1:1 Adduct Ion Formation of Permethylated Monosaccharides with Organic Cations in FAB Mass Spectrometry[1)]

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The 1:1 adduct ion formation between a series of permethylated monosaccharides (M; $\mathbf{I_a}$—$\mathbf{I_h}$) and an organic or a metallic cation (A^+) has been examined in quantitative FAB mass spectrometry. In a careful comparison under the same FABMS conditions, the relative $(M+A)^+$ peak intensities increase in the following order of the monosaccharides, and further increase at nearly the same extents in spite of using three different cations such as octylammonium, (methoxycarbonyl)methylammonium, and potassium ions:

$$\beta\text{-Glc} < \alpha\text{-Glc} < \alpha\text{-Gal} < \beta\text{-Gal} \leq \alpha\text{-Man} < \beta\text{-Man} < \alpha\text{-Tal} < \beta\text{-Tal}.$$

The order and cation-independency clearly indicate the OCH_3 configurational effects of M on $(M+A)^+$ adduct ion formation. The findings can be interpreted in terms of multisite electrostatic interaction of oxygens with the cation. Coupled with the results of gas-phase behavior by FABMS/MS(CAD), solution behavior by ^1H NMR, and model calculations by MNDO, the characteristic structure of the 1:1 adduct ion is deduced as host-guest type association between permethylated monosaccharides and cationic species in particular cases, at least β-Tal and α-Tal cases.

Elucidation of stereochemical effects of organic compounds on mass spectra has been a challenging problem for a long time. Much attention has been paid to this problem on the basis of fragmentation patterns in EIMS (electron impact mass spectrometry)[2)] or peak intensities of quasi-molecular ions in CIMS (chemical ionization mass spectrometry),[3,4)] etc.[5)] Recently, Puzo et al. reported epimer-identification of underivatized monosaccharides with metallic cations in FAB/MIKES (Fast Atom Bombardment)/(Mass Analyzed Ion Kinetic Energy Spectroscopy).[6)] However, there are almost no systematic studies concerning stereochemical effects on conventional FABMS.[7-12)] We now focus our attention on the structural factors which promote the 1:1 adduct ion formation in quantitative FABMS.

In the present study, permethylated monosaccharides, $\mathbf{I_a}$—$\mathbf{I_h}$ (family **I**), are chosen as the substrates (M), and compared with monomethylated monosaccharides, $\mathbf{II_a}$—$\mathbf{II_h}$ (family **II**), as the references.[13)] The permethylated monosaccharides can avoid the effects of intramolecular hydrogen bonding of M,[14)] and further intermoleculer hydrogen bonding of M with matrix (for example, glycerol; G).[15)] The monomethylated monosaccharides can prevent α, β isomerization during the preparation of sample solutions of M.

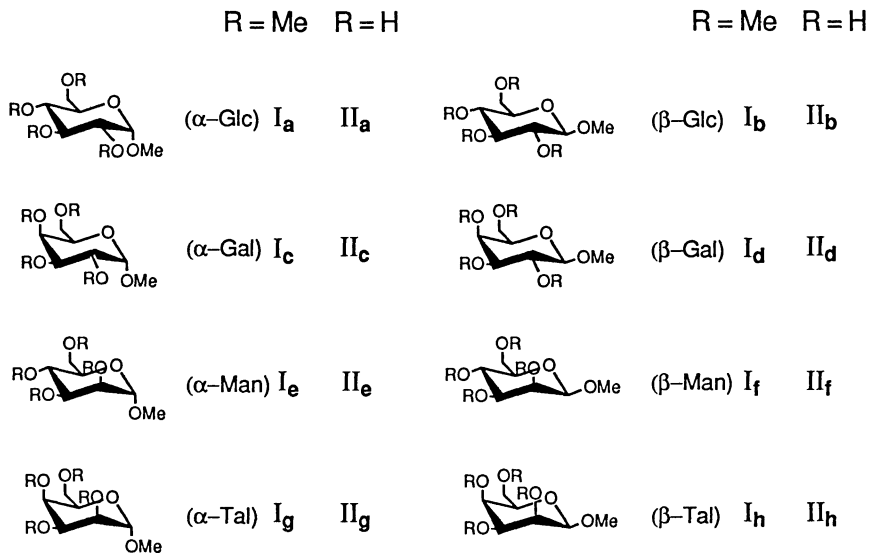

Chart 1.

Complexations of metallic cations with underivatized monosaccharides were extensively studied by Angyal et al. mainly on the basis of ^{1}H NMR and X-ray crystal data, where importance of axial-equatorial-axial arrangement of three sequential OH groups was explored for strong 1 : 1 complexation.[16] This paper describes primarily the stereochemical effects of permethylated monosaccharides (M) on $(M+A)^+$ adduct ion formation with organic cations (A^+) in conventional FABMS, and deduces the structure as host-guest type association in particular cases.[17–19]

Results

Selection of Cations. Table 1 shows the results of qualitative FABMS experiments for searching good cations (A^+), where $\mathbf{I}_f$ (β-Man) is fixed as a common substrate (M). From the relative intensity values, ($I[M+A]^+/I[M+1]^+$), it was found that primary and secondary alkylammonium ions with relatively long alkyl chains, and metallic cations with one positive charge, are suitable for generating abundant $(M+A)^+$ adduct ions. Figure 1 is a typical example of a FAB mass spectrum for the case of adding octylammonium ion salt.

