lized even by rechromatography. An alkaline permanganate streak on the extruded column indicated two zones at 40 and 50 mm. from the column top. The eluate from the top zone provided α-maltose heptaacetate after solvent removal and recrystallization from acetone: yield 50 mg., m.p. 149–151° unchanged on admixture with the product obtained from β-maltose heptaacetate, $[\alpha]^{20}D$ +128° (*c* 0.77, chloroform).

β-Maltose hexaacetate was obtained from the eluate of the second zone: yield 40 mg., m.p. 163–165° (after recrystallization from absolute ethanol). This product was identical with the above-mentioned β-maltose hexaacetate by mixture melting point and X-ray powder diffraction pattern.

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Mass Spectrometry in Carbohydrate Chemistry. Dithioacetals of Common Monosaccharides

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The mass spectra of the dialkyl and ethylene dithioacetals of D-glucose, D-arabinose, 2-deoxy-D-glucose, 6-deoxy-L-galactose, 6-deoxy-L-mannose, 2-*O*-methyl-D-glucose, and 3-*O*-methyl-D-glucose are interpreted in terms of structural differences among the compounds. The dialkyl dithioacetals exhibit molecular-ion peaks ranging from 5–20% of the base peak; the ethylene dithioacetals exhibit no discernible molecular-ion peak or one of very low intensity relative to the base peak. It is possible to relate peaks in the mass spectra to the position of deoxy and methoxyl groups in these molecules.

A frequently employed procedure for the isolation of carbohydrate residues which are present in complex molecules is hydrolysis of glycosidic bonds with a thiol and a strong acid. This mercaptolysis technique has been useful in determining the structure of streptomycin,[1] lincomycin,[2] and other compounds containing sugar moieties.

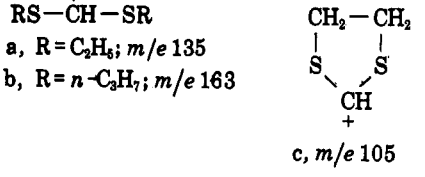
Recently mass spectral studies of monosaccharide diethyl dithioacetal[3] and ethylene dithioacetal[4] peracetates have been reported. The mass spectra of these peracetyl derivatives of mercaptolysis products can be readily interpreted in terms of structural differences such as molecular weight and substitution. Only a fraction of a milligram of material is required for obtaining this wealth of information. These considerations lead one to anticipate that mass spectrometry combined with mercaptolysis may soon be a powerful tool in the structure elucidation of the carbohydrate portion of complex molecules.

With the advent of commercially available mass spectrometers equipped with inlet systems which allow insertion of samples of relatively low vapor pressure directly into the ion source, the dithioacetal derivatives of carbohydrates can be studied without having to acetylate to increase volatility. The mass spectra of a number of common dithioacetal monosaccharide derivatives have been obtained using this direct-insertion technique and are discussed here. Their mass spectra are much less complex than those of the acetylated analogs, yet they are very sensitive to structural differences.

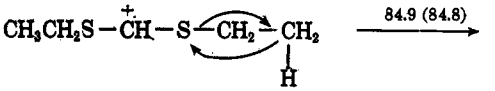
Results

Molecular-Ion Peaks.—The mass spectra of D-arabinose diethyl dithioacetal (Figure 1) and di-*n*-propyl dithioacetal (Figure 2), of D-glucose diethyl dithioacetal (Figure 3) and di-*n*-propyl dithioacetal (spectrum not shown), of 2-deoxy-D-glucose diethyl dithioacetal (Figure 4), of 6-deoxy-L-mannose diethyl dithioacetal (Figure 5), of 3-*O*-methyl-D-glucose diethyl dithioacetal (Figure 6) and di-*n*-propyl dithioacetal (Figure 7), and of 2-*O*-methyl-D-glucose diethyl dithioacetal (Figure 8) exhibit molecular-ion peaks ranging from 5–20% of the base peak in the spectra; from their mass spectra, their molecular weight can be directly determined. In contrast, the mass spectra of D-arabinose ethylene dithioacetal (Figure 9), D-glucose ethylene dithioacetal (Figure 10), 2-deoxy-D-glucose ethylene dithioacetal (Figure 11), and 6-deoxy-L-galactose ethylene dithioacetal (Figure 12) either exhibit no discernible molecular-ion peak or one of intensity 0.03–1.5% of the base peak.

The Dithioacetal Fragments.—In all of the mass spectra studied, except that of 2-deoxy-D-glucose diethyl dithioacetal (Figure 4), the base peak results from C-1–C-2 cleavage with charge retention on the dithioacetal carbon atom, C-1, where it is stabilized by two adjacent sulfur atoms. This fragment is characteristic of C-1 and its substituents.



Metastable-ion peaks and peak shifts from changes in the R groups show that the dialkyl dithioacetal



$$CH_3CH_2S-\overset{+}{C}H-S-H + CH_2=CH_2 \quad (1)$$

m/e 107

(1) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink, and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2096 (1946).

(2) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *ibid.*, **86**, 4223 (1964).

(3) D. C. DeJongh, *ibid.*, **86**, 3149 (1964).

(4) D. C. DeJongh, *ibid.*, **86**, 4027 (1964).

