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Mass Spectra of N-Salicylidene Aminosugars[1]

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Mass spectra of the N-salicylidene derivatives of twenty aminosugars and related compounds including mono- and diaminosugars, monoaminoalcohols and diaminocyclitol were studied. The spectra were characterized by the appearance of strong molecular ion peaks, most of them being the base peak. Fragmentation of the N-salicylidene aminosugars was discussed in terms of the structures, in particular, the substituted positions of amino groups, and compared with fragmentation of the parent free aminosugars.

Mass spectra of carbohydrates so far reported have been largely recorded on the volatile derivatives such as methyl and trimethylsilyl ethers, acetates and acetonides.[3] These spectra suffer from certain limitations. For example, a molecular ion peak (M^+) is extremely weak, and in many cases not recognized, except for sugar mercaptals that gave well recognized M^+.[4] In addition, the spectra of carbohydrates are rather complex, and in many instances significant fragment ions are of low abundance. As a result, correlation of fragmentation pattern with the structure often becomes difficult.

In the past, several attempts have been made to overcome these disadvantages. The field ionization mass spectrometry introduced recently has provided one of solutions.[5] It gives relatively intense M^+ and simplified spectra, as compared to the electron impact ionization mass spectrometry. Another approach using the more popular electron impact type is to incorporate an aromatic nucleus into carbohydrates, thereby stabilizing the molecular ion and directing fragmentation. Ito[6] studied mass spectra of the open chain phenylosazone derivatives for this purpose, and Johnson and co-workers[7] utilized the open chain 1-phenylflavazol derivatives prepared *via* phenylosazone. But there has been no report on the aminosugar derivatives studied on this line.

In the present paper, we would like to show that N-salicylidene Schiff bases are suitable derivatives for mass spectrometry of aminosugars. N-Salicylidene Schiff bases have been utilized for characterization of aminosugars, and for stereochemical determination of an amino group by the use of optical rotatory dispersion.[8] The Schiff bases can be easily prepared on a micro scale by adding excess of salicylaldehyde into a solution of an aminosugar followed by removal of the excess reagent by distillation. Mass spectra of N-salicylidene Schiff bases thus obtained showed the distinct M^+ and characteristic fragment ions reflecting the structure as described below.

Molecular Ions (M^+)

Table I summarizes relative abundance of the molecular ion peaks of the N-salicylidene derivatives and the parent aminosugars. Fig. 1 and 2 illustrate the mass spectra of methyl 3-salicylideneimino-3-deoxy-α-D-glucopyranoside (**1**) and its parent free base (**1a**).

1) Preliminary report: S. Inouye, *Sci. Reports, Meiji Seika Kaisha*, **11**, 102 (1970).
2) Location: *Morooka, Kohoku-ku, Yokohama.*
3) N.K. Kochetkov and O.S. Chizlov, *Adv. in Carbohydrate Chem.*, **21**, 39 (1966).
4) D.C. DeJongh and S. Hanessian, *J. Am. Chem. Soc.*, **87**, 1408 (1965).
5) H.D. Beckey, *Angew. Chem.*, **81**, 662 (1969).
6) T. Ito, *Agr. Biol. Chem.*, **33**, 1217 (1969).
7) G.S. Johnson, W.S. Ruliffson and R.G. Cooks, *Carbohyd. Res.*, **18**, 233, 243 (1971).
8) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **15**, 1557 (1967).

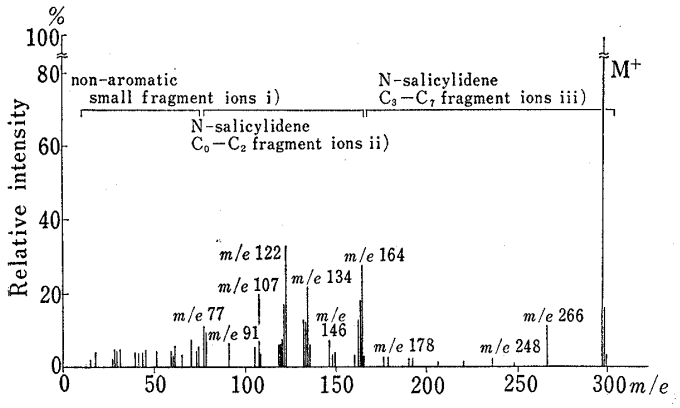


Fig. 1. Mass Spectrum of Methyl 3-Salicylideneimino-3-deoxy-α-D-glucopyranoside (**1**) at 150°

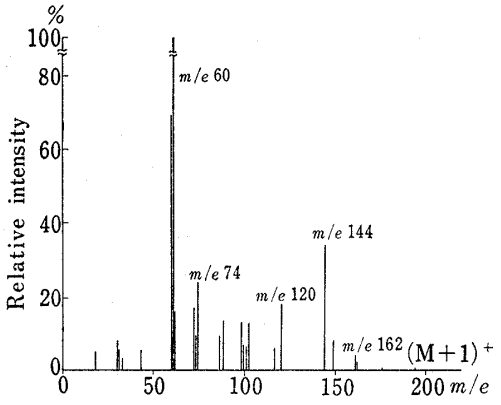


Fig. 2. Mass Spectrum of Methyl 3-Amino-3-deoxy-α-D-glucopyranoside (**1a**) at 130°

Comparison of both spectra clearly shows that the intensity of the M^+ is markedly increased in the N-salicylidene derivative than that of the free base. Intensification of the M^+ in going from free bases to N-salicylidene Schiff bases was observed widely in other aminosugars as shown in Table I, and the M^+ of a number of the Schiff bases became the base peak. In contrast, the spectra of parent aminosugars showed very weak or no molecular ion peak. In many cases, the $(M \text{ plus } 1)^+$ appeared in stead of the M^+ as seen in the spectra of **1a**, **3a**, **11a**, **17a**, **18a**, **19a** and **20a**, and the intensity of the $(M \text{ plus } 1)^+$ increased upon raising temperature.

