62.5 (19-methyl), doublet at 112 having J = 2.5 c.p.s. (21-methyl), 122.5 (acetate methyl), doublet at 325 (6-H), and a multiplet at 365 c.p.s. (allenic H).

Anal. Calcd. for $C_{25}H_{35}ClO_2$: C, 74.53; H, 8.69; Cl, 8.82. Found: C, 75.04; H, 8.95; Cl, 8.43.

The second fraction (250 mg.) was a mixture of XIX and V.

The third fraction (750 mg.) was mostly V. It was rechromatographed and crystallized three times from methanol to give 600 mg. of pure V.

The fourth fraction (100 mg.) was crystallized twice from methanol, m.p. 137–139°. It was identified as VII. It had infrared bands at 3.05 and 4.8 μ; $\lambda_{max}^{methanol}$ 233 mμ (ϵ 15,000); n.m.r. peaks 55 (18-methyl), 62.5 (19-methyl), 105 with half-band width of 2 c.p.s. (21-methyl), 177.5 (ethynyl H), and doublet at 322.5 c.p.s. having J = 4.5 c.p.s. (6-H).

Anal. Calcd. for $C_{25}H_{34}O_2$: C, 81.92; H, 9.35. Found: C, 81.78; H, 9.05.

The fifth fraction (125 mg.) was crystallized from methanol: m.p. 145–147°; $\lambda_{max}^{methanol}$ 233 mμ (ϵ 15,000). It was identified as VI. It had infrared bands at 3.05 and 4.8 μ; n.m.r. peaks at 55 (18-methyl), 62.5 (19-methyl), triplet at 113 having J = 1.6 c.p.s. (21-methyl), 182.5 (ethynyl H), and doublet at 322 c.p.s. having J = 4.5 c.p.s.

Anal. Calcd. for $C_{25}H_{34}O_2$: C, 81.92; H, 9.35. Found: C, 81.72; H, 9.41.

20-Acetyl-5,20(21)-pregnadien-3β-ol Acetate (XV). A catalyst was prepared by warming together 0.25 g. of red mercuric oxide, 0.1 ml. of boron trifluoride etherate, 5 mg. of trichloroacetic acid, and 1 ml. of methanol. A solution of 500 mg. of the vinylacetylene V in 20 ml. of methanol was then added to it and the mixture stirred at room temperature for 3 hr., after which time it was poured into dilute sulfuric acid and the product was isolated by extraction with ethyl acetate. The residue obtained after removal of ethyl acetate was acetylated with 3 ml. of acetic anhydride and 6 ml. of pyridine at room temperature for 16 hr. The product, obtained after working up, was purified by chromatography over alumina. The product (300 mg.) eluted from the column was crystallized from methanol: m.p. 210–211°; $\lambda_{max}^{methanol}$ 227 mμ (ϵ 9500); infrared bands at 5.78 (acetate), 5.98 (conjugated ketone), and 6.15 μ (conjugated C=C).

Anal. Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 77.95; H, 9.26.

Hydration of the Enynes VI and VII. To 1 ml. of 97% formic acid was added 50 mg. of the enyne (VI or VII) and then it was heated on a steam bath for 3 min. when it went into solution and turned pink. It was then worked up by diluting with water and extracting with methylene chloride. After removal of solvent, the crude residue was purified by crystallization. The ketone obtained from VI had the same characteristics as IV, and that from VII was identical with III.

Characterization of Amino Sugars by Mass Spectrometry

Don C. DeJongh and Stephen Hanessian

Contribution from the Chemistry Department, Wayne State University, Detroit, Michigan, and Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan. *Received April 10, 1965*

Amino sugar dithioacetals in the form of the N-acetates are suitable derivatives for characterization by mass spectrometry. The classes investigated include derivatives of 2-amino-2-deoxy-, 2,6-diamino-2,6-dideoxy-, 3,6-diamino-3,6-dideoxy-, 3-amino-3,6-dideoxy-, 6-amino-6-deoxyhexoses, and 3-amino-3-deoxy-, 4-amino-4,5-dideoxy-, 5-amino-5-deoxy-, and 4,5-diamino-4,5-dideoxypentoses. The mass spectra are discussed and interpreted in terms of the structures of the acetamido-deoxyaldose diethyl dithioacetals, in terms of the peak shifts in the spectra of the di-n-propyl dithioacetals and the acetamido-d_3 analogs, and in terms of metastable ion peaks.

Introduction

The application of mass spectrometry to carbohydrate chemistry should prove increasingly valuable to the biological field. The limitation of volatility has been overcome by the availability of mass spectrometers with inlet systems that allow direct introduction of the sample into the ion source. The great variety of structure found in the carbohydrate class has also limited utilization of mass spectrometry in structural carbohydrate chemistry. It has been necessary to systematically study the electron-impact behavior of model compounds, a goal toward which steps have been undertaken during the past 2 years.[1]

Initial efforts by Reed and co-workers[2] were directed to the study of some disaccharides, oligosaccharides, and glycoside derivatives. More detailed studies on the acetates,[3,4] acetates and methyl ethers,[5] and isopropylidene derivatives[6] of pentoses and hex-

(1) For recent reviews on this subject see K. Biemann, *Angew. Chem.*, **74**, 102 (1962); K. Biemann, "Mass Spectrometry," McGraw Hill Book Co., Inc., New York, N. Y., 1962; H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, p. 203; R. J. Ferrier and N. R. Williams, *Chem. Ind.* (London), 1696 (1964).

(2) P. A. Finan, R. I. Reed, and W. Snedden, *Chem. Ind.* (London), 1172 (1958); P. A. Finan and R. I. Reed, *Nature*, **184**, 1866 (1959); R. I. Reed, W. K. Reid, and J. M. Wilson "Symposium on Mass Spectrometry," Oxford University Press, London, Sept. 1961; P. A. Finan, R. I. Reed, W. Snedden, and J. M. Wilson, *J. Chem. Soc.*, 5945 (1963).

(3) K. Biemann, H. K. Schnoes, and J. A. McCloskey, *Chem. Ind.* (London), 448 (1963).

(4) K. Biemann, D. C. DeJongh, and H. K. Schnoes, *J. Am. Chem. Soc.*, **85**, 1763 (1963).

(5) D. C. DeJongh and K. Biemann, *ibid.*, **85**, 2289 (1963).

oses and nucleosides[7] were reported by Biemann and co-workers. Carbohydrate methyl ethers have been investigated by Kochetkov and collaborators.[8] The study of methyl ethers of common glycosides and peracetylated sugars was also reported by Heyns and co-workers.[9,10] More recently, carbohydrates in the form of their acetylated dithioacetals have been studied.[11,12] The spectra of the dithioacetals of common monosaccharides, which are less complex than the acetylated analogs, have been obtained and interpreted in detail with respect to structural differences.[13]

A preliminary communication dealing with the study of dithioacetals of amino sugars was reported.[14]

Discussion

The present paper is concerned with the detailed interpretation of the mass spectra of the dithioacetals previously studied[14] and a number of additional dithioacetals of amino sugars.