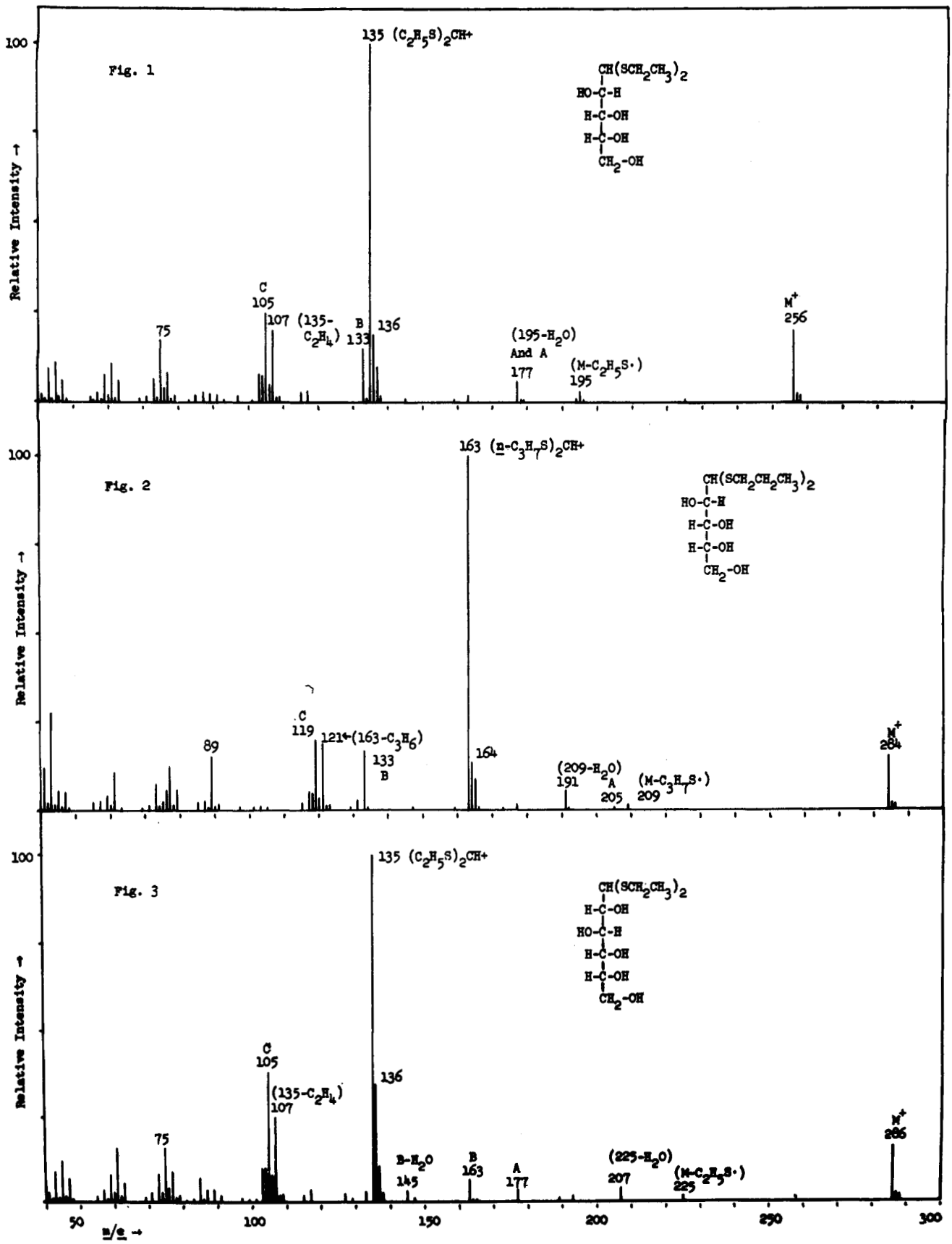


Figure 1.—Mass spectrum of D-arabinose diethyl dithioacetal
Figure 2.—Mass spectrum of D-arabinose di-*n*-propyl dithioacetal.
Figure 3.—Mass spectrum of D-glucose diethyl dithioacetal.