Selectivity of Permethylated Monosaccharides. Based on the data in Table 1, octylammonium (A_1^+), (methoxycarbonyl)methylammonium, $CH_3OCOCH_2NH_3^+$ (A_2^+), and potassium ions (A_3^+) were chosen as better cationic species in the present quantitative

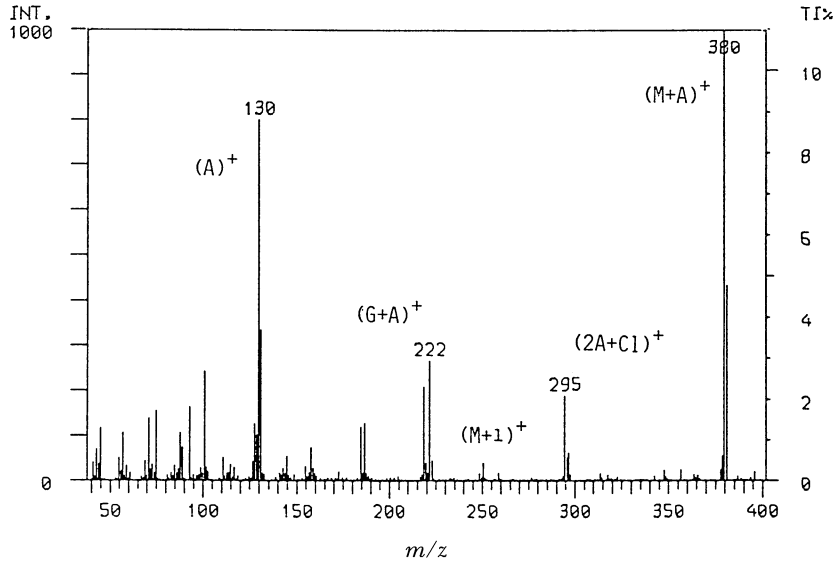

Fig. 1. A FAB mass spectrum of M with A^+X^- (glycerol matrix): M=$\mathbf{I}_f$ (β-Man), A^+X^-=n-$C_8H_{17}NH_3^+Cl^-$ (M: A^+X^-=1 : 1).

Table 1. Search Experiments for Good Additives by Using FABMS (Glycerol Matrix)

additive A^+X^-	Molar ratio $A^+ : M^{a)}$	Relative peak intensity $I[M+A]^{+b)}$
$CH_3CH_2NH_3^+Cl^-$	1 : 1	16
$CH_3(CH_2)_3NH_3^+Cl^-$	1 : 1	68 (3)
$CH_3(CH_2)_7NH_3^+Cl^-$	1 : 1	>1000 (40)
$CH_3(CH_2)_9NH_3^+Cl^-$	1 : 1	>1000 (36)
$PhCH_2CH_2NH_3^+Cl^-$	2 : 1	59 (4)
$PhCH_2CH_2NH_2^+(CH_3)Cl^-$	2 : 1	47 (4)
$PhCH_2NH^+(CH_3)_2Cl^-$	2 : 1	8 (<1)
$CH_3OCOCH_2NH_3^+Cl^-$	2 : 1	68
$NH_4^+Cl^-$	5 : 1	36
$HOOCCH_2CH_2CH(COOH)NH_3^+Cl^-$	1 : 1	2
$CH_3CH(CH_3)CH(COO^-)NH_3^+$	1 : 1	9
Na^+Cl^-	2 : 1	29
K^+Cl^-	2 : 1	280
Rb^+Cl^-	2 : 1	240
Cs^+Cl^-	2 : 1	170
Ag^+Cl^-	5 : 1	26

a) M=$\mathbf{I}_f$. b) Normalized to $I[M+1]^+$=10. In parenthesis, neutral amine was used as an additive compound.

study. Affinities of a series of permethylated monosaccharides (family **I**) toward the cation were determined by measuring relative peak intensities of the relevant $(M+A)^+$ adduct ions. Internal standard technique was employed for quantitative measurements so as to get highly reliable peak intensity data in FABMS.[20-22] Deuterated monosaccharide, $\mathbf{I}_e$-d_{12} (methyl tetra-*O*-methyl-d_{12}-α-D-mannoside), was used as an internal standard (R), and competitive conditions[23] where an equimolar amount of M and R was included in a matrix (glycerol) were employed. The relative peak intensity value, $I(M+A)^+/I(R+A)^+$, was taken as a measure of $(M+A)^+$ adduct ion formation. It should be noted here that measuring conditions of a mass spectrometer and concentration conditions of all sample solutions were kept constant throughout the experiments of getting a set of relative peak intensity values.

Different affinities were observed for the set of monosaccharides. By contrast, they were very close to each other for the three sets of cations (A_1^+, A_2^+, and A_3^+; Table 2). The $(M+A)^+$ peak of $\mathbf{I}_h$ (β-Tal) was almost 100 times more intense than that of $\mathbf{I}_b$ (β-Glc). The characteristic ordering of the resulting relative affinities is, β-Tal>α-Tal>β-Man>α-Man≥β-Gal>α-Gal>α-Glc>β-Glc. This indicates that the relative peak intensities of $(M+A)^+$ ions are strongly related with the configuration of the OCH_3 groups in the family **I**.

Table 2. Relative Peak Intensities of $(M+A)^+$ Adduct Ions for Permethylated Monosaccharides in FABMS (Glycerol Matrix)

Monosaccharide M (R)[a]	$I[M+A]^+/I[R+A]^+$ value		
	(A_1^+)[b]	(A_2^+)[c]	(A_3^+)[d]
$\mathbf{I}_a$	0.4	0.5	0.5
$\mathbf{I}_b$	0.3	0.3	0.2
$\mathbf{I}_c$	0.6	0.8	0.7
$\mathbf{I}_d$	1.0	0.9	1.0
$\mathbf{I}_e$ ($\mathbf{I}_e$-d_{12})	1.0	1.0	1.0
$\mathbf{I}_f$	5.8	4.2	3.7
$\mathbf{I}_g$	9.0	6.1	12
$\mathbf{I}_h$	36	22	18

a) R=$\mathbf{I}_e$-d_{12} (α-Man-d_{12}). See text. b) $A_1^+X^-$=CH_3-$(CH_2)_7NH_3^+Cl^-$. c) $A_2^+X^-$=$CH_3OCOCH_2NH_3^+Cl^-$. d) $A_3^+X^-$=K^+Cl^-.