These derivatives have been found to be very suitable for the present study and provide spectra which can be easily correlated with structure without complications associated with cyclic forms. Such advantages have been observed and discussed previously.[11-13]

In addition, it has been found that relatively small amounts of many amino sugar derivatives can be conveniently converted to their dithioacetals and the products crystallized directly or after purification by thin layer chromatography. Since the amount required for a spectrum is less than a milligram, as little as 7–10 mg. of an acetamido sugar or 10–20 mg. of its glycoside can be used initially. Amino sugars of varied structural complexity can be directly obtained from the many biological substances of which they are a part by mercaptalation with a suitable alkyl mercaptan and hydrochloric acid. The resulting amino sugars are not affected by this treatment and invariably give crystalline dithioacetals. This, coupled with the technique of obtaining and interpreting their mass spectra, makes the structure elucidation of amino sugars by mass spectrometry an attractive tool.

A complementary procedure to the structure determination of carbohydrates is the deduction of stereochemistry and the partial or total configuration of a certain sugar. To this end the use of amino sugar dithioacetals presents an added asset in that the degradative procedures which provide the stereochemistry at the asymmetric carbon atoms (except C-2)[15] in many amino sugars utilize the corresponding dithioacetals. The MacDonald-Fischer degradation[16] has been

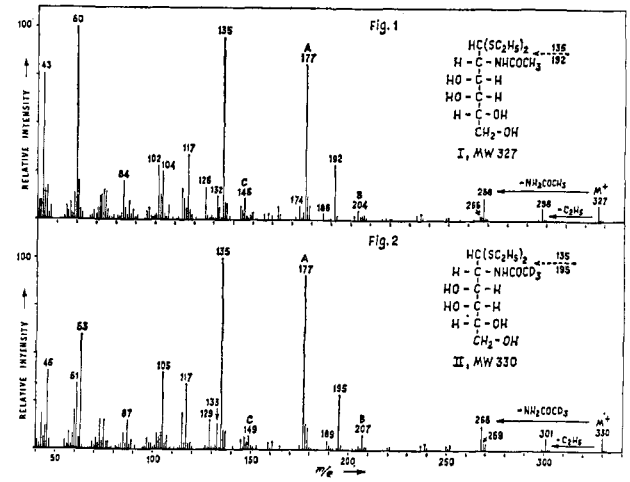


Figure 1. Mass spectrum of 2-acetamido-2-deoxy-D-galactose diethyl dithioacetal (I).
Figure 2. Mass spectrum of 2-acetamido-d_3-2-deoxy-D-galactose diethyl dithioacetal (II).

adapted to 2-amino-2-deoxy-D-glucose diethyl dithioacetal,[17] 3-amino-3-deoxy-D-allose,[18] and D-altrose[19] diethyl dithioacetals, and more recently to 2,6-diacetamido-2,6-dideoxy-L-idose diethyl dithioacetal[20] and 5-acetamido-5-deoxy-L-arabinose diethyl dithioacetal.[21,22] By a combination of mass spectrometry and a two-step chemical reaction, information otherwise difficultly accessible regarding the structure and configuration of an amino sugar can be obtained from a small amount of a common intermediate.

The diethyl dithioacetals of 2-acetamido-2-deoxy-D-glucose and -D-galactose (I) were prepared according to the general method of Wolfrom and Yosizawa.[23]

By application of the same procedure[23] it was possible to prepare the following new crystalline derivatives: 2-acetamido-2-deoxy-D-idose diethyl dithioacetal, 2-acetamido-2-deoxy-D-gulose diethyl dithioacetal, and 2-acetamido-2-deoxy-D-glucose di-*n*-propyl dithioacetal. The galactose derivative I is considered a representative of the 2-amino sugar derivatives and its spectrum is shown in Figure 1. The amino sugars D-glucosamine and D-galactosamine are constituents of biologically important substances,[24] while D-gulosamine is a constituent of the streptothricin family of antibiotics.[25]

In the 3-amino sugar series, the new 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal (III, Figure 3) and 3-acetamido-3,6-dideoxy-L-idose diethyl dithioacetal (IV, Figure 4) were obtained in crystalline condition. The occurrence of 3-amino sugars such as 3-amino-3-deoxy-D-ribose, 3-amino-3-deoxy-D-glucose, and 3-amino-3,6-dideoxy-D-mannose in the antibiotics

(6) D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86**, 67 (1964).
(7) K. Biemann and J. A. McCloskey, *ibid.*, **84**, 2005 (1962).
(8) N. K. Kochetkov, N. S. Wulfson, O. S. Chizov, and B. M. Zolotarev, *Tetrahedron*, **19**, 2209 (1963); N. K. Kochetkov and O. S. Chizov, *Biochim. Biophys. Acta*, **83**, 134 (1964).
(9) K. Heyns and H. Scharmann, *Ann.*, **667**, 183 (1963); *Tetrahedron*, **21**, 507 (1965).
(10) K. Heyns and D. Muller, *ibid.*, **21**, 55 (1965).
(11) D. C. DeJongh, *J. Am. Chem. Soc.*, **86**, 3149 (1964).
(12) D. C. DeJongh, *ibid.*, **86**, 4027 (1964).
(13) D. C. DeJongh, *J. Org. Chem.*, **30**, 1563 (1965).
(14) D. C. DeJongh and S. Hanessian, *J. Am. Chem. Soc.*, **87**, 1408 (1965).
(15) The configuration at C-2 in 2-acetamido-2-deoxyaldose dithioacetals can also be determined by converting the dithioacetal to a 1-deoxyalditol by reduction, oxidation of the latter, and isolation of D- or L-alanine resulting from C-1, C-2, and C-3 as described by M. L. Wolfrom, R. U. Lemieux, and S. M. Olin, *ibid.*, **71**, 2870 (1949).
(16) D. L. MacDonald and H. O. L. Fischer, *ibid.*, **74**, 2087 (1952).
(17) L. Hough and M. Taha, *J. Chem. Soc.*, 3564 (1957).
(18) B. Coxon and L. Hough, *ibid.*, 1463 (1961).
(19) B. Coxon and L. Hough, *ibid.*, 1643 (1961).
(20) T. H. Haskell and S. Hanessian, *J. Org. Chem.*, **28**, 2598 (1963).
(21) S. Hanessian and T. H. Haskell, *ibid.*, **28**, 2604 (1963).
(22) S. Hanessian and T. H. Haskell, *J. Heterocyclic Chem.*, **1**, 57 (1964).
(23) M. L. Wolfrom and Z. Yosizawa, *J. Am. Chem. Soc.*, **81**, 3474 (1959).
(24) For a general review see R. W. Jeanloz in "Comprehensive Biochemistry," Vol. 5, Elsevier Publishing Co., New York, N. Y., p. 262; D. Horton in "The Amino Sugars," Vol. 1, R. W. Jeanloz and E. A. Balasz, Ed., Academic Press Inc., New York, N. Y., 1965.
(25) For a recent review on amino sugars from antibiotics see J. D. Dutcher, *Advan. Carbohydrate Chem.*, **18**, 259 (1963).

