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ANALYSIS OF SUGAR DERIVATIVES BY CHEMICAL-IONIZATION MASS-SPECTROMETRY*

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ABSTRACT

D-Glucose diethyl dithioacetal (**1**), its penta-*O*-acetyl derivative (**2**), penta-*O*-acetyl-*aldehydo*-D-glucose (**3**), L-*xylo*-hexulose phenylosotriazole (**4**), 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**5**), 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**6**), 1,2-*O*-isopropylidene-α-D-glucofuranose (**7**) and its triacetate (**8**), 1,6-anhydro-β-D-galactopyranose (**9**) and its triacetate (**10**), D-glucopyranose (**11**), methyl β-D-glucopyranoside tetraacetate (**12**), 1-thio-β-D-glucopyranose pentaacetate (**13**), β-D-fructofuranose pentaacetate (**14**), and raffinose hendecaacetate (**15**) have been examined by chemical-ionization mass-spectrometry with both isobutane and ammonia as ionizing intermediates. Extreme simplicity characterizes these spectra, and, in most instances, molecular-weight data are available from intact, proton—or NH_4^+—capture ions; the limited fragmentation that occurs corresponds in large measure to simple dehydration or substituent-cleavage processes, and is strongly dependent upon the groups present, so that considerable information about the substituent groups in the sugar molecule may be inferred.

INTRODUCTION

Electron-impact mass-spectrometry (e.i.–m.s.) is an established tool for obtaining structural information on submilligram quantities of carbohydrate derivatives[2–4]; the peracetates and per(trimethylsilyl) derivatives are particularly useful for this purpose, and can afford detectable molecular ions for oligosaccharides at least as large as tetrasaccharides, together with structurally significant fragment-ions[5]. However, the high energies used for ionization oftentimes cause extensive

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fragmentation, so that spectra may be dominated by small, stable fragment-ions at the expense of the large ions, which are structurally more meaningful.

The technique of chemical-ionization mass-spectrometry (c.i.–m.s.) offers a useful complement to e.i.–m.s. as an analytical tool in the carbohydrate field. In c.i.–m.s., a dilute (~0.1%) gaseous solution of the sample in an appropriate reagent gas is bombarded by electrons. The reagent gas experiences most of the ionization by electron impact, and the primary ions thus formed undergo a number of deactivating collisions before effecting a Lewis acid–Lewis base type of reaction to transfer a small, charged species to or from the neutral sample-molecule, which is thereby converted into a relatively low-energy, even-electron ion. The identity of the species transferred in the initial step is dependent upon the ionizing gas and upon features within the sample molecule. Some indication of the scope of c.i.–m.s. has been provided by studies[6] of unsubstituted D-glucopyranose and methyl α-D-glucopyranoside with methane and ammonia as the ionizing gases, the macrolide-sugar antibiotic erythromycin B (ref. 7), the aminoacyl thioglycoside antibiotics celesticetin[8] and lincomycin[9] (with ammonia and isobutane as ionizing gases), and a number of nucleosides[10]. The spectra are characteristically simple and clear cut, and feature as the principal peak an intact ion formed by capture of a cationic species; this behavior offers great potential in analytical applications, especially for examining the composition of mixtures or for monitoring the isolation of a natural product. Other peaks in the spectra are attributable either to simple products of thermal decomposition or to products formed from the initial capture ions by a very few, low-energy pathways of decomposition that can frequently be correlated with metastable ions observed in the spectra.

To assess the broad utility of c.i.–m.s. with especial regard to current programs of synthetic carbohydrate chemistry in this laboratory, we have examined the behavior, under ionization by ammonia [c.i. (NH_3)] and by isobutane [c.i. (C_4H_{10})], of a range of typical derivatives frequently used in synthesis, or encountered in reaction-productmixtures. Acyclic sugar derivatives include an aldose dithioacetal[11] and its peracetate[12], an *aldehydo* sugar peracetate[13], a sugar phenylosotriazole[14], and an isopropylidene acetal of an alditol[15]. Isopropylidene acetals of cyclic sugars are represented by a pyranoid example[16], a furanoid example[17,18], and an acetylated derivative of the latter[19]. A 1,6-anhydrohexopyranose and its triacetate are included as examples of the bicyclic, full acetal structure[20,21] and compared with a free aldohexopyranose, a methyl hexopyranoside (and its acetate), and the peracetates of a 1-thioaldopyranose[22] and a hexulofuranose[5,23]. As a preliminary exploration in the oligosaccharide area[5], the survey also includes an acetylated trisaccharide.

It is shown that, in all of these examples, the chemical-ionization process principally involves transfer of H^+ or NH_4^+ from ammonia, or of H^+ or $C_4H_9^+$ from isobutane, to a heteroatom (generally N or O; S appears less reactive) having nonbonded, Lewis-basic electron pairs in the molecule. The results permit broad generalizations and a predictive rationale regarding the influence of structural features in the molecule on its behavior in c.i.–m.s. They illustrate the utility of the technique in

analytical applications and in the assignment of structural features from observed fragmentation processes.

EXPERIMENTAL

Chemical-ionization mass-spectra were recorded on two instruments; an AEI MS-9 equipped with an SRIC CIS-2 chemical-ionization source, and a Finnigan 1015 quadrupole mass spectrometer, also equipped with a chemical-ionization source. The spectra obtained from the two instruments were consistent for each compound. Figures 1–6 are reproductions of the spectra obtained on the quadrupole mass spectrometer. All metastable-ion data were obtained from the oscillographic recordings obtained on the magnetic sector instrument. Samples were introduced into the mass spectrometers *via* direct-insertion probes, and heated until strong spectra were observed on an oscilloscope. The ion-source temperatures were maintained at 180 °C and a reagent-gas pressure of 0.5–1 torr. The MS-9 operating conditions were: electron energy 450 eV, ion-repeller 0 V, filament emission 150 μA, and an accelerating voltage of 8 kV. The quadrupole operating conditions were: electron energy 100 eV, ion-repeller 3 V, filament emission 100 μA, lens voltage 30 V, and ion energy 3 V.

DISCUSSION

In this section, data are presented and considered in turn for each example in this study. Only a few, important pathways for decomposition prevail throughout the entire series, and these are mapped for each derivative, with the component undergoing elimination in each step indicated above the arrow that leads from the ion decomposed to the ion produced; metastable ions are relatively abundant, and the fragmentation steps that are accompanied by intense metastable ions are indicated by an asterisk beside the arrow. Intensities of ions are referenced to that of the most prominent ion in the same spectrum (the base peak, assigned the arbitrary intensity value 100%) and are expressed as a percent of the intensity of the base peak. The mass spectra (Figs. 1–6) plotted begin at *m/e* 60, so that reactant-gas ions are ignored.

D-*Glucose diethyl dithioacetal*[24] (**1**). — Under conditions of chemical ionization by ammonia [c.i. (NH_3)], the mass spectrum (Fig. 1a) of D-glucose diethyl dithioacetal (**1**) exhibits two prominent peaks and seven other peaks of considerably lesser intensity. A weak ion ($[M+NH_4]^+$, 10% of the base peak) formed by capture of an ammonium ion is observed at *m/e* 304, and the two principal peaks, at *m/e* 242 (base peak, 100%) and *m/e* 180 (80%), correspond to ions formed by sequential elimination from $[M+NH_4]^+$ of two components that each has a mass of 62 daltons, specifically, two molecules of ethanethiol; such behavior could be used to identify the alkyl groups in an unknown dithioacetal. The remaining ions correspond mainly to successive losses of water molecules. Ions at *m/e* 332 and *m/e* 270 are

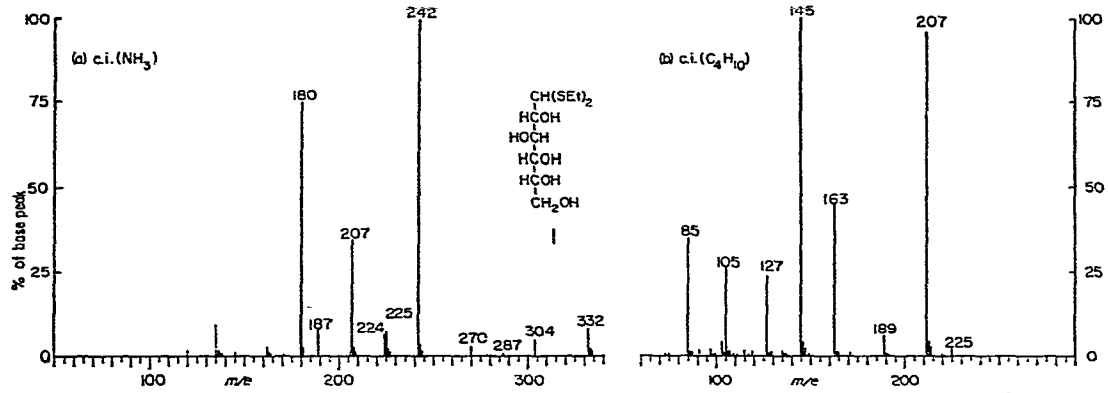
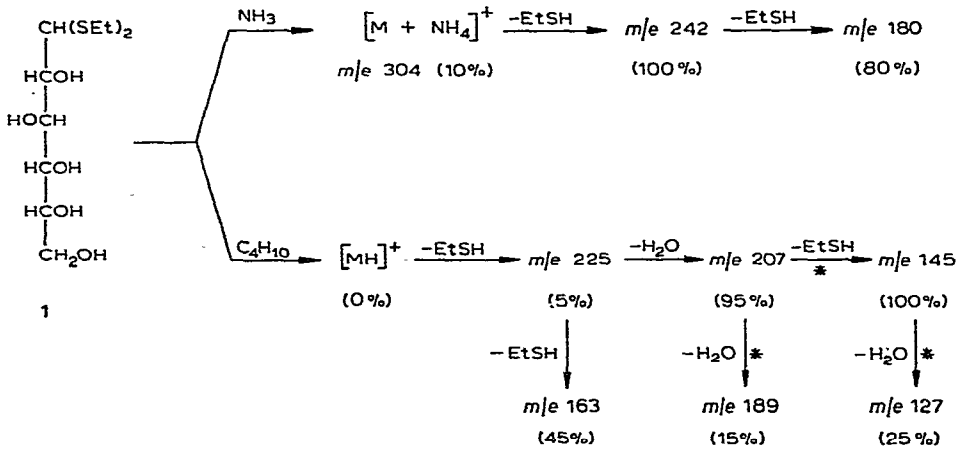


