The Structure of Amicetin

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Methanolysis of amicetin (I) yielded, besides cytimidine (II),[2,3] the methyl glycosides (VI) of a neutral sugar, amicetose (VII), and of an amino sugar, amosamine (IX). Periodate fission of the 2,4-dinitrophenylhydrazone of amicetose (VIII) gave acetaldehyde and a derivative of succindialdehyde, leading to structure VII for the neutral sugar. Various degradations of amosamine and its derivatives were conducted and the results showed the amino sugar to be IX. Evidence is presented for the mode of linkage of amosamine, amicetose, and cytimidine in amicetin as shown in structure I.

The structure of the antibiotic amicetin (I), isolated from *Steptomyces plicatus*[4] and *Steptomyces vinaceus-drappus*,[5] was first studied by Flynn and co-workers.[2] Upon brief acid hydrolysis of amicetin, these authors isolated a C_{15} fragment called cytimidine, for which they established structure II. They adduced further evidence in support of the partial formulation of amicetin (I) as having a a C_{14} residue attached to position 1 of cytosine in the cytimidine moiety (II). Alkaline hydrolysis of amicetin (I) cleaved the dipeptide portion to give cytosamine (IV), $C_{18}H_{30}N_4O_6$, which these authors obtained as a monohydrate. Cytosamine (IV) was later[3] isolated in the anhydrous state. The C_{14} fragment, amicetamine (III), was isolated in the later work[3] in a pure state, assigned the empirical formula $C_{14}H_{27}NO_6$, and shown to have two N-methyl and two C-methyl groups. From the periodate fission of amicetamine (III), dimethylamine, formic acid, and glyoxal were isolated.[3] A preliminary communication[6] from our group assigned a tentative structural formula for amicetin. After a more detailed study, made possible especially by the availability of a larger quantity of the antibiotic, this structure assignment has been revised and evidence is presented in this paper that amicetin is better represented as I.

Methanolysis of amicetin (I) was achieved by saturating a methanolic suspension with dry hydrogen chloride gas below 0°. The resulting solution, on standing at room temperature for several hours, deposited a precipitate of cytimidine hydrochloride in quantitative yield. The mother liquor, after evaporation of the solvent, was separated by ion exchange techniques into neutral and basic portions. The neutral fragment was the methyl glycoside of *amicetose* (VI) and was obtained in 56% yield. The basic product from the methanolysis amounted to 80% and represented an anomeric mixture of the methyl glycosides of amosamine (X). These compounds were also obtained in fair to good yields by the methanolysis of amicetamine (III) or cytosamine (IV).

Structure of Amicetose (VII).—The methyl glycoside of amicetose (VI) had the empirical formula $C_7H_{14}O_3$ and showed the presence of an hydroxyl group but no carbonyl group in the infrared spectrum. Group analysis indicated the presence of one C-methyl and one methoxyl group. The methyl glycoside gave no reducing tests and only a very slight iodoform reaction. It reduced a negligible amount of sodium metaperiodate at pH 7 in twelve hours. Treatment with 3 *N* hydrochloric acid at room temperature caused immediate hydrolysis to amicetose (VII) as indicated by a fall in rotation of the solution. Thereafter the rotation changed very little during several hours. The neutralized solution gave a good iodoform reaction, a sluggish reducing test with Benedict's reagent under stronger alkaline conditions than normally used, and consumed one mole of periodate. The free sugar isolated from this hydrolysis could be distilled to give a viscous oil which did not give a satisfactory analysis. Vapor phase chromatographic analysis of the methyl glycoside showed two major peaks and a minor one, indicating that it was not homogeneous. However, it was shown to be mostly a mixture of anomers, since it gave a single crystalline 2,4-dinitrophenylhydrazone (VIII), m.p. 152–153°, in 85% yield. The same 2,4-dinitrophenylhydrazone was also obtained from the free sugar. The 2,4-dinitrophenylhydrazone had the empirical formula $C_{12}H_{16}N_4O_6$ (corresponding to $C_6H_{12}O_3$ for amicetose) and showed the presence of hydroxyl absorption bands in the infrared, at least one C-methyl group in the Kuhn-Roth estimation, and no methoxyl groups by the Zeisel method. It formed a crystalline di-*O*-acetate lacking hydroxyl bands in the infrared spectrum. Quantitative periodate cleavage studies[7] on the 2,4-dinitrophenylhydrazone showed consumption of

(1) Parke, Davis and Co., Research Laboratories, Ann Arbor, Michigan.

(2) E. H. Flynn, J. W. Hinman, E. L. Caron, and D. O. Woolf, Jr., *J. Am. Chem. Soc.*, **75**, 5867 (1953).

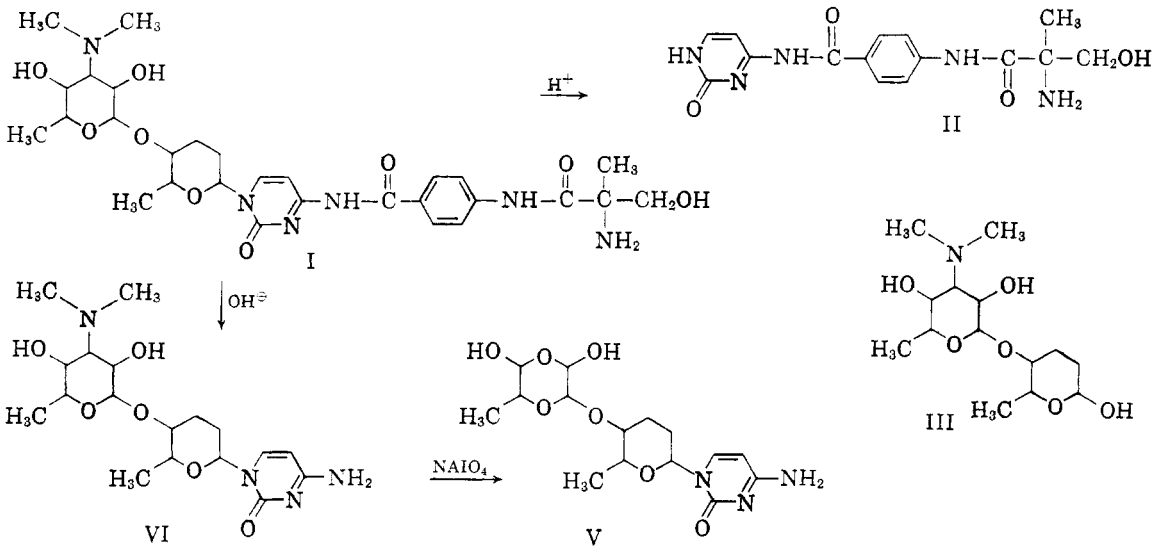
(3) T. H. Haskell, *ibid.*, **80**, 747 (1958).

(4) T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, Z. L. Jakubowski, and Q. R. Bartz, *ibid.*, **80**, 743 (1958).

(5) J. W. Hinman, E. L. Caron, and C. DeBoer, *ibid.*, **75**, 5864 (1953).

(6) C. L. Stevens, R. J. Gasser, T. K. Mukherjee, and T. H. Haskell, *ibid.*, **78**, 6212 (1956).

(7) Acetone 2,4-dinitrophenylhydrazone, used as a control for these studies, could be quantitatively recovered from acetylation conditions. Further it reduced only a negligible amount of sodium metaperiodate in twenty-four hours.



a little over one mole of the reagent in fifteen minutes and the uptake did not increase significantly during a period of three hours. From the cleavage, acetaldehyde was isolated as the volatile product in 51% yield and identified as its 2,4-dinitrophenylhydrazone. The nonvolatile residue was a yellow crystalline compound, m.p. 130–132°, obtained in 80% yield. It had correct analysis for the mono-2,4-dinitrophenylhydrazone of a C_4 dicarbonyl compound. Further, treatment of this compound with an excess of Brady's reagent gave a sparingly soluble derivative, m.p. 265° dec., identical with succindialdehyde bis-2,4-dinitrophenylhydrazone. These results establish the structure of 4,5-dihydroxyhexanal (VII) for amicetose and (VIII) for the 2,4-dinitrophenylhydrazone. Amicetose (VII) had no carbonyl absorption band in the infrared region and should therefore exist in the cyclic hemiacetal form. In analogy with many sugars, it is represented in the pyranose form. Since the methyl glycoside precursor gave but a slight iodoform reaction, it must exist largely as a mixture of the pyranoside anomers (VI).

Structure of Amosamine (IX).—Fractional crystallization of the mixture of basic glycosides from the methanolysis of amicetin (I) gave α-methyl amosaminide (X)[8] as a crystalline compound, m.p. 93–94°, which formed a hydrochloride, m.p. 195–196° (dec.), and a hydriodide, m.p. 193–194°. The base had the empirical formula $C_9H_{19}NO_4$ and $[\alpha]^{25}D$ +138°. Group analysis indicated the presence of one C-methyl and one methoxyl group. Benedict's reagent and the iodoform reaction gave negative results, but the base reduced two moles of sodium metaperiodate, undergoing slight over-oxidation subsequently. About 0.75 mole of a volatile acid was isolated from the cleavage solution. Reduction of the nonvolatile product with sodium borohydride and treatment with acidic 2,4-dinitrophenylhydrazine gave the osazone derivative of glyoxal. Oxidation of the glycoside (X) by periodic acid was slow at room temperature, requiring twenty-four hours for an uptake of two moles. At 5°, the reaction was even slower, only one mole of the reagent being reduced in seventeen hours. However, there was no selectivity in cleavage of the molecule, since paper chromatographic analysis of the mixture after one mole reaction indicated the presence of a significant amount of uncleaved α-methyl amosaminide (X). With carbobenzyloxychloride in chloroform solution at room temperature, the glycoside gave a monocarbobenzyloxy ester isolated as the hydrochloride, m.p. 157–159° (dec.), having pK_a' 5.4, compared to 7.2 for the starting material.

Although the β-methyl amosaminide (X) was a glass, it could be isolated readily as its crystalline hydrochloride from the mother liquors of the crystallization of the alpha isomer. The hydrochloride, m.p. 209–210° (dec.), had pK_a' 7.2, $[\alpha]^{25}D$ −32°, and analysis correct for the formula $C_9H_{19}NO_4 \cdot HCl$. Like the alpha isomer, it had one C-methyl and one methoxyl group and showed negative reducing tests and negative iodoform reaction. It readily consumed two moles of sodium metaperiodate, and then underwent over-oxidation to a somewhat greater extent than the alpha isomer. From the fission, 0.76 mole of formic acid was isolated; after sodium borohydride reduction of the nonvolatile product, followed by acid hydrolysis, glyoxal was recovered as its 2,4-dinitrophenylosazone in 34% yield. Extraction of of the periodate cleavage solution of β-methyl amosaminide (X) directly into acidic 2,4-dinitrophenylhydrazine gave a product considered to be a mixture of pyruvaldehyde and glyoxal derivatives.

Amosamine (IX) was easily prepared by refluxing the crude anomeric mixture of methyl amosaminides (X) from the methanolysis of

(8) The terms alpha and beta are used for the anomers because of the correspondence of their respective specific rotations with those reported for the methyl glycoside pairs of glucosamine and glucose by A. Neuberger and R. P. Rivers, *J. Chem. Soc.*, 122 (1939).

amicetin (I) with 3 *N* hydrochloric acid for four hours. Evaporation and crystallization of the residue from alcohol gave amosamine hydrochloride in 40% yield. A somewhat more tedious procedure which gave a similar yield was the hydrolysis of amicetamine (III) using Dowex 50 acid resin,[6] but the neutral fragment was largely destroyed in the process; only a small amount could be isolated as the 2,4-dinitrophenylhydrazone. Acid hydrolysis of pure β-methyl amosaminide (X) was also shown to give amosamine (IX). The relationship of amosamine (IX) to the two glycosides was further established by saturation of a methanolic solution of amosamine with hydrogen chloride, followed by isolation of α-methyl amosaminide as the free base in 39% yield and the beta anomer as its hydrochloride in 9% yield.

Amosamine hydrochloride had analysis correct for the formula $C_8H_{17}NO_4 \cdot HCl$ and contained one C-methyl group. A Herzig-Meyer determination showed less than one *N*-methyl group to be present. The present work showed amosamine (IX) to be the only basic moiety of amicetamine (III) from which dimethylamine had been previously isolated in good yield after fission with periodate.[3] Because of this conflict, it was felt desirable to adduce further proof that amosamine was a *N*-dimethylamino sugar. By refluxing amosamine hydrochloride with 3 *N* sodium hydroxide solution for several hours,[9,10] dimethylamine was liberated. It was isolated as its *p*-hydroxyazobenzene-*p'*-sulfonic acid salt[3] in a crude yield of 70%, the yield of the analytically pure sample being 47%. This experiment indicates that the results of Herzig-Meyer estimation of *N*-methyl groups on dimethylamino sugars is sometimes not reliable.[11]

Amosamine hydrochloride gave a positive iodoform reaction and reduced Benedict's reagent readily. Its infrared spectrum showed three different absorption bands in the hydroxyl region but none for a carbonyl group. Thus, the carbonyl group was masked as a cyclic hemiacetal. Amosamine (IX) reduced about four moles of sodium metaperiodate in forty-eight hours. From the fission, 2.7 moles of volatile acid, 0.33 mole of a volatile amine, and a poor yield of acetaldehyde were isolated. No formaldehyde could be detected by using the sensitive chromotropic acid test, although about 0.1 mole of this aldehyde was formed when the cleavage was carried out in the presence of excess sodium bicarbonate. Reduction of amosamine (IX) with sodium borohydride gave a good yield of amosaminol (XI), isolated as its crystalline hydrochloride, $C_8H_{19}NO_4 \cdot HCl$, m.p. 141–143°. Amosaminol (XI) readily consumed about 4 moles of sodium metaperiodate with the production of one mole of formaldehyde. These results showed that amosamine had a 6,X-dideoxy-X-*N*,*N*-dimethylaminoaldohexose structure (IX, XVII, or XV). Since the two methyl amosaminides (X) gave negative iodoform reactions and readily reduced two moles of periodate, they must be pyranosides.