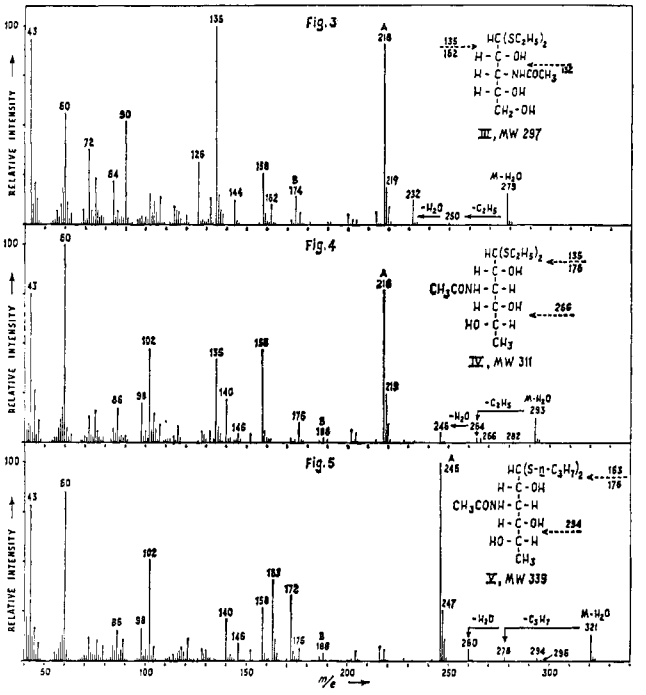


Figure 3. Mass spectrum of 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal (III).
Figure 4. Mass spectrum of 3-acetamido-3,6-dideoxy-L-idose diethyl dithioacetal (IV).
Figure 5. Mass spectrum of 3-acetamido-3,6-dideoxy-L-idose di-*n*-propyl dithioacetal (V).

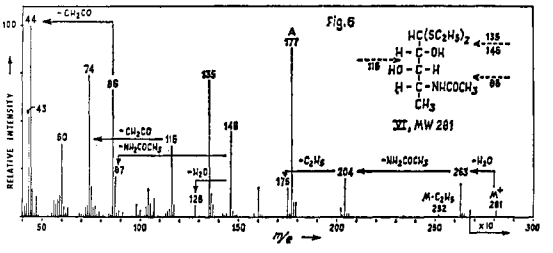


Figure 6. Mass spectrum of 4-acetamido-4,5-dideoxy-D-xylose diethyl dithioacetal (VI).

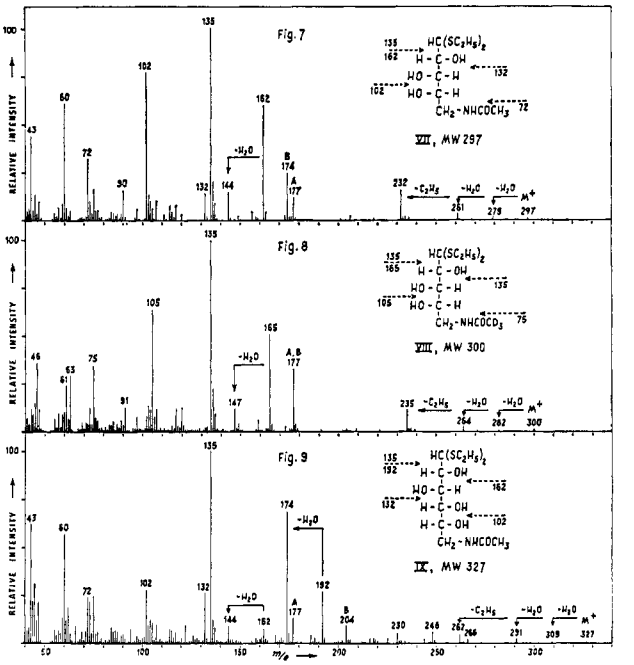


Figure 7. Mass spectrum of 5-acetamido-5-deoxy-L-arabinose diethyl dithioacetal (VIII).
Figure 8. Mass spectrum of 5-acetamido-d_3-5-deoxy-L-arabinose diethyl dithioacetal (VIII).
Figure 9. Mass spectrum of 6-acetamido-6-deoxy-D-glucose diethyl dithioacetal (IX).

puromycin, kanamycin, and nystatin, respectively, is noteworthy.[25]

The terminal aminodeoxyhexose dithioacetals were prepared by mercaptolysis of the corresponding methyl 6-acetamido-6-deoxy-D-glycosides while 5-acetamido-5-deoxy-L-arabinose diethyl dithioacetal and its d_3-analog was prepared as described previously.[21] The occurrence of 6-amino-6-deoxy-D-glucose in the antibiotic kanamycin has been shown.[25]

As representative of a 4-amino sugar derivative, 4-acetamido-4,5-dideoxy-D-xylose diethyl dithioacetal[26] (VI, Figure 6) was included in the series. The isolation of several 4-amino sugar derivatives from biological sources[27] and the fact that model synthetic sugars of this class are not numerous and have not been well studied make their characterization by mass spectrometric techniques quite rewarding.

In the diamino sugar series, 2,6-diacetamido-2,6-dideoxy-L-idose diethyl dithioacetal[20] (XI, Figure 10) was chosen since the parent diamino sugar is one of two such diamino sugars encountered in the antibiotics neomycin,[28] paromomycin,[29] and kanamycin B.[30] Compound XI was obtained from the original antibiotic by mercaptolysis.[20]

The 3,6-diamino sugar derivative[31] 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithioacetal (XII, Figure 11), which belongs to a new class of diamino sugars,[32] was studied as a model to corroborate the findings with 3- and 6-amino sugar derivatives.

Finally the effect of vicinal amino functions on the fragmentation pattern of aldose dithioacetals was studied with 4,5-diacetamido-4,5-dideoxy-L-xylose diethyl dithioacetal[26] (XIII, Figure 12).