Fig. 1. Chemical-ionization mass spectra of D-glucose diethyl dithioacetal (1) determined with (*a*) ammonia or (*b*) isobutane as the reagent gas.

artifacts caused by slight contamination of the ammonia by a 2-carbon alkylamine; these are also found in the spectra of most of the other samples, and are not mentioned again in this article.



The corresponding spectrum (Fig. 1b) of **1** that was procured under conditions of chemical ionization by isobutane [c.i. (C_4H_{10})] likewise exhibits peaks for two principal ions, together with five additional peaks of slightly lesser prominence and a very weak (5%) peak at highest mass number. The last peak, *m/e* 225, is 62 mass numbers smaller than the proton-capture ion $[MH]^+$ anticipated, and indicates that the $[MH]^+$ ion is produced in such an excited condition that it does not survive transit through the spectrometer without fragmentation by loss of a molecule of ethanethiol. The two principal peaks, *m/e* 207 (95%) and *m/e* 145 (100%), are formed by sequential loss of fragments of 18 (water) and then 62 daltons (ethanethiol) from *m/e* 225; two less-prominent peaks at *m/e* 189 (15%) and at *m/e* 127 (25%) correspond to the loss of 18 mass units (water) from *m/e* 207 and *m/e* 145, respectively. The

third-most abundant ion, *m/e* 163 (45%), is formed from *m/e* 225 by the loss of ethanethiol, whereas the remaining two ions, *m/e* 105 (30%) and *m/e* 85 (35%), are evidently formed by processes that involve the breakage[6] of carbon–carbon bonds along the skeleton of the molecule.

Partial interpretations[25] of the electron-impact (e.i.) mass spectrum of **1** suffice to identify several fragments, including a molecular ion, from which the molecular weight of **1** and the identity of the thiol groups may likewise be determined. The location of substituents on the polyhydroxyalkyl side-chain of dithioacetals may also be deduced, in favorable instances, by identification[26] of products of skeletal-cleavage reactions occurring subsequent to electron impact.

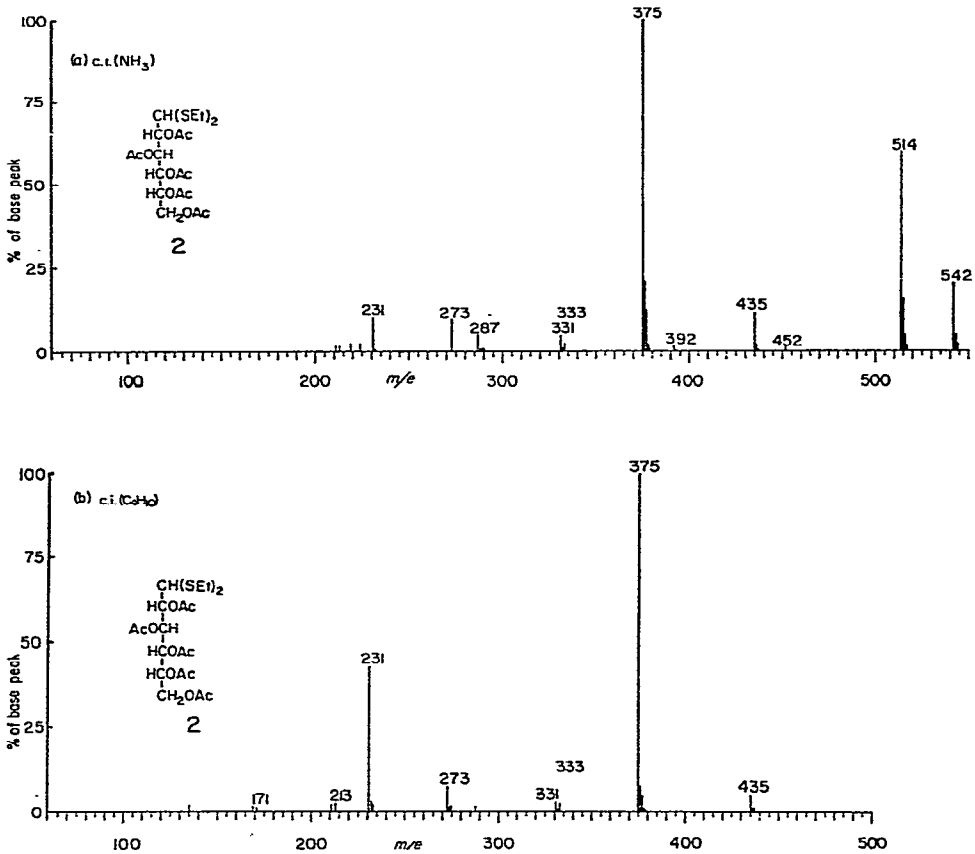


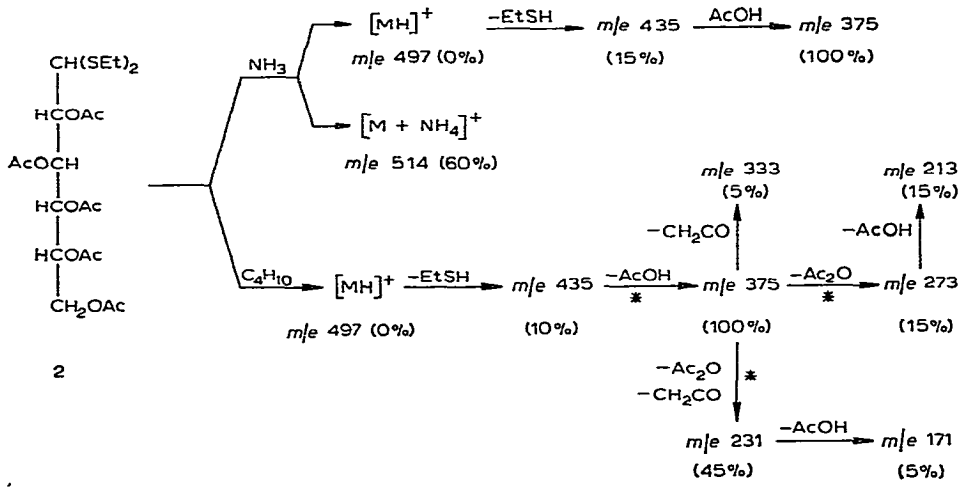
Fig. 2. Chemical-ionization mass spectra of penta-*O*-acetyl-D-glucose diethyl dithioacetal (**2**) determined with (*a*) ammonia or (*b*) isobutane as the reagent gas.

Penta-O-acetyl-D-glucose diethyl dithioacetal[27] (**2**). — Both the c.i. (NH_3) (Fig. 2a) and the c.i. (C_4H_{10}) (Fig. 2b) mass spectra undergo profound changes as a consequence of the introduction of the acetyl substituents onto **1** to give **2**. The former

spectrum [c.i. (NH_3)] features two prominent ions, *m/e* 514 (60%) and *m/e* 375 (100%), together with a single, less-intense ion of *m/e* 435 (15%). The largest-mass ion ($[M+NH_4]^+$ *m/e* 514) is formed by the usual process of capture of the gaseous NH_4^+ ion, although, in contrast with the corresponding ion $[\mathbf{1}+NH_4]^+$ from **1**, the ion $[\mathbf{2}+NH_4]^+$ appears to undergo no further fragmentation. The other two ions, *m/e* 435 and *m/e* 375, appear to be derived from the undetected $[MH]^+$ ion (*m/e* 497) by the loss of fragments of 62 and then 60 daltons, corresponding to sequential expulsion of ethanethiol and acetic acid (although a contribution to these two by the loss of NH_3·ethanethiol cannot be positively excluded).

An initial concern in the use of ammonia as a reagent gas for the c.i. of this and other acetylated molecules is the known susceptibility of ester groups toward cleavage by ammonolysis; there is, however, no evidence whatsoever from the spectrum (Fig. 2a) for the occurrence of any chemical reaction between the uncharged reagent gas and the ester groups, except for the processes of capture that produce the initial ions.

