

Letters to the Editor

Dear Sir

Use of Sodium Bicarbonate for *in situ* Generation of Reagent Gas for Negative Chemical Ionization in Electron Impact Source†

Application of negative ion mass spectrometry has been increasing rapidly in the past few years. However, useful electron impact negative ion spectra are obtained only in a limited number of compounds having electron-capturing groups.¹ It has been shown by Brandenberger that, unlike positive ion chemical ionization mass spectrometry (CIMS), negative ion CIMS requires lower pressures and that this can be achieved in conventional EI sources.^{2,3} A sample mixed with NH_4Cl has been reported to give chloride ion attachment spectra in a CI source.⁴ We have observed formation of $[\text{M}+\text{Cl}]^-$ ion in an EI source from samples mixed with NH_4Cl .⁵ Caldwell and Bartmess have reported *in situ* generation of alkyl nitrite for alkoxide ion negative chemical ionization.⁶ Among the different reagent gases used for negative chemical ionization, CO_2 has been found to generate negative ions from most organic compounds due to the production of both secondary electrons and O^- ions^{2,3} which can react with the sample molecule to produce $[\text{M}]^-$ or $[\text{M}-\text{H}]^-$ ions, respectively.

This communication describes a method of recording negative ion spectra of compounds which do not give negative ion spectra under normal EI conditions, using NaHCO_3 as the reagent for *in situ* generation of CO_2 . The results obtained with compounds of different classes, such as alcohols, amines, steroids, terpenoids and alkaloids, are shown in Table 1. All the compounds examined give an $[\text{M}-\text{H}]^-$ ion as the base peak. These spectra were recorded on a Jeol D-300 mass spectrometer using the EI source. The sample mixed with a small amount (c. 1 mg) of NaHCO_3 is introduced into the source (200 °C) and heated to about 190–200 °C (uncorrected). As the evolution of CO_2 starts the source pressure, as indicated by a pressure gauge c. 25 cm away from the source, slowly rises and reaches 1×10^{-5} Torr. Figure 1 shows the TIM trace obtained in such an experiment using cholesterol. Several spectra can be recorded during this period. Heating NaHCO_3 alone under the same conditions gives the spectra

Table 1. Relative abundances of major ions in negative ion spectra using NaHCO_3 ^a

| Compound | $[\text{M}]^-$ | $[\text{M}-\text{H}]^-$ | $[\text{M}-\text{H}+\text{CO}_2]^-$ | Other ions |
|--|---------------------|-------------------------|-------------------------------------|---|
| 2-Adamantanol | 152 (11) | 151 (100) | | 39 25 (2) (3) |
| Nonacosan-10-ol (ginnol) | 424 (31) | 423 (100) | 467 (5) | 422, 421, 295, 282, 253, 155, (5) (7) (13) (5) (7) (34) 45, 43, 32, 26, 25 (13) (23) (117) (45) (40) |
| Cholesterol | 386 (28) | 385 (100) | 429 (14) | 399, 383, 367, 125, 111, (4) (8) (16) (6) (8) 99, 59, 57, 32, 26 (11) (22) (18) (34) (11) |
| β -Sitosterol | 414 (36) | 413 (100) | 457 (8) | 412, 411, 399, 269, 117, 107 (9) (15) (7) (7) (29) (11) 57, 43, 39 (13) (21) (15) |
| Estrone | 270 (20) | 269 (100) | | 268, 253, 240, 171, 145, 117, (14) (5) (3) (4) (4) (6) |
| Progesterone | 314 (26) | 313 (100) | | 297, 147, 117, 115, 57, 39 (2) (27) (8) (8) (11) (8) |
| Cycloartenol ^b | 426 (34) | 425 (100) | 469 (8) | 441, 439, 424, 423, 246, 192 (23) (9) (12) (10) (25) (10) 166, 125, 85, 71, 59, 57 (18) (15) (89) (36) (47) (22) |
| α -Amyrin acetate | 468 (28) | 467 (100) | 511 (16) | 466, 451, 425, 59, 41, 32 (5) (4) (5) (33) (82) (11) |
| 6- α -Hydroxy-7- deacetylazadirone | 410 (28) | 409 (100) | 453 (2) | 426, 195, 165, 121, 112, 70, 59 (8) (14) (16) (18) (65) (16) (40) |
| Indoleacetic acid | 175 (12) | 174 (100) | | 129, 26 (5) (13) |
| Valine | 117 (6) | 116 (100) | | 26 (2) |
| Primaquine diphosphate | base 259 (20) | 258 (100) | | 245, 244, 173, 158, 143, 130, (5) (7) (14) (11) (59) (34) 115, 26 (18) (81) |
| Ephedrine hydrochloride ^b | base 165 (11) | 164 (100) | | 202, 200, 107 (12) (38) (52) |
| Morphine | 285 (29) | 284 (100) | | 283, 269, 225, 209, 146 (12) (11) (15) (20) (12) 26 (41) |
| Tetrahydro palmatine | 355 (26) | 354 (100) | | 352, 340, 339, 190 (10) (16) (15) (26) 174, 173, 161, 135, (44) (50) (25) (31) 133, 45, 42, 26 (36) (23) (15) (80) |