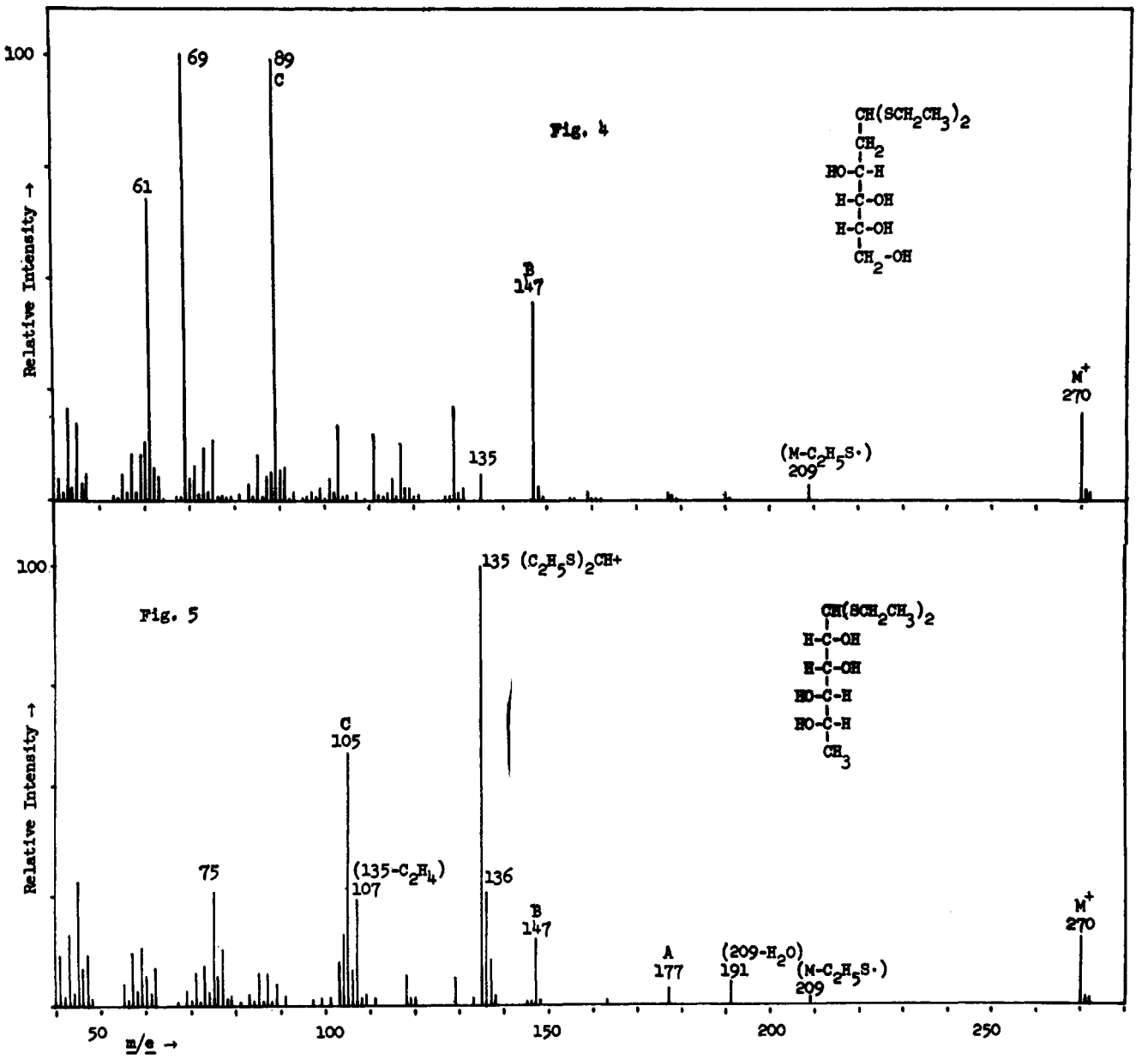
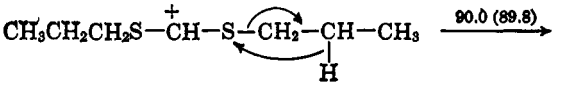
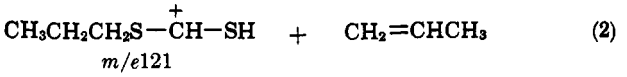


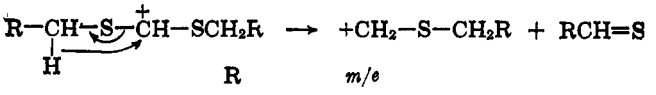
Figure 4.—Mass spectrum of 2-deoxy-D-glucose diethyl dithioacetal.
Figure 5.—Mass spectrum of 6-deoxy-L-mannose diethyl dithioacetal.





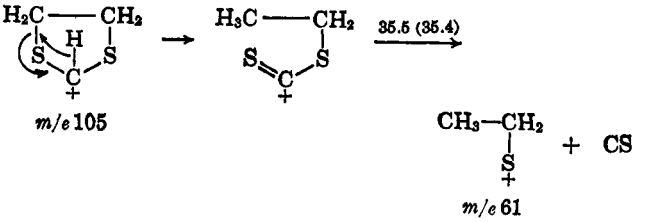
(2)

cation fragments further. Olefin expulsion with hydrogen migration is one example. (See eq. 1 and 2.) Thioethers also commonly eliminate olefin upon electron impact.[5] Another fragmentation appears to be thioaldehyde elimination. Although no metastable-ion peak supports this process, the mass spectra of the peracetylated analogs[3] and the peak shifts when R is varied do indicate that the elimination occurs.



R	*m/e*
CH_3	75
C_2H_5	89

The ethylene dithioacetal cation expels carbon monosulfide to give a peak at *m/e* 61 characteristic of that cyclic type of dithioacetal.



C-1–C-2 cleavage with hydrogen transfer is responsible for the nonisotopic portion of the peaks at *m/e* 136 in Figures 1, 3, 5, 6, and 8; at *m/e* 164 in Figures 2 and 7, and the mass spectrum of D-glucose di-*n*-propyl

(5) E. J. Levy and W. A. Stahl, *Anal. Chem.*, **33**, 707 (1961).