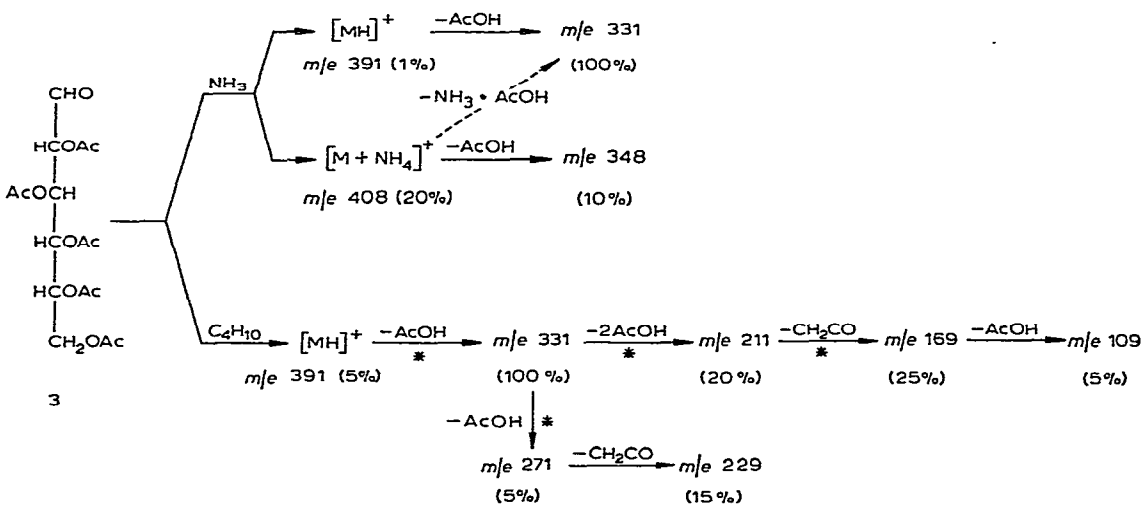
The obviously highly-favored process of loss of ethanethiol from the unstable, proton-capture ion $[MH]^+$ (*m/e* 497, unobserved) appears also to provide the highest-mass ion found in the c.i. mass spectrum of **2** with isobutane as the reagent gas. Two prominent fragments are detected, namely, *m/e* 375 (100%), which is presumably



formed by loss of acetic acid from *m/e* 435, and *m/e* 231 (45%), which is shown, by the presence of a metastable ion, to be formed from *m/e* 375 in essentially a single step by loss of the elements both of ketene and of acetic anhydride. Other fragments, at *m/e* 333 (5%), *m/e* 273 (15%), and *m/e* 213 (15%), can be accounted for by the usual acetate decomposition-processes through loss of ketene or acetic anhydride, followed by acetic acid, from *m/e* 375. The very weak (5%), but clearly distinguishable, ion at *m/e* 171 is instructive, because its formation occurs with the loss of all five acetyl groups, and thus signifies the presence of not fewer than five acetoxyl groups in **2**.

The tendency for more-extensive fragmentation after c.i. (C_4H_{10}) than after c.i. (NH_3) suggests that the former constitutes a considerably more energetic mode of ionization. For both **1** and **2**, the loss of one molecule of ethanethiol seems to be highly favored, although excess energy present in the fragment from **2** after this initial decomposition step is observed to be expended exclusively in fragmentation reactions involving the acetyl substituents. Somewhat greater simplicity characterizes c.i.–mass-spectral (NH_3 or C_4H_{10}) decomposition of **2** than that shown by the e.i.–mass spectrum, although molecular ions are a fairly common feature of e.i.–mass spectra of dithioacetal peracetates[28], and detailed analysis of fragmentations in the latter spectra allows the deduction of structural information, albeit less efficiently than by e.i.–m.s. of **1** or c.i.–m.s. of **2**.

Penta-O-*acetyl*-aldehydo-D-*glucose*[29] (**3**). — The c.i. (NH_3) mass spectrum of **3** is dominated by a single ion, *m/e* 331, although three ions of considerably lower intensity, *m/e* 408 (20%), *m/e* 391 (1%), and *m/e* 348 (10%), can also be discerned. The higher-mass ions, *m/e* 408 $[M+NH_4]^+$ and *m/e* 391 $[MH]^+$, correspond to capture ions, and *m/e* 348 is derived from $[M+NH_4]^+$ by loss of acetic acid; the base peak, *m/e* 331, arises by loss of acetic acid from $[MH]^+$, and, possibly, also by loss of the elements of ammonia·acetic acid from $[M+NH_4]^+$. As with the preceding example, however, fragmentation after c.i. (NH_3) is very slight and is limited to losses of acetic acid.

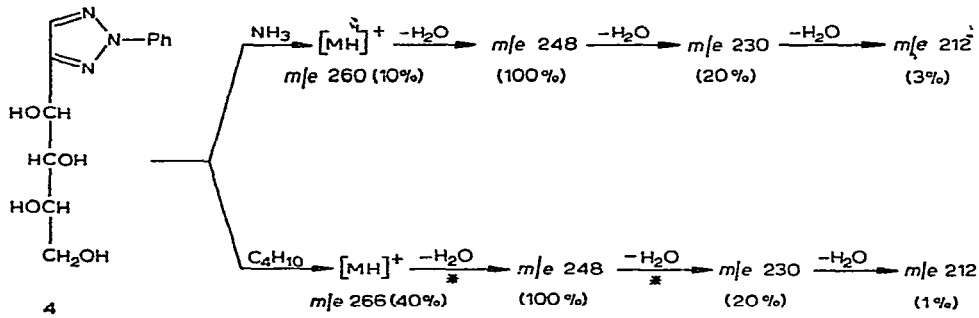


The c.i. (C_4H_{10}) mass spectrum of **3** is precisely that anticipated from the preceding examples; *m/e* 391 (5%), $[MH]^+$, and *m/e* 331 (100%), $[MH]^+$ – acetic acid, are the peaks at highest mass number, and the greater energy imparted during this mode of ionization causes more-extensive fragmentation, with generation of *m/e* 271 (5%), *m/e* 229 (15%), *m/e* 211 (20%), *m/e* 169 (25%), and *m/e* 109 (5%), by decomposition of acetate groups, the first two being formed by sequential loss of acetic acid

and ketene from *m/e* 331, and the last three presumably arising by elimination: first, of two molecules of acetic acid; then, one of ketene; and finally, of the last acetate group as acetic acid, as indicated in the chart. The latter sequence of fragmentations proceeds from $[MH]^+$ by sequential loss of all five acetate groups and, as with **2**, provides an accurate accounting of the number of acetoxyl groups present in the intact molecule.

The c.i.–mass spectrum of **3** is much easier to interpret than the e.i.[30] spectrum, which features a number of skeletal-cleavage reactions. More germane to the general discussion, however, is the point that the c.i. (NH_3) mass spectrum again displays no evidence suggesting that ammonolysis of ester groups is caused by the reagent gas. 1,1-Bis(acetamido)-1-deoxyalditols and glycosylamine derivatives result from ammonolysis, in solution, of **3** and its *arabino* homolog[31], and such a compound could, in principle, be formed from **3** by the action of gaseous ammonia. However, there is no evidence indicating the presence of any such ammonolysis products in the c.i. (NH_3) mass spectrum of **3**, and so it may be concluded that even esters of fairly low stability should not be chemically altered by the relatively brief exposure to ammonia in the ion source of the mass spectrometer.

L-xylo-*Hexulose phenylosotriazole*[32] (**4**). — The c.i. (NH_3) mass spectrum of **4** is essentially identical to its c.i. (C_4H_{10}) mass spectrum, except for an exceedingly minor (1%) ion at *m/e* 283 $[M+NH_4]^+$ in the former. The proton-capture ion $[MH]^+$ at *m/e* 266 is present (10%, NH_3; 40%, C_4H_{10}); despite its even mass-number, this is an even-electron ion, because of the presence of an odd number of nitrogen atoms. The base peak of both spectra is *m/e* 248, which corresponds to loss of water from $[MH]^+$. Less-intense ions at *m/e* 230 (2%, NH_3; 20%, C_4H_{10}) and *m/e* 212 (<5% in each) are formed by further, sequential loss of two more water molecules, whereas *m/e* 188 (10%, NH_3; 20%, C_4H_{10}) may arise from *m/e* 248 by cleavage of the C-4–C-5 bond.



The most noteworthy feature of the c.i. mass spectrum of **4** is the virtual absence, from the c.i. (NH_3) mass spectrum, of the $[M+NH_4]^+$ ion, which is relatively prominent in the c.i. mass spectra (NH_3) of **1–3**. $[M+NH_4]^+$ ions are not produced in significant amounts by c.i. (NH_3) of the nitrogen-containing, carbohydrate anti-

biotics lincomycin[9], clindamycin[9], and celesticetin[8], and it has been proposed[1] that the absence of an $[M+NH_4]^+$ ion constitutes valid presumptive evidence for the presence of nitrogen atoms in the molecule. As proton transfer by NH_4^+ occurs only to basic[10a] nitrogen atoms, an observed $[M+NH_4]^+$ ion should not be interpreted as indicating that nitrogen is necessarily absent.

