside **3**. Similarly, the ions at *m/z* 102, 100 (ion **k'**), and 60 have respectively shifted to one a.m.u. higher. The aforementioned shifts of the deuterium-labeled isomer **4** reinforce the proposed fragmentation pattern of methyl glycoside **3**, and prove beyond doubt the non-viability of the fragmentation route that presumably yielded the fragment-ions **h'**, **j**, and **k**.

The (methane) c.i.-mass spectrum of the methyl glycoside **3** showed the protonated molecular-ion $[MH]^+$ at *m/z* 232 (see Fig. 1d), and the ions $[M + 29]^+$ and $[M + 41]^+$ at *m/z* 260 and 272, respectively. The primary fragment-ion at *m/z* 200 (base peak) is generated by the loss of a molecule of methanol from the protonated molecular-ion. Contrary to its corresponding ion in the c.i.-mass spectrum of the diastereomeric methyl glycoside **1**, the ion at *m/z* 200 loses a molecule of acetamide (instead of a molecule of acetic acid), to form the secondary fragment-ion at *m/z* 141. Although the last feature is the only diagnostic characteristic that permits differentiation between the c.i.-mass spectra of the diastereomeric methyl glycosides **3** and **1**, the fragmentation pattern of the aforementioned glycosides is governed by the same consecutive loss of the C-1 and C-3 substituents, respectively.

The simple and well established behavior of alditol acetates upon electron impact[12,13] makes these derivatives suitable for the identification of these novel aminodideoxy sugars. The e.i.-mass spectra of 2-acetamido-1,3,5-tri-*O*-acetyl-1-deuterio-2,4-dideoxy-DL-*threo*-pentitol (**5**) and 3-acetamido-1,2,4-tri-*O*-acetyl-1-deuterio-3,4-dideoxy-DL-*threo*-pentitol (**6**), shown in Fig. 4, were found to obey the same fragmentation rules as other alditol acetates of amino sugars[12]. As expected, the fragmentation pattern of alditol acetates **5** and **6** was governed by fission between C-2 and C-3 of the alditol chains, to afford the primary fragment-ions at *m/z* 145 and 158, respectively.

In conclusion, the relevant data obtained from the e.i.- and c.i.-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, and were used without purification.

Synthesis of the methyl glycosides. — The methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-α-DL-*threo*-pentopyranoside (**1**) and methyl 3-acetamido-2-*O*-acetyl-3,4-dideoxy-β-DL-*threo*-pentopyranoside (**3**) were obtained from their precursor *N*-(trifluoroacetyl)ated derivatives[2,14,15] in the following ways. *N*-De(trifluoroacetyl)ation was effected by dissolving the corresponding *N*-(trifluoroacetyl)ated methyl glycosides in 50% ethanol, and treating with an ~2-fold excess of Dowex-1 (OH^-) resin, suspended in 50% ethanol[16]. *N*-De(trifluoroacetyl)ation was complete within 1 h; the resin was filtered off, and the filtrate evaporated to dryness. The methyl glycosides obtained were acetylated with 1:1 acetic anhydride–pyridine for 1 h at

100°, and the solutions evaporated to dryness. The methyl glycosides **1** and **3** were *O*-deacetylated with 1% sodium methoxide in methanol for 3 h at room temperature, the base neutralized with Dry Ice, the solution evaporated, and the product (trideuterioacetyl)ated with trideuterioacetic anhydride in pyridine for 1 h at 100°.

Synthesis of the alditol acetates. — The methyl glycosides **1** and **3** were hydrolyzed with M hydrochloric acid for 1 h at 100°, followed by evaporation to dryness. The free amino sugars resulting were reduced with sodium borodeuteride in water for 1 h at room temperature, followed by neutralization with dil. acetic acid, evaporation to dryness, and co-distillation of traces of solvents with methanol–acetic acid. The corresponding alditols **5** and **6** were acetylated with 1:1 acetic anhydride–pyridine for 1 h at 100°, and the solution evaporated.

Gas-liquid chromatography–mass spectrometry. — Combined gas-liquid chromatography–electron-impact mass spectrometry was performed in a Hewlett–Packard model 5985A 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. Combined gas-liquid chromatography–(methane) chemical-ionization mass spectrometry was performed in the same instrument. C.i. spectra were recorded at a source pressure of 120 Pa, using methane 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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