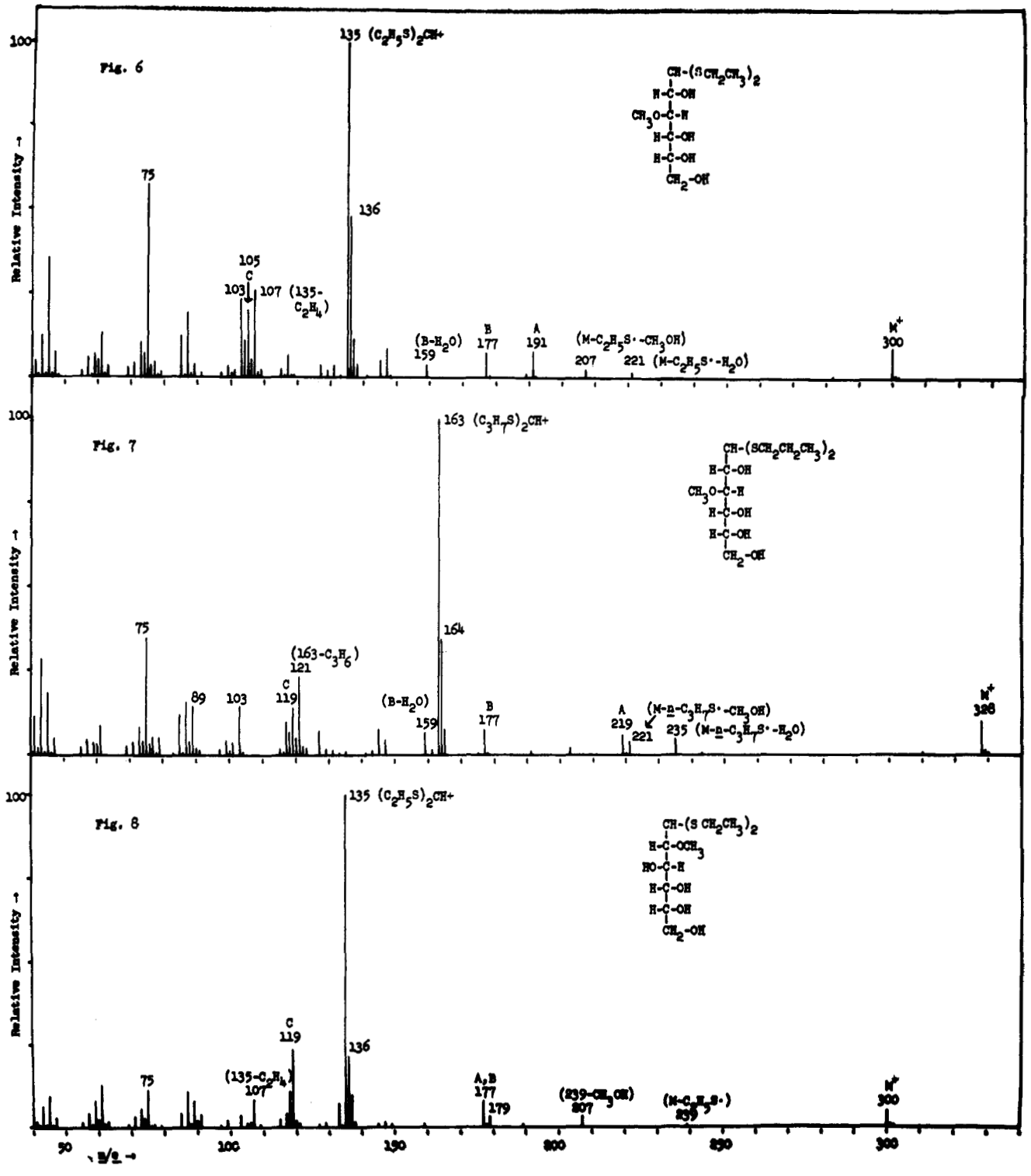


Figure 6.—Mass spectrum of 3-*O*-methyl-D-glucose diethyl dithioacetal.
Figure 7.—Mass spectrum of 3-*O*-methyl-D-glucose di-*n*-propyl dithioacetal.
Figure 8.—Mass spectrum of 2-*O*-methyl-D-glucose diethyl dithioacetal.

dithioacetal; and at *m/e* 106 in Figures 9, 10, and 12. This hydrogen transfer is inhibited if C-2 is unsubstituted, *e.g.*, Figures 4 and 11, or if the molecule is peracetylated.[3,4] The presence of 2- and 3-*O*-methyl groups does not interfere with the formation of this species. This transfer can be used to differentiate the 2- and 6-deoxy isomers in the ethylene dithioacetal series.

Carbon–Sulfur Bond Cleavage.—C-1–S bond cleavage with charge retention on C-1 is characteristic of all the dialkyl dithioacetal derivatives studied. A molecule of water is eliminated from the fragment formed, placing a carbon–carbon double bond in conjugation with the charge on carbon. The fact that the M − RS· fragment from the 3-*O*-methyl-D-glucose derivative (Figure 6) loses both methanol and water, whereas the 2-*O*-methyl analog (Figure 8) loses only methanol, supports the suggested preferential elimination of the C-2 substituent.

The ethylene dithioacetal compounds cannot eliminate fragments by carbon–sulfur bond cleavage. Peaks