Comparative study of the rate of elimination of amines from amino sugars has been utilized by Hochstein and Regna[12] for assigning position 3 to the dimethylamino group in mycaminose. The rate of deaminolysis of amosamine (IX) was studied in 3 *N* aqueous sodium hydroxide at 50° and compared with the rates found by us for desosamine,[9] glucosamine, and *N*,*N*-dimethylglucosamine (XX) besides with the one reported for mycaminose.[12] It was found that, unlike mycaminose and desosamine with half-lives of one-half hour, amosamine (IX) had a half-life of about twenty-four hours for elimination of amine. This figure was somewhat smaller than the one observed for glucosamine but a little larger than the one for dimethylglucosamine (XX). Unfortunately, unlike the cases of mycaminose[12] and desosamine,[13] the corresponding sugar with one less carbon atom could not be isolated from amosamine (IX) (see below). The results of deaminolysis experiments supported a 2-dimethylamino structure, XV, for amosamine. Such a structure would account for the somewhat reduced basicity of amosamine (pK_a' 7.4) and its derivatives as compared to mycaminose[10] (pK_a' 8.5) and desosamine[9] (pK_a' 8.6) of established 3-aminohexose structures. However, structure XV was deemed unlikely for amosamine for the following weightier considerations.

1) Acid hydrolysis of methyl amosaminide (X) to amosamine (IX) was readily accomplished, which compared with the behavior of glycosides of 3-aminosugars[9,14] and was in contrast to the behavior of glucosamine derivatives[8,15] where the proximity of the positive charge on the protonated nitrogen at position 2 exerts a considerable retarding effect on the rate of hydrolysis. Conversely, amosamine (IX) was readily converted into the methyl glycosides (X) in fair yield, which again paralleled the behavior of 3-amino sugars like desosamine[9] and mycaminose,[10] rather than that of 2-amino sugars. In fact, the conversion of glucosamine to its methyl glucoside is satisfactorily

(9) *Cf.*, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, and K. Gerzon, *J. Am. Chem. Soc.*, **76**, 3121 (1954).

(10) *Cf.*, F. A. Hochstein and K. Murai, *ibid.*, **76**, 5080 (1954).

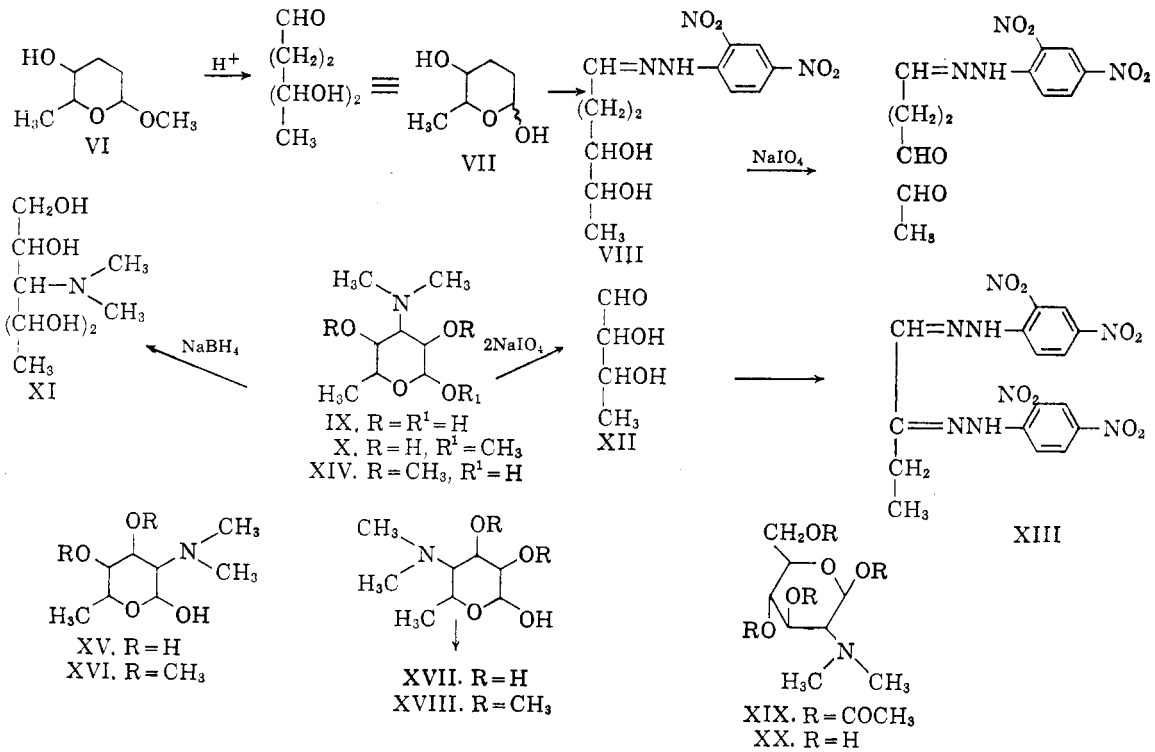
(11) In fact, the estimate of *N*-methyl groups (reported in the experimental section) was poor from several dimethylamino sugar derivatives studied; the list comprised, besides amosamine (IX), the anomeric pair of methyl amosaminides (X) and their mono and dimethyl ethers, di-*O*-methylamosamine (XIV), amicetamine (III), cytosamine (IV), mycaminose,[10] and *N*,*N*-dimethylglucosamine (XX). The maximum recovery was less than 1.5 groups and the minimum about 0.3 group.

(12) F. A. Hochstein and P. P. Regna, *J. Am. Chem. Soc.*, **77**, 3353 (1955).

(13) H. Brockmann, H. Konig, and R. Oster, *Ber.*, **87**, 856 (1954) (picrocin has been identified with desosamine).

(14) R. E. Schaub and M. J. Weiss, *J. Am. Chem. Soc.*, **80**, 4683 (1958).

(15) R. C. G. Moggridge and A. Neuberger, *J. Chem. Soc.*, 745 (1938); *Cf.* W. Pigman, "Chemistry of the Carbohydrates," Academic Press, Inc., New York, 1957, pp. 472–473.



achieved only indirectly by first blocking the amino group.[15,16]

Thus, glucosamine pentaacetate, on refluxing with 2.2% methanolic hydrogen chloride for two hours, gave *N*-acetylmethylglucosaminide in 76% yield, whereas β-tetra-*O*-acetylglucosamine hydrochloride under the same conditions gave a good yield of glucosamine hydrochloride.[15] Another case studied was tetra-*O*-acetyl-*N*,*N*-dimethylglucosamine hydrochloride (XIX).[17] Under the conditions used for converting amosamine (IX), amicetamine (III), cytosamine (IV), or amicetin (I) to methyl amosaminide (X), XIX gave as the only isolable product *N*,*N*-dimethylglucosamine (XX) in 50% yield. XX was also obtained by aqueous acid hydrolysis of XIX in about the same yield.

2) The rate of formaldehyde production in the periodate cleavage of amosaminol (XI), *N*,*N*-dimethylglucosaminol, and a few relevant models was studied. Amosaminol (XI) readily consumed about four moles of periodate in one hour and produced one mole of formaldehyde. There was no significant change in these figures after twenty-four hours. Desosamine, mycaminose, and glucose, upon sodium borohydride reduction and cleavage with sodium metaperiodate, all liberated the theoretical amount of formaldehyde (one, one, and two moles, respectively) within one to twenty-four hours. However, a terminal 1,2-dimethylaminoalcohol grouping was cleaved much more slowly. For example, *N*,*N*-dimethylglucosaminol under the same conditions gave a maximum of about 0.5 mole of formaldehyde in fifty hours.

The results discussed so far did not vigorously permit the assignment of the amino group in amosamine to any one of the three available positions, represented by structures IX, XV, and XVII. The behavior of the dimethyl ether of amosamine (XIV) (with an unblocked hydroxyl group at position 1) toward periodate was expected to be of use in the choice of one structure, since XIV would theoretically consume no periodate, while XVI and XVIII should each reduce one mole, with the formation of formic acid and acetaldehyde, respectively. The borohydride reduction product of XIV again would be unaffected by periodate, while the ones from XVI and XVIII would be cleaved to give formaldehyde and acetaldehyde, respectively. A source of encouragement in attempting the methylation was that methyl amosaminide (X) did not quaternize with methyl iodide readily and under forcing conditions gave only the hydriodide salt, the acid presumably being formed by some solvolysis of methyl iodide.

The anomeric mixture of methyl amosaminides (X) was used because in the final step of hydrolysis of the glycoside, both should give the same reducing sugar. Alkylation methods such as the use of methyl iodide, sodium, and liquid ammonia[18] and Haworth's procedure[19] were of little avail. The use of sodium naphthalenide[20] was not explored nor was the recently discovered application

(16) M. Viscontini and J. Meier, *Helv. Chim. Acta*, **35**, 807 (1952). S. Akiya and T. Osawa, *Chem. Abstr.*, **51**, 17763g (1957); *Yakugaku Zasshi*, **77**, 726 (1957).

(17) C. L. Stevens and K. Nagarajan, paper to be published.

(18) I. E. Muskat, *J. Am. Chem. Soc.*, **56**, 2449 (1934).

(19) W. N. Haworth, *J. Chem. Soc.*, **107**, 13 (1915).

(20) N. D. Scott, J. F. Walker, and V. L. Hansley, U. S. Patent 2,171,867; *Chem. Abstr.*, **34**, 115⁹ (1940).

of diazomethane in the presence of an acid catalyst such as boron trifluoride[21] or fluoboric acid,[22] since the amino group in the sugar would be expected to neutralize the acid and precipitate as the salt. Satisfactory results were obtained by the use of Purdie's reagent. By refluxing the methyl amosaminide mixture with methyl iodide in ether in the presence of silver oxide for eighteen hours, a liquid was obtained in 75% yield which had correct analysis for a monomethyl ether. Chromatographic analysis in an *n*-butyl alcohol–water system showed a single spot.[23] It seemed likely that one of the two hydroxyl groups had been selectively converted into the methyl ether. That the second hydroxyl group was not readily converted to the ether was shown by the fact that continuation of the reaction in refluxing ether for a longer period formed only 30% of the dimethyl ether and left 70% of unchanged monomethyl ether (as shown by paper chromatography and combustion results[24]). The monomethyl ether showed a single absorption band in the hydroxyl region in the infrared spectrum. Acetylation gave an oily monoacetate which again traveled as a single spot in an *n*-butyl alcohol–water system.[23] It had no hydroxyl absorption band in the infrared but had bands at 5.75 μ and 8.0 μ due to the acetate ester. It had a lower basicity (pK_a' 5.4) compared to the unacetylated monomethyl ether (pK_a' 6.8).[25]

The dimethyl ether of methyl amosaminide was finally prepared by the action of methyl iodide and silver oxide on the monomethyl ether in refluxing 1,2-dimethoxyethane. After four days, the supernatant liquid in the reaction mixture contained no basic material. Extraction of the silver sludge with methanol and evaporation gave a quaternary hydroxide corresponding to the dimethyl ether. (Quaternization must have occurred after the free hydroxyl had been methylated. This observation coupled with the resistance of methyl amosaminide (X) itself to quaternization would suggest some interaction in the latter compound between the dimethylamino group and adjacent hydroxyl groups.) Pyrolysis of the quaternary hydroxide *in vacuo* at 150° gave liquid products which were separated by means of dilute acid into basic and neutral fractions. The neutral fraction with empirical formula $C_8H_{14}O_4$ corresponded to the product of elimination, followed by acid hydrolysis of the resulting vinyl ether. The basic component, obtained in 16% yield, was the desired dimethyl ether of methyl amosaminide, as shown by elemental analysis, infrared spectrum, and identity of its paper chromatographic behavior with that of the more mobile component in the mixture obtained in the earlier methylation experiments in refluxing ether.[26] The dimethyl ether was formed by displacement by hydroxyl ion on a methyl group of the quaternary nitrogen. The occurrence of displacement as a reaction competing with elimination has been discussed in the literature and shown to be dependent on stereochemical features.[27]

Hydrolysis of di-*O*-methyl methyl amosaminide with boiling 3 *N* hydrochloric acid for four hours gave di-*O*-methyl-amosamine (XIV) in 53% yield as its crystalline hydrochloride, m.p. 209–210° (dec.), giving correct analysis for $C_{10}H_{21}NO_4 \cdot HCl$ and positive reducing tests. The infrared spectrum showed a single band in the hydroxyl region and none in the carbonyl region. Using an excess of 0.08 *N* sodium metaperiodate at room temperature, di-*O*-methylamosamine hydrochloride was found to reduce 0.14, 0.25, 0.37, and 0.71 moles of the reagent in one, twenty-four, fifty, and one hundred and twenty hours, respectively. This behavior looked more like a general, nonspecific oxidation of the molecule rather than the specific cleavage of a vicinal amino alcohol system. Furthermore, at least part of the reagent was utilized for the production of formaldehyde (0.1 mole in one hundred and twenty hours), which could not arise from a normal cleavage.[26] The results of the periodate fission of the borohydride reduction product were 0.15, 0.20, and 0.30 mole uptake of oxidant in one, twenty-five, and ninety-six hours, respectively, with a maximum of 0.07 mole of formaldehyde being detectable. It would thus seem that the dimethylamino group in di-*O*-methyl amosamine (XIV) was not adjacent to the single hydroxyl group present and that it should hence be represented by structure XIV, leading to IX for amosamine, and X and XI, respectively, for

(21) E. Muller and W. Rundel, *Angew. Chem.*, **70**, 105 (1958).