Selectivity of Monomethylated Monosaccharides. Affinities of a series of monomethylated monosaccharides (family **II**) toward the cation were determined by the same method as that mentioned above (Table 3). Here, methyl-d_3 α-D-glucopyranoside ($\mathbf{II}_a$-d_3) was employed as an internal standard (R). As seen in the FABMS columns of Table 3, the differences in affinities toward each cation largely diminished or disappeared. For example, the $(M+A)^+$ peak of $\mathbf{II}_h$ (β-Tal) was at most 2—5 times more intense than that of $\mathbf{II}_a$ (α-Glc), being in marked contrast to a much larger selectivity of the family **I**.

$(M+A)^+$ Adduct Ions Detected by FABMS/MS(CAD). FABMS/MS (mass spectrometry/mass spectrometry) is useful to know purely gas-phase ion phenomena. MS/MS operating principle is (1) a selected ion is directed into a second mass spectrometer, and (2) the ion (precursor ion) is decomposed typically by argon collision, and (3) the resulting fragment ions are analyzed.[24] The MS/MS has been a rapidly growing technique for studying the order of relative binding energies,[25] and for analysing organic ions in the gas-phase.[31] Our precursor ions used were Puzo's three component ions,[6a] $(M+K+DEA)^+$ (m/z 338), which were composed of the family **II**, K^+, and diethanolamine (DEA) (Chart 2).

Relative area intensities of the $(M+K)^+$ adduct ions (m/z 233) to the reference one $(DEA+K)^+$ (m/z 144) were measured under the same experimental conditions,[26] and tabulated in the FABMS/MS column of Table 3. The present FABMS/MS area intensity data

Table 3. Relative Peak Intensities of $(M+A)^+$ Adduct Ions for Monomethylated Monosaccharides in FABMS

M (R)[a]	$I[M+A]^+/I[R+A]^+$ FABMS				$A[M+A]^+/A[R+A]^+$	
	(A_4^+)[b] (G)[e]	(A_2^+)[c] (G)[e]	(A_3^+)[d] (G)[e]	(A_3^+)[d] (DEA)[f]	FABMS/MS (A_3^+)[d] (DEA)[f]	FAB/MIKES (A_3^+)[d] (DEA)[f]
$\mathbf{II}_a$ ($\mathbf{II}_a$-d_{12})	1.0	1.0	1.0	0.3	0.9	(0.2)[h]
$\mathbf{II}_b$	0.8		1.0	0.3	0.9	
$\mathbf{II}_c$	0.9			0.4	1.3	(0.7)[h]
$\mathbf{II}_d$	1.2			0.8	2.1	
$\mathbf{II}_e$	1.5	1.8	2.5	1.0[g]	1.5	(1.0)[h]
$\mathbf{II}_f$	1.7				3.6	
$\mathbf{II}_g$	1.6	1.7	1.8	1.3	3.7	(3.7)[h]
$\mathbf{II}_h$	2.0	1.8	2.2	1.6	4.8	

a) R=$\mathbf{II}_a$-d_3 (α-Glc-d_3). See text. b) $A_4^+X^-$=$PhCH_2CH_2NH_3^+Cl^-$. c) $A_2^+X^-$=CH_3-$OCOCH_2NH_3^+Cl^-$. d) $A_3^+X^-$=K^+Cl^-. e) Glycerol matrix. f) Diethanolamine matrix. g) As an internal standard (R), $\mathbf{II}_e$-d_3 (α-Man-d_3) was used. h) Anomers for underivatized monosaccharides were not differentiated.[6a]