A second point of interest in the c.i. mass spectrum of **4** is the absence of fragmentation processes involving rupture of the two (triazole and phenyl) rings. In view of the limited energy imparted on ionizing by this method, coupled with the considerable stability associated with ring compounds having delocalized π-electrons, this observation is understandable. In c.i. studies of molecules possessing aromatic ring-systems and also polyhydroxyalkyl side-chains, it seems reasonable to regard the aryl rings as inert substituents.

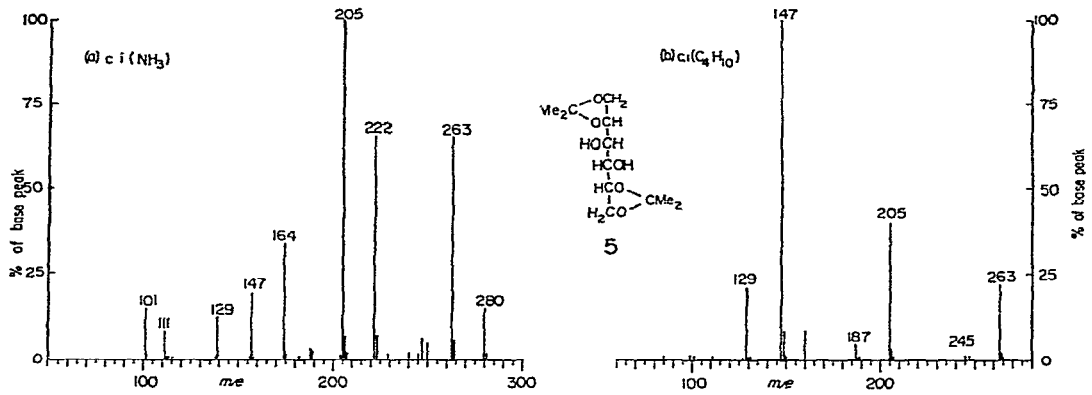
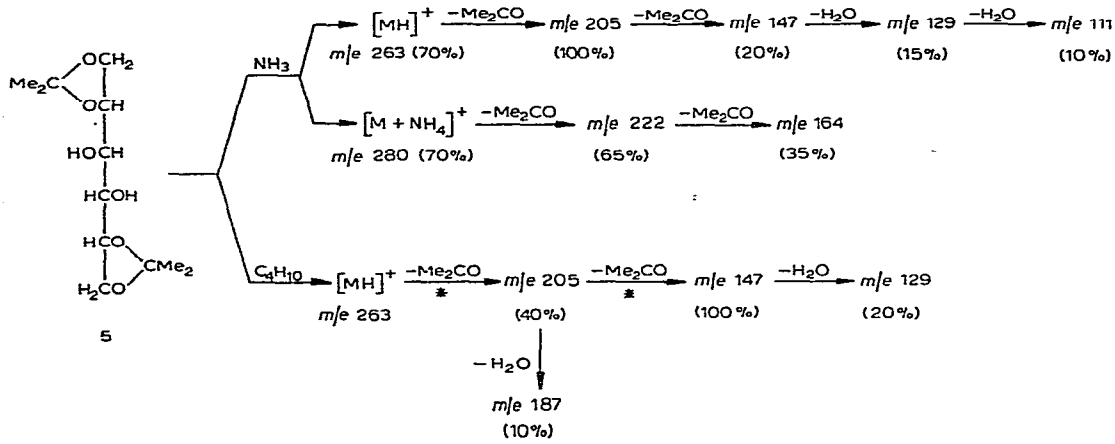


Fig. 3. Chemical-ionization mass spectra of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**5**) determined with (*a*) ammonia or (*b*) isobutane as the reagent gas.

1,2:5,6-Di-O-isopropylidene-D-mannitol[33] (**5**). — The c.i. (NH_3) mass spectrum (Fig. 3a) of **5** exhibits three principal ions, *m/e* 263 (70%), *m/e* 222 (65%), and *m/e* 205 (100%), and five lesser ions, *m/e* 280 (20%), *m/e* 164 (35%), *m/e* 147 (20%), *m/e* 129 (15%), and *m/e* 111 (10%); the ion at *m/e* 308 is an artifact, as noted in the discussion of **1**, and *m/e* 101 (15%) probably arises through skeletal carbon–carbon bond breakage. The two large-mass ions, *m/e* 280 and *m/e* 263, correspond to $[M+NH_4]^+$ and $[MH]^+$, respectively, and establish that the molecular weight of this acetal is 262 daltons; *m/e* 222 and *m/e* 164, and *m/e* 205 and *m/e* 147 are related to $[M+NH_4]^+$ and $[MH]^+$, respectively, by the sequential loss of two components having a mass of 58, which corresponds to acetone. The remaining two ions are derived from *m/e* 147 by the stepwise loss of two molecules of water.

The c.i. (C_4H_{10}) mass spectrum (Fig. 3b) of **5** displays *m/e* 263 (20%), *m/e* 205 (40%), *m/e* 147 (100%), and *m/e* 129 (20%), all of which arise by the same steps from the proton-capture ion *m/e* 263; the only additional fragment is a weak (10%) ion at *m/e* 187, which may be interpreted as resulting from loss of water from *m/e* 205.

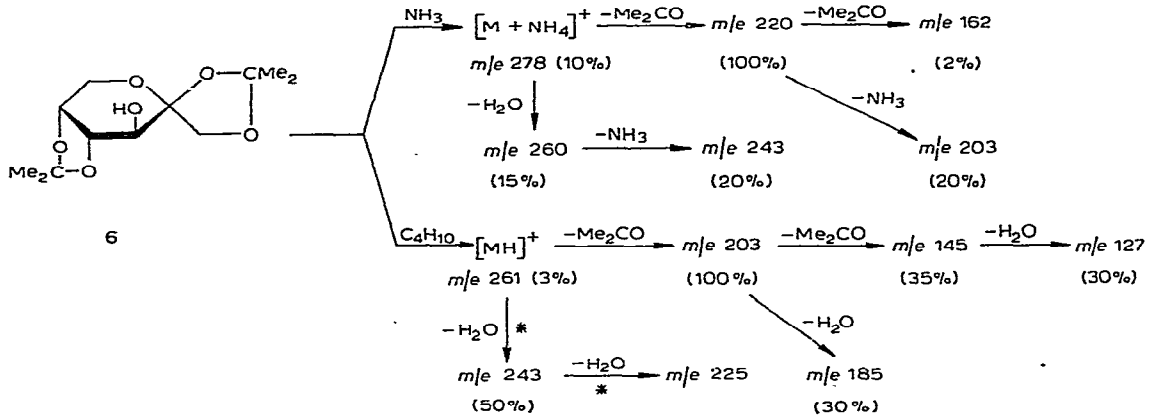


It is noteworthy that, as with the acetates **2** and **3**, the carbonyl-derived substituents effectively control the modes of decomposition of **5**, so that this initial, directed fragmentation provides a clear and unambiguous determination of the number of isopropylidene groups present in the molecule.

*1,2:4,5-Di-*O*-isopropylidene-β-*D*-fructopyranose*[34] (**6**). — The milder condition of c.i. (NH_3) acts upon **6** to produce a mass spectrum dominated by a single ion, *m/e* 220, accompanied by only four significant ions, having much lesser abundance, namely, *m/e* 278 (10%), 260 (15%), 243 (20%), and 203 (20%). These peaks are readily identified as the capture ion $[M+MH_4]^+$ (*m/e* 278) and fragment ions derived (*a*) from $[M+NH_4]^+$ by loss of 18 mass units (water) to give *m/e* 260, followed by 17 mass units (ammonia) to give *m/e* 243, and (*b*) by loss from *m/e* 278 of 58 mass units (acetone) to give the base peak at *m/e* 220; loss of another 17 mass units (ammonia) gives *m/e* 203. An exceedingly weak (2%) ion at *m/e* 162 presumably arises from *m/e* 220 by loss of the second isopropylidene group as acetone, but the weak intensity of this latter ion suggests that the presence of the second isopropylidene group might not be readily detected by c.i.–m.s. (NH_3).

The more energetic c.i. (C_4H_{10}) process leads to one principal ion, *m/e* 203 (100%), together with four prominent, lesser ions, *m/e* 243 (50%), 185 (30%), 145 (35%), and 127 (30%), and two minor, significant peaks at *m/e* 261 (3%) and 225 (10%); peaks at *m/e* 117 (25%) and 85 (10%) appear to arise by processes involving breakage of carbon–carbon bonds. The former seven ions are accounted for by formation of the proton-capture ion $[MH]^+$ (*m/e* 261) and by loss of (*a*) one (*m/e* 243) and a second (*m/e* 225) molecule of water (which must involve opening of one acetal ring) or (*b*) of acetone (*m/e* 203) from $[MH]^+$; subsequent loss of water (*m/e* 185) or of acetone (*m/e* 145) followed by water (*m/e* 127) from *m/e* 203 completes the fragmentation.