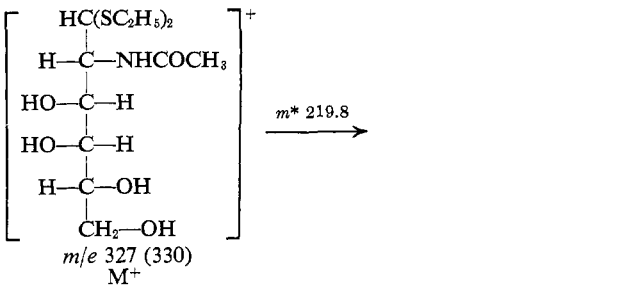
Mass Spectra of Amino Sugars

2-Acetamido-2-deoxyaldose Dialkyl Dithioacetals. The mass spectra of the 2-acetamido-2-deoxy diethyl dithioacetal derivatives of D-gulose, D-galactose (I, Figure 1), D-idose, and D-glucose are identical except for minor relative intensity differences. The major

(26) S. Hanessian, *Carbohydrate Res.*, **1**, in press.
(27) See R. W. Wheat, E. L. Rollins, and J. M. Leatherwood, *Biochem. Biophys. Res. Commun.*, **9**, 120 (1962); C. L. Stevens, P. Blumbergs, F. Daniher, J. L. Strominger, M. Matsuhashi, D. N. Dietzler, S. Suzuki, T. Okazaki, K. Sugimoto, and R. Okazaki, *J. Am. Chem. Soc.*, **86**, 2939 (1964); C. L. Stevens, P. Blumbergs, D. H. Otterbach, J. L. Strominger, M. Matsuhashi, and D. N. Dietzler, *ibid.*, **86**, 2938 (1964); C. L. Stevens, P. Blumbergs, and F. A. Daniher, *ibid.*, **85**, 1552 (1963).
(28) K. L. Rinehart, Jr., "The Neomycins and Related Antibiotics," John Wiley and Sons, Inc., New York, N. Y., 1964, p. 36.
(29) Parke, Davis and Co., U. S. Patent 2,916,485 (Dec. 8, 1959); T. H. Haskell, J. C. French, and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3480 (1959).
(30) T. Ito, N. Nishio, and H. Ogawa, *J. Antibiotics* (Tokyo), **27**, 189 (1964).
(31) M. L. Wolfrom, D. Horton, and Yen-Lung Hung, Abstracts of Papers, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 20. The stereochemistry was communicated privately by Dr. D. Horton.
(32) S. Hanessian and T. H. Haskell, *J. Org. Chem.*, **30**, 1080 (1965)

peaks in their mass spectra will be discussed in terms of their structure, peak shifts in the mass spectrum of 2-acetamido-d_3-2-deoxy-D-galactose diethyl dithioacetal (II, Figure 2), and metastable ion peaks. Their molecular weights can be determined directly from molecular ion peaks, *m/e* 327 (330),[33] the intensity of which is approximately 10% of the intensity of the base peak.

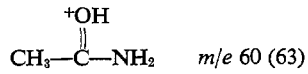
Elimination of a molecule of acetamide from the molecular ion is characteristic of the 2-acetamido



m/e 327 (330) M^+

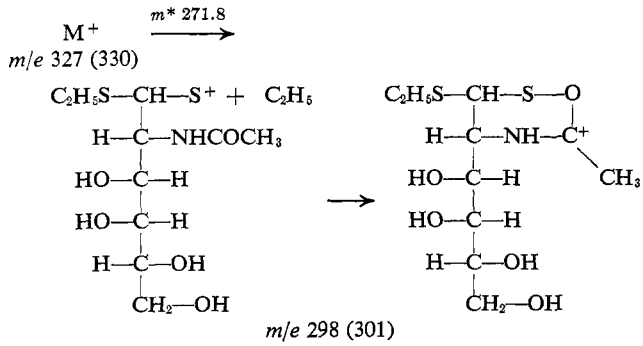
m/e 268 (268) $M - NH_2COCH_3$

substituent. The metastable ion peak indicates that an electron impact rather than a thermal phenomenon is being observed. If the elimination is accompanied by rearrangement of a proton from somewhere in the molecule to the departing acetamide molecule, the charge remains on the protonated acetamide fragment. This ion accounts for the partial shift of *m/e* 60 (Figure 1) to *m/e* 63 (Figure 2).



m/e 60 (63)

Another molecular ion fragmentation characteristic of the 2-acetamido-2-deoxyhexose diethyl dithioacetals is the loss of an ethyl radical by cleavage of a carbon–sulfur bond in a thioethyl group. Stabilization of the charge on the sulfur atom by participation



m/e 298 (301)

of the acetamido group may account for the relatively large abundance of this ion.

(33) The *m/e* value placed in parentheses is the location of the peak in the mass spectrum of the N-acetyl-d_3 analog.

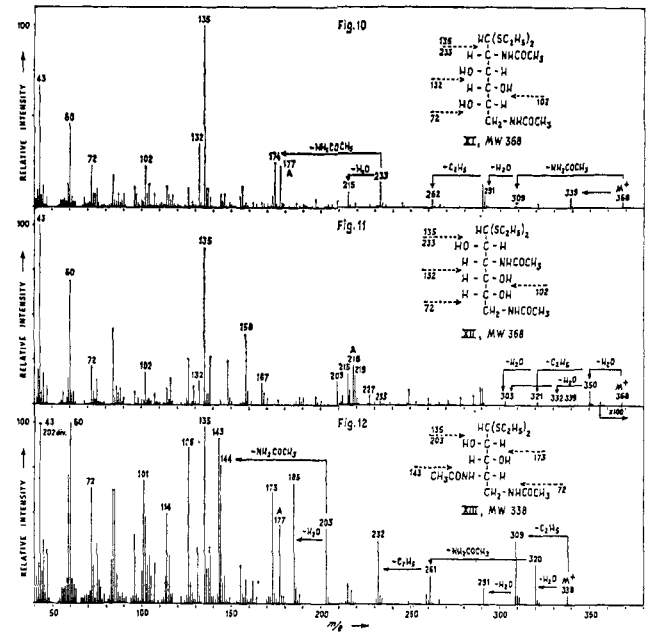
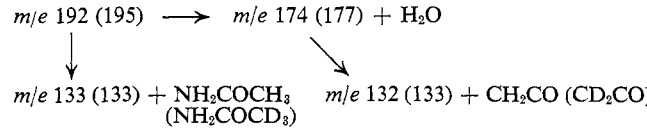


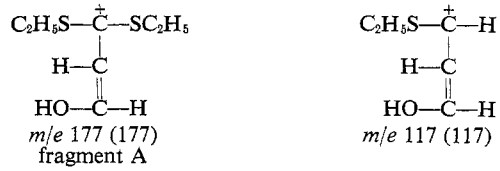
Figure 10. Mass spectrum of 2,6-diacetamido-2,6-dideoxy-L-idose diethyl dithioacetal (XI).
Figure 11. Mass spectrum of 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithioacetal (XII).
Figure 12. Mass spectrum of 4,5-diacetamido-4,5-dideoxy-L-xylose diethyl dithioacetal (XIII).