(22) M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron*, **6**, 36 (1959).

(23) In this system, there was very little difference in the R_f values of α- and β-methylamosaminides; hence one spot for the methyl ether of the anomeric glycoside mixture.

(24) Calculated for a mixture of mono- and di-*O*-methyl ethers in the ratio 7:3: C, 55.31; H, 9.73; N, 6.27; OCH_3, 31.78. Found: C, 55.04; H, 9.95; N, 6.03; OCH_3, 32.81.

(25) Acid hydrolysis of the monomethyl ether of the methyl amosaminide mixture with refluxing 3 *N* hydrochloric acid for eight hours followed by paper chromatographic analysis of the product showed two spots, instead of one for the single reducing sugar expected. One of the two spots corresponded to what might be expected for this reducing sugar and the other one with greater R_f was similar to the one observed for the starting material. It is not unlikely that one anomer was more readily hydrolyzed than the other. Such differences in the ratio of hydrolysis of anomeric pairs of glycosides have been recorded and discussed.[8,14]

(26) Unexpectedly the dimethyl ether reduced 2.3 moles of sodium metaperiodate at room temperature in twenty-four hours, although theoretically it should be unaffected. The anomalous uptake was probably due to *N*-demethylation, since one mole of formaldehyde was detected in the solution. The acetate ester of the monomethyl ether likewise reduced one mole of periodate in twenty-four hours with concomitant formation of 0.5 mole of formaldehyde. Similar production of formaldehyde was also noted in the periodate cleavage of the monomethyl ether free base but was almost completely suppressed when the crude hydrochloride salt was used instead. It would thus seem that anomalous reduction of periodate may occur with dimethylamino sugars when there is no cleavable group or when the amino alcohol system is hindered, the extent of the anomalous uptake being pH dependent.

(27) D. Y. Curtin, R. D. Stolow, and W. Maya, *J. Am. Chem. Soc.*, **81**, 3330 (1959), and reference cited therein.

methyl amosaminide and amosaminol. The same conclusion would arise by eliminating the 2-dimethylamino structure, XV, for amosamine for reasons discussed earlier and eliminating the 4-dimethylamino structure, XVII, because it would not be expected to suffer loss of amine at any appreciable rate under basic conditions. However, the results of periodate treatment of di-*O*-methylamosamine (XIV) could only be interpreted as excellent negative evidence for structure XIV. The specific uptake of one mole would have ruled out XIV in preference to XVI or XVIII, but the observed periodate uptake, even if interpreted as negligible and nonspecific, would not rule out XVI or XVIII entirely in favor of XIV, since examples are known in the literature of a vicinal dimethylaminoalcohol system (methyl mycaminoside[12]) or a vicinal glycol (cladinose[28]) being resistant to periodate cleavage.

The oxidation of amosamine (IX) with limited amounts of periodate lent additional evidence in support of structure IX. Unlike mycaminose[12] or desosamine,[13] amosamine could not be cleaved by one mole of periodate to a C_7 sugar. One mole of periodic acid was readily reduced by amosamine hydrochloride at room temperature, but paper chromatographic examination of the products showed the presence of an appreciable amount of starting material. After cleavage of amosamine hydrochloride with one mole of sodium metaperiodate at room temperature, 0.80 mole of volatile acid could be distilled and identified as formic acid through its *p*-bromophenacyl ester. However, the cleavage had not been selective for the carbon-carbon bond at 1–2, since pure amosamine hydrochloride could be recovered in 11% yield from the mixture. Paper chromatographic examination of the mother liquors revealed the presence of more amosamine. A C_4 carbonyl compound was also produced in this reaction and was identified as the 2,4-dinitrophenylosazone (XIII) of 1,2-butanedione. These results indicated that cleavage was rapid for the carbon bonds between positions 1,2 and 2,3 and that further reaction was less rapid. This was confirmed by running the fission with two moles of sodium metaperiodate on amosamine hydrochloride at 5°. Paper chromatographic analysis using acid-base indicator showed that there was no amosamine or other nonvolatile basic components in the reaction mixture. Distillation of the solution yielded 1.9 moles of volatile acid, presumably formic acid, and the neutralized distillate gave a poor yield of the osazone XIII. By continuously extracting a different batch of periodate fission into a hot hydrochloric acid solution of 2,4-dinitrophenylhydrazine, crude XIII was obtained in 36% yield, giving an analytically pure specimen in 24% yield. The derivative was identified with a synthetic specimen by mixture m.p., comparison of ultraviolet, and infrared spectra and X-ray powder patterns. The aqueous layer from the continuous extraction was shown to contain 0.48 mole of volatile base, presumably dimethyl amine. The 1,2-butanedione derivative arose from 2,3 - dihydroxybutyraldehyde (XII) formed by selective cleavage at carbon atoms 2 and 3, which established structure IX for amosamine. The literature does not describe the behavior of 2,3 - dihydroxybutyraldehyde (XII)[29,30] towards acidic 2,4 - dinitrophenylhydrazine. However, the lower homolog, glyceraldehyde, has been shown to yield pyruvaldehyde under warm, acidic conditions.[31,32] In fact, the action of acidic 2,4-dinitrophenylhydrazine on glyceraldehyde yields mainly the pyruvaldehyde osazone and only traces of the glyceraldehyde derivative.[33a,b] The 1,2-butanedione derivative XIII could conceivably be formed from 2-dimethylamino-3-hydroxybutyraldehyde produced by selective cleavage between positions 1 and 2 and 2 and 3 of the hypothetical 4-dimethylamino structure XVII. However, this was not the case, since there was no basic compound apart from dimethylamine in the mixture obtained from amosamine with two moles of periodate. The possibility that the dimethylamino aldehyde initially formed rapidly underwent beta elimination of water and that the resultant vinylamine was irreversibly hydrolysed to 1,2-butanedione was considered very unlikely[34] since 2,3-dihydroxybutyraldehyde (XII) is known to be stable in the presence of an excess of 0.1 *N* sulfuric acid for several days.[29] The dehydration of 2-dimethylamino-3-hydroxybutyraldehyde may be expected to be less facile.

With the exception of the rate of deaminolysis, all of the data were best interpreted in terms of the 3-dimethylamino-3,6-dideoxyaldohexose structure IX for amosamine.[35] The elimination probably

(28) P. F. Wiley and O. Weaver, *J. Am. Chem. Soc*, **78**, 808 (1956).

(29) A. Wohl and F. Frank, *Ber.*, **35**, 1908 (1902).

(30) J. W. E. Glattfeld and W. G. Straitiff, *J. Am. Chem. Soc.*, **60**, 1384 (1938).

(31) C. Neuberg, E. Farber, A. Levite, and E. Schwenk, *Bio. Zeitschrift*, **83**, 263 (1917).

(32) S. Akiya, S. Okui, and S. Suzuki, *J. Pharm. Soc. Japan*, **72**, 785 (1952).

(33) (a) H. Reich and B. K. Samuels, *J. Org. Chem.*, **21**, 68 (1956); (b) G. Matthiessen and H. Hagedorn, *Mikrochemie ver Mikrochim. Acta*, **29**, 55 (1941), claim to have prepared the dinitrophenylosazone of glyceraldehyde directly from the aldehyde. They did not report any analysis and obviously had only the pyruvaldehyde derivative, since their melting point is in agreement with that of pyruvaldehyde 2,4-dinitrophenylosazone and not with the one reported for the glyceraldehyde osazone.[33a]

(34) Further evidence that the periodate fission solution, prior to ether extraction into acidic 2,4-dinitrophenylhydrazine solution, indeed contained 2,3-dihydroxybutyraldehyde (XII) and not 1,2-butanedione was that after addition of excess periodate at this stage, a further uptake of 1.6 moles of the oxidant was noticed.

(35) The pyranose structure is assigned in analogy with other hexoses. The gross structure arrived at for amosamine (IX) is the same as the one that has been proposed for mycaminose. However, the two sugars are definitely different as could be seen by comparing the physical constants and reactions of amosamine and the glycosides with those reported for mycaminose and its derivatives. Paper chromatographic examination of the two sugars also confirmed this difference. Amosamine (IX) is therefore a stereoisomer of mycaminose.

proceeded on the open chain form rather than on the cyclic one, since methyl mycaminoside[12] and alpha-methyl amosaminide (X) suffered very little loss of dimethylamine under standard conditions. The difference in behavior of amosamine (IX) in this respect from the other two known 3-dimethylamino sugars may be due to the difference in stereochemistry of the 2,3-substituents. The validity of this speculation is currently being investigated using 3-amino sugars of known stereochemistry.

A few experiments were initiated on elucidating the stereochemistry of amosamine. The methyl amosaminides, being aminopyranosides, should undergo normal cleavage with periodate.[17,36] The isolation and identification of a methoxymethyldiglycollic aldehyde[37-39] from this cleavage would establish the configuration of position 5. However, it has not yet been possible to isolate a crystalline compound from the fission of either methyl amosaminide (X), although the expected dialdehyde V[40] was obtained as a crystalline picrate from the fission of cytosamine (IV).

Structure of Amicetamine (III), Cytosamine (IV), and Amicetin (I).—Amicetamine (III) readily reduced two moles of periodate to yield formic acid and a derivative of glyoxal,[3] showing that it was a glycoside of amosamine (IX) with one of the two hydroxyl groups in the neutral sugar, amicetose (VII). This was further supported by the qualitative observation of the formation of methyl amosaminide (X) from the methanolysis of amicetaminol, the sodium borohydride reduction product of amicetamine (III). Amicetamine (III) gave a positive iodoform reaction and reduced Benedict's reagent, although only very sluggishly and under strongly basic conditions. The hydroxyl group at position 4 in amicetose (VII) was therefore involved in the glycosidic link with amosamine (IX) to give amicetamine (III), thus establishing structure III for it. Earlier work[2] showed that amicetin (I) and hence cytosamine (IV) had the C_{14} fragment (amicetamine) attached at position 1 of the cytosine ring. Hence, structures IV and I follow for cytosamine and amicetin, respectively.[41] Cytosamine (IV) consumed two moles of periodate readily and unlike amicetamine (III) gave a negative iodoform reaction and did not reduce Benedict's reagent under any condition. These facts fully supported structure IV for cytosamine. The ease of cleavage of amicetin or cytosamine under conditions of hydrolysis or methanolysis at the sugar–pyrimidine nucleoside link would be consistent with this formulation rather than with an alternate one, wherein amosamine and not amicetose was bound to cytosine, since no cleavage of the nucleoside link occurs under these conditions in pyrimidine nucleosides of glucose,[42] glucosamine,[17] or 3-amino-3-deoxyribose.[43] On the other hand, cleavage of a nucleosidic link with a polydeoxysugar like amicetose (VII) is more likely to occur and has been in fact observed by the action of even a mild acid such as acetic acid on a 2-deoxyribose-pyrimidine nucleoside.[44]

Experimental

Methanolysis of Amicetin (I).—A suspension of 6 g. of amicetin (I) in 120 ml. of reagent grade methanol was cooled in a Dry Ice–acetone bath and saturated with dry hydrogen chloride gas. The resulting solution was left at room temperature and after a few hours a crystalline precipitate separated. After 36 hr., this was collected and washed with a little methanol to give 3.35 g. (94%) of cytimidine (II) hydrochloride, m.p. 260–263° (dec.). The filtrate was evaporated at room temperature *in vacuo* to a very small volume and the residual sirup dissolved in 50 ml. of methanol and poured on to a 2.5 × 40 cm. column of Dowex 50 (25–50 mesh) suspended in methanol.[45] The column was washed with about 500 ml. of methanol until the eluate was neutral. The eluate was then freed from hydrochloric acid by passing it through a 4 × 75 cm. column of Amberlite IR 45.[46] The column was washed with 500 ml. of methanol and the combined eluates were evaporated to dryness *in vacuo* at room temperature. The residual sirup was dissolved in ether and the ether solution decanted from some insoluble material, dried over sodium sulfate, and evaporated to leave 1.1 g. of a thin oil.