^a Relative abundance (not corrected for ^{13}C abundance) is shown against m/z values (above m/z 20). Halide ions observed in some of the spectra have been excluded.

^b Above m/z 50.

shown in Fig. 2. The formation of OH^- is apparently due to reaction of O^- and H_2O .⁷ It is obvious that the facile ionization of the sample molecules is due to their interaction

with O^- and OH^- generated from CO_2 and H_2O formed by the decomposition of NaHCO_3 . Apart from $[\text{M}-\text{H}]^-$ ion, fragment ions and the adduct ion of $[\text{M}-\text{H}]^-$

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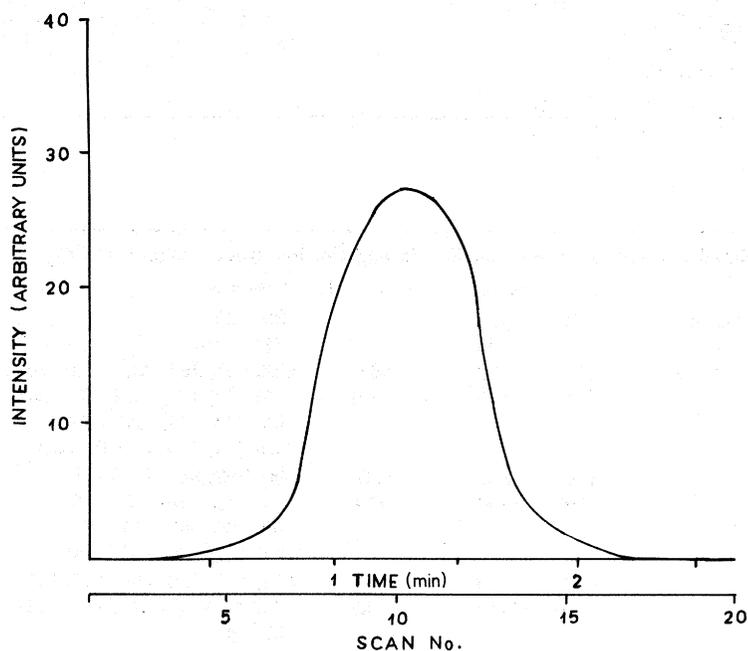


Figure 1. Total ion current plot of cholesterol negative ion spectrum using NaHCO_3 .

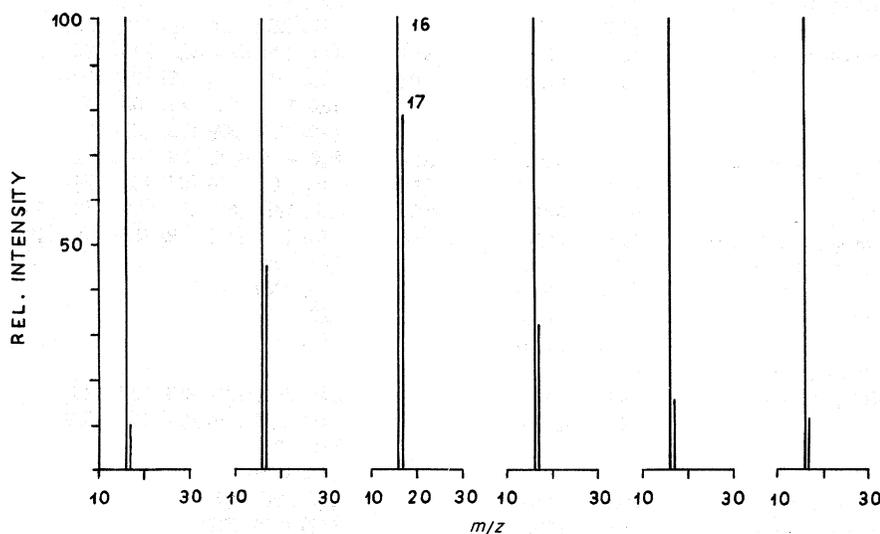


Figure 2. Negative ion spectra obtained during the evolution of CO_2 from NaHCO_3 .