It has been reported that common sugar dithioacetals show relatively strong M^+,[4] but when the M^+ of the dithioacetal (**10a**) and its N-salicylidene derivative (**10**) were compared, the relative abundance of the latter based on the base peak was increased 13 times more than that of the former. 1,2-O-Isopropylidene aminosugars show the weak M^+ and moderately strong $(M \text{ minus } 15)^+$ due to a stable methylcarbonium cation, and the latter peak was used for the estimation of molecular weight.[9] It is now apparent that the strong M^+ of 1,2-O-isopropylidene-5-salicylideneimino-5-deoxy-α-D-glucofuranose (**12**) allowed the easy recognition of molecular weight. Similarly, the M^+ of 5-salicylideneimino-5-deoxy-3-O-benzyl-1,2-O-isopropylidene-6-O-trityl-α-D-glucofuranose (**13**) was observed with 19% intensity, in contrast to the weak M^+ (0.2%) of the parent aminosugar (**13a**). This indicated that an aromatic moiety such as a trityl or benzyl group introduced into **13a** did not stabilize the M^+. High resolution mass analysis revealed that a 6-trityl and 3-benzyl groups were easily splitted off, yielding a stable trityl and benzyl (or tropium) cation, as evidenced by the strong peaks at *m/e* 243 with an elemental composition $C_{19}H_{15}$ and *m/e* 91 with C_7H_7. It is generally known that a carbon-nitrogen bond is more stable against the electron impact than a carbon-oxygen bond, and this difference seemed to be responsible at least partly for the stabilization of the M^+ by the N-salicylidene moiety.

Fragmentation of N-Salicylideneiminosugars

Fragment ions appeared in the mass spectra of N-salicylidene Schiff bases can conveniently divided into three groups, *i.e.*, i) small fragment ions that do not contain the aromatic moiety, ii) small fragment ions that do contain the aromatic nucleus, and iii) large fragment ions containing the N-salicylidene C_3—C_7 fragments (see Fig. 1). The ions belonged to group i) appeared below *m/e* 75. Since they are generally weak, their contribution to the structural information was greatly diminished. Fragment group ii) appeared between *m/e* 77 and 164, and their relative intensities were reflected the position of amino groups in the sugar

9) D.C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86**, 67 (1964).

TABLE I. Relative Abundance of Molecular Ions and Small Fragment Ions ii) of N-Salicylidene Aminosugars, and Molecular Ions of Aminosugar Free Bases

N-Salicylidene derivatives of	N-Salicylidene schiff bases								Free aminosugars		
	Temp.	M^+ (abund.)	Base peak	Small fragment ions ii)					Temp.	M^+ (abund.)	Base peak
				*m/e*164	*m/e*163	*m/e*134	*m/e*122	*m/e*121			
Methyl 3-amino-α-D-glucopyranoside (**1**)	150°	*m/e*297 (100%)	*m/e*297	28%	13%	22%	33%	17%	130°	*m/e*194[a] (0.4%)	*m/e*60
Methyl 3-amino-β-L-glucopyranoside (**2**)	150	297 (100)	297	42	28	28	39	25			
Methyl 3-amino-α-D-mannopyranoside (**3**)	150	297 (100)	297	35	24	23	27	14	140	194[a] (2.5)	58
Methyl 6-amino-α-D-glucopyranoside (**4**)	150	297 (100)	297	18	2	82	62	32	120	193 (10)	73
5-Amino-1,2-O-isopropylidene-α-D-xylofuranose (**5**)	130	293 (95)	134	50	10	100	28	30			
Methyl 2-amino-α-D-glucopyranoside (**6**)	130	297 (100)	297	75	65	51	20	26			
Methyl 2-amino-β-D-glucopyranoside (**7**)	150	297 (100)	297	85	70	80	19	30			
2-Amino-D-glucopyranose (**8**)	140	283 (23)	163	50	100	89	20	21			
2-Amino-D-glucitol (**9**)	170	285 (43)	164	100	3	14	42	27			
2-Amino-D-glucose diethyl dithio acetal (**10**)	150	389 (9)	135	10	2	5	49	14	130	285 (0.7)	150
2,6-Diamino-D-glucose diethyl dithioacetal (**11**)	150	492 (7)	135	25	9	81	53	42	100	285[a] (2.3)	149
5-Amino-1,2-O-isopropylidene-α-D-glucofuranose (**12**)	140	323 (80)	164	100	6	17	37	29	150	220[a] (0.7)	60
5-Amino-3-O-benzyl-1,2-O-isopropylidene-6-O-trityl-α-D-glucofuranose (**13**)	190	655 (19)	243	6	1	3	2	3	150	551 (0.2)	243
5-Amino-3-O-benzyl-1,2-O-isopropylidene-6-O-trityl-β-L-idofuranose (**14**)	180	655 (17)	243	4	1	2	1	2	160	551 (0.4)	243
D-Glucosylamine (**15**)	130	283 (47)	121	2	3	7	42	100	150	179 (0)	18
Tris-hydroxymethylaminomethane (**16**)	150	225 (81)	194	10	3	6	13	18			
Methyl 3,6-diamino-α-D-glucopyranoside (**17**)	160	400 (100)	400	72	14	36	57	25	130	193[a] (1.1)	102
Methyl 3,6-diamino-α-D-mannopyranoside (**18**)	150	400 (100)	400	77	17	40	66	29			
Methyl 3,6-diamino-α-D-altropyranoside (**19**)	170	400 (100)	400	82	18	51	80	39	130	193[a] (1.5)	60
2-Deoxystreptamine (**20**)	190	370 (99)	122	52	5	13	100	24	140	162 (0.4)[b]	71

a) (M plus 1) peak *b*) (M plus 1) peak at *m/e* 163 (0.4%)

chains. Fragment group iii) that appeared above *m/e* 164 were most closely related with the whole structure of aminosugars.