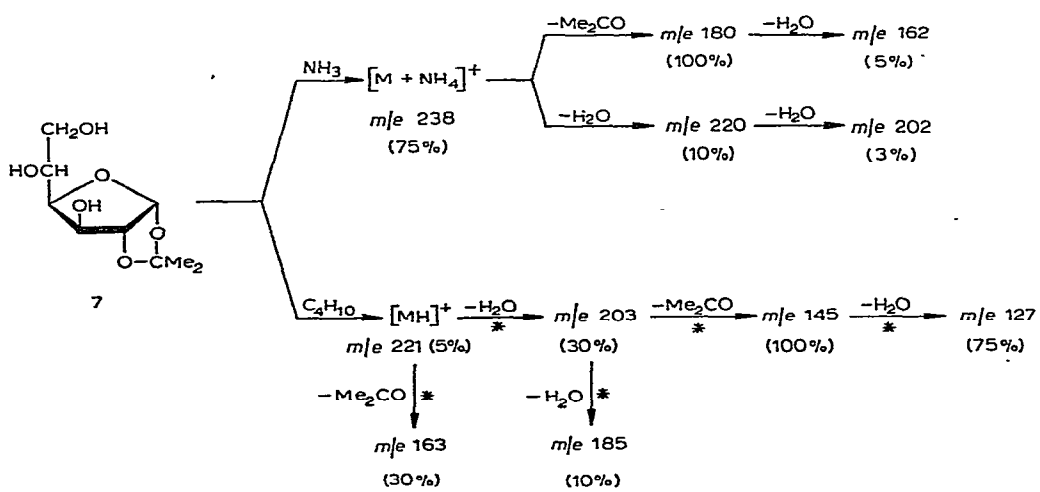
It is at once apparent that c.i. (C_4H_{10}) provides a more sensitive indication than does c.i. (NH_3) of the number of isopropylidene acetal groups present, just as it does for acetates.



The e.i.–mass spectrum[35] of **6** exhibits the anticipated $M^{+\cdot}-15$ ion, from which the molecular weight may be deduced, and low-intensity fragments corresponding to losses of acetone, acetone+water, and acetic acid from $M^{+\cdot}-15$. Thus, similar information is accessible under both modes of ionization, although the greater selectivity in c.i.-induced fragmentation of **6** minimizes the production of extra ions in this instance.

1,2-O-Isopropylidene-α-D-glucofuranose[33] (**7**). — The c.i. (NH_3) mass spectrum of **7** features two principal ions, *m/e* 238 (75%, $[M+NH_4]^+$) together with *m/e* 180 (100%); the latter arises from the former by the anticipated loss of acetone. Also formed are three very minor fragments, at *m/e* 220 (10%), 202 (3%), and 162 (5%) which correspond to loss of one molecule, and two molecules, of water from the NH_4^+-capture ion, and one from *m/e* 180, respectively.

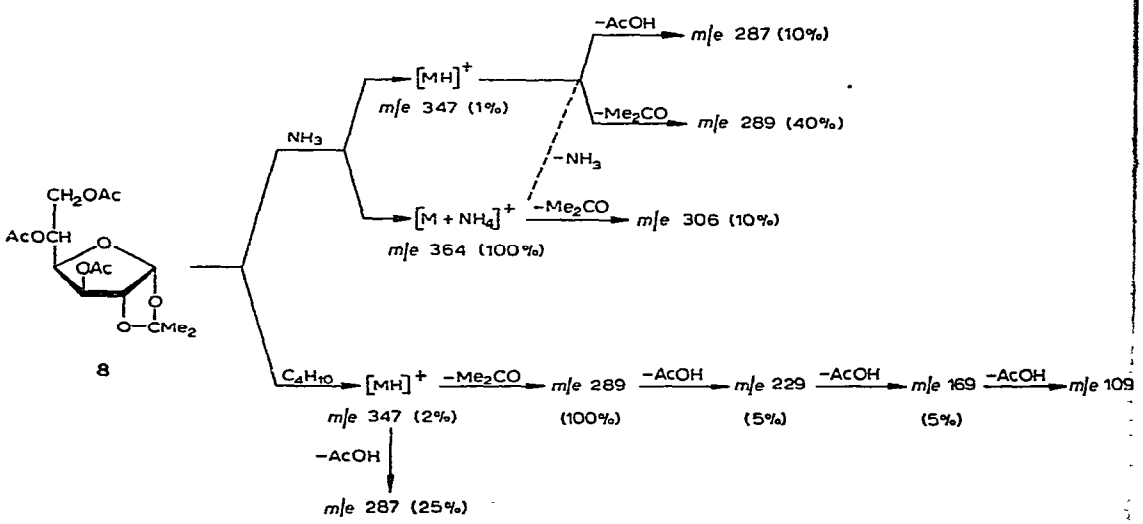
The c.i. (C_4H_{10}) mass spectrum of **7** is likewise elementary, consisting of prominent fragments having *m/e* 145 (100%) and 127 (75%), together with less-intense



ions at *m/e* 221 (5%), 203 (20%), 185 (10%), and 163 (30%). These are readily assigned as the proton-capture ion (*m/e* 221) $[MH]^+$, which loses either a first (*m/e* 203) and a second molecule (*m/e* 185) of water or a molecule of acetone (*m/e* 163), and products of the loss of acetone (*m/e* 145) and then water (*m/e* 127) from *m/e* 203. An ion of *m/e* 85 (40%) is a relatively common feature in the c.i. (C_4H_{10}) mass spectra of the hexose derivatives in this study; it is probably formed by the process of rearrangement elucidated by Hogg and Nagabhushan[6].

*3,5,6-Tri-*O*-acetyl-1,2-*O*-isopropylidene-α-*D*-glucofuranose*[36] (**8**). — This molecule was selected because, in the same system, it incorporates two structural features that have been shown to exert, individually, a dominant effect in directing fragmentation; the e.i. mass spectrum[19] of **8** displays insufficient evidence to permit identification of either functional group as dominant in this respect.

The c.i. (NH_3) mass spectrum of **8** consists of a prominent ion at *m/e* 364, a vestigial (1%) ion at *m/e* 347, weak fragments at *m/e* 306 (10%) and 287 (10%), and a fairly intense (40%) fragment at *m/e* 289. Three ions, *m/e* 364 ($[M+NH_4]^+$), 347 ($[MH]^+$), and 306 (which is derived from $[M+NH_4]^+$ by loss of acetone) may be identified at once; the origin of the remaining two fragments is ambiguous, in that *m/e* 289 may arise by loss of acetone from $[MH]^+$ or by loss of acetone·ammonia from $[M+NH_4]^+$, whereas *m/e* 287 may result from the corresponding loss of acetic acid from $[MH]^+$ or of acetic acid·ammonia from $[M+NH_4]^+$. Comparison with the c.i. (C_4H_{10}) mass spectrum provided support for the former process, because the intensity ratios of *m/e* 287, 289, and 347 are very similar in both spectra; much of the information affordable by these two ions may, however, be educed without reference to their exact mode of formation. The deacetonation fragment *m/e* 289 is seen to exceed in abundance the corresponding deacetylation product (*m/e* 287) of the $[MH]^+$ ion by a factor of ~4, which indicates a clear (but not overwhelming), energetic favoring of the former process.



More-extensive fragmentation is evident in the c.i. (C_4H_{10}) mass spectrum of **8**, which exhibits, in addition to *m/e* 347 (2%, $[MH]^+$) and its deacetonation (*m/e* 289, 100%) and deacetylation products (*m/e* 287, 25%), a family of three weak ions at *m/e* 229 (5%), 169 (5%), and 109 (2%) which are separated from *m/e* 289 in steps of 60 mass numbers. Thus, these three ions result from stepwise expulsion of all three of the acetate groups, with the reassuring consequence that the proposed use of c.i. (C_4H_{10}) for indicating the number of acetoxyl groups present in the molecule is not invalidated by the presence of the isopropylidene acetal group, as it causes no interference.