and CO_2 are also observed in many of the cases (Table 1). Highly volatile or non-volatile compounds fail to give spectra under these conditions as approximate coincidence of sample evaporation and CO_2 evolution seems to be a requisite for obtaining these spectra. The advantage of this method lies in the fact that compounds for which normal negative ion EI spectra are not obtained can be made to yield useful spectra under these conditions. Studies of the applications of this technique to various other classes of compounds are presently in progress.

Yours

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Dear Sir

Electron Impact Mass Spectra of Permethy- lated *N*-methyl,*N*-phenyl(glucopyranosyl- 1-deoxyglucitol-1-yl)amines

Due to the fact that electron impact (EI) mass spectra of sugars are characterized by the absence of molecular ions and a multiplicity of fragmentation pathways,¹ recent applications of mass spectrometry to carbohydrate analysis^{1,2} and oligosaccharide sequencing^{3,4} are generally conducted in the chemical ionization or field desorption⁵ mode. Our interest in the biological significance of natural oligo- and poly-saccharides has led one of us (S.O.) to develop an alternate method for determining structures of these biomolecules.⁶ This procedure in-

cludes the tagging of the reducing end of a saccharide by reductive amination with aniline and sodium cyanoborohydride, followed by permethylation with dimsyl K, partial hydrolysis and separation of the partial hydrolysates by high-pressure liquid chromatography.

In this report we present the 70 eV EI mass spectra of two glucosylalditol derivatives prepared by this newly developed polysaccharide sequencing procedure. These spectra are of interest because they show that it may be possible to study sugars by EI mass spectrometry using this derivative. The mass spectra of the (1→4) and (1→3) linked compounds, namely, permethylated *N*-methyl,*N*-phenyl(4-*O*- α -D-glucopyranosyl-1-deoxy-D-glucitol-1-yl)amine, which was

derived from maltose, and permethylated *N*-methyl,*N*-phenyl(3-*O*- β -D-glucopyranosyl-1-deoxyglucitol-1-yl)amine, which was derived from laminarin, a β -(1→3)-glucan, are shown in Fig. 1.

The ions m/z 545 and 120 observed for both compounds represent the molecular ion and an ion generated by cleavage β to the amine group of the alditol portion, respectively, while the m/z 187 ion is derived from cleavage of the glucosyl portion with a subsequent loss of methanol.³ These three ions serve to derive the general structure of the terminal two units of the reducing end of the polymer under investigation. The presence of ions m/z 326 in Fig. 1(a) and m/z 218 in Fig. 1(b), which are both derived from the alditol portion of the molecule,