Fragment group ii) consisted of relatively strong peaks at *m/e* 164, 163, 134, 122, 121 and 107, and medium to weak peaks at *m/e* 91 and 77. The peaks at *m/e* 164 and 163 were N-salicylidene C_2 ions (a_I and a_{II}) with elemental compositions $C_9H_{10}O_2N$ and $C_9H_9O_2N$, respectively. Their formation could be explained by α-cleavage of a nitrogen atom accompanied with or without a hydrogen transfer as shown in Chart 1. Another N-salicylidene C_2 fragment was observed at *m/e* 146 with a composition C_9H_8ON. But, its intensity was weak in many cases, and of little value as a diagnostic ion.

The composition of the peak at *m/e* 134 (b_I) was hydroxymethine less than that of the *m/e* 164 peak, and assigned to the N-salicylidene C_1 ion. The peak at *m/e* 122 (c_I) consisted of two fragment ions, of which the major ion had C_7H_8ON, and was the salicylideneimino cation formed by the cleavage of a carbon-nitrogen bond accompanied with two hydrogens transfer. The minor ion was the salicylaldehyde cation ($C_7H_6O_2$), probably arising from decomposition of a Schiff base. The *m/e* 121 (c_{II}) was a hydrogen less from c_I. The peaks at *m/e* 107 (d), 91 and 77 were nitrogen free, and assigned to a protonated salicyl or hydroxytropium cation (C_7H_7O), protonated tropium cation (C_7H_7) and benzene cation (C_6H_6), respectively. These peaks were found in all the N-salicylidene Schiff bases so far examined, and the relative intensities of them, in particular a, b and c ions depended on the position of the salicylideneimino group in the aminosugars. Accordingly, the relative intensities of these peaks along with the fragments belonged to group iii) were discussed below in terms of the structure of aminosugars.

3-Salicylideneiminosugars (1, 2 and 3)

The mass spectra of methyl 3-salicylideneimino-3-deoxy-α-D-glucopyranoside (**1**), -β-L-glucopyranoside (**2**) and -α-D-mannopyranoside (**3**) were characterized by the extremely strong M^+. Indeed, the M^+ of all 3-salicylideneiminosugars became the base peak. The a_I and c_I ions were moderately strong, but the b_I and d ions were much weaker. Another significant fragment ion of **1**, **2** and **3** was a peak at *m/e* 266, which was formed by loss of a methoxyl radical, as shown in Chart 2. The spectral similarity of three compounds indicated that stereochemical difference is not seriously reflected on fragmentation.

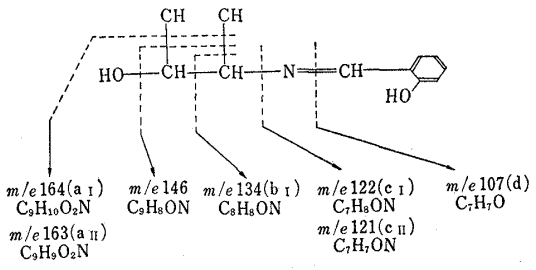
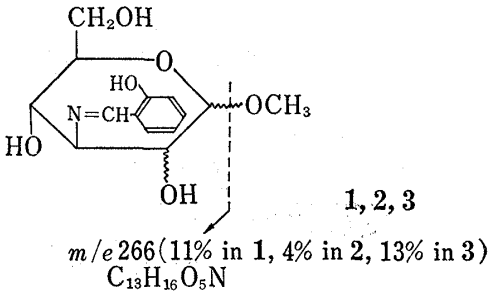


Chart 1



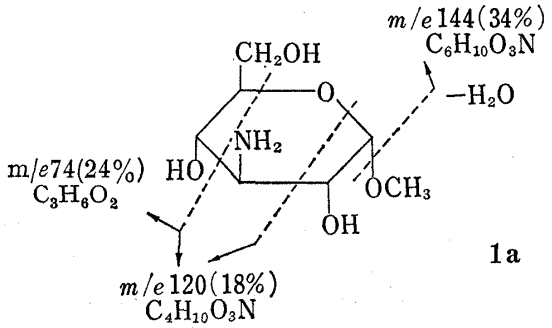


Chart 2

In order to find characteristic feature of the fragmentation of the Schiff base, the spectra were compared with those of the parent free bases. Since first ionization of the Schiff base probably occurs on the conjugated π electron system, its fragmentation may be different from that of the free base, where a charged amino group would be the driving force for the fragmentation. As was expected, the free aminosugar (**1a**) showed relatively strong peaks at