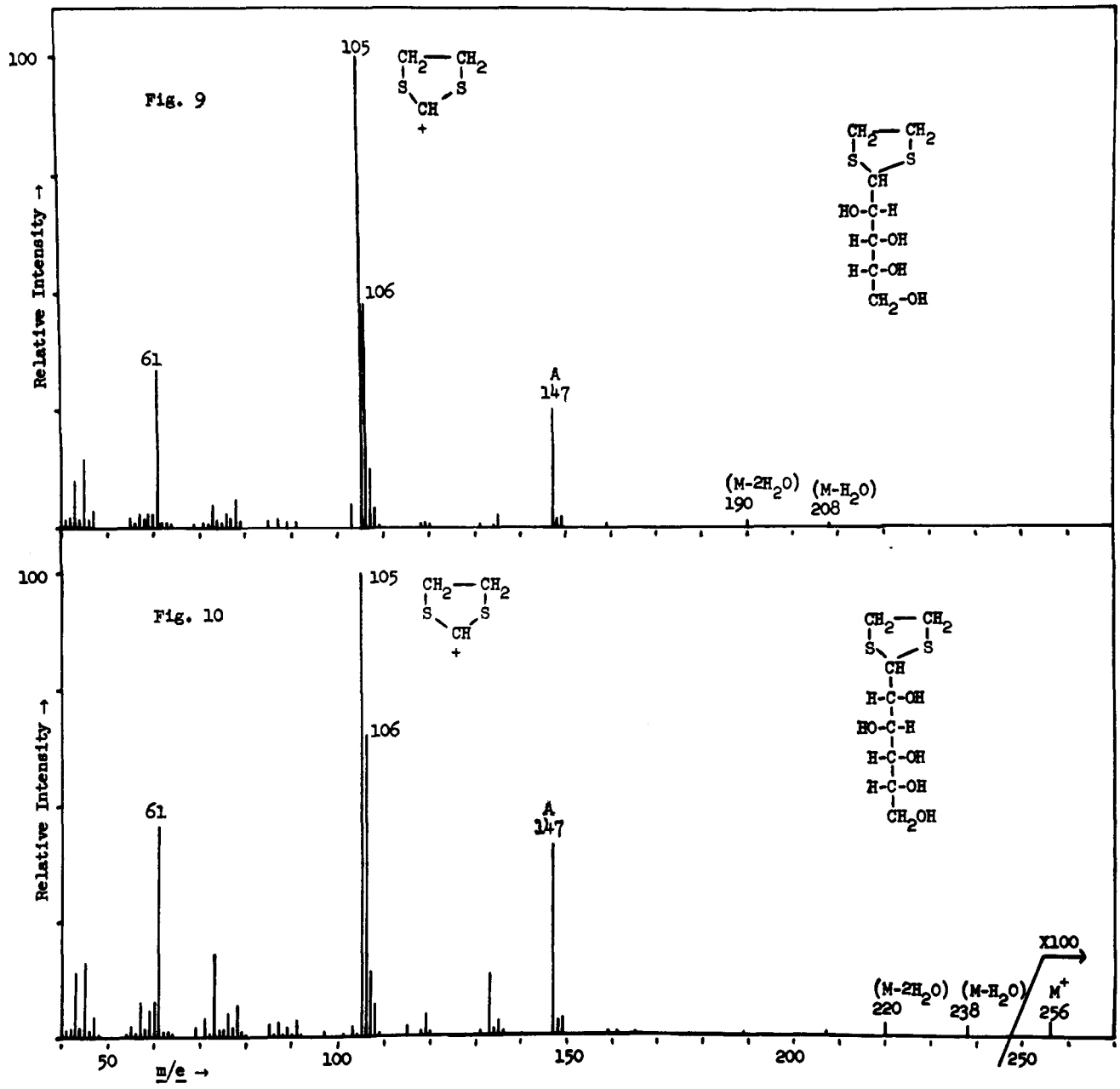
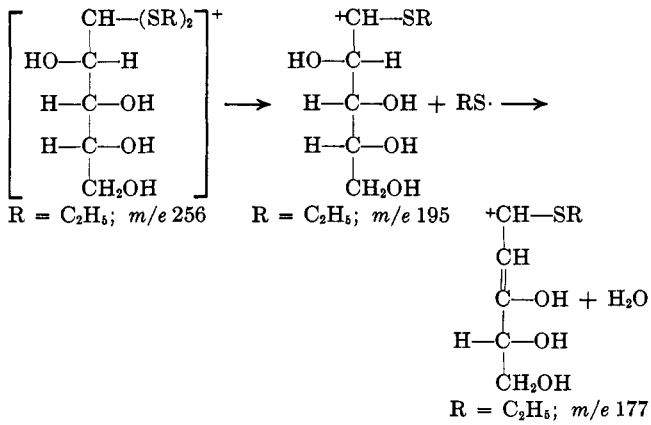
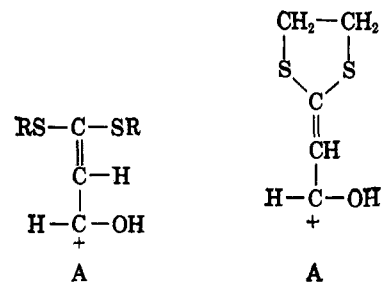


Figure 9.—Mass spectrum of D-arabinose ethylene dithioacetal.
Figure 10.—Mass spectrum of D-glucose ethylene dithioacetal.



in the high *m/e* region of their spectra result from expulsion of one and two molecules of water from their molecular ions.

Fragment A.—The mass spectra of the dithioacetal derivatives indicate that a fragment is formed by fission of the C-3–C-4 bond of the monosaccharide and elimination of C-2 substituent along with a hydrogen atom at C-1. Fragment A is not present in the mass spectrum of 2-deoxy-D-glucose diethyl dithioacetal (Figure 4).