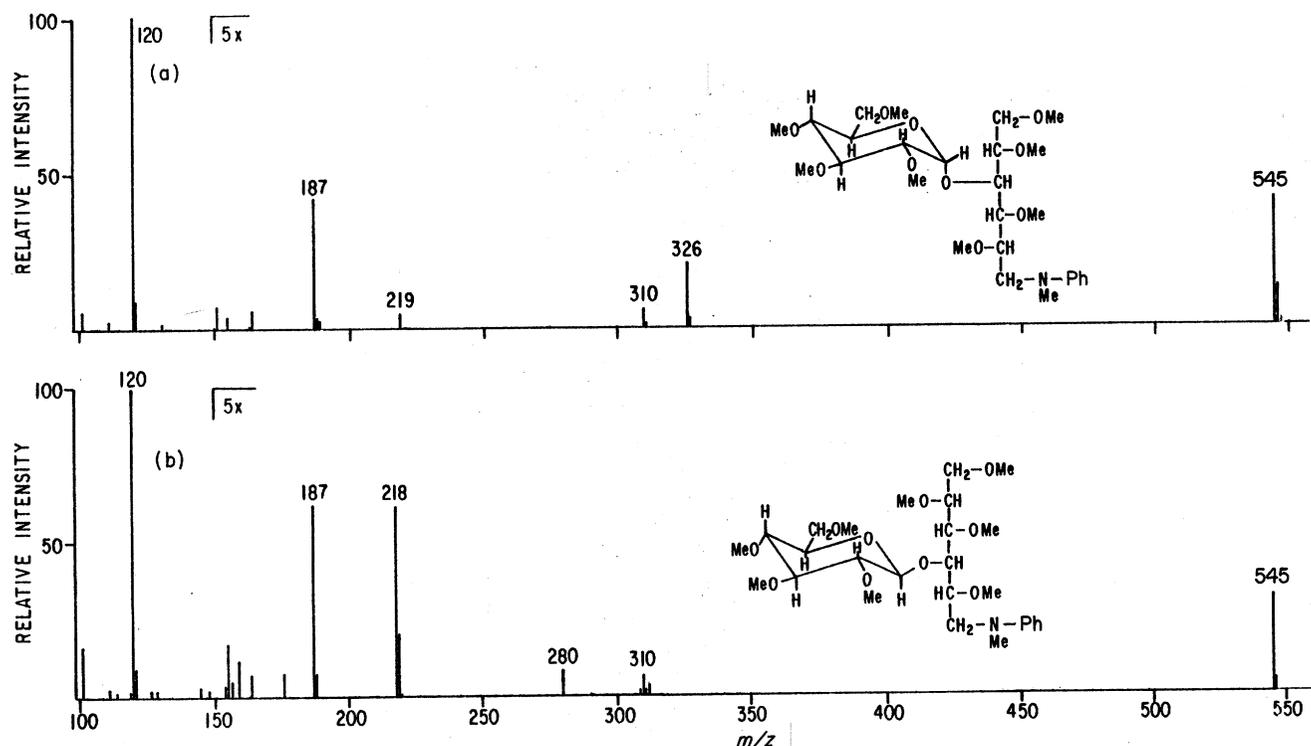


Figure 1. 70 eV EI mass spectra of (a) permethylated *N*-methyl, *N*-phenyl(4-*O*- α -D-glucopyranosyl-1-deoxy-D-glucitol-1-yl)amine and (b) permethylated *N*-methyl, *N*-phenyl(3-*O*- β -D-glucopyranosyl-1-deoxyglucitol-1-yl)amine.

clearly differentiates the (1 \rightarrow 4) from the (1 \rightarrow 3) linkage. The accurate mass of the *m/z* 218 ion was measured and found to be 218.1195 \pm 0.001. This compares favorably to mass 218.1181 of C₁₃H₁₆O₂N, which is the empirical formula of the alditol portion -2[O(CH₃)₂]. Whether the formation of this ion is also related to the rarely studied linkage conformation (α -, β -linkage)⁷ is not clear.

Compared to other derivatization processes, permethylation of oligosaccharides offers the advantage of relatively low molecular weights, which makes mass spectrometric studies easier. However, EI mass spectrometric investigations of these derivatives are often hindered by the absence of an abundant molecular ion.⁸ Reduction^{4,9} of the reducing end of the oligosaccharide provides an effective labeling of the reducing terminal sugar, and prevents the possible complication of anomer formation. The attachment of an *N*-phenyl, *N*-methyl group as a result of the amination by aniline and subsequent permethylation is shown here to provide a significant abundance of molecu-

lar ions and to simplify the fragmentation pathway under 70 eV EI mass spectrometric conditions.

Further studies on the effect of linkage position,^{3,10} linkage conformation and higher saccharides are in progress and will be reported in a full paper.

Yours

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Dear Sir

The Ring Opening of the Benzocyclobutene Radical Cation

Neutral benzocyclobutene (1,2-dihydrocyclobutabenzene) is known to undergo a thermal electrocyclic ring opening to *o*-xylylene (*o*-quinodimethane) at 200 °C.¹ The corresponding valence isomerization of the radical anion is more facile and appears

to occur in a conrotatory manner.^{2,3} However, nothing is known about the valence isomerization of the radical cation, which is the subject addressed here. Related systems, cyclobutene and phenyl-substituted cyclobutenes, do show an energy advantage for the electrocyclic reaction if the reactant exists as the radical cation.^{4,5}

Ionization of benzocyclobutene in a high-

pressure (c. 1 Torr) mass spectrometer source by using a low-energy charge exchange reaction with [CS₂]⁺ produced a [C₈H₈]⁺ ion which can be trapped by reaction with neutral styrene- β -d₂. The [C₁₆H₁₄D₂]⁺ adduct was then submitted to collisional activation by selecting it with MS-I of a triple analyzer mass spectrometer and admitting the ion to a collision cell