Evaporative distillation of the oil at 5 mm. gave, as the major fraction, 0.8 g. (50%) of the methyl glycoside of amicetose (VI), b.p. 55–60° (bath temperature). Another 0.1 g. of a viscous liquid collected over a period of 20 hr. at 55–66° (3 mm.), gave 85 mg. (3% based upon amicetin) of the 2,4-dinitrophenylhydrazone of amicetose (VIII) (see below), m.p. 142–145°. The undistillable residue of 0.1 g. was discarded. The Dowex column was washed with 1 l. of methanolic ammonia (10 ml. of concentrated aqueous ammonium hydroxide added to each 90 ml. of methanol). The eluate was evaporated to dryness and the gummy residue extracted repeatedly with warm, dry ether. The ether extracts were filtered from a small amount of insoluble material (cytimidine) and the filtrate evaporated to afford 1.6 g. (80%) of the anomeric mixture of methyl amosaminides (X) as a gum.

Methanolysis of amicetamine (III) or cytosamine (IV) under the same conditions gave similar results.

Amicetose. Methyl Glycoside (VI).—The methyl glycoside of the neutral sugar from the above experiment was redistilled at the same temperature and pressure for an

(36) M. J. Weiss, J. P. Joseph, H. M. Kissman, A. M. Small, R. E. Schaub, and F. J. McEvoy, *J. Am. Chem. Soc.*, **81**, 4050 (1959).

(37) W. D. Maclay, R. M. Hann, and C. S. Hudson, *ibid.*, **61**, 1660 (1939).

(38) I. J. Goldstein, B. A. Lewis, and F. Smith, *ibid.*, **80**, 939 (1958).

(39) D. R. Walters, J. D. Dutcher, and O. Wintersteiner, *ibid.*, **79**, 5076 (1957).

(40) The dialdehydes from 6-deoxy sugar derivatives are known to hold a molecule of water to form similar lactal structures.[38,39]

(41) Representations of amicetin and its fragments pertain only to gross structures with no implications of stereochemistry.

(42) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930).

(43) H. M. Kissman and A. J. Weiss, *ibid.*, **80**, 2575 (1958).

(44) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 34 (1954).

(45) The resin was prepared as follows: Commercial Dowex 50 was washed with 3 *N* hydrochloric acid, then with water, and finally with methanol. The resin was then left to soak in methanol for a day and made into a column.

(46) The commercial sample was washed with 10% sodium hydroxide solution until free from chloride ion, then with water, and finally with methanol. After standing in more methanol overnight, the resin was made into a column and the old solvent replaced by fresh.

analytical sample; $n^{25}D$ 1.4484; $[\alpha]^{25}D$ +75.1 (*c* 0.87 in H_2O). The compound gave a negligible iodoform test[47]; its infrared spectrum in chloroform solution showed bands at 2.75 (shoulder) and 2.9 μ for a hydroxyl group and at 7.25 μ for a C-methyl group.

Anal. Calcd. for $C_7H_{14}O_3$: C, 57.50; H, 9.65; 1-OCH_3, 21.24; 1 C-methyl, 10.3. Found: C, 57.83; H, 9.63; OCH_3, 19.9; C—CH_3, 9.00.

Amicetose 2,4-Dinitrophenylhydrazone (VIII).—To a warm solution of 0.32 g. of 2,4-dinitrophenylhydrazine in 50 ml. of 2 *N* hydrochloric acid was added 0.2 g. of the methyl glycoside (VI). A crystalline precipitate separated immediately. The solution was allowed to stand at room temperature for 30 min. and the precipitate was collected and washed with a little water to yield 0.365 g. (85%) of amicetose 2,4-dinitrophenylhydrazone (VIII), m.p. 148–150°. A sample recrystallized from methanol–benzene formed small yellow needles, m.p. 152–153°; $[\alpha]^{25}D$ −10.0° (*c* 0.86 in pyridine); λ_{max}^{EtOH} 225 mμ (ϵ 14,100), 357 mμ (ϵ 20,500); the infrared spectrum of a Nujol mull showed bands at 2.98 (weak; OH), 3.05 (NH), and 6.1 μ (C=N).

Anal. Calcd. for $C_{12}H_{16}N_4O_6$: C, 46.14; H, 5.16; N, 17.94; 1 C—CH_3, 4.82. Found: C, 46.24, 46.36; H, 5.54, 5.38; N, 17.85; C—CH_3,[48] 5.92, 6.62, 7.10; O—CH_3, 0.

A solution of 51 mg. of the dinitrophenylhydrazone (VIII) in 1 ml. of pyridine was mixed with 1 ml. of acetic anhydride and heated at 40° for 16 hr. The solution was then evaporated to dryness *in vacuo* at room temperature and the residue rubbed with two 5-ml. portions of cold water to leave a crystalline mass. Recrystallization from aqueous methanol gave 24 mg. of shining yellow needles, m.p. 93–94°. A second crystallization from ether–petroleum ether gave the analytical sample, m.p. 95–96°; λ_{max}^{EtOH} 226 mμ (ϵ 13,000), 356 mμ (ϵ 20,400); bands at 3.05 (NH), 5.75 (ester C=O), 6.15 (C=N), and 8.0 μ (acetate confirming) were observed in the infrared spectrum of its chloroform solution.

Anal. Calcd. for $C_{16}H_{20}N_4O_8$: C, 48.49; H, 5.09. Found: C, 48.36; H, 5.29.

Periodate Fission of Amicetose 2,4-Dinitrophenylhydrazone (VIII). (A) Quantitative Estimation.—A solution of 14.2 mg. of the 2,4-dinitrophenylhydrazone (VIII) in 5 ml. of pure dioxane was mixed with 5 ml. of 0.201 *N* sodium metaperiodate solution. Two-milliliter aliquots were withdrawn at intervals and the periodate uptake determined by standard procedure.[49] It was found that the compound reduced 1.25, 1.25, 1.25, and 1.35 moles of periodate in 15, 30, 60, and 180 min., respectively.

(B) **Isolation of Products.**—A solution of 0.14 g. (4.5 mmoles) of the dinitrophenylhydrazone (VIII) in 5 ml. of dioxane was mixed with 5 ml. of 0.201 *N* (5 mmoles) of aqueous sodium metaperiodate. After standing at room temperature for 2.5 hr., 40 ml. of water was added to the solution, whereupon a yellow precipitate separated. The mixture was evaporated *in vacuo* at room temperature using a water aspirator. The volatile products were drawn through a trap cooled to 0° and containing a solution of 0.25 g. of 2,4-dinitrophenylhydrazine in 50 ml. of 3 *N* hydrochloric acid. After a few seconds a yellow precipitate began to separate in the trap. After the periodate mixture had evaporated to near dryness, the trap was disconnected and the contents were filtered to give 65 mg. (65%) of derivative, m.p. 141–144°. One crystallization from 95% ethanol gave 51 mg. (51%) of yellow needles, m.p. 148–149°, undepressed by admixture with an authentic sample of acetaldehyde 2,4-dinitrophenylhydrazone, which, when prepared in the same way, had m.p. 148–149°, corresponding to the lower melting point recorded in the literature.[50] Identity was further confirmed by comparison of infrared spectra.

The residue from the evaporation of the cleavage solution was triturated with water, left at 0° for some time, and the mixture filtered to afford 95 mg. (80%) of succindialdehyde mono 2,4-dinitrophenylhydrazone as yellow crystals, m.p. 130–133°, with previous sintering. Recrystallization from methanol gave an analytical sample, m.p. 130–132°; λ_{max}^{EtOH} 225 mμ (ϵ 14,100), 356 mμ (ϵ 21,000), having bands at 3.05 (NH), 5.80 (aldehyde C=O, medium intensity), and 6.12 μ (C=N) in the infrared spectrum (Nujol mull).

Anal. Calcd. for $C_{10}H_{10}N_4O_5$: C, 45.11; H, 3.79; N, 21.04. Found: C, 45.04; H, 3.87; N, 20.73, 20.66.

In some preparations, when recrystallization was effected from a methanol–benzene mixture, the crystals exhibited a double melting point: melting with frothing at about 105°, resolidifying, and melting again at 133°.

Addition of excess Brady's reagent to a warm ethanol solution of this compound gave a sparingly soluble compound as a fine orange precipitate in quantitative yield. Crystallization from dimethylformamide afforded orange-yellow needles, m.p. 265–273° (dec.) (depending upon rate of heating); λ_{max}^{DMF} 372 mμ (ϵ 27,500). The mixture melting point with succindialdehyde bis-2,4-dinitrophenylhydrazone[51] was undepressed. The ultraviolet and infrared spectra and X-ray powder patterns of the two samples were superimposable.

Anal. Calcd. for $C_{16}H_{14}N_8O_8$: C, 43.05; H, 3.16; N, 25.11. Found: C, 43.16; 43.09; H, 3.39, 3.24; N, 25.24, 25.07.

Periodate Fission of Amicetose Methyl Glycoside (VI).—To a solution of 12.06 mg. of the methyl glycoside (VI) in 3 ml. of water was added a saturated solution of sodium bicarbonate and 5 ml. of 0.2 *N* aqueous sodium metaperiodate. The resulting solution was diluted to 10 ml. and found to have approximately pH 7. At the end of 12 hr., 0.055 mole of periodate (duplicate determination) had been reduced.

Acid Hydrolysis of Amicetose Methyl Glycoside (VI).—To 52.2 mg. of the methyl glycoside (VI) was added 8 ml. of 3 *N* hydrochloric acid. The hydrolysis was followed at room temperature in a polarimeter tube. The rotation fell within a few minutes and remained constant over a period of 16 hr. The specific rotation at this time was +24°, calculated for free amicetose (VII) present in the solution. After neutralization with 10% aqueous sodium hydroxide, the hydrolysis solution gave a definite iodoform test, and reduced Benedict's solution slowly when excess alkali was added.

Periodate Fission of Amicetose (VII).—The methyl glycoside (VI) (20.1 mg.) was hydrolyzed with 7 ml. of 3 *N* hydrochloric acid. After 15 hr., most of the acid was neutralized by the addition of 4.1 ml. of 5 *N* aqueous sodium hydroxide. To the resulting solution of amicetose (VII) was added 10 ml. of 0.2 *N* aqueous metaperiodate and the solution diluted to 25 ml. The uptake of the oxidant was estimated against a blank at various intervals and found to be 0.99 mole in 0.5 hr., 1.02 moles in 2.5 hr., and 1.06 moles in 25 hr.

A 7-ml. aliquot of this solution was evaporated *in vacuo*, drawing the distillate through an acid solution of 2,4-dinitrophenylhydrazine. The derivative that separated amounted to 5 mg. and had m.p. 260–265° (dec.), with much preliminary shrinking. This was probably a mixture of succindialdehyde and acetaldehyde derivatives and a separation was not attempted.

(47) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, 1956, p. 156.

(48) The three C-methyl determinations were carried out by three different analytical laboratories and all were higher than the expected value for one C-methyl. This is somewhat puzzling in the light of values obtained for three model dinitrophenylhydrazones. Acetaldehyde dinitrophenylhydrazone showed 0.86 group for a theoretical of 1 while the 5-hydroxypentanal derivative showed none, as it should.

(49) E. L. Jackson, *Org. Reactions*, 341 (1944).

(50) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, 1956, p. 283.

(51) A. C. Cope and R. T. Keller, *J. Org. Chem.*, **21**, 141 (1956).

Isolation of Amicetose (VII).—A solution of 0.67 g. of the methyl glycoside (VI) was dissolved in 20 ml. of 3 *N* hydrochloric acid at room temperature. After 12 hr., most of the acid was neutralized by the addition of 30% sodium hydroxide solution with cooling. The solution was saturated with sodium chloride and extracted five times with 100-ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness to give 0.38 g. (63%) of a viscous oil, which gave a slow reducing test with Benedict's reagent in the presence of added alkali. Evaporative distillation at 0.3 mm. gave 0.16 g. (26%) of a colorless, thick viscous oil, b.p. 65–70° (bath temperature), $n^{25}D$ 1.4689; $[\alpha]^{25}D$ +28.6 (*c* 1.2 in $CHCl_3$). The oil showed OH and C-methyl absorption bands in the infrared and no C=O absorption. With a warm solution of 2,4-dinitrophenylhydrazine in 3 *N* hydrochloric acid, it gave a slow precipitate, but eventually a quantitative yield of amicetose 2,4-dinitrophenylhydrazone (VIII), m.p. and mixture m.p. 148–150°, was formed. Satisfactory analysis was not obtained for the free sugar.

By the addition of an acidic solution of 2,4-dinitrophenylhydrazine to the aqueous layer from the ether extraction, 0.16 g. (11%) of amicetose 2,4-dinitrophenylhydrazone (VIII), m.p. 148–150°, could be recovered.

Amosamine (IX). Separation of Methyl Amosaminides (X).—A solution of 1.6 g. of the anomeric mixture of methyl amosaminides (X) from the methanolysis of amicetin (I) in ether was filtered from traces of amorphous material and concentrated to a small volume. Hexane was added to this solution until turbidity occurred. Crystallization usually began after rubbing with a glass rod, especially after adding a seed. After a few hours at room temperature, the crystals were collected and washed with ether–petroleum ether mixture to afford 0.160 g., m.p. 91–94° of the alpha anomer.

The mother liquors were evaporated and converted into the hydrochloride salt by the addition of ethereal hydrogen chloride. The gummy salt, on rubbing with absolute ethanol, afforded crystals which were collected by filtration and washed with a little ethanol to give 0.290 g. of β-methyl amosaminide (X) hydrochloride, m.p. 208–209° (dec.).