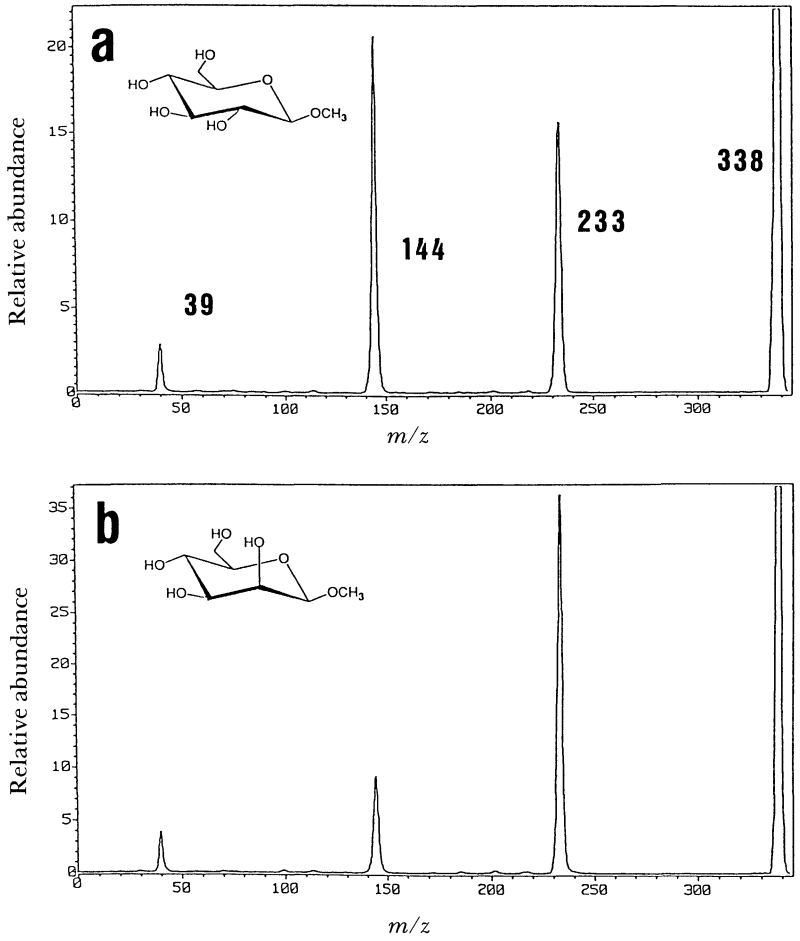

Fig. 2. FABMS/MS (CAD) spectra of the precursor ion $(M+K+DEA)^+$; *m/z* 338. (a) M=**II**$_b$ (β-Glc), (b) M=**II**$_f$ (β-Man).

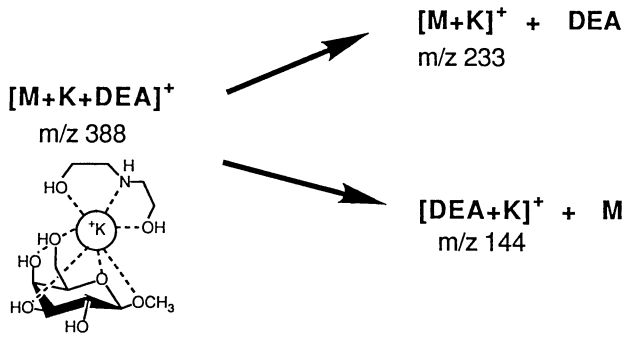

Chart 2.

via CAD (collisionally activated decomposition) process are consistent with earlier FAB/MIKES data via unimolecular decomposition process.[6a] The resulting ordering of the binding energies for $(M+K)^+$ adduct ions is, β-Tal>α-Tal>β-Man>-β-Gal>α-Man>α-Gal>α-Glc=β-Glc, which is in good agreement with the affinity ordering of the family **I** in FABMS.

$(M+A)^+$ Adduct Ions Supported by ^{1}H NMR. ^{1}H NMR experiments were carried out to see if the $(M+A)^+$ adduct ions between the family **I** and organic cations in solution can be detected or if existence of equilibrium (Eq. 1) can be established by means of diamagnetic induced chemical shifts (ICS).[16,27] Because of solubility problems of the alkylammonium ion salts employed, acetonitrile-d_3 was utilized as a solvent.

$$M + A^+ \rightleftarrows (M+A)^+ \quad (1)$$

Typical ^{1}H NMR spectra before and after addition of 2-phenylethylammonium hexafluorophosphate ($A_4^+PF_6^-$) are compared in Fig. 3 ([M]=0.014 M, $[A_4^+PF_6^-]$=0.008 M). Here, M is **I**$_h$ (β-Tal), because it gave the highest $(M+A)^+$ peak in Table 2. Position-dependent ICS was clearly observed after the addition. That is, (1) the broad ammonium proton signal moves to a downfield direction to the greatest extent (ca. 0.15 ppm), (2) the monosaccharide ring proton signals also undergo downfield shifts to different extents, and (3) some of methyl proton signals (around δ=3.4) show downfield shifts. These observations in both sides of A^+ and M suggest that $(M+A)^+$ adduct ion formation is in an fast equilibrium on the NMR time scale, and that apparent ICS