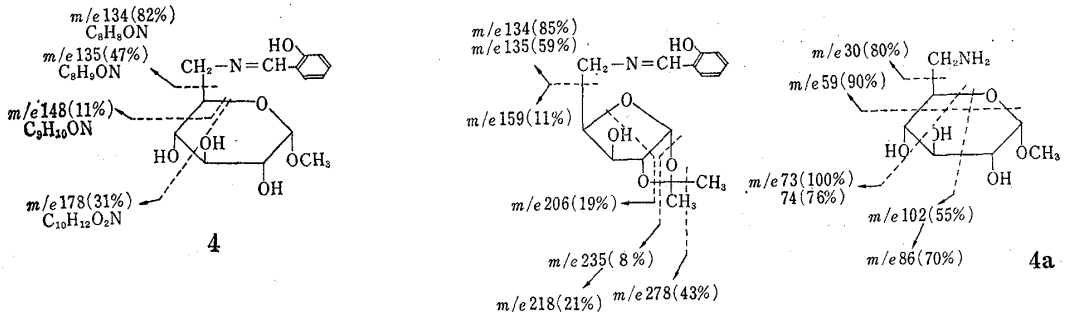
m/e 74 and 120 arising from the cleavage of a pyranose ring, and the dehydrated peak at *m/e* 144, but the corresponding N-salicylidene ions were not present or very weak in the Schiff base (**1**).

It was further noted in this comparative study that many peaks in **1a** consisted of multiple fragment ions of different composition. This complicated the interpretation of the low-resolution spectrum. For example, a base peak at *m/e* 60 consisted of two fragment ions, the strong one containing a nitrogen (C_2H_6ON) and the other with medium intensity nitrogen free ($C_2H_4O_2$). Such an overlapping of many ions could be avoided in the Schiff base, since nitrogen-containing ions were shifted into higher mass region.

Terminal Salicylideneiminosugars (4 and 5)

The mass spectra of methyl 6-salicylideneimino-6-deoxy-α-D-glucopyranoside (**4**) and 5-salicylideneimino-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**5**) showed strong N-salicylidene C_1 ions at *m/e* 135 and 134 (b). Of these, the *m/e* 135 peak seemed to be most diagnostic for the terminal aminosugars, because this peak appeared also in the spectra of 3,6-disalicylideneiminosugars (**17**, **18** and **19**) with moderate intensity, but no other aminosugars that do not contain terminal amino group showed the *m/e* 135 peak with comparable intensity.

In the cleavage of the C-5 and C-6 bond (**4**), or the C-4 and C-5 bond (**5**), a positive charge was retained predominantly in the leaving N-salicylidene C_1 ions, and distributed in small ratio to the sugar ion having one less carbon chain at *m/e* 163 or 159 (Chart 3). Other fragment ions noted in the spectrum of **4** included peaks at *m/e* 148 and 178. The former was a N-salicylidene C_2 fragment with an elemental composition $C_9H_{10}ON$, and the latter a N-salicylidene C_3 fragment with $C_{10}H_{12}O_2N$, both arising from the cleavage of a pyranose ring.



4 **5** **4a**

Chart 3

The mass spectrum of the free base (**4a**) showed relatively strong peaks at *m/e* 73 and 74, which seemed to be related with a peak at *m/e* 178 (73 plus 104 plus 1, 74 plus 104) in the Schiff base (**4**). The formation of the strong N-salicylidene C_1 ions in **4** coincided with the appearance of the C_1 fragment at *m/e* 30 in **4a**. Therefore, the free base and its N-salicylidene derivative appeared to be degraded in parallel, though the cleavage of a pyranose ring was less favorable in the latter. Major fragmentation of compound **5** was shown in Chart 3.

2-Salicylideneiminosugars (6, 7 and 8)

The spectra of methyl 2-salicylideneimino-2-deoxy-α-D-glucopyranoside (**6**) and -β-D-glucopyranoside (**7**) were characterized by the appearance of the strong a and b ions, and moderately strong non-nitrogenous C_6 fragment ion at *m/e* 145, and the weakness of the c ions. The *m/e* 145 ion had a composition $C_6H_9O_4$, and its formation was explained by ready losses of a salicylideneimine and methoxyl group as shown in Chart 4. In the cleavage of the C-2 and nitrogen bond, a cationic charge was mainly retained in the sugar fragment in **6**, but distributed more in the salicylideneimino fragment in **7** (Chart 4). In this connection, it

was noted that the c_{II} ion was more stronger than the c_I. Other strong fragment ions in **6** and **7** included the demethanolated fragment at *m/e* 265 and the N-salicylidene C_3 fragment at *m/e* 176.

Fragmentation of 2-salicylideneimino-2-deoxy-D-glucose (**8**) was considerably different from those of **6** and **7**. In compound **8**, the a_{II} ion was more stronger than the a_I. This is probably due to the preferred fission of the C-2 and C-3 bond. Bond fission between C-4 and C-5 was also characteristic in **8**, giving rise to ions at *m/e* 224 and 205 (Chart 4). This fission was not observed in **6** and **7**.

2-Salicylideneimino-alditol (9)

The mass spectrum of 2-salicylideneimino-2-deoxy-D-glucitol (**9**) showed the a_I ion as the base peak, but the b and c ions were weak. This result coupled with the weakness of the (M minus 31) peak at *m/e* 254 indicated that α-cleavage occurred preferentially between C-2 and C-3, rather than between C-1 and C-2.