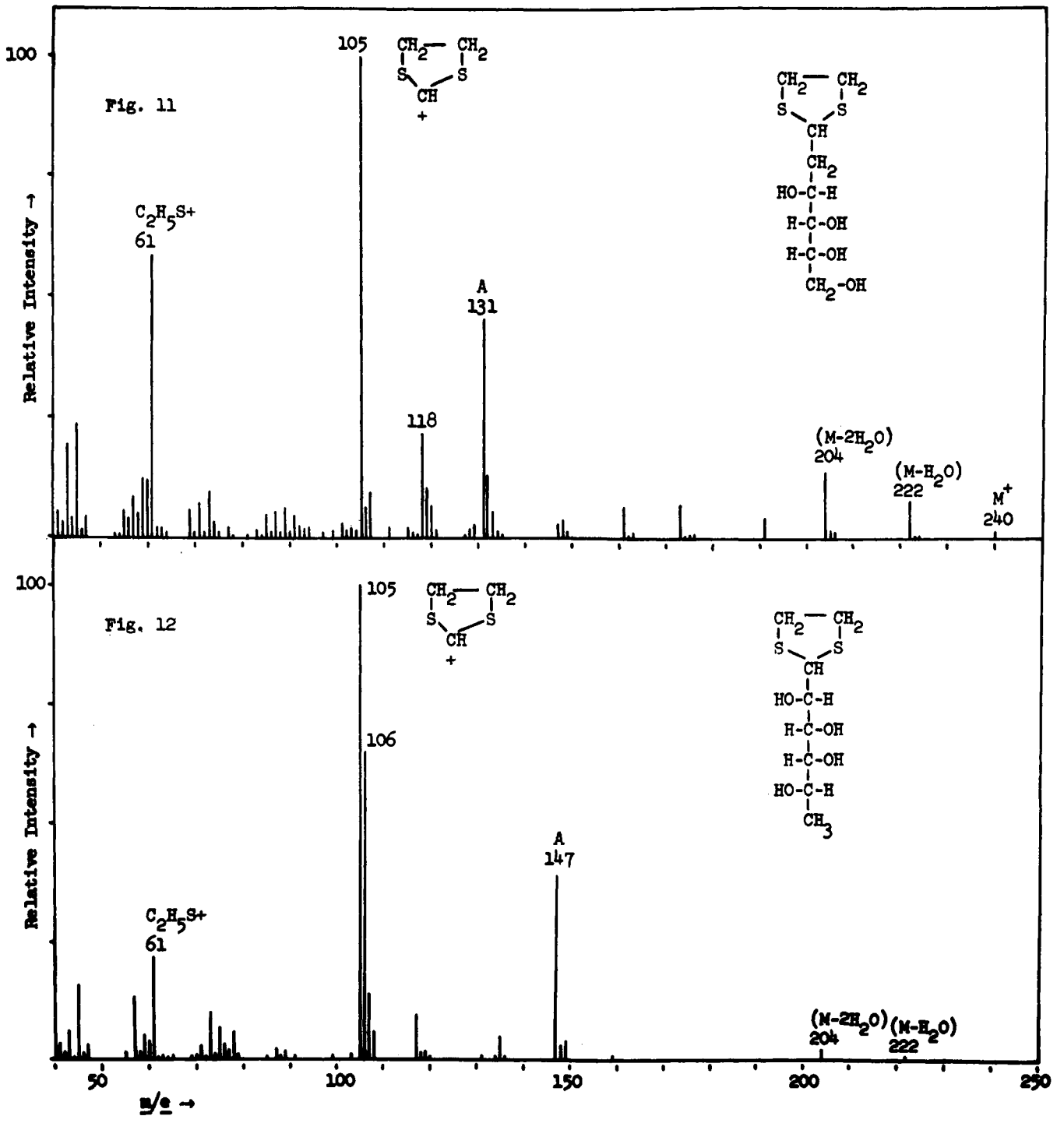
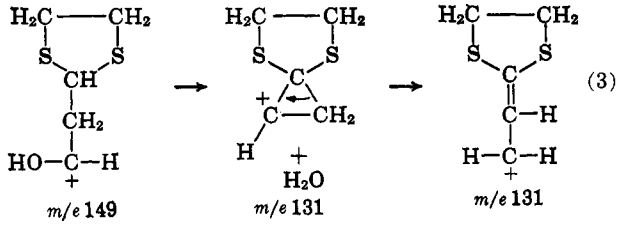
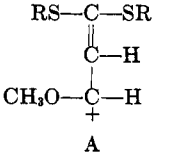


Figure 11.—Mass spectrum of 2-deoxy-D-glucose ethylene dithioacetal.
Figure 12.—Mass spectrum of 6-deoxy-L-galactose ethylene dithioacetal.

The relatively minor peaks at *m/e* 149, 148, and 147 in the mass spectrum of 2-deoxy-D-glucose ethylene dithioacetal (Figure 11) indicate that fragment A, *m/e* 147, is formed from C-3–C-4 bond cleavage followed by elimination of one and then two hydrogen atoms or of a hydrogen molecule. The major fragment of type A is found at *m/e* 131 arising from C-3–C-4 bond cleavage and loss of the C-3 hydroxyl group with a hydrogen atom from C-1 or C-2 (see eq. 3).



(3)



R	*m/e*
C_2H_5	191
n-C_3H_7	219

A

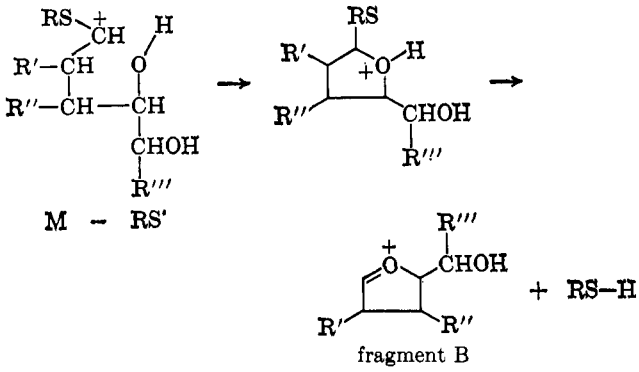
A comparison of the mass spectra of the 2- and 3-*O*-methyl dialkyl dithioacetal derivatives, Figures 6–8, shows that fragment A above indicates the presence of

substitution on C-3. It is found 14 mass units higher in the spectra of the 3-*O*-methyl derivatives.