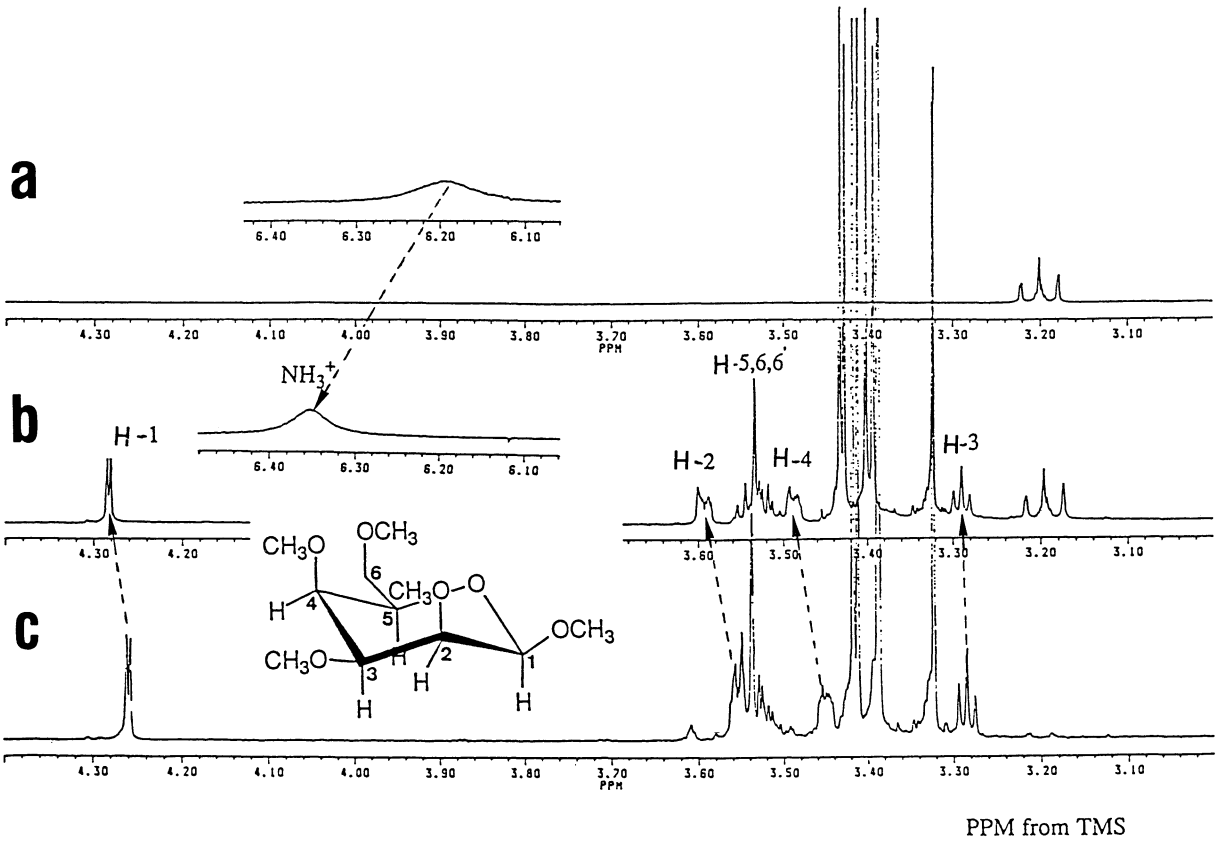

Fig. 3. ^{1}H NMR spectra (360 MHz) of M before and after an addition of A^+X^- in CD_3CN: M=$\mathbf{I_h}$ (β-Tal), A^+X^-=$PhCH_2CH_2NH_3^+PF_6^-$. A solution of (a) A^+X^-, (b) M+A^+X^-, or (c) M.

values reflect weighted averages of the existing M (or A^+) and $(M+A)^+$ species.

(a) Equilibrium Constant in Solution: From the concentration-dependent ICS of the ammonium proton signal, the equilibrium constant (K_s for Eq. 1) could be determined by using a Benesi-Hildebrand equation.[28] The K_s for $(M+A)^+$ adduct ion formation between $\mathbf{I_h}$ and 2-phenylethylammonium ion ($A_4^+PF_6^-$) is estimated to be 9 M^{-1} at 30 °C in CD_3CN ([M]/[$A_4^+PF_6^-$]=10—23, [$A_4^+PF_6^-$]$_{fix}$=0.0045 M, ICS range=113—195 Hz).[29,30]

(b) Position-Dependent Induced Chemical Shifts in Solution: ICS values for different pairs of M and A^+X^- in CD_3CN could be determined by using ^{1}H NMR. Spectral assignment was done by irradiation techniques and in light of the reported assignments.[32] In spite of different cationic species such as 2-phenylethylammonium, ammonium, and potassium ions, similar ICS patterns of M were observed for $\mathbf{I_h}$: that is, (1) the ICS ordering for the ring protons is H_2=H_4>H_1>H_3, (2) three of five methyl proton signals show more downfield shifts than the others (not assigned).

Compared with the results for $\mathbf{I_h}$, those for $\mathbf{I_g}$ are characterized by the decrease of the H_1 ICS value, and the decrease of the number of more downfield methyl proton signsls. On the other hand, $\mathbf{I_f}$ seems to give nearly isotropic shift pattern, and $\mathbf{I_a}$ shows no NMR changes to detectable extents. These position-dependent ICS data are deposited as Document No. 9109 at the office of Bull. Chem. Soc. Jpn.

MNDO Calculations of Model Systems. MNDO optimized structures were obtained by using the simplest model system, where Li^+ was used instead of K^+ (or RNH_3^+) to elucidate the essential complexation sites and relative binding energies of M toward the cation in the gas-phase. As schematically shown in Chart 3, in the cases of $(M+A)^+$ ions for $\mathbf{I_h}$ and $\mathbf{I_g}$, the cationic centers are located at almost equal distance (2.3 Å±0.1) from the three oxygens of the O-2, O-4, and the ring-O.[33] The distance corresponds to a sum of oxygen Van der Waals radius (1.6 Å) and Li^+ radius (0.6 Å).

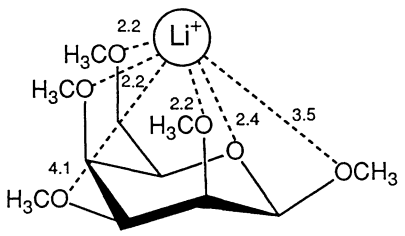

Chart 3.

Discussion

Relative Peak Intensity in FABMS vs. Gas-Phase Stability for $(M+A)^+$ Adduct Ion Formation. An illustration of the importance of gas-phase phenomena for the relative peak intensities in FABMS is shown in Fig. 4. The results are similar to those of Kebarle et al.[34,35] Here, the logarithm of the relative peak intensity, $\log[I(M+K)^+/I(R+K)^+]$, for the family **I** is plotted against that of the corresponding relative area intensity, $\log[A(M+K)^+/A(R+K)^+]$, in FABMS/MS (CAD). The latter quantities are taken as a reference measure of the relative stabilities (relative binding energies) of the relevant $(M+K)^+$ ions in the gas-phase.