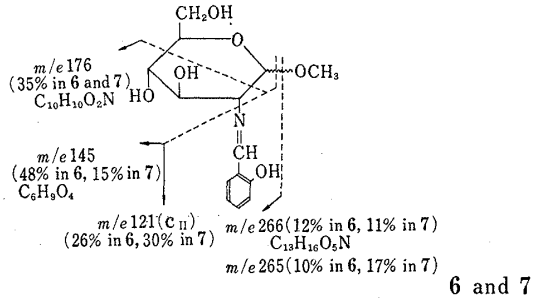


Chart 4

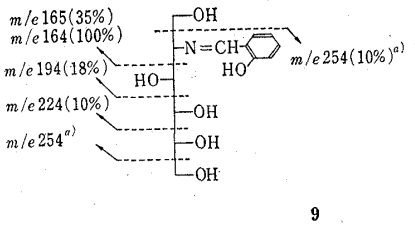


Chart 5

a) Accumulated relative intensities of two or more fragment ions.

The diagnostic fragment ion of **9** was found at *m/e* 135. Since this peak appeared only in the open chain aminoglycols such as **9** and **12**, its formation was suggestive of the presence of the $\left[\begin{matrix}CH_2OH\\|\\CHN{=}CH-\end{matrix}\right]$ group. Other significant ions of **9** included fragments at *m/e* 224 (M minus C-5 and C-6), *m/e* 194 (M minus C-4, C-5 and C-6), indicating the regular splitting of the open carbon chain from the terminal position. Accidentially, the C-1 and C-6 groups have the same mass units (*m/e* 31), and both (M minus C-6) and (M minus C-1) ions were overlapped at *m/e* 254 (Chart 5).

2-Salicylideneimino- and 2,6-Disalicylideneiminohexose Diethyl Dithioacetals (10 and 11)

Fragmentation of aminosugar diethyl dithioacetals in the form of N-acetyl derivatives was studied in detail by DeJongh and Hanessian,[10] and it has been shown that main bond cleavage occurred between C-1 and C-2, giving rise to fragments at *m/e* 135 (diethyl dithioacetal ion) as the base peak, and the C-1 less sugar ion (M minus 135) with medium or weak intensity.

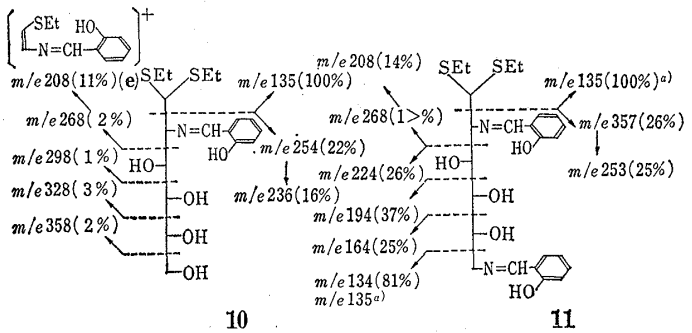
Similar to acetamidosugar derivatives, diethyl dithioacetals of 2-salicylideneimino-2-deoxy-D-glucose (**10**) and 2,6-disalicylideneimino-2,6-dideoxy-D-glucose (**11**) gave the base peak at *m/e* 135 and a medium peak at *m/e* 254 (**10**) or 357 (**11**) (M minus 135). In contrast,

10) D.C. DeJongh and S. Hanessian, *J. Am. Chem. Soc.*, **87**, 3744 (1965).

the parent aminosugar dithioacetals (**10a** and **11a**) afforded the base peak at *m/e* (M minus 135), *i.e.*, 150 (**10a**) and 149 (**11a**), whereas the *m/e* 135 peak amounted only to 12% in both cases (Chart 6). Other diagnostic fragment ion for the 2-salicylideneiminosugars was observed at *m/e* 208, to which the structure e was proposed in Chart 6.

Compound **11** contains two salicylidene groups in its molecule, and it was of interest to see which group retained a positive charge. As shown in Chart 6, fragment ions arising from the cleavage of the open carbon chain appeared at *m/e* 224 and 194. Since these ions were not observed in **10**, they were assigned to fragments involving the terminal salicylideneimino moiety. Similar retaining of a charge by the terminal amino group of **11a** was seen in peaks at *m/e* 120, 102, 90 and 30 (Chart 6).

Comparative study on the free bases and the Schiff bases further revealed that the dehydrated peaks such as the *m/e* 131 and 102 peaks in **11a** were no more remarkable in the Schiff bases. This was the phenomenon widely recognized in other compounds, indicating the stabilization of a cationic charge by dehydration in case of absence of an aromatic nucleus such as a salicylidene group.



a) Accumulated relative intensities of two or more fragment ions.

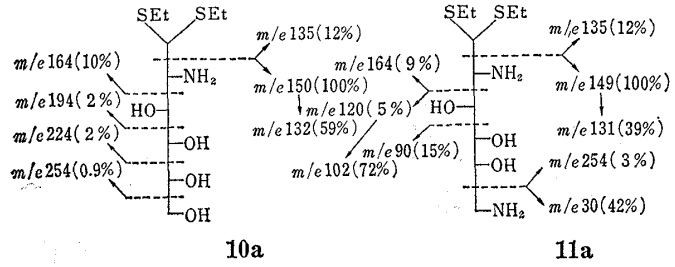
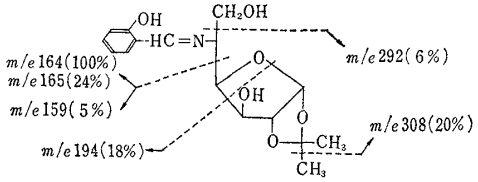