The alcoholic mother liquor from the crystallization showed a high positive rotation and thus evidently contained a considerable amount of the alpha anomer. The alcohol solution was deacidified by shaking with purified, dry Amberlite IR 45 (hydroxide form) and evaporated to give 0.90 g. of a gum. Crystallization of the gum from ether–petroleum ether mixture gave 0.465 g. of α-methyl amosaminide (X), m.p. 90–93°.

A total of 0.625 g. (31%) of α-methyl amosaminide (X) and 0.290 g. (12%) of β-methyl amosaminide (X) hydrochloride was thus obtained in a fair state of purity from methanolysis of 6 g. of amicetin (I). The residual gummy mixture of bases was shown to be only a mixture of the two anomers by paper chromatography and could be used for experiments such as hydrolysis and methylation. The relative proportions of the two anomers that could be isolated varied from batch to batch, but essentially the same procedure could be applied for their separation.

α-Methyl Amosaminide (X).[52]—Recrystallization of the anomer, m.p. 90–93°, from ether–hexane gave a sample as long colorless needles, m.p. 92–93°, which when further purified by sublimation at 75° (0.5 mm.) gave an analytical sample as minute needles, m.p. 93–94°; $[\alpha]^{25}D$ +138.2° (*c* 0.5 in H_2O), +146° (*c* 1.1 in 0.1 *N* HCl, unchanged after 24 hr.); pK_a' 7.2 (in 50% MeOH). Its infrared spectrum in chloroform solution had a broad band at 2.8–2.9 μ (OH) and one at 7.25–7.28 (C-methyl).

Anal. Calcd. for $C_9H_{19}NO_4$: C, 52.65; H, 9.33; N, 6.82; O, 31.20; 1 OCH_3, 15.12; 2 *N*-methyl, 14.66; 1 C-methyl, 7.33. Found: C, 52.75; H, 9.40; N, 6.91, 6.95; O, 31.83; OCH_3, 15.10; N-methyl, 7.77; C-methyl, 7.8, 8.1.

The glycoside (X) was readily soluble in water, alcohol, chloroform, ether, and benzene and insoluble in hexane. It gave a negative iodoform reaction and did not reduce Benedict's reagent.

The hydrochloride was formed in quantitative yield in ether solution and on crystallization from an ethanol–ether mixture, was obtained as needles, m.p. 195–196° (dec. and frothing); $[\alpha]^{25}D$ +113.7 (*c* 0.7 in H_2O); absorption bands at 3.0 μ (broad, OH) and 3.63 μ (salt, NH) in the infrared spectrum (Nujol mull).

Anal. Calcd. for $C_9H_{20}ClNO_4$: C, 44.73; H, 8.34; N, 5.80; Cl, 14.67. Found: C, 44.74; H, 8.09; N, 5.79; Cl, 14.62.

An attempt to make the methiodide by refluxing the base with methyl iodide in methanol gave a poor yield of the hydriodide,[52] m.p. 193–194°, from which the base could be recovered in quantitative yield by the use of Amberlite IR 45 (hydroxide form).

Anal. Calcd. for $C_9H_{20}INO_4$: C, 32.45; H, 6.05; N, 4.21. Found: C, 32.17, 32.32; H, 6.36, 6.27; N, 4.21.

Fission of α-Methyl Amosaminide (X). (A) Quantitative Estimation.—The free base (0.1 mmole) was dissolved in 10 ml. of 0.2 *N* sodium metaperiodate solution, alone or in the presence of one equivalent of dilute sulfuric acid. The solution was made up to 25 ml. and left at room temperature. Measured aliquots were withdrawn at intervals and the periodate uptake determined by the standard procedure. The following table gives the number of moles of periodate reduced at various intervals, by the base and the sulfate.

Compound	15 min.	1 hr.	5 hr.	24 hr.	48 hr.
Base	2.35	...	2.40	...	...
Sulfate	2.35	2.30	2.30	2.34	2.67

When the fission was run at room temperature using 51.3 mg. (0.25 mmole) of alpha-methyl amosaminide (X) and 5 ml. of 0.2 *N* paraperiodic acid (0.5 mmole), the uptake of the oxidant was 0.69, 0.80, 1.10, and 2.06 moles, respectively, at the end of 0.25 hr., 1 hr., 3 hr., and 24 hr., respectively. Using the same quantities but running the reaction at 5°, 0.46, 0.50, 0.56, and 0.92 mole of periodic acid was reduced at the end of 5 min., 15 min., 1 hr. and 17 hr., respectively. An aliquot was treated, after 17 hr., with a slight excess of barium hydroxide to remove iodate and periodate ions. After passing carbon dioxide into the mixture to remove excess barium, the mixture was filtered and the filtrate evaporated *in vacuo* to dryness. The residue was shown by paper chromatography to contain only α-methyl amosaminide (X) as the basic component.

(B) Isolation of Products.—In a quantitative experiment, it was shown that α-methyl amosaminide hydrochloride reduced 2.25 moles of sodium metaperiodate in 48 hr. and that no formaldehyde was present in the mixture (chromotropic acid test[53]). For isolation of products, 48.86 mg. (0.202 mmole) of the hydrochloride was added to 10 ml. of water containing 0.1 g. (0.46 mmole) of sodium metaperiodate. After standing at room temperature for 48 hr., the solution was evaporated to dryness (residue A) at room temperature *in vacuo*. The distillate was collected in an efficient Dry Ice trap and, after allowing to come to room temperature, titrated against standard sodium hydroxide solution (approximately 0.06 *N*) to phenolphthalein end point. After allowing for a blank titer value obtained by evaporating 10 ml. of plain sodium metaperiodate solution alone under the same conditions, 0.74 mole of volatile

(52) The isolation of this compound from the methanolysis of amicetin (I) or cytosamine (IV) and the characterization of the free base and its hydriodide, were first worked out in this laboratory by Dr. E. D. Parker to whom we are thankful.

(53) W. R. Frisell and C. G. Mackenzie, "Methods of Biochemical Analysis," Vol. VI, 1958, p. 63.

acid was found to be produced in the fission.[54] The neutralized solution gave negative tests for chloride, iodate, or periodate ions and for carbonyl compounds.

Residue A was repeatedly extracted with hot ether. The ether extracts, on evaporation, left about 30 mg. of a yellowish gum that failed to crystallize. An aqueous solution of the gum was then reduced with 50 mg. of sodium borohydride during 16 hr. and then acidified with dilute hydrochloric acid. Addition of a 3 *N* hydrochloric acid solution of 2,4-dinitrophenylhydrazine and warming on the steam bath gave a red precipitate which was collected and washed with methanol to afford 15 mg. of a product, m.p. 310° (dec.). Recrystallization from dimethylformamide gave red needles, m.p. 322° (dec.), identical with glyoxal 2,4-dinitrophenylosazone (mixture m.p.).

The white residue from the ether extraction was extracted with 15 ml. of cold ethanol, leaving 45 mg. of inorganic salts containing mostly sodium iodate and a trace of periodate. The alcohol extract, on evaporation to a small volume and addition of ether, gave about 5 mg. of dimethylamine hydrochloride.

Mono-*O*-carbobenzyloxy-α-methyl Amosaminide.—To a solution of 0.103 g. (0.5 mmole) of α-methyl amosaminide (X) in 5 ml. of chloroform was added 0.7 g. (1.5 mmoles) of a 30% solution of carbobenzyloxy chloride in toluene and 0.21 g. (2.5 mmoles) of powdered anhydrous sodium bicarbonate. The mixture was shaken occasionally at room temperature and after 15 hr. filtered. The filtrate was evaporated *in vacuo* to remove the solvents and as much of the excess acid chloride as possible. The residual sirup was dissolved in ether and mixed with an ether solution of dry hydrogen chloride gas. The resulting precipitate was thoroughly washed with ether, dissolved in 5 ml. of hot chloroform, and filtered from some insoluble crystalline material. The filtrate was concentrated and, on adding ether to it to turbidity, crystals began to separate. These were collected and washed with ether to afford 41 mg. (21%) of the hydrochloride of the monocarbobenzyloxy derivative, m.p. 157–159° (dec.), shrinking above 148°. Recrystallization from chloroform–ether mixture gave a sample, m.p. 157–159° (dec.); pK_a' 5.4 (in 50% MeOH); the infrared spectrum as a Nujol mull had bands at 2.9 (shoulder) and 3.1 μ (OH), 3.75 (salt NH) and 5.7 μ (ester C=O).[55] Analysis indicated it to be a monohydrate. In 18 hr., the compound reduced only 0.23 mole of sodium metaperiodate.

Anal. Calcd. for $C_{17}H_{26}ClNO_6 \cdot H_2O$: C, 51.83; H, 7.17; N, 3.56. Found: C, 52.16, 52.01; H, 7.37, 7.35; N, 3.81.

The chloroform-insoluble material from the reaction amounted to about 15 mg. and on recrystallization from ethanol–ether had m.p. 195–196° (dec.). Mixture melting point with α-methyl amosaminide hydrochloride was undepressed.

β-Methyl Amosaminide (X).—Recrystallization of 0.45 g. of the hydrochloride (m.p. 208–209°) from the methanolysis of amicetin (I) from ethanol–ether gave 0.31 g. of a pure sample as colorless thick cubes, m.p. 209–210° (dec.); $[\alpha]^{25}D$ −32.4° (*c* 0.5 in water); pK_a' 7.2 (in 50% methanol). A mixture melting point with the alpha anomer was depressed; as a Nujol mull, it had bands at 2.95 (OH), 3.15 (OH), and 3.70 μ (salt NH) in the infrared.

Anal. Calcd. for $C_9H_{20}ClNO_4$: C, 44.73; H, 8.34; N, 5.80; Cl, 14.67; 1 methoxyl, 12.85; 2 *N*-methyl, 12.44; 1 C-methyl, 6.22. Found: C, 44.79, 44.56; H, 7.97, 8.21; N, 5.79, 5.74; Cl, 14.51; methoxyl, 13.00, 13.20; N-methyl, 5.85, 4.2; C-methyl, 7.00.

The compound gave negative iodoform and Benedict's tests.

Addition of excess sodium bicarbonate to an aqueous solution of 0.18 g. of the hydrochloride and ether extraction gave the free base which was sublimed at 80° (1 mm.) to afford 0.11 g. (72%) of a glass. Satisfactory analysis was not obtained for this compound.

Anal. Calcd. for $C_9H_{19}NO_4$: N, 6.82; methoxyl, 15.12. Found: N, 7.25; methoxyl, 16.66.

Periodate Fission of β-Methyl amosaminide (X).—Quantitative experiments using the hydrochloride showed that it reduced 1.97, 2.41, 2.7, and 2.8 moles, respectively, of sodium metaperiodate at the end of 0.5, 2, 24, and 45 hr. No formaldehyde could be detected in the product mixture.

In one experiment 48 mg. (0.2 mmole) of the hydrochloride was dissolved in 10 ml. of water containing 0.10 g. (0.46 mmole) of sodium metaperiodate. After 48 hr. at room temperature, the solution had no excess periodate and was evaporated to dryness *in vacuo* to leave a white residue (A). The distillate was trapped at −80°; titration with standard sodium hydroxide to phenolphthalein end point showed the presence of 0.76 mole of volatile acid. The neutralized solution did not give any precipitate with acidic 2,4-dinitrophenylhydrazine solution. Extraction of the residue (A) gave a gum which afforded, in aqueous hydrochloric acid solution, 36 mg. of a red 2,4-dinitrophenylhydrazone, shrinking above 260° and melting with decomposition at about 300°. This mixture, presumably of glyoxal and pyruvaldehyde derivatives, on one crystallization from dimethylformamide, afforded 15 mg., shrinking above 290° and decomposing around 315°. The residue from the ether extraction, on treatment with warm, aqueous sodium hydroxide solution, evolved a volatile base smelling of dimethylamine.

In another experiment, 0.121 g. (0.5 mmole) of beta-methyl amosaminide (X) hydrochloride was cleaved with 0.269 g. (1.25 mmoles) of sodium metaperiodate in 5 ml. of water. After 12 hr., an aqueous solution of barium hydroxide was added until the solution had pH 7 and the precipitated barium salts were removed by filtration. To the filtrate was added 0.2 g. of sodium borohydride. The solution was allowed to stand at room temperature for 5 hr., made slightly acidic by the dropwise addition of concentrated sulfuric acid, and then continuously extracted with ether into a reservoir containing 0.3 g. of 2,4-dinitrophenylhydrazine in 30 ml. of warm 4 *N* hydrochloric acid. After 14 hr., the contents of the reservoir were filtered to yield 70 mg. (34%), of a red osazone, m.p. 320° (dec.). Recrystallization from dimethylformamide afforded 50 mg. (24%) of glyoxal 2,4-dinitrophenylosazone, m.p. 323–324° (dec.), λ_{max}^{DMF} 585 mμ (ε 54,400), identified by mixture m.p., infrared and ultraviolet spectra, and X-ray powder pattern.

Anal. Calcd. for $C_{14}H_{10}N_8O_8$: C, 40.22; H, 2.41; N, 26.80. Found: C, 40.27; H, 2.68; N, 26.5.