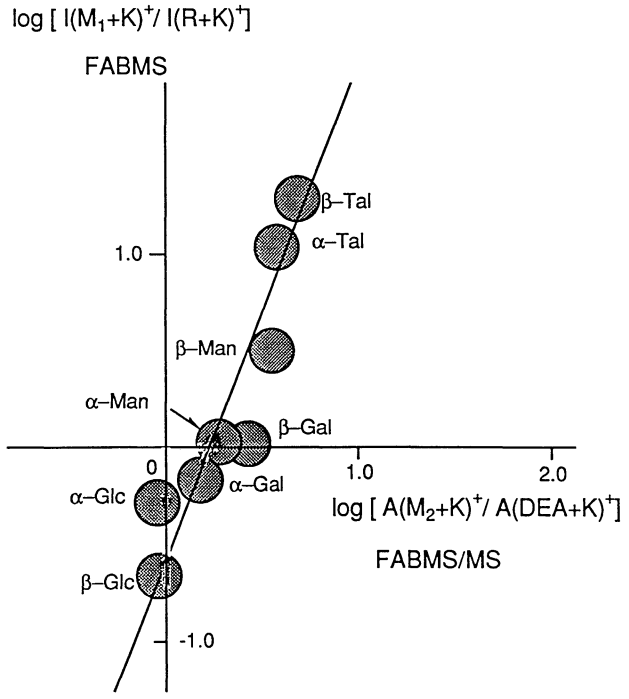

Fig. 4. Relative $(M_1+K)^+$ peak intensity data on FABMS are plotted against relative $(M_2+K)^+$ area intensity data on FABMS/MS(CAD). M_1 is the family **I**, and M_2 is the family **II**.

A good correlation is observed for the family **I**, but not clearly observed for the family **II** because of a narrow range of the relative peak intensities (not shown). This illustrates that the relative peak intensities of the family **I** in FABMS reflect characteristically the relative stabilities of $(M+A)^+$ ions in the gas-phase.[23-25] Recently, there has appeared the other aspect that relative peak intensities in FABMS reflect just solution phenomena on the basis of the results for 18-crown-6 complexations,[20,36] etc.[37] Unfortunately, we can not estimate the K_s values in solution except for $\mathbf{I}_h$. They are too small to be estimated for the entire series of the family **I** by ^{1}H NMR ICS methods.[29] Therefore, there still remains the possibility that the gas-phase stability is essentially parallel to the solution-phase stabilities for $(M+A)^+$ ions of the family **I**. In the family **II**, intramolecular hydrogen bonding toward transannually located oxygens may mask such selective complexations. One of the relevant examples has been seen in the complexation of a modified crown ether.[14]

The demonstration that the family **I**, compared with the family **II**, shows much larger selectivity toward an organic (or metallic) cation is practically important for wider applications of FABMS. Permethylation of saccharides would also provide the increase of electron densities on the oxygen atoms for good complexation. The finding of such a larger selectivity will open a new practical approach that the relative stabilities of saccharide-cation adduct ions can

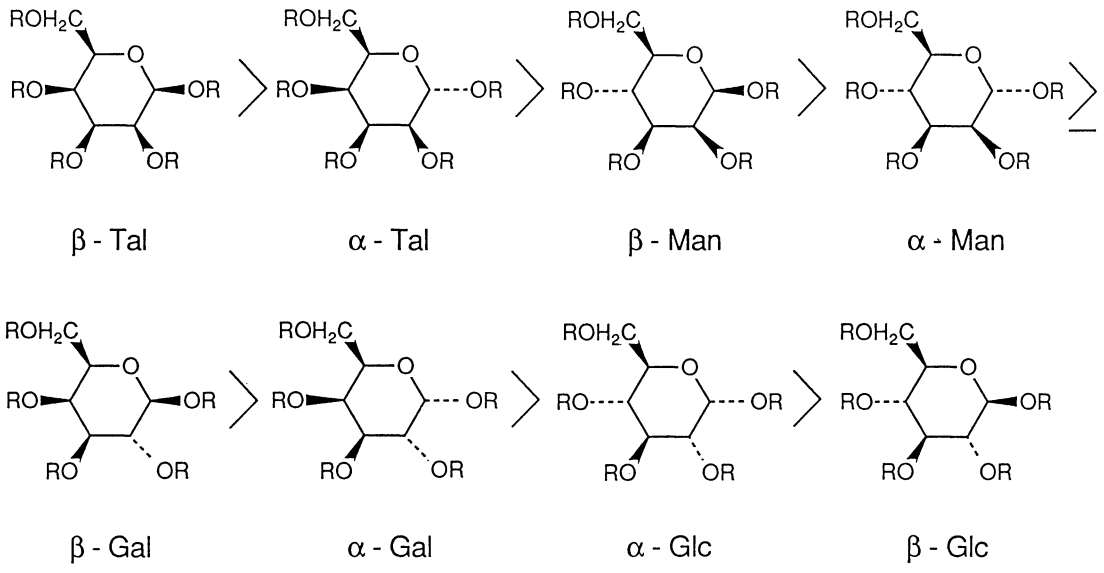

Chart 4.

Table 4. Effects of Alkylammonium Ions on Relative Peak Intensities in FABMS (Glycerol Matrix)

Substrate M	Relative peak intensity			
	$[M+A_1]^{+}$ a)	$[M+A_2]^{+}$ b)	$[M+A_3]^{+}$ c)	$[R+1]^{+}$ d)
I$_f$ (β-Man)	32	23	5	100
I$_h$ (β-Tal)	120	69	8	100
15-crown-5	120	12	—	100
18-crown-6	400	8	—	100

a) $A_1^+X^-$=$PhCH_2CH_2NH_3^+Cl^-$. b) $A_2^+X^-$=$PhCH_2NH_2^+(CH_3)Cl^-$. c) $A_3^+X^-$=$PhCH_2NH^+(CH_3)_2Cl^-$. d) R^+X^-=$(n\text{-}C_4H_9)_4N^+Cl^-$ (internal reference).

be easily judged by the corresponding relative peak intensities in conventional FABMS, (1) if saccharides are permethylated or more generally peralkylated, and (2) if FABMS is applied to a proper stereoisomeric series of saccharides in a careful comparison.