Chart 6



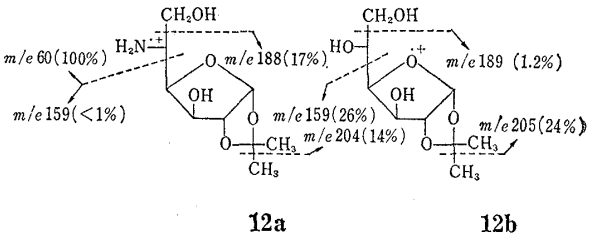


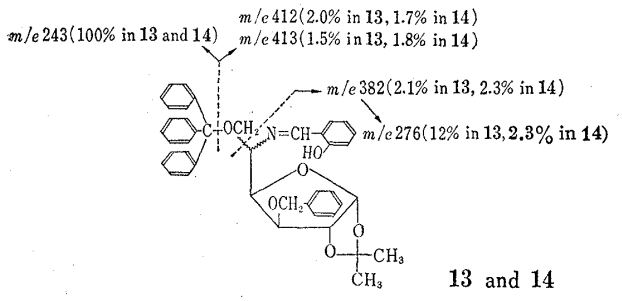
Chart 7

5-Salicylideneiminosugars (12, 13 and 14)

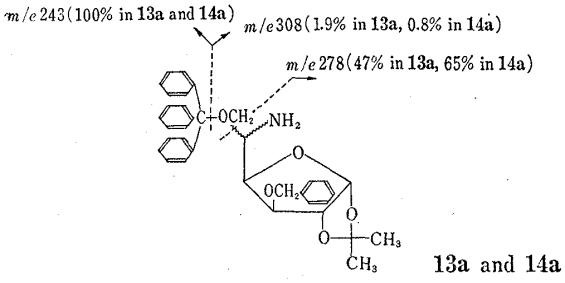
The mass spectrum of 5-salicylideneimino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**12**) showed, similar to that of compound **9** described above, the strong N-salicylidene C_2 fragments at *m/e* 164 (a_I) and 165 due to the preferential cleavage of the C-4 and C-5 bond. A cationic charge was retained little on the sugar fragment at *m/e* 159 (5%), which was formed simultaneously in this cleavage. An alternative α-cleavage of the C-5 and C-6 bond occurred as well, but in less case as evidenced by the weakness of the *m/e* 292 peak (6%) and the b_I ion (17%).

Fragmentation of the parent free base (**12a**) was analogous to that of **12**. Thus, α-cleavage of an amino group gave a major ion at *m/e* 60 (100%) and a minor at *m/e* 188 (17%). In a similar α-cleavage of the 5-hydroxy derivative (**12b**), however, a positive charge was considerably retained to the sugar fragment ion at *m/e* 159 (26%) due to the initial ionization of the ether oxygen atom. Other important fragmentation of **12**, **12a** and **12b** was shown in Chart 7.

In the case of 3-O-benzyl-5-salicylideneimino-5-deoxy-6-O-trityl derivatives (**13** and **14**), the spectra became complicated, and the direction of fragmentation was drastically changed, due to the presence of other aromatic nuclei. Fragment ion arising from fission of the C-4 and C-5 bond was no more observed, and ions from fission of the C-5 and C-6 bond, together with an ion from fission of the ether oxygen and trityl bond became dominant (Chart 8). Even more favorable fission between C-5 and C-6 was observed in the parent aminosugars (**13a** and **14a**), yielding strong fragment ions at *m/e* 278. Thus, the relative ease of cleavage between the C-4 and C-5 bond, and the C-5 and C-6 bond were greatly affected by the nature of substituents at C-5 and C-6.[11] Other significant fragmentation of **13**, **14**, **13a**, and **14a** were shown in Chart 8.

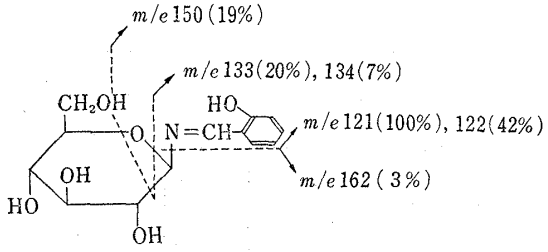


13 and **14**



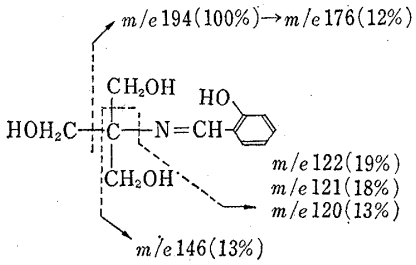
13a and **14a**

Chart 8



15

Chart 9



16

Chart 10

1-Salicylideneiminosugar (15)

Differing from other N-salicylidene aminosugars, the spectrum of N-salicylidene D-glucosylamine (**15**) showed the c_{II} ion as the base peak, together with the intense c_I ion. This suggested that the C-1 and nitrogen bond is quite unstable against the electron impact, giving rise to the major salicylideneimino cations (c_I and c_{II}) and the minor sugar fragment (*m/e* 162). Other diagnostic fragment ion was found at *m/e* 150, whose formation was rationalized by the simultaneous cleavages of the C-1 and C-2 bond, and the ether oxygen and C-5 bond as shown in Chart 9. The b ions (*m/e* 134 and 133) had medium intensity, but the a ions were extremely weak.