Amosamine (IX). (A) By Hydrolysis of Anomeric Mixture of Methyl Amosaminides (X).—The gummy mixture of methyl amosaminides (X) from methanolysis of amicetin (I) was used for this experiment without purification. A solution of 1.35 g. of the glycoside in 30 ml. of 3 *N* hydrochloric acid was refluxed for 4 hr., after which time it gave a good reducing test with Benedict's reagent. The solution was decolorized with charcoal, filtered, and the filtrate evaporated to dryness *in vacuo* at room temperature. After drying further for several hours *in vacuo* to ensure that traces of moisture and acid had been removed, the residue was triturated with 10 ml. of absolute ethanol, whereupon crystallization began. The solution was cooled in the ice chest overnight and the solid collected and washed with a little ethanol to afford 0.60 g.[56] (40%) of amosamine (IX) hydro-

(54) Control experiments were carried out on a similar scale on α-methyl glucoside, desosamine hydrochloride,[9] and glucosamine hydrochloride. α-Methyl glucoside produced 0.86 mole of volatile acid for a theoretical 1 mole. From fission of desosamine, 1.9 moles of volatile acid (theoretical 2 moles) and a poor yield of crotonaldehyde[9] were obtained. Glucosamine hydrochloride liberated 93% of the expected 5 moles of volatile acid.

(55) The amide C=O in 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-carbobenzoxyamino-β-D-glucose appears at 5.88 μ in the infrared region.

(56) This represents the maximum yield obtained under favorable conditions. On this scale, yields varied from 0.40 g. to 0.60 g.

chloride, m.p. 180–182° (dec.). Recrystallization from alcohol gave a sample of the same melting point; $[\alpha]^{25}D$ +44.5° (*c* 0.51 in H_2O; equilibrium value); pK_a' 7.2 (in 50% MeOH). The melting point of amosamine hydrochloride varied from one batch to another. Melting points ranging from 170–185° were obtained for different preparations and were also dependent upon rate of heating. Paper chromatograms showed these samples were homogeneous and identical, showing an R_f value of 0.58 as free base (IX) in *n*-butyl alcohol–water, compared to R_f 0.73 for methyl amosaminides (X).

Combustion values were also in agreement with one another. Infrared spectrum as a Nujol mull showed hydroxyl absorption at 2.8, 2.9, and 3.1 μ, salt NH absorption at 3.6 μ and no carboxyl absorption.

Anal. Calcd. for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97; N, 6.15; Cl, 15.57; 1 C—CH_3, 6.61; 2 N—CH_3, 13.22. Found: C, 42.51, 42.56; H, 8.18, 8.17; N, 6.27; Cl, 15.04; C—CH_3, 7.0, 7.6; N—CH_3, 2.70.

Amosamine (IX) hydrochloride gave a positive reducing test with Benedict's reagent and a slow iodoform reaction.

(B) By Hydrolysis of β-Methyl Amosaminide (X).—Hydrolysis of 0.1 g. of the hydrochloride by refluxing with 5 ml. of 3 *N* hydrochloric acid and working up as before gave 23 mg. (10%) of pure amosamine (IX) hydrochloride, m.p. and mixture m.p. 180–182° (dec.), having identical infrared spectrum and paper chromatographic behavior as the amosamine hydrochloride described above.

(C) By Cleavage of Amicetamine (III) on Acidic Resin.—A solution of 1.61 g. of amicetamine (III) hydrochloride was dissolved in 10 ml. of distilled water and placed on a 2 × 12 cm. column of purified Dowex 50 resin (25–50 mesh; acid cycle). The column was washed with water until the eluate was free of chloride ion (silver nitrate test). The resin was then transferred to a flask with the aid of 50 ml. of water and the mixture refluxed for 72 hr. The mixture was then repacked into a column and washed with water until the eluate (A) was neutral. The resin was then eluted with 1.5 *N* ammonium hydroxide and a total of 500 ml. of eluate was collected. The ammoniacal eluate was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml. of water, made acidic by the addition of dilute hydrochloric acid and the solution, after decolorization with charcoal, evaporated to dryness *in vacuo* at room temperature. The residue on crystallization from absolute ethanol afforded 0.41–0.61 g. (38–57%) of amosamine (IX) hydrochloride, m.p. 183–185° (dec.), $[\alpha]^{25}D$ +45.5° (*c* 1 in H_2O), pK_a' 7.4, having identical infrared spectrum and paper chromatographic behavior described before.

Anal. Calcd. for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97; N, 6.15; ·Cl, 15.57, neut. equiv., 227.7. Found: C, 42.09; H, 8.13; N, 6.13; Cl, 15.18 (ionic); neutralization equivalent, 222.0.

Eluate (A) from the resin hydrolysis was mixed with a warm hydrochloric acid solution of 2,4-dinitrophenylhydrazine. The crude derivative that separated, on crystallization from methanol–benzene mixture, gave 75 mg. of amicetose 2,4-dinitrophenylhydrazone (VIII), m.p. and mixture m.p. 148–150°, and a second crop amounted to 60 mg., m.p. 135–140°.

Conversion of Amosamine (IX) to the Methyl Glycosides (X).—A solution of 0.2 g. of amosamine (IX) hydrochloride in 20 ml. of methanol was saturated with dry hydrogen chloride gas below 0° and then left aside for 25 hr. at room temperature. The solution was then evaporated to dryness *in vacuo* and the residue rubbed with 2 ml. of ethanol, whereupon crystals separated. The crystallization medium was placed in the ice chest for 16 hr. and the crystals were then collected by filtration and washed with ethanol–ether mixture to afford 18 mg. (85%) of β-methyl amosaminide (X) hydrochloride, m.p. and mixture m.p. 209–210° (dec.). The mother liquors were evaporated and the residue dissolved in a little water. Addition of excess sodium bicarbonate and repeated extraction with ether yielded 0.10 g. (56%) of a gum. Crystallization from ether–petroleum ether mixture afforded 70 mg. (39%) of pure α-methyl amosaminide (X) as stout needles, m.p. and mixture m.p. 93–94°.

Dimethylamine from Amosamine (IX) Hydrochloride.—A solution of 0.114 g. (0.5 mmole) of amosamine (IX) hydrochloride in 10 ml. of a 3 *N* solution of sodium hydroxide in 50% aqueous methanol was refluxed for 16 hr. and the escaping gases were absorbed in a wash bottle containing 0.136 g. (0.49 mmole) of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 10 ml. of methanol. At the end of this period, nitrogen gas was bubbled into the alkaline solution for 2 hr. to sweep out any volatile amine present. To insure complete removal of dimethylamine, about 5 g. of solid sodium hydroxide was added to the alkaline solution and the solution was then concentrated *in vacuo* to one third its volume, the volatile distillate being drawn through the sulfonic acid solution. Evaporation of the latter and crystallization of the orange residue from ethanol–ether gave a total of 114 mg. (75%) of fairly pure dimethylamine salt, m.p. 210° (dec.), with blackening above 200°. One recrystallization from ethanol gave 72 mg. (47%) of orange needles, m.p. 216–217° (dec.), undepressed by admixture with an authentic sample of the dimethylamine salt[3] of *p*-hydroxyazobenzene-*p'*-sulfonic acid. Identity was confirmed by comparison of infrared spectra and analysis.

Anal. Calcd. for $C_{14}H_{17}N_3O_4S$: C, 51.96; H, 5.30; N, 13.00. Found: C, 51.76; H, 5.23; N, 12.93.

Rate of Deaminolysis of Amosamine (IX) and Other Amino Sugars.—The procedure of Hochstein and Regna[12] was followed. About 1 mmole of the amino sugar was accurately weighed into a 25-ml. volumetric flask and dissolved in 5 ml. of water. The flask and contents were warmed to 50° and 20 ml. of 4 *N* sodium hydroxide solution heated to the same temperature was pipetted into the flask, which was then stoppered tightly and kept in a bath maintained at 50° ±2°. At various intervals, 5-ml. aliquots were removed and evaporated to a volume of about 0.5 ml., during 8–10 min. *in vacuo* at room temperature. The volatile components were collected in a series of two traps cooled by a Dry Ice–acetone mixture. At the end of the evaporation, the distillates collected in the two traps were combined and titrated with standard (approximately 0.05 *N*) sulfuric acid or hydrochloric acid, using methyl red indicator. A blank was run by evaporating 5 ml. of plain sodium hydroxide solution under the same conditions and no titrable base could be detected in the distillate. Control experiments were carried out by using 1-mmole quantities of hydrochlorides of low molecular weight amines. Recoveries of 80–85% were obtained for diethylamine and 80–90% for dimethylamine. The following table shows the rate of evolution of amine in moles at various intervals from the aminosugars studies and represents data collected rom one set of experiments. The results could be essentially duplicated in a second run.

Periodate Cleavage of Amosamine (IX). (A) Total Fission.—About 0.1 mmole of amosamine (IX) hydrochloride, 10 ml. (10 mmoles) of 0.2 *N* sodium metaperiodate solution, and 15 ml. of water were used for the experiment which was conducted at room temperature; 2-ml. aliquots were withdrawn for titrations.

Times in hr.	0.08	0.25	1.0	2.0	5.0	24	48
Moles periodate reduced	2.76	2.96	3.35	3.54	3.73	3.80	3.92

When only a 5% excess of sodium metaperiodate (4.2 moles for 1 mole of amosamine) was used, the rate of uptake was a little slower and reached 3.6 moles in 48 hr. When the cleavage was run in a bicarbonate buffered medium, using 1 mmole of amosamine (IX) hydrochloride, 5 ml. of saturated bicarbonate solution, 10 ml. of 0.2 *N* sodium metaperiodate, and 15 ml. of water, it was found that 4 moles of

RATE OF DEAMINOLYSIS OF AMINO SUGARS

Amino sugar	0.5 hr.	1 hr.	2 hr.	4.5 hr.	24 hr.
Amosamine (IX)	0.05	0.07	0.12	0.20	0.47
Desosamine[a]	0.44	0.66	0.76	0.77	0.66
Mycaminose[b]	0.40–0.45			0.80	0.80
Glucosamine	0.04	0.05	0.10	0.29	0.36
N,N-Dimethylglucosamine (XX)	0.08			0.45	
				0.64 (11 hr.)	
α-Methylamosaminide (X)			0		0.07
					0 (60 hr.)

[a] See ref. 57. [b] See ref. 12.

periodate were reduced in the course of 1 hr. Readings could not be taken after this period, since sodium paraperiodate began to separate. By using the chromotropic acid procedure,[53] 0.07 and 0.11 mole of formaldehyde, respectively, could be detected in the bicarbonate-buffered fission of amosamine hydrochloride at the end of 1 and 24 hr., and none in the unbuffered metaperiodate cleavage.

In another experiment, 91 mg. (0.4 mmole) of amosamine (IX) hydrochloride and 0.428 g. (2 mmoles) of sodium metaperiodate were dissolved in 50 ml. of water. After standing for 50 hr. at room temperature, the solution was evaporated to dryness *in vacuo*. Water (10 ml.) was added to the residue and the solution again evaporated. The distillates were trapped at −80° and titration with 0.05 *N* sodium hydroxide showed that 2.68 moles of volatile acid had been produced in the fission. The neutralized solution gave negative tests for chloride, iodate, and periodate ions. The residue from the evaporation was dissolved in 25 ml. of 4 *N* sodium hydroxide solution and on concentration to 5 ml. at room temperature *in vacuo* gave 0.325 mole of volatile amine.

In an experiment designed for the isolation of acetaldehyde, 91 mg. (0.4 mmole) of amosamine (IX) hydrochloride was dissolved in 40 ml. (4 mmoles) of 0.2 *N* aqueous sodium metaperiodate. The solution was allowed to stand 24 hr. at room temperature and then evaporated to near dryness *in vacuo* at room temperature, the distillate being drawn into a trap containing a hydrochloric acid solution of 2,4-dinitrophenylhydrazine. The derivative that separated was filtered and washed with water to afford 14 mg. of a solid, m.p. 135–140°, whose m.p. was raised by one crystallization for aqueous ethanol to 141–144°. Mixture melting point with acetaldehyde 2,4-dinitrophenylhydrazone (m.p. 148–149°) was 143–147° and with the derivative of formaldehyde, 110–120°. The residue from the evaporation of acetaldehyde was dissolved in water and poured onto a small column of Dowex 50. The column was washed with water until the eluate was neutral and then rinsed with 200 ml. of 2.5 *N* hydrochloric acid. This eluate was evaporated to dryness. By addition of alkali to the residue followed by distillation, dimethylamine was produced and was characterized as its *p*-hydroxyazobenzene-*p'*-sulfonic acid salt (yield about 5 mg.), m.p. and mixture m.p. 216–217° (dec.).

(B) **With 1 Mole of Periodic Acid or Sodium Metaperiodate.**—A solution of 22.8 mg. (0.1 mmole) of amosamine (IX) hydrochloride in 1 ml. of 0.2 *N* paraperiodic acid (0.1 mmole) was left at room temperature. A test portion showed no periodate ion to be present at the end of 15 min. After 1 hr., iodate ion was removed by the use of barium and the solution subjected to paper chromatography in a butanol–water system. A strong spot corresponding to starting material was obtained in addition to faint ones with less mobility.