The fragments at *m/e* 135 (135) and 192 (195) form when the C-1–C-2 bond cleaves with charge retention on C-1 and on C-2, respectively. A series of peaks results from the 192 fragment by elimination of water, acetamide, and water followed by ketene. Loss of



ketene has been observed previously from N-acetyl[34] as well as from O-acetyl[4] groups.

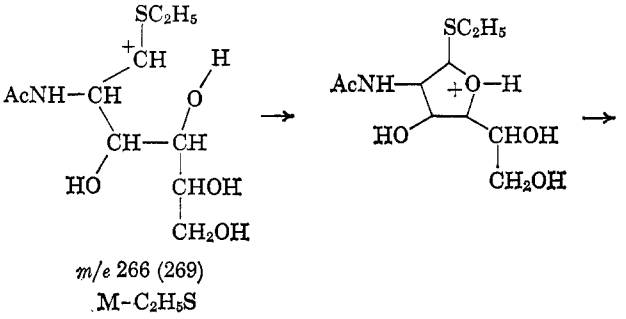
The peaks labeled A, B, and C in the figures correspond to similarly labeled fragments present in the mass spectrum of D-glucose diethyl dithioacetal.[13] Fragment A retains C-1, C-2, and C-3 of the monosaccharide chain and the substituent on C-3. It may result directly from the M – CH_3CONH_2 fragment by cleavage

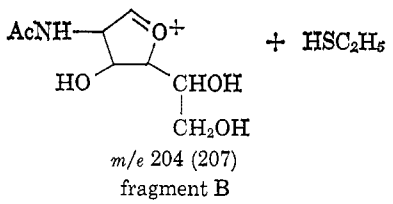


of the C-3–C-4 bond with charge retention on C-3. Fragment 117 also appears to retain the 3-substituent. It is found 14 mass units higher, at *m/e* 131, in the mass spectrum[13] of D-arabinose di-*n*-propyl dithioacetal, as would be expected if one *n*-propyl group were present.

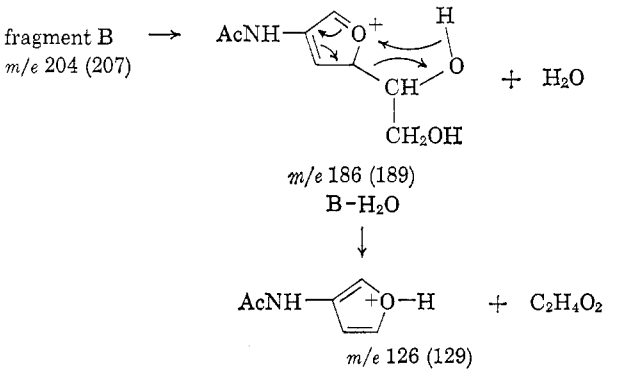
The fragment which is labeled B in the mass spectrum[13] of D-glucose diethyl dithioacetal is found 41 mass units higher, at *m/e* 204, in Figure 1, corresponding to an acetamido group in place of an hydroxyl group.

(34) Z. Pelah, M. A. Kielczewski, J. M. Wilson, M. Ohaski, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2470 (1963).



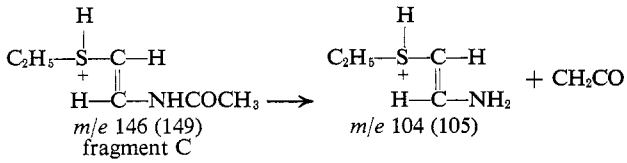


Fragment B eliminates a molecule of water and the C-5–C-6 group to form *m/e* 186 (189) and 126 (129),



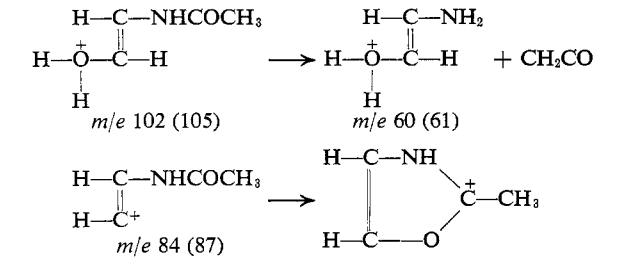
respectively. Since fragment B contains all six carbon atoms of the monosaccharide, it does not provide much information on the location of substituents in the molecule.

Fragment C is a very important indicator of substitution on C-2. It is located at *m/e* 146 (149) in the mass spectra of the 2-acetamido derivatives. It



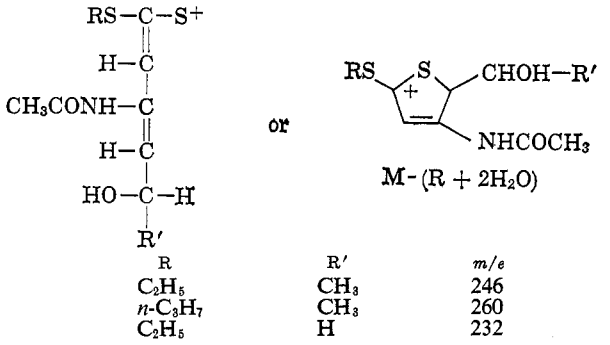
apparently fragments further by eliminating ketene. Data are not available to specify the origin of the extra hydrogen atom in this fragment (tentatively placed on the sulfur atom).

Shifts for the fragments at *m/e* 102, 84, and part of 60 in Figure 1 when N-acetyl is replaced with N-acetyl-d_3 (Figure 2) indicate that they retain the acetamido substituent. Protonated and fragmented olefins consisting of adjacent carbon atoms of the chain can be proposed to explain these peak shifts. The major fragment contained in the *m/e* 43 peak is the acetyl ion, CH_3CO^+ (CD_3CO^+), which shifts to *m/e* 46.



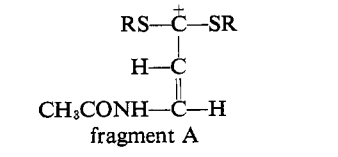
3-Acetamido-3-deoxyaldose Dialkyl Dithioacetals. The lack of a molecular ion peak and the ready loss of a molecule of water from the molecular ion are characteristic of the 3-acetamido derivatives. A metastable ion peak at *m/e* 262.3 in the mass spectrum of 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal (III, Figure 3) relates the molecular ion to the M − H_2O fragment. Only at very high sample pressures does compound III exhibit a small molecular ion peak in its mass spectrum.