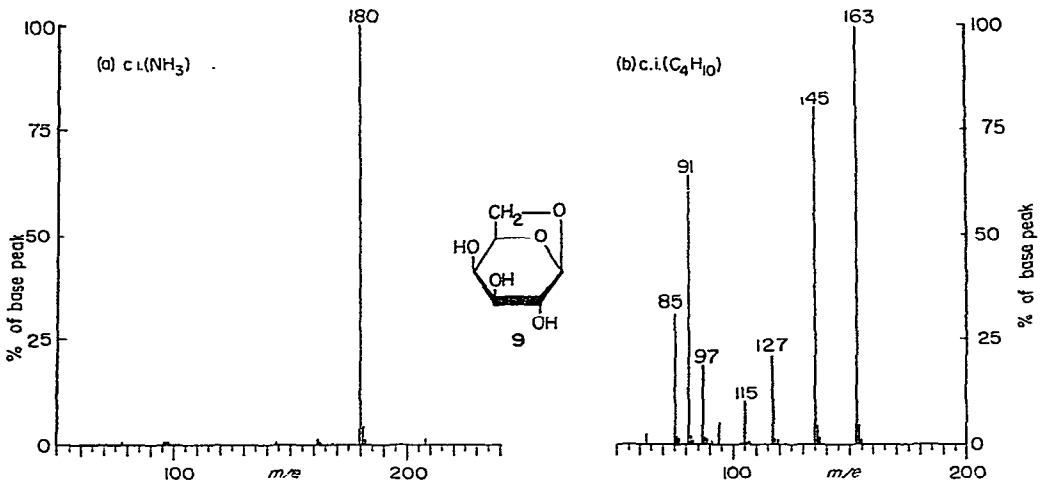
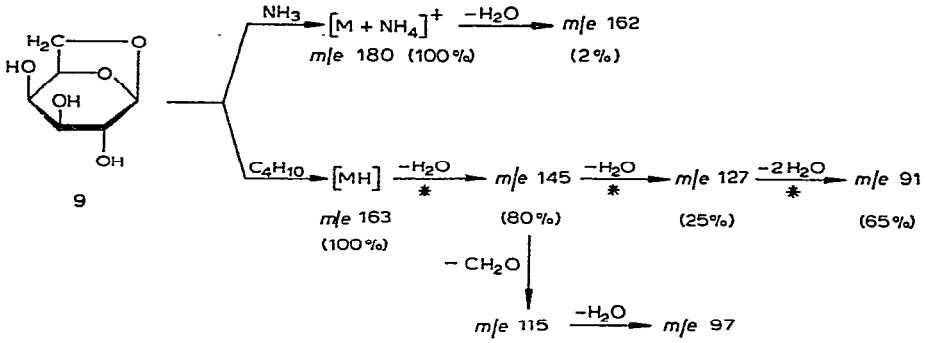


Fig. 4. Chemical-ionization mass spectra of 1,6-anhydro-β-D-galactopyranose (**9**) determined with (*a*) ammonia or (*b*) isobutane as the reagent gas.

1,6-Anhydro-β-D-galactopyranose[37] (**9**). — This molecule was selected to permit examination of the effect of incorporating the acetal group into a ring structure. The c.i. (NH_3) mass spectrum (Fig. 4a) consists essentially of a single peak at *m/e* 180 ($[M+NH_4]^+$), which demonstrates convincingly that the energy needed to fragment this bicyclic acetal exceeds that imparted by c.i. (NH_3); a feeble (2%) fragment at *m/e* 162 is formed from $[M+NH_4]^+$ by loss of water.

The c.i. (C_4H_{10}) mass spectrum (Fig. 4b) of **9** exhibits seven ions of 10% or greater intensity, the most prominent, *m/e* 163, being the proton-capture ion $[MH]^+$ which appears to be rather stable even after the more-energetic ionization conditions. Separated from *m/e* 163 by multiples of 18 mass numbers, the fragments at *m/e* 145 (80%), 127 (25%), and 91 (65%) arise by loss of one molecule, a second molecule, and finally two more molecules of water, respectively, from $[MH]^+$; the fragment at *m/e* 115 evidently arises by expulsion of H_2C-6 and O-6 as formaldehyde from *m/e* 145, and *m/e* 97 can be formed from *m/e* 115 by loss of water. The remaining ion, *m/e* 85, has already been recognized as a common feature of the c.i. (C_4H_{10}) mass spectra of aldohexose derivatives.

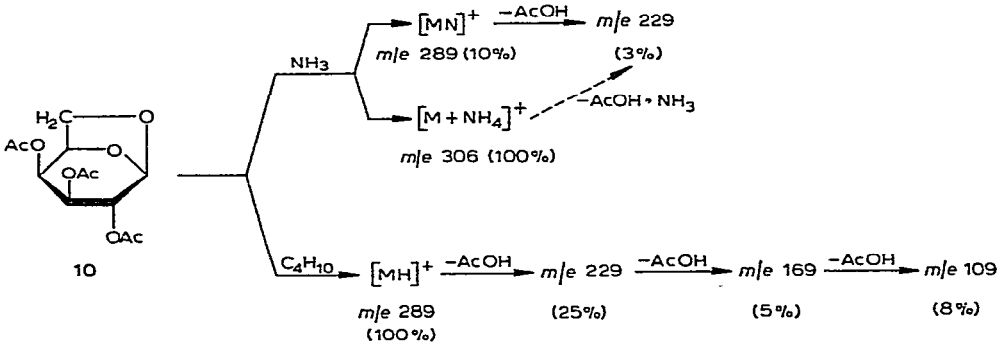


Accurate inferences as to the molecular weight may be drawn from either kind of c.i.–m.s. of **9**; however, the occurrence of a ring-opening reaction in the decomposition sequence of **9** subsequent to c.i. (C_4H_{10}) liberates a fourth oxygen atom for expulsion as a water molecule, and creates information that could lead to an erroneous conclusion concerning the number of hydroxyl groups present in **9** prior to ionization. Heyns and Scharmann[36] alluded to the e.i.–mass spectrum of the D-*ido* isomer of **9**, but included no primary data in their report.

2,3,4-Tri-O-*acetyl-1,6-anhydro-β*-D-*galactopyranose*[35] (**10**). — This molecule was selected in order to illustrate the utilization of the fragmentation-directing proclivities of acetate groups as a means of suppressing the more intricate processes of skeletal cleavage that complicate interpretation of the c.i. (C_4H_{10}) mass spectrum of **9**. The c.i. (NH_3) mass spectrum of **10** is also very simple, consisting of a very prominent ion at *m/e* 306 (100%), a weak ion at *m/e* 289 (10%), and a minuscule peak at *m/e* 229 (3%), which correspond, respectively, to a very stable NH_4^+-capture ion $[M+NH_4]^+$, a comparatively stable proton-capture ion $[MH]^+$ whose formation is much less favored, and the product of loss of acetic acid from $[MH]^+$ or, less probably, of acetic acid·ammonia from $[M+NH_4]^+$.

The c.i. (C_4H_{10}) mass spectrum of **10** consists of four ions, *m/e* 289 (100%, $[MH]^+$), *m/e* 229 (25%), *m/e* 169 (5%), and *m/e* 109 (8%), which are mutually separated by 60 mass numbers and thus correspond to sequential loss of one, two, and all three acetate groups, in the form of acetic acid, as the exclusive fragmentation processes of $[MH]^+$. This spectrum contains essentially no redundant or potentially misleading information, and allows the molecular weight and the number of acetoxyl substituents present in **10** prior to ionization to be specified precisely; this information thus also provides the number of hydroxyl groups present in **9** (prior to acetylation to give **10**). Similarly elegant simplicity characterizes the c.i. (C_4H_{10}) mass spectrum of 1,6-anhydro-3,4-*O*-isopropylidene-*β*-D-talopyranose, which forms a detectable $[MH]^+$ ion that decomposes only by elimination of acetone and then of water[39], so that the principle of diverting energy into fragmentationally labile substituents to prevent excessive fragmentation appears to be general.

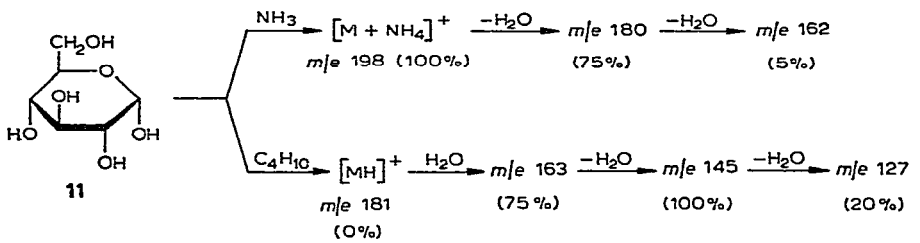
Another point of interest is that, despite the apparent complexity of **10**, the



capture ions $[\mathbf{10}+H]^+$ and $[\mathbf{10}+NH_4]^+$ appear to be extremely stable. This feature will also be noted later, especially in the mass spectra of **14** and **15**, and the evidence suggests that, unless there is a particularly favored mode of decomposition accessible to a capture ion, an increase in complexity (that is to say, an increase in the number of bonds over which the excess energy acquired in the process of ionization may be partitioned) is accompanied by some enhancement of the intensity (stability) of this ion.

The e.i.–mass spectrum of the D-*ido* analog of **10** has been recorded and interpreted in some detail[38]; greater complexity obtains in the e.i. spectrum, but isotopic substitution and derivatization experiments were employed successfully to elucidate prominent decomposition-modes.