In another experiment 0.228 g. (1 mmole) of amosamine (IX) hydrochloride and 0.214 g. (1 mmole) of sodium metaperiodate were dissolved in 50 ml. of water and left at room temperature. After 60 hr., the solution was evaporated to dryness *in vacuo* to leave a white residue (A). The distillate was trapped at −80° and on titration consumed 0.8 mole of sodium hydroxide. The neutralized solution was again evaporated *in vacuo* to leave a residue of sodium formate. The distillate was mixed with a hydrochloric acid solution of 2,4-dinitrophenylhydrazine and on warming gave 20 mg. of derivative. This was digested with hot methanol and the insoluble part crystallized from dimethylformamide to yield about 3 mg. of 1,2-butanedione 2,4-dinitrophenylosazone (XIII) (see below), m.p. and mixture m.p. 231–233° (dec.). The sodium formate residue was converted to *p*-bromophenacylformate according to a published procedure.[58] Crystallization from methanol afforded 30 mg. of the ester as shining plates, m.p. 95–99°, undepressed by admixture with an authentic specimen. The infrared spectrum showed a little *p*-bromophenacyl bromide to be present but was otherwise identical with that of the synthetic specimen. The residue (A) from the periodate fission was extracted with three 10-ml. portions of cold ethanol and the extract evaporated to dryness to leave 0.155 g. of froth which, on crystallization from ethanol, afforded 25 mg. (11%) of amosamine hydrochloride, m.p. 183–184° (dec.), identified by mixture melting point and paper chromatography. A small portion of the mother liquor from the crystallization was neutralized with sodium bicarbonate and subjected to paper chromatography in a butanol–water system. Using Bromocresol Green–Methyl Red indicator, a strong spot corresponding to amosamine was obtained, in addition to very faint ones of slower moving basic materials. Using aniline–hydrogen phthalate indicator, besides amosamine (IX), a more mobile reducing substance was detected. From the remaining portion of the ethanolic mother liquor, using Brady's reagent, about 2–3 mg. of 1,2-butanedione 2,4-dinitrophenylosazone (XIII), m.p. 231–233°, could be obtained.

(C) **With 2 Moles of Sodium Metaperiodate at 5°.**—A solution of 0.214 g. (1 mmole) of sodium metaperiodate in 25 ml. of water was cooled to 5°. To the cooled solution was added 0.114 g. (0.5 mmole) of amosamine (IX) hydrochloride. The solution, after standing at 5° for 42 hr. had no periodate present and was then evaporated to dryness *in vacuo* at room temperature. The distillate, trapped at −80° and titrated with 0.05 *N* sodium hydroxide, was found to contain 1.87 moles of volatile acid. The neutralized solution was worked up as usual for 2,4-dinitrophenylhydrazine derivatives and afforded 17 mg. of a red osazone, m.p. 220–230° (dec.), or 231–233° after two recrystallizations from dimethylformamide–methanol mixture. The residue from the original evaporation was extracted with cold alcohol. An aliquot of the extract was neutralized with sodium bicarbonate and then subjected to paper chromatography in a butanol–water system. On spraying the chromatogram with the base indicator, no spot was detected. With aniline–hydrogen phthalate indicator, however, faint spots were seen. On treatment of the rest of the ethanol extract with acidic 2,4-dinitrophenylhydrazine, a very small amount of the osazone, m.p. 231–233° (dec.), was obtained.

For a preparative experiment, 0.171 g. (0.75 mmole) of amosamine (IX) hydrochloride was dissolved in 40 ml. of

(57) We are very grateful to Dr. E. H. Flynn of the Lilly Research Laboratories, Eli Lilly & Company, for a generous sample of desosamine hydrochloride.

(58) T. Kubota and T. Matsura, *J. Inst. Polytech., Osaka City Univ., Series C.* **1**, 49 (1950).

water containing 321 mg. (1.5 mmoles) of sodium metaperiodate, precooled to 5°. The solution was allowed to stand at 5° for 40 hr. and then was continuously extracted with ether into a reservoir containing a warm solution of 300 mg. of 2,4-dinitrophenylhydrazine in 120 ml. of 3 *N* hydrochloric acid. At the end of 18 hr., a deep red precipitate had formed in the reservoir. This was collected and washed with water and then with ether to afford 0.12 g. (36%) of a red osazone, m.p. 225–230° (dec.). One recrystallization from acetic anhydride gave 80 mg. (24%) of deep red, glistening needles, m.p. 231–233° (dec.), giving a deep blue fluorescent solution, characteristic of 2,4-dinitrophenylosazones,[59] with alcoholic sodium hydroxide. This was identified as 1,2-butanedione 2,4-dinitrophenylosazone (XIII) (see below) by mixture melting point and comparison of infrared and ultraviolet spectra and X-ray powder patterns; λ_{max}^{DMF} 580 mμ (ε 42,600).

Anal. Calcd. for $C_{16}H_{14}N_8O_8$: C, 43.05; H, 3.16; N, 25.11. Found: C, 43.18; H, 3.03; N, 25.15.

The aqueous layer from the continuous extraction was treated with a slight excess of barium hydroxide to precipitate iodate ion and filtered. The filtrate was acidified with dilute hydrochloric acid and evaporated to dryness. The residue was reconstituted in 25 ml. of 4 *N* sodium hydroxide solution which was then evaporated *in vacuo* to a small volume, during 30 min. at room temperature. The volatile products were collected in a Dry Ice–acetone cooled trap. Titration with 0.05 *N* hydrochloric acid showed 0.48 mole of volatile amine to be present in the distillate.

Amosaminol (XI).—To a solution of 0.228 g. (1 mmole) of amosamine (IX) hydrochloride in 10 ml. of water was added 0.10 g. of sodium borohydride. The solution was allowed to stand at 5° for 12 hr. and then an additional 50 mg. of sodium borohydride was added. After another 12 hr., about 20 g. of purified Dowex 50 (25–50 mesh) resin was added to the solution to decompose the excess hydride and to make the solution acidic. The mixture was made into a column and washed with water until the eluate was neutral. The column was then rinsed with 100 ml. of 1 *N* aqueous ammonium hydroxide, followed by 400 ml. of 1 *N* methanolic ammonium hydroxide. These were combined and evaporated *in vacuo* to dryness. The residual froth was converted to the hydrochloride which, on crystallization from ethanol–ether, afforded 0.145 g. (63%) of amosaminol (XI) hydrochloride, m.p. 138–141°, with softening above 135°. One recrystallization from the same solvent pair gave an analytical sample as shining needle clusters, m.p. 141–143°; $[\alpha]^{25}D$ −8.8° (*c* 1.6 in H_2O); pK_a' 8.7 (in 50% MeOH), giving positive iodoform reaction and negative reducing tests. The hydrochloride was somewhat hygroscopic.

Anal. Calcd. for $C_8H_{20}ClNO_4$: C, 41.83; H, 8.78; N, 6.10. Found: C, 42.00; H, 8.85; N, 5.88.

(A) About 1 mmole of amosaminol (XI) hydrochloride was accurately weighed and dissolved in 10 ml. of 0.2 *N* sodium metaperiodate and the solution diluted to 25 ml. Aliquots were withdrawn at appropriate intervals for estimation of periodate uptake. The formaldehyde produced at these intervals was measured spectrophotometrically using the chromotropic acid procedure[53] with a dulcitol standard. The data obtained are compared with corresponding data for *N,N*-dimethylglucosamine (XX) and *N,N*-dimethylglucosaminol hydrochloride (see below) in the following table:

	1 hr.		24 hr.	
	Moles IO_4^-	Moles CH_2O	Moles IO_4^-	Moles CH_2O
Amosaminol (XI)	3.72	0.93	3.66	0.92
N,N-Dimethylglucosaminol	4.45	1.10	4.77	1.30
N,N-Dimethylglucosamine (XX)	4.18	0.62	6.00	1.08

(B) The following procedure for comparison of the rate of formaldehyde production eliminated the necessity of isolating the sugar alcohols and gave satisfactory results: About 0.1 mmole of the sugar was accurately weighed into a 25-ml. volumetric flask and dissolved in 5 ml. of water. To this solution was added 0.1 g. of sodium borohydride. The solution was allowed to stand at room temperature for 17 hr. and then acidified with 5 ml. of a stock solution of approximately 0.5 *N* hydrochloric acid to destroy excess borohydride and bring the pH to 4–5. Ten milliliters of 0.2 *N* sodium metaperiodate solution was added and the solution made up to 25 ml. At appropriate intervals, 5-ml. aliquots were withdrawn for estimating periodate uptake and 2-ml. aliquots for estimating formaldehyde produced (using the chromotropic acid procedure). In the following comparative table the compounds are named as the alcohols derived from the respective sugars:

	1 hr.		25 hr.		50 hr.	
Compound	Moles IO_4^-	Moles CH_2O	Moles IO_4^-	Moles CH_2O	Moles IO_4^-	Moles CH_2O
Amosaminol (XI)	3.18	1.01	4.14	1.09	4.25	1.09
Glucitol	4.86	1.9	5.10	2.13		
Desosaminol	1.04	0.97	1.33	1.03		
Mycaminosol[a]	1.31	0.77	2.44	0.84		
N,N-Dimethylglucosaminol	4.17	1.11	4.82	1.50	4.84	1.53

[a] See ref. 60.

Methyl Amosaminide Monomethyl Ether.—A solution of 0.82 g. (4 mmoles) of an anomeric mixture of methyl amosaminides (X) and 2 ml. of methyl iodide in 20 ml. of dry ether was refluxed with 2 g. of silver oxide for 18 hr. The mixture was filtered and the silver residues washed thoroughly with boiling ether. The ether filtrates were evaporated to give 0.895 g. of oil which, on evaporative distillation (0.2 mm.) afforded 0.65 g. (74%) of the monomethyl ether as a colorless, viscous liquid, b.p. 55° (bath temperature); $n^{25}D$ 1.4594; pK_a' 6.8 (in 50% MeOH), showing OH absorption at 2.87 μ in the infrared spectrum. In an *n*-butyl alcohol–water system, it gave a single spot with R_f 0.85, compared to 0.73 for the methyl amosaminides (X).

Anal. Calcd. for $C_{10}H_{21}NO_4$: C, 54.76; H, 9.65; N, 6.39; 2-methoxyl, 28.3; 2 *N*-methyl, 13.72. Found: C, 54.68; H, 9.71; N, 6.28; methoxyl, 27.15; *N*-methyl, 6.58.

Using 26 mg. of the monomethyl ether, 10 ml. of 0.2 *N* sodium metaperiodate solution, and 15 ml. of water, the periodate uptake was 0.46, 0.87, and 1.40 moles, respectively, at the end of 0.5, 2, and 24 hr. For the same intervals, the production of formaldehyde was 0.23, 0.46 and 0.72 mole, respectively. The hydrochloride of the anomeric mixture was made but efforts to isolate either anomer were unsuccessful. Cleavage of 0.1 mmole of the unpurified salt under the same conditions as before led to reduction of 0.27, 0.51 and 0.54 mole, respectively, of the oxidant at the end of 1, 24, and 50 hr., with concomitant formation of 0.03, 0.04, and 0.065 mole of formaldehyde.

A liquid *O*-acetate was prepared in quantitative yield by heating the monomethyl ether with a mixture of acetic anhydride and pyridine for 12 hr. at 60–70°. Purification by evaporative distillation (0.2 mm.) afforded the acetate in 61% yield as a colorless liquid, b.p. 60° (bath temperature), $n^{26}D$ 1.4517, pK_a' 5.4 (in 50% MeOH), showing no absorption in the hydroxyl region of the infrared but strong bands at 5.75 and 8.0 μ (acetate).

Anal. Calcd. for $C_{12}H_{23}NO_5$: C, 55.12; H, 8.87; N, 5.36. Found: C, 55.35; H, 8.99; N, 5.19.

(59) C. K. Ingold, G. J. Pritchard, and H. G. Smith, *J. Chem. Soc.*, 79 (1934).

(60) We are grateful to Dr. F. A. Hochstein of Chas. Pfizer & Co. Inc., for a sample of mycaminose hydrochloride.

With sodium metaperiodate under the usual conditions, the acetate consumed 0.49 and 1.05 moles of the oxidant, with concomitant production of 0.34 and 0.53 moles of formaldehyde, respectively, in 2 and 24 hr.

Methyl 2,5-Di-*O*-methylamosaminide.—A solution of 1.8 g. of methyl amosaminide monomethyl ether and 5 ml. of methyl iodide in 35 ml. of distilled 1,2-dimethoxyethane was refluxed with 5 g. of silver oxide on a steam bath for 16 hr.; 3 more ml. of methyl iodide and 2 g. of silver oxide were then added and the refluxing continued for another 10 hr. A paper chromatogram of the reaction product in *n*-butyl alcohol–water system was sprayed with Bromocresol Green–Methyl Red indicator. Two spots of nearly equal intensity were obtained, one with R_f value of 0.85 representing starting material and another R_f 0.90, corresponding to the dimethyl ether. Hence, the reaction was continued for another 3 days with addition of 2-ml. portions of methyl iodide and 2 g. of silver oxide every 24 hr. At the end of this time, the supernatant liquid from the reaction mixture had no basic material in it. The mixture was filtered and the silver residue extracted with 100 ml. of boiling ether. The combined extracts on evaporation left about 0.35 g. of oil which, on partitioning between ether and 2 *N* hydrochloric acid, gave 0.23 g. of neutral oil and a negligible amount representing the basic fraction, both of which were rejected. The silver residues from the reaction were extracted thoroughly with boiling methanol until the extracts were neutral. The methanol extracts amounted to 1 l. and on evaporation to dryness *in vacuo* at room temperature gave about 1.7 g. of a gum which turned from light yellow to dark brown in the last stages of drying. The gum was strongly basic and contained no halide ion. With silver nitrate, it gave a precipitate of silver oxide. It was readily soluble in methanol, ethanol and water and insoluble in ether or benzene. No decomposition occurred when this quaternary hydroxide was pyrolysed at 100° at 0.5 mm. pressure. However, when it was heated at 150° and 0.5 mm. pressure, decomposition took place readily and was judged to be complete in 2 hr. The liquid products from the reaction were collected in an ice water–cooled receiver. The gaseous products were trapped at −80° and smelled strongly of trimethylamine but were not characterized. The pyrolysis flask and the water-cooled receiver were rinsed with 200 ml. of ether to give 0.842 g. of an ether-soluble oil and 0.33 g. of an ether-insoluble sludge. The oil was partitioned between ether and 2 *N* hydrochloric acid to separate the basic and nonbasic fractions. The nonbasic oil amounted to 0.265 g. which, on distillation, yielded 0.13 g. of colorless oil, b.p. 55 (0.3 mm.), $n^{25}D$ 1.4582. The infrared spectrum had no absorption band in the OH, NH region, but had a strong carbonyl band at 5.75 μ, a weak one at 6.15 μ, shoulders at 5.85 and 5.95 μ, besides broad absorption in the 8–10-μ region.