Salicylideneimino-trishydroxymethylmethane (16)

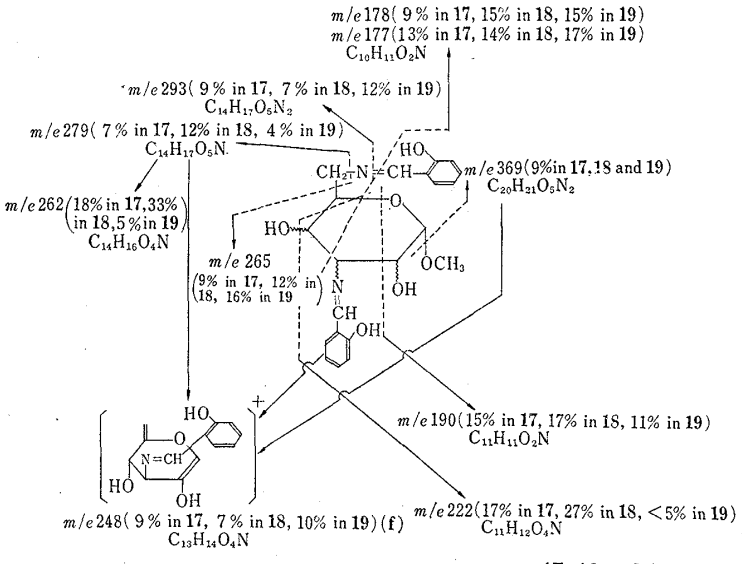
Although compound (**16**) is not an aminosugar derivative, its spectrum demonstrates the fragmentation pattern of a N-salicylidene aminoalcohol, where an amino group is substituted to a tertiary carbon. As was expected, one hydroxymethyl group of **16** was easily expeled from the tertiary carbon to yield a stable ion at *m/e* 194 (100%). The a and b ions were weak. The c ions were moderately observed at *m/e* 120, 121 and 122, of which the *m/e* 121

11) Fragmentations of **12a**, **12b**, **13a**, **14a** and a number of related compounds have been reported in detail.[12]
12) S. Inouye, *Sci. Reports, Meiji Seika Kaisha*, **11**, 52 (1970).

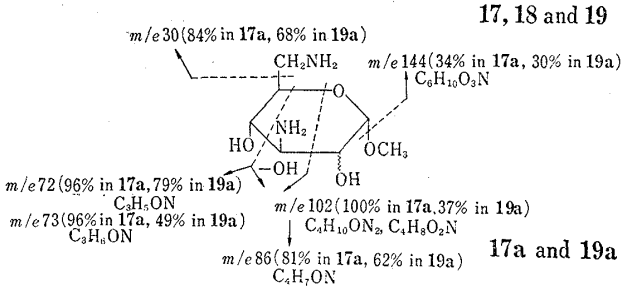
peak (c_{II}) was most prominent (Chart 10). Other noteworthy fragments included ions at *m/e* 176, 146 and 60 (35%). The formation of the *m/e* 60 ion could be rationalized only on the assumption of rearrangement of a hydroxymethine or hydroxyl group to form a $\left[\begin{matrix}CHOH\\ \|\\ CHOH\end{matrix}\right]^+$ cation.

3,6-Disalicylideneiminosugars (17, 18 and 19)

The spectra of methyl 3,6-disalicylideneimino-3,6-dideoxy-α-D-glucopyranoside (**17**), -mannopyranoside (**18**) and -altropyranoside (**19**) showed the M^+ and the $(M \text{ minus } 31)^+$ containing two salicylidene groups at *m/e* 400 and 369. Most of other fragment ions, however, contained only one salicylidene moiety, owing to ready release of one of two aromatic nuclei. Release of the salicylidene group from the M^+ took place *via* three fragments, *i.e.*, salicyl, salicylideneimino and salicylideneiminomethine groups, yielding ions at *m/e* 293, 279 and 265, respectively. In view of the extraordinarily stable M^+ of 3-salicylideneimino sugars (**1**, **2** and **3**) over the 6-salicylideneiminosugar (**4**), the leaving salicylidene group may be reasonably ascribed to that at the C-6 position. Further degradation of these ions yielded fragments at *m/e* 262 and 248 (structure f in Chart 11).



17, 18 and 19



17a and 19a

Chart 11

Cleavage of a pyranose ring gave the N-salicylidene C_4 fragments at *m/e* 222 and 190, and the N-salicylidene C_3 fragments at *m/e* 178 and 177. All of these fragmentations were supported by metastable ions and elemental compositions.

We have observed no significant difference in the spectra of **17** and **18**, but compound (**19**) was differentiated from the other two by the weakness of the *m/e* 262 and 222 peaks, and the intense *m/e* 161 and 176 peaks (not shown in Chart 11).

When the spectra of the N-salicylidene derivatives (**17** and **19**) were compared with those of the parent free bases (**17a** and **19a**), favorable cleavage of a pyranose ring in the free aminosugars was again noted. Thus, in compound (**17a**), preferential cleavage of the C-2 and C-3 bond was indicated by the appearance of the base peak at *m/e* 102. Cleavage of the C-3 and C-4 bond was suggested by the second strongest peak at *m/e* 72 and 73. In the salicylidene Schiff base (**17**), on the other hand, the intensities of ions arising from the cleavage of a pyranose ring were greatly reduced. The spectrum of **19a** was similar to that of **17a**, though the relative peak intensities were varied.

N-Salicylidene 2-Deoxystreptamine (20)

The mass spectrum of N-salicylidene 2-deoxystreptamine (**20**) was characterized by the appearance of two strong peaks due to the M^+ at *m/e* 370 (99%) and the c_I cation (100%).

Except for the a_I ion (52%), no other peak exceeded 30% intensity. Formation of the strong M^+ and weak N-salicylidene C_3—C_5 fragments indicated that the framework of 2-deoxystreptamine was stable against the electron impact fragmentation. Other ions typical for **20** included peaks at *m/e* 266, 250, 234, 231, 214, 213 and 190, and their formations were shown in Chart 12.