Stereochemical Effects of Permethylated Monosaccharides on $(M+A)^+$ Peak Intenstities. It is obviously found from Table 2 that the relative $(M+A)^+$ peak intensities for the family **I** show a close relationship with the configuration effect of the CH_3O groups included. The ordering is pictured by using Mills formulae (Chart 4). From the comparison among epimers, the more CH_3O groups are located on the same side, the larger the $(M+A)^+$ ion peak is. From the comparison between anomers, the $(M+A)^+$ ion peak for β-anomer is essentially larger than that for α-one.

Let us consider a series of β-anomers for the pyranose set. Here, the O-1, O-3, O-6, and the ring-O are common in the series. In the case of the highest peak-intensity (β-Tal), the monosaccharide has two upward axial oxygens (O-2, O-4). In the case of middle one (β-Man or β-Gal), M has one upword axial oxygen (O-2 or O-4, respectively). In the lowest case (β-Glc), there is no upward axial oxygen. These characteristics are consistent with the fact that the upward axial oxygen is very effective for stabilizing $(M+A)^+$ adduct ions. Further, the β-anomer preference is also consistent with the fact that the upward equatrial oxygen has also a contribution for stabilizing $(M+A)^+$ ions somewhat less than the upward axial one. These findings are in good agreement with some MNDO predictions of the model systems. The intensity difference of β-Man from β-Gal informs that the axial O-2 can act more effectively for stabilization than the axial O-4.

The affinity ordering of different permethylated monosaccharides for the cation by generating $(M+A)^+$ ions can be reasonably interpreted in terms of multisite electrostatic interaction for effective binding.[38,39] The cooperative multisite binding is one of the main interaction mechanisms for the well-known crown ether-cation complexation.[38] A complexation site of the organic (or metallic) cation can be deduced from MNDO model calculations, at least for the cases of **I**$_h$ and **I**$_g$, where the cationic centers are located at nearly equal distance from the O-2, O-4, and the ring-O: that is, the Li^+ cation stands in contact with the three oxygens.[33]

^{1}H NMR ICS data also support this type of structures with organic (or metallic) cations. The picture is just consistent with the host-guest type association, as seen, for example, in the crystal structure of the complex between benzo-15-crown-5 and Ca^{2+} ion.[40] On the other hand, a different complexation site in the complex between epi-inositol and La^{3+} (D_2O) has been reported.[16] The ^{1}H NMR limiting shift values[16c,41] of H-3 are completely different each other, supporting the different complexation sites with cations between the two complexes in solutions.

Affinities toward primary, secondary, and tertiary alkylammonium ions are informative (Table 4). In the case of crown ethers (18C6, 15C5), the primary alkylammonium ion provides the highest $(M+A)^+$ peak, and the tertiary one shows no $(M+A)^+$ peak. The affinity ordering (prim.≫sec.≫tert.) is corresponding to the number of $^+$N-H hydrogen atoms which can exert hydrogen bondings with host oxygens. Similarly, in the case of the permethylated monosaccharides (β-Tal, β-Man), the ordering is prim.>sec.>tert. Hydrogen bonding between the permethylated monosaccharide and alkylammonium ion may be effective to lesser extents in these complexations.

Experimental

General. ^{1}H NMR spectra were determined on a Bruker AM360 spectrometer. Chemical shifts are given in units in ppm relative to TMS as an internal standard (δ=0). Liquid column chromatography was carried out on a Yamazen LC apparatus using a column (50×1.5 cm) packed with Merck LiChroprep Si 60 (0.025—0.040 mm) under medium-pressure (1.5—2 kg cm^{-2}, flow rate 1.5 mL min^{-1}); 1 : 1 hexane/ethyl acetate was used as a typical eluent. For separation of aldopyranose/aldofuranose or its anomer derivative, a fraction collector (Gilson model 203) was employed with continuous TLC checking or RI monitoring (Yamazen, RI-31). The TLC checking was carried out on a piece (size 10×2. 5 cm) of Merck Kieselgel 60 F_{254} sheet. The TLC plates were sprayed with an 10% aqueous H_2SO_4 solution containing 1% $Ce(SO_4)_2$, and then heated on a hot plate until the spots become visible in blown (2—3 min).

Gas chromatography was performed on a Shimadzu GC-

9A instrument using a conventional glass column packed with Silicone OV 17/Chromosorb W or SE 30 (detection by FID mode, data reduction by C-R5A chromatopac). IR spectra were measured with a Hitachi 345 IR or an Analect RFX65 FTIR spectrometer. Mass spectra were recorded with a JEOL DX300 instrument under FAB and/or EI mode. MS/MS spectra were obtained with a JEOL JMS-HX100 instrument having E/B/E-type operation.[26]

Purity of the materials prepared was verified by ^{1}H NMR, EIMS, FABMS, and FTIR spectra. These spectra of the family **I** and the family **II** have been stored in the total computer system designated as 'TASMAC', ISIR, Osaka Univ.[43]

Materials. KF-alumina reagent was prepared according to the published procedure by Ando et al.[44] Alumina (Al_2O_3, Merck Aluminiumoxid 90 aktiv, neutral, 70—230 mesh, 60 g) was poured into an aqueous solution (70 ml) of KF (40 g) and fully shaked and stirred. After then, the water was removed at 50—60 °C with a rotary evaporator in vacuo. The impregnated alumina was further dried in a vacuum oven (15—20 mmHg) for 7 h at 70—80 °C (yield 102 g).