Fragment B.—A study of the spectra of the dithioacetals indicates that a fragment forms which is independent of the type of dithioacetal as long as it is not the cyclic ethylene type. The information on this fragment is summarized as follows.

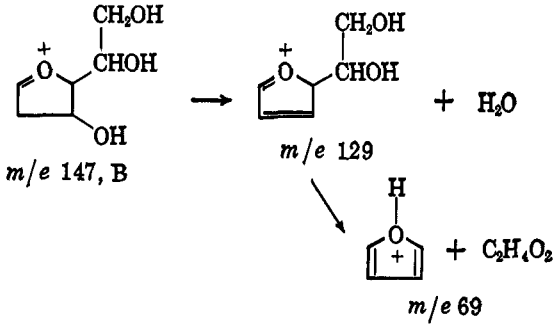
$(RS)_2CH$	R	R′	R″	R‴	*m/e*
CH—R′	C_2H_5	OH	OH	H	133
CH—R″	*n*-C_3H_7	OH	OH	H	133
CHOH	C_2H_5	OH	OH	CH_3	147
CHOH	C_2H_5	H	OH	CH_2OH	147
R‴	C_2H_5	OH	OH	CH_2OH	163
	C_2H_5	OH	OCH_3	CH_2OH	177
	n-C_3H_7	OH	OCH_3	CH_2OH	177
	C_2H_5	OCH_3	OH	CH_2OH	177

The following scheme can account for this data. A six-membered ring analog could be formed by using the C-5 hydroxyl group to help accommodate the charge on C-1 rather than the C-4 hydroxyl group as shown. The analog of fragment B is formed in the mass spectra of the corresponding peracetyl and perdeuterioacetyl derivatives.[3]



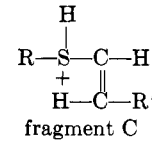
fragment B

Metastable-ion peaks are found in the mass spectrum of 2-deoxy-D-glucose diethyl dithioacetal (Figure 4) for the loss of ethanethiol from the M − C_2H_5S fragment to give fragment B, and for the loss of one and two molecules of water from fragment B to give *m/e* 129 and *m/e* 111, respectively. The intense peak at *m/e* 69, Figure 4, is most likely a protonated furan ion and may arise in the following manner. The



stability of the ion could be the driving force for its formation.

Fragment C.—A fragment which retains C-2 and its substituent appears in all the mass spectra of the dialkyl dithioacetals. A suggested structure which explains the shifts upon changes in R and changes in substitution on C-2 is shown. At present it is not possible to specify which hydrogen appears at the sulfur atom; fragment C may be formed from the M − RS fragment

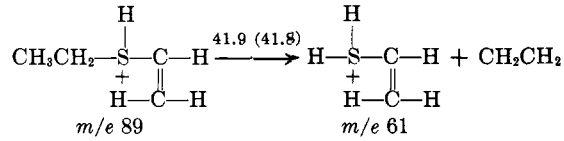


fragment C

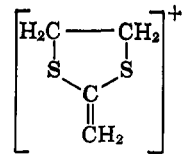
R	R′	*m/e*
C_2H_5	OH	105
n-C_3H_7	OH	119
C_2H_5	H	89
C_2H_5	OCH_3	119

by cleavage of the C-2–C-3 bond with transfer of a hydrogen atom to sulfur from somewhere in the molecule. The fact that this fragment is found as the unprotonated form in the mass spectra of the corresponding peracetylated analogs[3] suggests that the hydrogen atom transferred may come from a hydroxyl group.

In the mass spectrum of 2-deoxy-D-glucose diethyl dithioacetal (Figure 4), a metastable-ion peak indicates that fragment C, *m/e* 89, eliminates a molecule of ethylene.

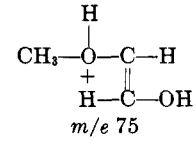


The mass spectrum of 2-deoxy-D-glucose ethylene dithioacetal has a peak at *m/e* 118 which can be considered as a type similar to C. Peak 118 is also prominent in the mass spectrum of the corresponding peracetyl derivative.[4]



m/e 118, C′

***O*-Methyl-D-glucose Dialkyl Dithioacetals.**—Besides the peaks due to the fragments discussed above, the mass spectra of the diethyl (Figure 6) and di-*n*-propyl (Figure 7) dithioacetals of 3-*O*-methyl-D-glucose contain fragments found at *m/e* 75 and 103 which are independent of the dithioacetal portion of the molecule. The part of *m/e* 75 in Figure 6 which shifts to *m/e* 89 in Figure 7 is $C_2H_5SCH_2^+$; the *m/e* 75 fragment which does not shift can be visualized as shown. This frag-



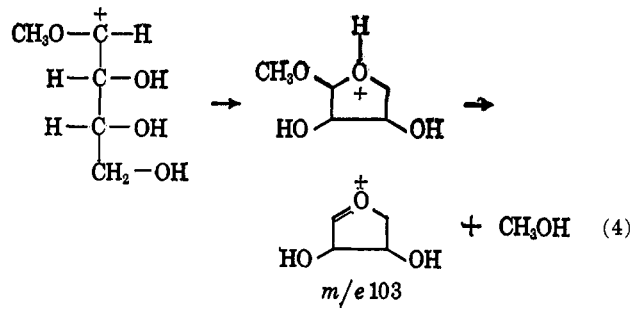
m/e 75

ment is found at *m/e* 75 in the mass spectra of partially methylated monosaccharides also.[6]

Cleavage of the C-2–C-3 bond adjacent to the methoxyl group followed by elimination of methanol leads to a fragment which would not shift with a change in the dialkyl dithioacetal portion of the molecule. A cyclization and elimination similar to the formation of fragment B can explain the peak at *m/e* 103 in Figures 6 and 7 (see eq. 4). A cleavage of the C-3–C-4 bond, also adjacent to the methoxyl group, with elimination of a molecule of water, leads to fragment A.