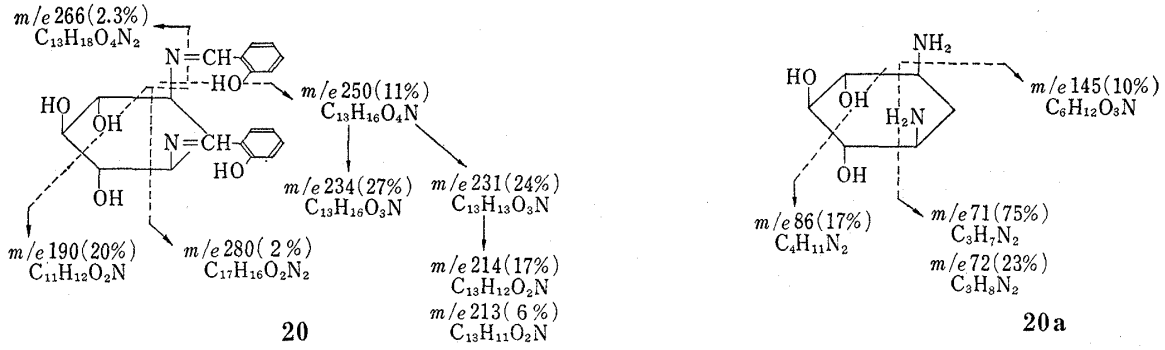


Chart 12

The base peak in the spectrum of 2-deoxystreptamine free base (**20a**) was found at *m/e* 71. This peak was a triplet, of which the main contributor was an ion with the highest mass number ($C_3H_7N_2$). Minor ions had the compositions, $C_3H_3O_2$ and C_3H_5ON. The formation of the $C_3H_7N_2$ ion indicated the preferential α-cleavage of both amino groups as shown in Chart 12. However, the corresponding N-salicylidene ion at *m/e* 280 (71 plus 208 plus 1) was very weak (2%) in **20**. Chart 12 showed other significant fragmentation of the free base (**20a**), yielding ions at *m/e* 145 and 86.

In conclusion, fragmentation of N-salicylidene aminosugars was summarized as follows.

a) The N-salicylidene aminosugars showed strong molecular ions due to the presence of an aromatic nucleus.

b) A salicylidene group was removed from the sugar portion mainly *via* three fragments, salicyl, salicylideneimino and salicylideneiminomethine groups, accompanied with one or two hydrogens transfer in general.

c) Relative intensities of the N-salicylidene C_2 ion at *m/e* 164 (a_I), the N-salicylidene C_1 ion at *m/e* 134 (b) and the salicylideneimino cation at *m/e* 122 (c_I) provided useful information with regards to the position of amino groups in carbohydrates.

i) The a_I ion was strong in the N-salicylidene 2-aminosugars (**6**, **7**, and **8**), 2-aminoalditol (**9**) and 5-aminosugars (**12**, **13**, and **14**). In addition, the a_{II} ion at *m/e* 163 was prominent in the N-salicylidene 2-aminosugars (**6**, **7**, and **8**), and the intense a_{III} ion (*m/e* 165) was characteristic for the latter two groups (**9**, **12**, **13**, and **14**).

ii) The strong b_I ion along with the moderate b_{II} ion (*m/e* 135) was diagnostic for the N-salicylidene 6-aminohexose (**4**) and 5-aminopentose (**5**) as well as 3,6-diaminohexoses (**17**, **18**, and **19**).

iii) The c_I and c_{II} (*m/e* 121) ions were prominent in the N-salicylidene 1-aminosugar (**15**) and 2-deoxystreptamine (**20**).

d) Among the 1-, 2-, 3-, 5-, and 6-aminohexose derivatives, the 3-aminosugar derivatives mostly resisted against the electron impact fragmentation, and gave the strongest M^+.

e) The (M minus salicylideneimino) ion was diagnostic for the 2-aminosugars.

f) Except for the terminal aminosugar derivatives, fragmentation of the N-salicylidene Schiff bases differed from the parent aminosugars. α-Cleavage of a nitrogen atom was dominated in the latter compounds, but in less case in the N-salicylidene derivatives.

g) Dehydrated peaks were less prominent in the spectra of the Schiff bases than the parent free bases. Except for the M^+, fragment ions of the Schiff bases are protonated in many cases.

Experimental

Materials——N-Salicylidene aminosugars employed in this work were prepared according to the procedures described in the literatures.[13–15] 2-Amino-2-deoxy-D-glucose diethyl dithioacetal (**10a**) and 2,6-diamino-2,6-dideoxy-D-glucose diethyl dithioacetal (**11a**) were prepared according to Inouye and coworkers.[16] Five mg of each dithioacetals was dissolved in methanol (0.2 ml), and excess of salicylaldehyde (0.02 ml) was added. The mixture was stood at room temperature for 1 hour, and then evaporated to dryness *in vacuo*. The residue was subjected to mass spectrometry without further purification.

Mass Spectra——Most of low-resolution mass spectra were determined on a Hitachi RMU-6 mass spectrometer at 70 eV by the direct introduction. High-resolution mass data and some of low-resolution spectra were taken with a JMS-01SG double-focusing mass spectrometer at 75 eV.

Metastable ions formed between the ion source and the electrostatic field were detected on the electric detection recorder by scanning the accelerating voltage.

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13) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **15**, 1540 (1967).
14) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **14**, 1112 (1966).
15) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **14**, 902 (1966).
16) S. Inouye, M.L. Wolfrom and D. Horton, to be pubished.