D-*Glucopyranose*[40] (**11**). — The c.i. (NH_3) mass spectrum of **11** consists essentially of two peaks, *m/e* 198 ($[M+NH_4]^+$) and a slightly less intense ion (75%) that is 18 mass numbers smaller, corresponding to the loss of water (presumably from

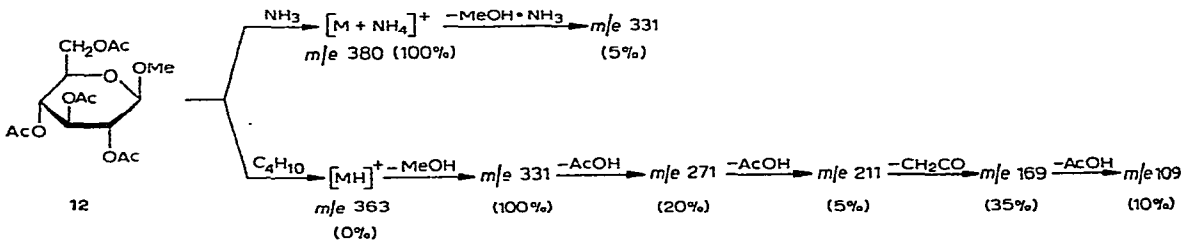


the anomeric position; see later), in accord with the data of Hogg and Nagabhushan[6]. The c.i. (C_4H_{10}) mass spectrum differs mainly in relative intensities from the c.i. (CH_4) mass spectrum reported[6] by the same authors; the largest-mass ion, *m/e* 163 (75%), derives from the (evidently unstable) proton-capture ion $[MH]^+$ by loss of water (presumably from the anomeric position, because of the possibility of resonance delocalization of the positive charge onto the glycosyl position[5]), whereas the base peak, *m/e* 145, is formed by loss of a second molecule of water in one or more processes that may also prove[6] to be specific. Fragments of lower mass are products

of presumed skeletal bond-rupture processes; *m/e* 85 (55%) has been rationalized[6] in terms of such a reaction, whereas peaks at *m/e* 91 (30%) and *m/e* 103 (25%) are apparently characteristic of c.i. (C_4H_{10}). Data in the report of Hogg and Nagabhushan[6] indicate that the c.i. decomposition of methyl α-D-glucopyranoside is quite analogous to that of **11**.

As in the case of **9**, the absence of fragmentationally labile substituents in **11** evidently causes sufficient localization of the excitation accompanying ionization to support skeletal bond-breakage; the greater complexity of such processes drastically limits the amount of structural information that may be inferred from a simple analysis, and suggests that substitution (as in **10**) of the hydroxyl groups may provide a more expedient derivative for examination by c.i.–mass spectrometry. Kochetkov and Chizhov have intimated[4], however, that even greater difficulties are encounted in examining free sugars (for example, **11**) by e.i.–mass spectrometry.

Methyl β-D-glucopyranoside tetraacetate[42] (**12**). — The c.i. (NH_3) mass spectrum of **12** (and also of the α-D anomer) consists of one peak at *m/e* 380, $[M+NH_4]^+$, and a very weak (5%) ion at *m/e* 331 that is, presumably, formed from the $[M+NH_4]^+$ ion by expulsion of the anomeric substituent as methanol·ammonia. Two observations merit consideration: firstly, the stereochemistry of the molecule being ionized is seen to be qualitatively without effect on the spectrum, so that conclusions drawn for specific examples in this study should be applicable to stereoisomers as well; secondly, the contribution of the anomeric substituent in the peracetylated glycoside to the molecular weight is calculable from the difference in mass number between the initial capture-ion and the glycosyl fragment-ion (see compound **13**).

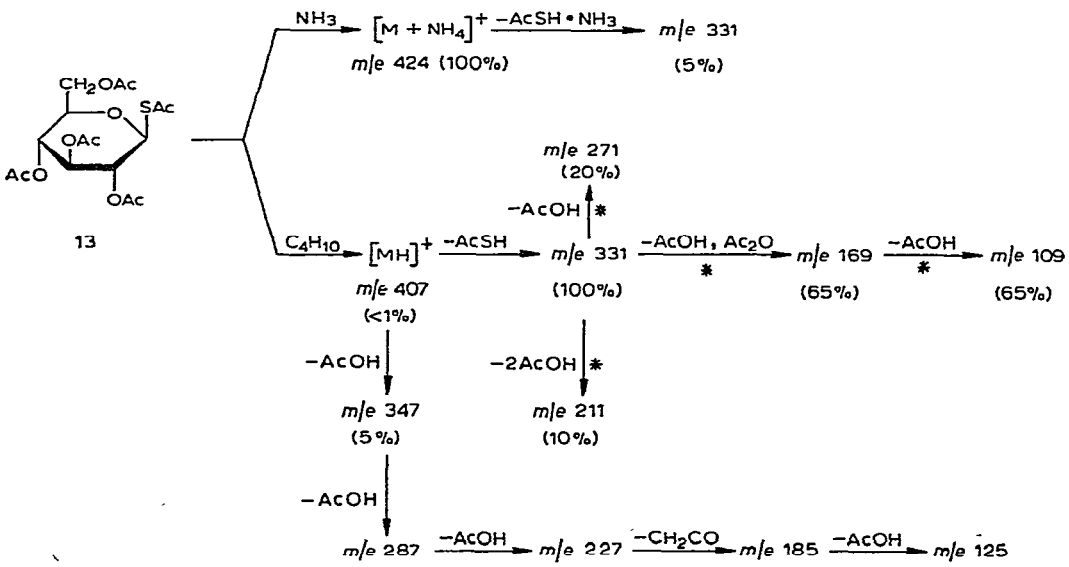


The c.i. (C_4H_{10}) mass spectrum of **12** is also very simple, consisting of a preponderant *m/e* 331 ion and four low-intensity ions at *m/e* 271 (20%), *m/e* 211 (5%), *m/e* 169 (35%), and *m/e* 109 (10%). The base peak (*m/e* 331) is formed in abundance from the $[MH]^+$ ion, because cleavage of the glycosyl bond is a very highly favored process, as discussed for the preceding and earlier[43] examples. The other four fragments may be interpreted as being formed from the base peak by ordinary acetate decomposition-processes of sequential loss of two molecules of acetic acid, one molecule of ketene, and finally another molecule of acetic acid, as indicated in the chart; this sequence of decompositions is identical with that deduced for c.i. (CH_4) of α-D-glucopyranose pentaacetate[6] and of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-

α-D-glucopyranose[44], and accounts for each of the individual substituents present in **12** (and its 1-*O*-acetyl analog), although the exact manner in which the acetate groups are lost from **12** cannot be specified without supporting analysis of associated metastable ions.

1-Thio-β-D-glucopyranose pentaacetate[45] (**13**). — The c.i. (NH_3) mass spectrum of **13** consists of two peaks, a prominent ion at *m/e* 424 $[M+NH_4]^+$ and a very weak fragment at *m/e* 331 (5%); from the discussion for **12**, the latter ion is evidently the glycosyl cation formed by elimination of the anomeric substituent plus the ammonium ion, whereas the mass difference (424 − 331 = 93 daltons) less 18 (the NH_4^+ ion), namely, 75, is the mass of the substituent group lost, which is, in this case, an acetylthio group.

The c.i. (C_4H_{10}) mass spectrum of **13** exhibits three principal ions, *m/e* 331 (100%), *m/e* 169 (65%), and *m/e* 109 (65%), together with eight lesser ions, *m/e* 407 (<1%), *m/e* 347 (5%), *m/e* 287 (1%), *m/e* 271 (20%), *m/e* 227 (10%), *m/e* 211 (10%), *m/e* 185 (5%), and *m/e* 125 (15%) that can be organized into two series of ions differing by 16 mass numbers. The first series, *m/e* 331, 271, 211, 169, and 109, corresponds to the decomposition of the glycosyl cation by sequential elimination of each of the acetate fragments as determined for **12**, whereas the second series, *m/e* 347, 287, 227, 185, and 125, results from a similar (but only 5% as abundant) sequence of eliminations in which the anomeric substituent is retained. As the relative stability of the glycosyl cation should be approximately independent of the identity of the anomeric group in the examples here cited, the second series of fragments presumably arises because of a lower effective basicity of sulfur than of oxygen in this process, which would facilitate competitive protonation of one or more of the numerous other heteroatoms, and initiate fragmentation at sites remote from the anomeric center.



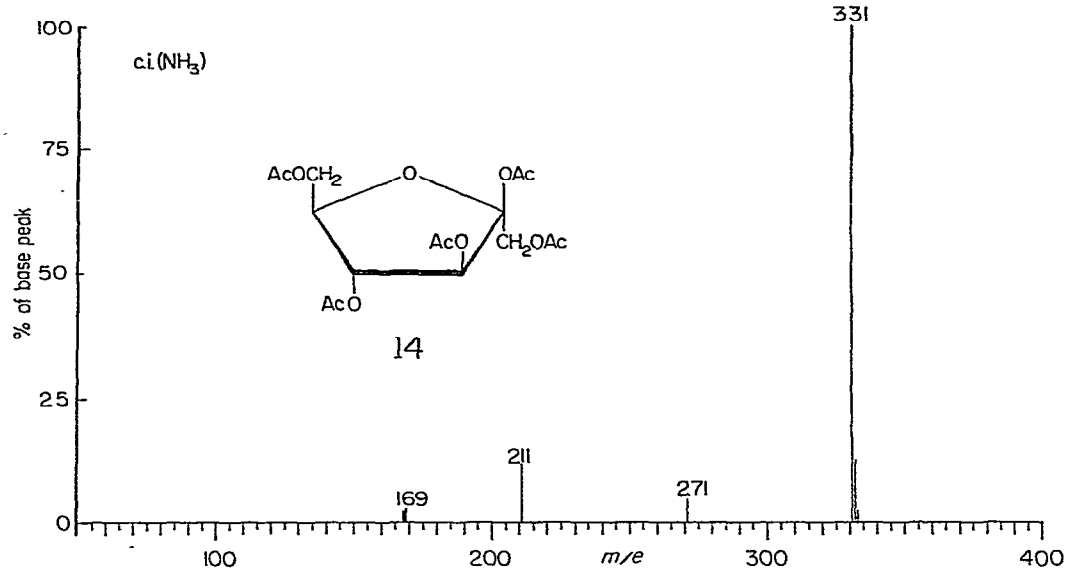


Fig. 5. Ammonia-mediated, chemical-ionization mass spectrum of β-D-fructofuranose pentaacetate (**14**).