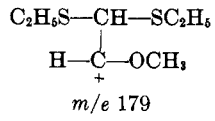
The peak at *m/e* 179 in the mass spectrum of the 2-*O*-methyl analog (Figure 8) likely arises from cleavage of the C-2–C-3 bond adjacent to a methoxyl group.

(6) N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Zolotarev, *Tetrahedron*, **19**, 2209 (1963).



m/e 103 (4)

The characteristic sulfur isotope peaks in the original spectrum support the presence of two sulfur atoms in this fragment; these isotope peaks do not appear in Figure 8 since only peaks >0.5% of the base peak are drawn.



m/e 179

Discussion

The dialkyl dithioacetals are superior to the ethylene dithioacetals as derivatives in the mass spectrometric study of carbohydrates owing to the presence of a molecular-ion peak in the mass spectra of the former. The acetylated dithioacetal derivatives are less desirable because successive losses of ketene and acetic acid from various fragments make their mass spectra more complex without providing any additional structural information.

Mercaptolysis of a carbohydrate with two different thiols provides two similar derivatives of the same compound whose mass spectra can be compared with substantiate proposed fragmentations. In the mass spectra of the dialkyl dithioacetals, the five main fragments formed are characteristic of the molecular weight, C-1 [$(RS_2)_2CH^+$, except for Figure 4], C-2 (fragment C), C-3 (fragment A), and the entire carbohydrate portion of the molecule (fragment B). The facts that mercaptolysis is often employed as a technique for characterizing carbohydrates and that considerable structural information is available from the mass spectrum obtained from as little as a fraction of a milligram of mercaptolysis product may make the combination of mass spectrometry and mercaptolysis an important development in the application of new physical methods to carbohydrate chemistry.

Experimental

Mass Spectra.—The mass spectra were determined with an Atlas–Werke CH4 mass spectrometer, ionizing potential 70 e.v., ionizing current 18 μa. The solid samples were ionized by electron bombardment after evaporation directly into the electron beam from a small furnace heated by a tungsten coil. A cathode with a tungsten wire of 0.15-mm. diameter was used; thereby, a minimum ion-source temperature of 80–90° was maintained.

Ethylene Dithioacetals.—D-Glucose ethylene dithioacetal, m.p. 143–145° (lit.[7] m.p. 143°), D-arabinose ethylene dithioacetal, m.p. 152–153° (lit.[8] m.p. 154.5°), 2-deoxy-D-glucose ethylene dithioacetal, m.p. 167.5–168.5°, and 6-deoxy-L-galactose ethylene dithioacetal, m.p. 190–190.5° (lit.[9] m.p. 191–191.5°), were prepared according to the procedure of Zinner[10] using 1,2-ethanedithiol in place of ethanethiol.

Dialkyl Dithioacetals.—D-Arabinose diethyl dithioacetal, m.p. 124.5–125.5° (lit.[10] m.p. 125.0–125.5°), D-arabinose di-*n*-propyl dithioacetal, m.p. 128–130° (lit.[11] m.p. 128°), D-glucose diethyl dithioacetal, m.p. 124–127° (lit.[10] m.p. 127–127.5), D-glucose di-*n*-propyl dithioacetal, m.p. 143–145.5 (lit.[12] m.p. 146°), 2-deoxy-D-glucose diethyl dithioacetal, m.p. 137.0° (lit.[13] m.p. 134.0°), and 6-deoxy-L-mannose diethyl dithioacetal, m.p. 134.5–136° (lit.[10] m.p. 136.5–137°), were prepared according to the procedure of Zinner.[10]

***O*-Methyl Dialkyl Dithioacetals.**—3-*O*-Methyl-D-glucose diethyl dithioacetal, m.p. 70–74° (lit.[14] m.p. 72–76°), and di-*n*-propyl dithioacetal were prepared according to the procedure of Hough and Richardson,[14] except that the reactants were shaken at 0° for 5 hr. before being shaken at room temperature for 2.5 hr.

2-*O*-Methyl-D-glucose diethyl dithioacetal, m.p. 176–177° (lit.[15] m.p. 177–178°), was prepared according to the procedure of Lieser and Leckzyck.[16]

Acknowledgment.—This investigation was supported by Public Health Service Research Grant GM 12328-01 from the National Institutes of Health. The mass spectrometer was purchased with funds from a Research Instrument Grant (GP-1474) from the National Science Foundation. The author wishes to thank Miss N. E. Roiba and Mr. U. G. Klopfer for preparing some of the compounds used in this investigation.

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(8) H. Zinner, H. Brandner, and G. Rembarz, *Chem. Ber.*, **89**, 800 (1956).
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(10) H. Zinner, *Chem. Ber.*, **84**, 780 (1951).
(11) Y. Maeda and Y. Uyeda, *Bull. Chem. Soc. Japan*, **1**, 181 (1926).
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