Anal. Calcd. for $C_8H_{14}O_4$: C, 55.16; H, 8.10; O, 36.74; methoxyl, 35.66. Found: C, 55.01; H, 8.07; O, 37.42; methoxyl, 37.03.

The basic product from the reaction was isolated by neutralization of the acid layer with sodium bicarbonate, saturation with sodium chloride, and exhaustive extraction with ether. Evaporative distillation *in vacuo* gave 0.3 g. (16%) of di-*O*-methyl-methyl amosaminide as a colorless, mobile oil, b.p. 55° (1.5 mm.), $n^{25}D$ 1.4500, pK_a' 6.5 (in 50% MeOH), R_f 0.90 in *n*-butyl alcohol–water. The compound had no absorption in the hydroxyl region in the infrared.

Anal. Calcd. for $C_{11}H_{23}NO_4$: C, 56.61; H, 9.93; N, 6.00; 3 methoxyl, 38.91; 2 *N*-methyl, 12.9. Found: C, 56.87; H, 9.99; N, 5.80; methoxyl, 37.85; *N*-methyl, 5.73.

In 24 hr., the dimethyl ether reduced 2.29 moles of sodium metaperiodate, with formation of 0.99 mole of formaldehyde.

2,4-Di-*O*-methylamosamine (XIV).—A solution of 0.3 g. of methylamosaminide dimethyl ether in 8 ml. of 3 *N* hydrochloric acid was refluxed for 4 hr. A test portion was neutralized at this time and found to give a good reducing test with Benedict's reagent. The rest of the solution was evaporated to dryness *in vacuo* at room temperature. On triturating the residue with 2 ml. of ethanol, crystallization occurred and was allowed to go to completion by addition of a little ether and storage in an ice chest. After 1 hr., the crystals were collected and washed with a little ethanol and then with ether to yield 0.175 g. (53%) of 2,4-di-*O*-methylamosamine (XIV) hydrochloride, m.p. 208–210° (dec.). Recrystallization from ethanol–ether mixture gave an analytical sample, m.p. 209–210° (dec.), $[\alpha]^{25}D$ +15.1° (*c* 0.75 in water, equilibrium value), pK_a' 7.2 (in 50% MeOH). The infrared spectrum had bands at 3.12 μ (OH) and 3.75 μ (salt NH), besides several characteristic ones in the 8–10 μ region. The compound gave a positive reaction with Benedict's reagent and in *n*-butyl alcohol–water systems, as the free base, had an R_f value of 0.855 compared to 0.90 for the starting methylglycoside and 0.58 for amosamine (IX).

Anal. Calcd. for $C_{10}H_{22}ClNO_4$: C, 46.94; H, 8.67; N, 5.48; Cl, 13.86; 2 methoxyl, 24.28; 2 *N*-methyl, 11.76. Found: C, 46.79; H, 8.82; N, 5.23; Cl, 13.72; methoxyl, 21.17; *N*-methyl, 4.30.

A solution of 37.5 mg. of the hydrochloride in 10 ml. of 0.2 *N* sodium metaperiodate and 15 ml. of water was used for measuring periodate uptake and concomitant formaldehyde production. The results are tabulated below:

Time in hr.	1	24	50	120
Moles IO_4^-	0.14	0.25	0.37	0.71
Moles CH_2O produced		0.06		0.10

The cleavage of di-*O*-methylamosaminol was carried out without isolating the alcohol itself according to procedure B for periodate fission of amosaminol (XI). The periodate uptake was 0.15, 0.20, and 0.30 mole, respectively, in 1, 25, and 96 hr. By the chromotropic acid procedure, 0.035 and 0.07 mole of formaldehyde were detected from the cleavage at 25 and 96 hr., respectively.

Periodate Fission of Cytosamine (IV).—Anhydrous cytosamine (IV) was prepared according to the directions of Haskell[3] and had m.p. 250–252°(dec.); $\lambda_{max}^{0.1\ N\ HCl}$ 278 mμ (ϵ 13,600). It gave a negative iodoform reaction and did not give reducing tests for sugars.

Anal. Calcd. for $C_{18}H_{30}N_4O_6$: C, 54.26; H, 7.59; N, 14.06; 2 C-methyl, 7.55; 2 *N*-methyl, 7.55. Found: C, 54.26; 54.25; H, 7.35, 7.46; N, 13.98; C-methyl, 5.30; *N*-methyl, 5.04.

For a quantitative estimation, 13.1 mg. of cytosamine (IV) was heated with 10 ml. of 0.1 *N* sodium metaperiodate solution and was found to reduce 2.23 moles of periodate in 16 hr. For isolation of the product, 0.2 g. of cytosamine (IV) (0.5 mmole), 0.30 g. of picric acid, and 0.642 g. (3 mmoles) of sodium metaperiodate were dissolved in 10 ml. of water and the solution kept at room temperature. A yellow gummy precipitate separated at once which, with occasional shaking, was gradually transformed into a fluffy precipitate. After 38 hr., the mixture was filtered and the residue washed well with water. The yellow product was dissolved in a small volume of hot water and the solution freed from a little gummy residue and set aside. On cooling, a yellow gelatinous picrate separated which was collected by filtration and washed with a little water. This filtrate was combined with the mother liquor from the fission and made up to 100 ml. Titration of an aliquot by standard procedure showed that 2.45 moles of periodate had been used for the cleavage. The gelatinous precipitate of dialdehyde V became a gritty, crystalline powder after drying and weighed 85 mg. (29%); m.p. 148–150° (dec.). An analytical sample was obtained by one more crystallization from water and had the same melting point.

Anal. Calcd. for $C_{21}H_{26}N_6O_{14}$; C, 43.00; H, 4.47; N, 14.33. Found: C, 42.95, 42.87; H, 4.56, 4.41; N, 14.35.

Amicetaminol.—To a solution of 0.342 g. (1 mmole) of amicetamine (III) hydrochloride[3] in 10 ml. of water was added 0.40 g. of sodium borohydride. The solution was allowed to stand at room temperature for 14 hr., acidified with dilute hydrochloric acid and the basic product isolated by absorption on Dowex 50 resin, elution with methanolic ammonium hydroxide and evaporation. The product did not give tests for reducing sugars. Neither the free base nor the hydrochloride could be crystallized. The latter was obtained, however, as a hygroscopic solid; yield, 0.11 g. Amicetaminol hydrochloride reduced 2.17 and 2.42 moles, respectively, of sodium metaperiodate in 1 and 25 hr. with negligible production of formaldehyde. Essentially the same results were obtained by reducing 0.1 mmole of amicetamine (III) hydrochloride with sodium borohydride and running the fission without isolation of the alcohol according to procedure B under "Periodate Fission of Amosaminol (XI)."

About 50 mg. of crude amicetaminol hydrochloride was subjected to methanolysis by dissolving it in 10 ml. of methanol and saturating the solution with hydrogen chloride gas below 0°. After 40 hr., a workup similar to the one used in "Methanolysis of Amicetin (I)" was applied. The basic product from the reaction was absorbed on 15 g. of Dowex 50 resin and eluted with methanolic ammonia. Evaporation left about 20 mg. of a gum, identified as methyl amosaminide (X) by paper chromatography in *n*-butyl alcohol–water system, using Bromocresol Green–Methyl Red indicator. Both this product and a sample of methyl amosaminide (X) (anomeric mixture) travelled at identical rates with an R_f value of 0.75; under the same conditions amosaminol (XI) had an R_f value of 0.55.

***N,N*-Dimethylglucosamine (XX).**—A solution of 0.824 g. (2 mmoles) of 1,3,4,6-tetra-*O*-acetyl-*N,N*-dimethylglucosamine (XIX)[17] in 20 ml. of methanol was cooled below 0° and saturated with dry hydrogen chloride gas, and then left at room temperature for 24 hr. The solution was then evaporated to dryness *in vacuo* at room temperature. A little benzene and more methanol were added to the residue and the solution again evaporated to dryness. Crystallization of the residue from dry ethanol gave 0.235 g. (48%) of *N,N*-dimethylglucosamine (XX) hydrochloride, m.p. 165–166° (dec.). About 50–100 mg. more of the same compound could be recovered from the mother liquors. Recrystallization from methanol–ethanol gave beautiful stellar clusters of needles, m.p. 165–166° (dec.), $[\alpha]^{25}D$ +83.5 (5 min.), +60.0° (17 hr.) (*c* 0.7 in H_2O); pK_a' 8.0 (in 50% MeOH). The compound reduced Benedict's solution readily.

Anal. Calcd. for $C_8H_{18}ClNO_5$: C, 39.44; H, 7.44; N, 5.75; 2 *N*-methyl, 12.34. Found: C, 39.48; H, 7.54; N, 5.83, 6.16; *N*-methyl, 3.84.

With aqueous 0.08 *N* sodium metaperiodate at room temperature, the compound consumed 1.70, 2.40, 3.44, 4.60, 5.07, and 5.50 moles, respectively, of the oxidant in 0.25, 0.5, 1, 2, 4, and 24 hr., respectively.

***N,N*-Dimethylglucosaminol.**—Reduction of 2.44 g. (1 mmole) of the dimethylamino sugar (XX) hydrochloride and isolation of the alcohol were carried out by the same prodedure as was used for amosaminol (XI). Crystallization of the initially gummy product from ethanol–ether gave 85 mg. (35%) of *N,N*-dimethylglucosaminol hydrochloride, which was recrystallized to give an analytical sample as stout needles (hygroscopic). In a sealed capillary, the compound melted to a gum at 124–126° but completely flowed only at about 182°, $[\alpha]^{25}D$ +1.9° (*c* 0.6 in water), pK_a' 8.9 (in 50% MeOH). The compound did not reduce Benedict's reagent. For analysis it was dried to constant weight *in vacuo*.

Anal. Calcd. for $C_8H_{20}ClNO_5$: C, 39.11; H, 8.21; N, 5.71. Found: C, 39.09; H, 8.42; N, 5.49.

1,2-Butanedione 2,4-Dinitrophenylosazone (XIII).—This was prepared from sorbic acid according to the procedure of Ingold and co-workers.[59] Details which are lacking in their publication are given here. Addition of bromine to an aqueous solution of 10 g. of sorbic acid led to a recovery of 1.18 g. of starting material and of 1.38 g. of 5-bromo-4-hydroxyhexen-2-oic acid, m.p. 103–105°. Into a cooled solution of 0.5 g. of the latter acid in 20 ml. of ethyl acetate, a vigorous stream of ozone was passed during the course of 15 min., until a blue color persisted. After allowing the solution to come to room temperature, 20 ml. of water was added and the mixture evaporated *in vacuo* with slight warming, the volatile products being drawn through a hydrochloric acid solution of 2,4-dinitrophenylhydrazine. An additional portion of 50 ml. of water was added to the reaction mixture and the solution again concentrated to half volume *in vacuo* on a steam bath during 1 hr., trapping the volatile aldehydes produced. The cloudy dinitrophenylhydrazine solution, on heating on the steam bath, deposited 0.1 g. of an orange-red precipitate which was extracted with a small volume of hot acetic anhydride. The insoluble fraction amounted to 80 mg. and on crystallization from dimethylformamide gave pyruvaldehyde 2,4-dinitrophenylosasone. The acetic anhydride soluble fraction gave only a few milligrams of the desired butanedione derivative. Hence, the residual solution from the evaporation was continuously extracted with ether into a reservoir containing a warm solution of 1 g. of 2,4-dinitrophenylhydrazine in 200 ml. of 2 *N* hydrochloric acid. After 18 hr., the reservoir was detached and the ether layer therein evaporated. The gummy residue that separated became a crystalline red precipitate on further digestion on a steam bath for 1 hr. This was collected and washed with water and then with ether to afford 0.6 g. of 1,2-butanedione 2,4-dinitrophenylosazone (XIII), m.p. 215–225° (dec.). One recrystallization from acetic anhydride gave 0.35 g. (33%) of glistening needles, m.p. 230–233° (dec.), raised by another crystallization to 231–233° (dec.); (literature[59] m.p. 247°); λ_{max}^{DMF} 580 mμ (ε 43,700).

Anal. Calcd. for $C_{16}H_{14}N_8O_8$: C, 43.05; H, 3.16; N, 25.11. Found: C, 43.18; H, 3.30; N, 25.22.

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