Peaks in the high *m/e* region in the mass spectra of compound III and of 3-acetamido-3,6-dideoxy-L-idose diethyl (IV, Figure 4) and di-*n*-propyl (V, Figure 5) dithioacetals include a relatively intense peak for the loss of a molecule of water from the molecular ion and a characteristic series of peaks for which the total loss is an alkyl radical and 2 molecules of water from M^+. The charge on sulfur can be delocalized by conjugation with the double bonds; it may also be stabilized by cyclization as shown.

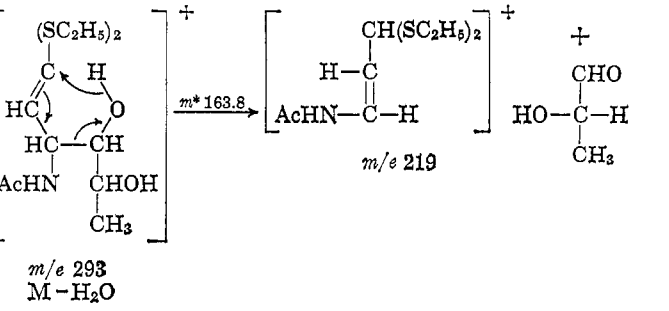


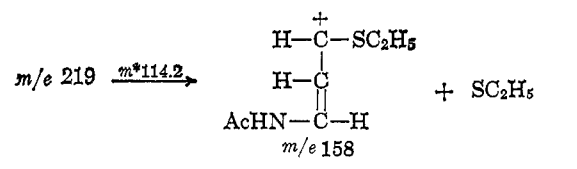
R	R′	*m/e*
C_2H_5	CH_3	246
n-C_3H_7	CH_3	260
C_2H_5	H	232

The shift of fragment A from *m/e* 177 in Figure 1 to *m/e* 218 in the mass spectra of the 3-acetamido-3-deoxyaldose diethyl dithioacetals (Figures 3 and 4) and to *m/e* 246 in the di-*n*-propyl analog (Figure 5) demon-



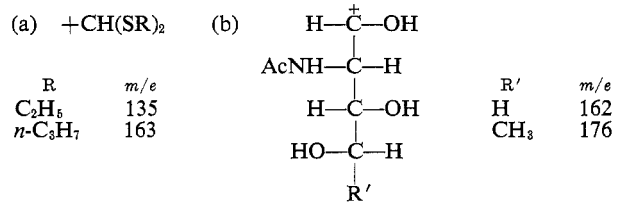
R	*m/e*
C_2H_5	218
n-C_3H_7	246



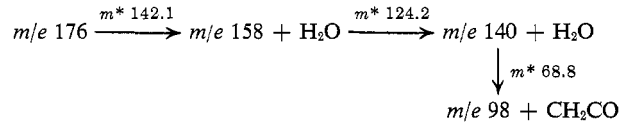


strates how readily a 3-acetamido substituent can be recognized in these mass spectra. Metastable ion peaks in the mass spectra of compounds III and IV relate the nonisotopic portion of *m/e* 219 to the M − H_2O fragment, and relate *m/e* 158 to *m/e* 219. The fragment at *m/e* 158 corresponds to *m/e* 117 discussed for compounds I and II, only an acetamido group has replaced the hydroxyl group on C-3. A plausible scheme can be proposed for formation of *m/e* 158. The 158 fragment is shifted to *m/e* 172 in Figure 5 due to the retention of one *n*-propyl group.

A series of peaks is formed by initial cleavage of the C-1–C-2 bond. Metastable ion peaks show that the

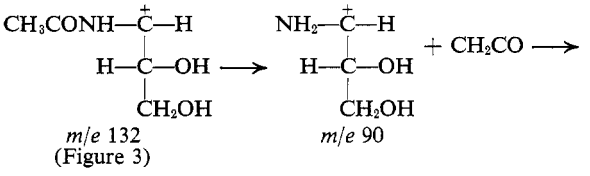


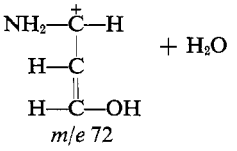
M − $CH(SR)_2$ fragment loses two molecules of water and ketene, *e.g.*



These peaks are found 14 mass units lower for pentose III, at *m/e* 162, 144, 126, and 84. The change from diethyl dithioacetal in Figure 4 to di-*n*-propyl in Figure 5 illustrates how the technique of substituent labeling has shown *m/e* 158 in the former to be composed of at least two fragments, found at *m/e* 158 and 172 in the latter.

The partial shift of *m/e* 132 and 72 in Figure 3 to *m/e* 146 and 86 in Figures 4 and 5 suggests that they may arise from a fragment formed by cleavage of the C-2–C-3 bond with charge retention on C-3.



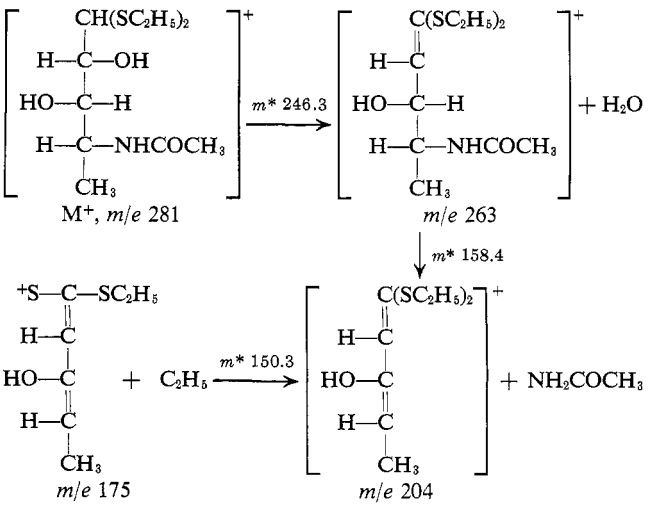


Elimination of ketene, 42 mass units, from fragment B, *m/e* 174 (Figure 3), may also contribute to *m/e* 132. Fragment B is found 30 mass units lower in Figure 3 than in Figure 2, and 16 mass units lower in Figures 4 and 5 than in Figure 2, corresponding to the difference in molecular weight of hexoses, pentoses, and deoxyhexoses.

Fragments at *m/e* 102, 60, and 43 most likely correspond to the fragments discussed for compound I.

*4-Acetamido-4,5-dideoxy-*D*-xylose Diethyl Dithioacetal* (*VI, Figure 6*). Compound VI displays a tendency to cleave successive carbon–carbon bonds of the monosaccharide chain upon electron impact, leading to fragments at *m/e* 146, 135, 116, and 86. Fragments 116 and 86 eliminate ketene, 42 mass units, accounting for the peaks at *m/e* 74 and 44, respectively. The peaks at *m/e* 128 and 87 result when fragment 146 losses water and acetamide, respectively.