β-D-Fructofuranose pentaacetate[46] (**14**). — The c.i. (NH_3) mass spectrum (Fig. 5) consists of one main peak, *m/e* 331, and three very minor fragment-ions at *m/e* 271 (5%), *m/e* 211 (10%), and *m/e* 169 (5%). The absence of an intact capture-ion $[M+NH_4]^+$ (*m/e* 424) is a very striking observation; it must be a consequence of enhanced stability of the tetrahydrofurylium ion (*m/e* 331) obtained after expulsion of the anomeric substituent as acetic acid·ammonia relative to the isomeric tetrahydropyrilium ion generated from **12** and **13**. It is known[47] from e.i. studies that greater stability is associated with the five-membered-ring oxonium ions than with the six-membered-ring homologs, and so the present example demonstrates that the difference in relative stability is quite substantial. The other three fragments are derived by loss of one molecule and two molecules of acetic acid and then one molecule of ketene, respectively, from *m/e* 331.

Raffinose hendecaacetate[48] (**15**). — The c.i. (NH_3) mass spectrum (Fig. 6) of the trisaccharide derivative **15** was recorded in an effort to ascertain the limits of the molecular-weight range over which the c.i.–m.s. technique is useful; the spectrum consists essentially of a single $[M+NH_4]^+$ peak at *m/e* 984. The stability of such a large ion contrasts with the e.i. behavior[5] of **15**, wherein the molecular ion is not detected, but it is particularly surprising, because a glycosyl cation, exactly analogous to the tetrahydrofurylium ion that dominates the c.i. mass spectrum (NH_3) of **14** and that appears prominently in the e.i. mass spectrum of **15**, could be formed by simple cleavage of the glycosyl bond of the acetylated D-fructofuranosyl group in **15**. To account for the stability of $[\mathbf{10}+NH_4]^+$ it was hypothesized (see earlier) that the large number of bonds in the molecule partitions the excess energy into enough modes

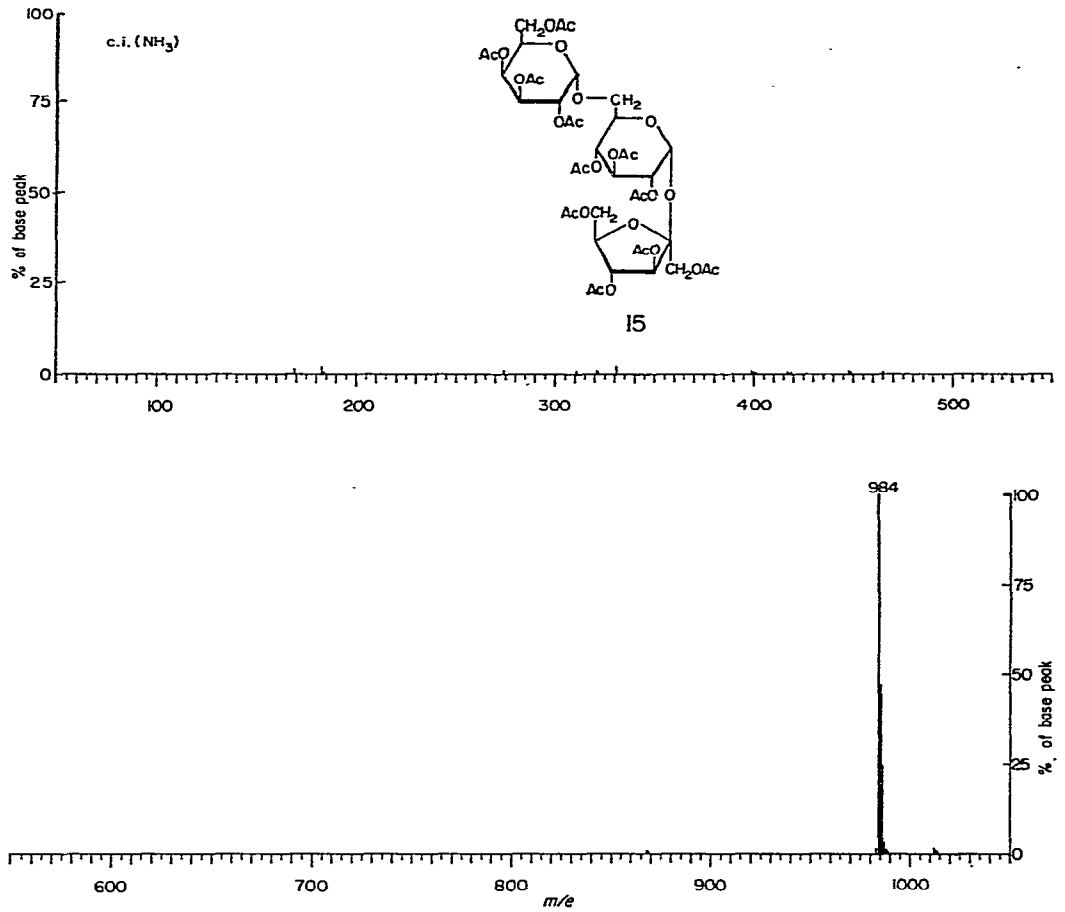


Fig. 6. Ammonia-mediated, chemical-ionization mass spectrum of raffinose hendecaacetate (**15**).

of internal motion that no bond is apportioned enough energy to cause it to rupture. The same explanation may account for the detection of only $[15+NH_4]^+$, although the multiplicity of potential sites for cation capture, and possible differences in effective basicity or steric accessibility of the anomeric oxygen atoms, may also contribute to the factors favoring preservation of the intact, capture-ion. The stability of the $[15+NH_4]^+$ ion suggests that detection of a substantial proportion of the initial ionization products as an intact capture-ion may be anticipated as a general phenomenon for related, large molecules, so that c.i. (NH_3) may prove a useful adjunct to e.i.–m.s. in physical structure-elucidation[5,23] of oligosaccharides as well.

CONCLUSIONS

Chemical-ionization mass-spectrometry is a technique that has great potential utility as a tool in the rapid, micro-scale characterization of sugars and their deriv-

atives, including oligosaccharides. The mild, ionizing conditions restrict molecular fragmentation to a few simple processes that mainly involve substituent groups only, and intact capture ions generally dominate the spectra, so that the technique has considerable potential for useful analytical application to mixtures, as well as to pure compounds. From the preceding examples, the following broad generalizations appear valid.

1. Ammonia supports an ionization process milder than that for isobutane, so that the tendency to afford intact capture ions ($[M+H]^+$ or $[M+NH_4]^+$) is greatly enhanced in this medium, which is thus an indicated choice for samples whose molecular weight is the primary concern.

2. Chemical ionization (NH_3) appears to transfer H^+ to basic nitrogen atoms[1,8,49] as the most favorable mode of ionization, so that the appearance of an $[MH]^+$ ion to the exclusion of the corresponding $[M+NH_4]^+$ ion constitutes presumptive evidence for the presence of basic nitrogen atoms in the sample.

3. Isopropylidene groups appear to exert a profound effect in determining the mode of fragmentation whenever they are present, and they are split off sequentially by c.i. (C_4H_{10}), so that the latter ionizing medium offers a method for determining the number of such groups present in the sample by analysis of the fragmentation sequence.

4. Acetate groups exert almost as strong an effect as the isopropylidene acetal group in determining modes of fragmentation, and dissipation of the initial ionization-energy through reaction of the acetate groups (loss of ketene, acetic acid, or acetic anhydride) can suppress more-energetic processes that involve other substituents or skeletal cleavage. The number of acetate groups present in a molecule may be determined by a simple perusal of the acetate-fragmentation reactions in the c.i. (C_4H_{10}) mass spectrum; reactive, unsubstituted hydroxyl groups might be acetylated or trideuteroacetylated and then counted by the same procedure.

5. Unsubstituted dithioacetals eliminate the alkylthio groups sequentially, although loss of the first one is a far more favored process than loss of the second; the presence of other substituents tends to inhibit fragmentation reactions involving loss of the second alkylthio group.

6. Carbon atoms that have more than one heteroatom attached to them are favored sites for fragmentation. The preceding items 3, 4, and, most especially, 5 derive from this generalization, which also includes the strong energetic favoring of expulsion of anomeric substituents from glycosyl derivatives to form the glycosyl ion.

7. Because of the greater number of bonds over which energy may be partitioned, large molecules that volatilize intact* appear to favor the generation of intact capture ions, particularly after c.i. (NH_3).

8. Skeletal fragmentation of the sugar is rarely observed under c.i. When such

*_Note added in proof:_ It has now been demonstrated[50], for a series of oligosaccharides, that intact molecular ions are commonly a prominent feature of the c.i. (NH_3) mass spectrum.

fission does occur, it appears that fission at exocyclic C–C bonds is favored, as against ring-opening reactions; rings having delocalized, multiple bonding are especially stable against internal bond-fission.

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