The very small molecular ion peak and the favorable loss of water from the molecular ion are characteristic of the 4-acetamido derivative. Also characteristic is



the loss of the 4-substituent from the M − H_2O fragment, followed by an ethyl radical. The loss of acetamide from the molecular ions of the 2-acetamido derivatives, the loss of water followed by an ethyl radical and water from the molecular ions of the 3-acetamido derivatives, and the loss of water followed by acetamide from the molecular ions of the 4-acetamido derivative VI indicate that the C-2 and the C-4 substituents are preferentially eliminated from the monosaccharide chain.

Fragment A with a hydroxyl group on C-3 is found at *m/e* 177. Placing an acetamido group on C-4 and a deoxy position at C-5 has blocked the formation of fragment B.

*5-Acetamido-5-deoxy-*L*-arabinose Diethyl Dithioacetal* (*VII, Figure 7*). Metastable ion peaks are found in the mass spectra of compound VII and its N-acetyl-d_3 analog VIII (Figure 8) for the ejection, from the molecular ion, of a molecule of water and then another molecule of water, and for the ejection of an ethyl radical from the M − $2H_2O$ fragment. The 5-acetamido group is not eliminated in any of the major fragmentation pathways of compounds VII and VIII; thus the C-5 substituent is preferentially retained as was the C-3 substituent in compounds III–V.

Fragment A has a C-3 hydroxyl group and is found at *m/e* 177 (177).[33] Fragment B is located at the same *m/e* value as it is for the isomeric compound III, at *m/e* 174 (177).

The intense peaks at *m/e* 162 (165), 135 (135), 132 (135), 102 (105), and 72 (75) indicate that the C-1–C-2, C-2–C-3, C-3–C-4, and C-4–C-5 bonds cleave upon electron impact. A *m/e* 132 (135), 90 (91) sequence is present, which is probably the same as described for isomeric compound III with the acetamido group at C-5 rather than C-3.

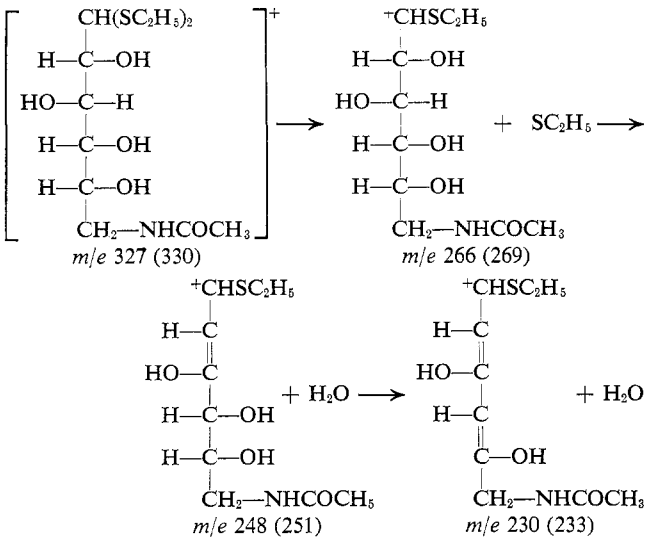
*6-Acetamido-6-deoxy-*D*-glucose Diethyl Dithioacetal* (*IX, Figure 9*). The mass spectra of 6-acetamido-6-deoxy-D-glucose diethyl dithioacetal (IX, Figure 9) and 6-acetamido-d_3-6-deoxy-D-galactose diethyl dithio-

acetal (X, spectrum not shown) are characterized by elimination of two molecules of water and an ethyl radical from the molecular ion, yielding peaks at *m/e* 327 (330),[33] 309 (312), 291 (294), and 262 (265). The failure of the 6-substituent to be eliminated as acetamide is in sharp contrast to the behavior of the 2-acetamido analogs, compounds I and II.

The series of peaks at *m/e* 192 (195), 162 (165), 132 (135), 102 (105), and 72 (75), differing successively by 30 mass units, suggests that the carbon–carbon bonds of the monosaccharide chain cleave with the charge retained on the nitrogen-containing fragment. This series of peaks is characteristic of the terminal acetamido group. Metastable ion peaks show that fragments 192 (195) and 162 (165) lose water, giving fragments 174 (177) and 144 (147), respectively. An elimination of acetamide, 59 mass units, from *m/e* 132 (135) would lead to the peak at *m/e* 73 (73).

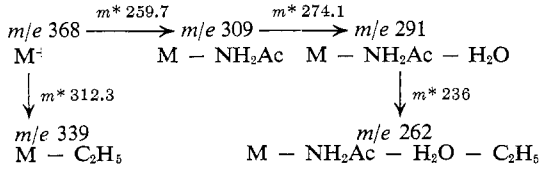
In the mass spectrum of compound X, the *m/e* 162 peak in Figure 9 shifts to *m/e* 163 as well as to *m/e* 165. Loss of ketene, CH_2CO (CD_2CO), from fragment B, *m/e* 204 (207), can account for the retention of the 1 deuterium atom in *m/e* 163. Fragment B is the same as discussed for compound I except that the acetamido group is on C-6 rather than on C-2. Fragment A is found at *m/e* 177 (177), but is of much lower relative intensity than in the mass spectra in Figures 1 and 2.

The series of peaks at *m/e* 266 (269), 248 (251), and 230 (233) results from fragmentation of the C-1 bond followed by loss of two molecules of water.



Mass Spectra of Diamino Sugars

*2,6-Diacetamido-2,6-dideoxy-*L*-idose Diethyl Dithioacetal* (*XI, Figure 10*). The relatively intense molecular ion and M − C_2H_5 peaks in Figure 10 and the elimination of a molecule of acetamide from the molecular ion are characteristic of the 2-acetamido group.



The fragments at *m/e* 233, 132, 102, and 72 from cleavage of the carbon–carbon bonds of the chain are characteristic of the 6-acetamido group. The 2-acetamido-2-deoxyhexose diethyl dithioacetals (Figure 1) eliminated both water and acetamide from the M − 135 fragment as does *m/e* 233 in Figure 10. Fragment A is located at *m/e* 177, confirming the hydroxyl group on C-3.

*3,6-Diacetamido-3,6-dideoxy-*D*-altrose Diethyl Dithioacetal* (*XII, Figure 11*). The mass spectrum of compound XII (Figure 11) is much more complex than the mass spectrum of its 2,6-diacetamido isomer (Figure 10). In spite of this complexity, the fragmentation pathways characteristic of the C-3 and the C-6 acetamido groups are readily recognizable.

The fragments at *m/e* 219, 218 (fragment A), and 158, and the absence of a peak at *m/e* 177 are dramatic evidence that a 3-acetamido group is present; a metastable ion peak at *m/e* 114.2 confirms the formation of *m/e* 158 from *m/e* 219. Also characteristic of the 3-substituent is the small molecular ion peak and the series of peaks resulting from elimination of water and an ethyl radical from the molecular ion. A metastable ion peak at *m/e* 333–334 confirms the formation of *m/e* 350, M − H_2O, from *m/e* 368, M^+.

The loss of water, *m/e* 215, from the M − 135 fragment, *m/e* 233 (*m** 198.5), and no loss of acetamide from *m/e* 233 are characteristic of those compounds with hydroxyl groups on C-2 and C-4. Fragment B is not seen at *m/e* 245, but peaks at *m/e* 227, 209, and 167 indicate it loses 2 molecules of water and ketene, as it does for compound III.

The peaks at *m/e* 233, 132, 102, and 72 are characteristic of the 6-acetamido group.

*4,5-Diacetamido-4,5-dideoxy-*L*-xylose Diethyl Dithioacetal* (*XIII, Figure 12*). The mass spectrum of compound XIII is very similar to the mass spectrum of compound VI with most peaks 57 mass units higher in Figure 12 than in Figure 6, corresponding to the difference between the C-5 substituents. The elimination of water from the molecular ion, followed by acetamide and an ethyl radical as indicated in Figure 12, is characteristic of a 4-acetamido group. The peaks at *m/e* 203, 173, 143, and 72 are characteristic of the terminal position of the other acetamido group. Fragment A is found at *m/e* 177 with a hydroxyl group on C-3.

Elimination of water (18 mass units), acetamide (59 mass units), or ketene (142 mass units) from the fragments discussed above accounts for such peaks as *m/e* 185, 144, 126, 114, and 101. The loss of both water and acetamide from the M − 135 fragment, *m/e* 203, is characteristic of the 4-substituent.

Experimental

Mass Spectra. The mass spectra were determined with an Atlas CH4 mass spectrometer at an ionizing potential of 70 e.v. and an ionizing current of 18 μa. The 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.

General. Melting points were determined with a Fisher-Johns-type apparatus and are uncorrected.

Infrared spectra were measured with a Beckman IR-7 spectrometer. Paper chromatography was carried out on Whatman No. 1 paper in 1-butanol–ethanol–water (3:1:1) (solvent A) and ethyl acetate–pyridine–water (120:50:40) (solvent B). Spots were detected with the alkaline silver nitrate reagent[35] (free sugars, dithioacetals) and with the bromine–methyl orange spray (dithioacetals).[36] Thin layer chromatography was carried out using Silica gel H (E. Merck, Darmstadt, Germany), with the solvent benzene–methanol (10:3) (solvent C). Spots were detected by the acid permanganate spray[37] and the bromine–methyl orange spray.[36] All the dithioacetals reported in this investigation had infrared spectra which were compatible with their structures and were chromatographically homogeneous solids.

Preparation of Intermediates. 2-Acetamido-2-deoxy-D-idose (a homogeneous sirup, solvent B) was obtained by partial acid hydrolysis (50% acetic acid, steam bath for 1.5 hr.) of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-idopyranoside, m.p. 197–199° (lit.[38] m.p. 201–202°).

2-Acetamido-2-deoxy-D-gulose was obtained as a chromatographically homogeneous white solid from crystalline 2-amino-2-deoxy-D-gulose[39] by N-acetylation.[40]

Methyl 6-acetamido-6-deoxy-α-D-glucopyranoside was prepared from the corresponding 6-*O*-*p*-toluenesulfonyl derivative[41] by refluxing for 18 hr. with sodium azide in Methyl Cellosolve containing 5% water. The resulting methyl 6-azido-6-deoxy-α-D-glucoside, a chromatographically homogeneous semicrystalline sirup, was isolated as the crystalline triacetate[41] and was regenerated by deacetylation with sodium methoxide in methanol. Hydrogenation of this product (Pd–C in methanol) followed by N-acetylation gave the crystalline 6-acetamido derivative.[41]

Methyl 6-acetamido-d_3-6-deoxy-α-D-galactoside was prepared by hydrogenation of crystalline methyl 6-azido-6-deoxy-α-D-galactopyranoside, m.p. 172–173° dec.,[42] as described above, followed by N-acetylation with acetic anhydride-d_3.

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Preparation of the Dithioacetals. A. From Free Amino Sugars. The general method of Wolfrom and Yosizawa[23] was used for the dithioacetals of free acetamido deoxy sugars. All the known dithioacetals thus prepared had melting points and physical constants in agreement with reported values in the literature. The acetyl functions containing deuterium were introduced in the amino sugars by treating methanol solutions of the free bases with acetic anhydride-d_6 or by Roseman and Ludowieg's method[40] in the case of the hydrochlorides. The dithioacetals were recrystallized from mixtures of methanol and ether. The following new dithioacetals were prepared: 2-acetamido-d_3-2-deoxy-D-glucose diethyl dithioacetal, m.p. 128–129°; 2-acetamido-2-deoxy-D-glucose di-*n*-propyl dithioacetal, m.p. 120–121°; 2-acetamido-d_3-2-deoxy-D-galactose diethyl dithioacetal (II, Figure 2), m.p. 161–162°; 2-acetamido-2-deoxy-D-idose diethyl dithioacetal, m.p. 100–101°; and 2-acetamido-2-deoxy-D-gulose diethyl dithioacetal, m.p. 147–148°.

B. From Methyl Glycosides. The N-acetylated methyl glycosides were stirred with an alkane thiol and concentrated hydrochloric acid at 0° for 18–24 hr., then processed in the usual way. Except for compound X which crystallized directly, preparative thin layer chromatography (solvent C) was used to obtain crystalline dithioacetals from small aliquots of the corresponding crude products. The following were prepared as described above: 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal (III, Figure 3), m.p. 91–92°; 6-acetamido-6-deoxy-D-glucose diethyl dithioacetal (IX, Figure 9, solid from methanol–ether); 6-acetamido-d_3-6-deoxy-D-galactose diethyl dithioacetal (X), m.p. 174–175°; 3-acetamido-3,6-dideoxy-L-idose diethyl dithioacetal[43] (IV, Figure 4), m.p. 138–139°; 3-acetamido-3,6-dideoxy-L-idose di-*n*-propyl dithioacetal[43] (V, Figure 5), m.p. 150–151°.

We thank Dr. D. Horton of Ohio State University for a generous gift of 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithioacetal.

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(42) S. Hanessian, unpublished.
(43) S. Hanessian, to be published.