General Preparation of Permethylated Monosaccharides (family I). The respective methyl α- or β-D-aldopyranoside (0.20 g, 1.0 mmol) was mixed with methyl iodide (0.5 mL, ca. 8 mmol), and KF-alumina reagent (2.75 g, ca. 20 mmol) in acetonitrile (20 ml), and magnetically stirred in a teflon sealed tube at room temperature for 2 or 3 d (tetramethylation). The solid material was filtered off and washed several times with acetonitrile. The combined acetonitrile solution was evaporated in vacuo to dryness. The residue was dissolved into chloroform, washed with water, and filtered. Purification by liquid column chromatography usually at atmospheric pressure using Merck Kieselgel 60 (0.040—0.063 mm (230—400 mesh), 15 cm or 50 cm column length, 1:1 hexane/ethyl acetate or 5:5:1 hexane/ethyl acetate/methanol eluent) gave the corresponding methyl tetra-*O*-methyl-D-aldopyranoside (family **I**) in a yield of 70—80% as a ^{1}H NMR pure sample. Examples of starting materials are methyl α-D-glucoside (Wako),[45] methyl β-D-glucoside (Wako),[45] methyl α-D-galactoside (Nakalai), methyl β-D-galactoside (Sigma), methyl α-D-mannopyranoside,[46] methyl α-D-talopyranoside,[47] methyl α-D-ribofuranoside, and methyl β-D-ribofuranoside.[47,48]

In the case of pentamethyl β-D-mannopyranoside ($\mathbf{I_f}$) or pentamethyl β-D-talopyranoside ($\mathbf{I_h}$), the corresponding underivatized D-aldose commercially available was directly methylated (pentamethylation) with KF-alumina reagent by the same method as described before and then, the desired permethylated derivative was separated from a mixture of aldopyranosides and aldofuranosides by using medium-pressure liquid column chromatography (see below).

Preparation of Methyl Tetra-*O*-methyl-β-D-mannopyranoside ($\mathbf{I_f}$). A mixture of D-mannose (Wako. 0.20 g), methyl iodide (0.5 ml), and KF-alumina reagent (2.75 g) in acetonitrile (30 mL) was magnetically stirred at room temperature for a prolonged period (7 d, pentamethylation). After filteration and evaporation, the products were dissolved into chloroform, washed with water. The solution was filtered and evaporated to dryness. TLC analyses showed four major spots suggesting four stereoisomers. By using medium-pressure liquid column chromatography with an eluent of 5:5:1 hexane/ethyl acetate/methanol (v/v), the fraction showing R_f value of 0.38 (the fourth spot from top) was collected as the major one. The ^{1}H NMR[32] and mass spectral data indicated the product being the desired $\mathbf{I_f}$ (40% yield); ^{1}H NMR ($CDCl_3$) δ=4.29 (d, 1H $J_{1,2}$=0.8 Hz, C_1-H), 3.69 (dd. 1H, $J_{6,6'}$=9.1 Hz, C_6-H), 3.67 (dd, 1H, $J_{2,3}$=3.2 Hz, C_2-H), 3.60 (dd, 1H, $J_{5,6'}$=5.6 Hz, $C_{6'}$-H), 3.35 (t, 1H, $J_{4,5}$=9.5 Hz, C_4-H), 3.29 (octet. 1H, $J_{5,6}$=2.0 Hz, C_5-H), 3.19 (dd, 1H, $J_{3,4}$=8.7 Hz, C_3-H), 3.618, 3.520, 3.518, 3.493, 3.407 (s, 15H, OMe).

Preparation of Methyl Tetra-*O*-methyl-β-D-talopyranoside ($\mathbf{I_h}$). $\mathbf{I_h}$ was prepared by the analogous way mentioned above. Direct pentamethylation of D-talose (Sigma, 0.20 g) gave four TLC spots. Three major materials could be separated. Among them, the fractions showing R_f value of 0.17 (the fourth from top) was collected as the major one (eluent 1:1 hexane/ethyl acetate or 5:5:1 hexane/ethyl acetate/methanol). The product was the desired $\mathbf{I_h}$ on the basis of ^{1}H NMR and mass spectral data (60 mg, 22% yield); ^{1}H NMR ($CDCl_3$) δ=4.33 (d, 1H, $J_{1,2}$=1.6 Hz, C_1-H), 3.75—3.70 (m, 2H), 3.63—3.54 (m, 3H), 3.265 (t, 1H, J=3.17 Hz), 3.593, 3.522, 3.507, 3.485, 3.404. In this pentamethylation process, *N*-methylacetamide was detected as a by-product (^{1}H NMR, IR, and GC analyses).

Quantitative FABMS Measurements. FAB mass spectra were obtained by using argon as the bombarding atom, with a beam energy of 6 keV. A stainless steel probe-tip was employed in each case. Mass conversion was carried out off-line on the basis of perfuruoro kerosene (PFK) calibration. In order to get higher reproducibility on peak intensity data, all the FAB spectra by quantitative measurements were averaged from at least 10 scans (1 scan=4 s) using a JMA-3100 data system.[22]

A typical preparation of sample solution is as the following. (1) The respective permethylated monosaccharide (16 μL) was dissolved into methanol (1 mL) (17.6 mg mL^{-1}; 0.07 M). (2) (Methoxycarbonyl)methylammonium chloride (0.88 g) was dissolved into methanol (10 mL) (8.8 mg mL^{-1}, 0.7 M). (3) Methyl tetra-*O*-methyl-d_{12}-α-D-mannopyranoside ($\mathbf{I_f}$-d_{12}) as an internal standard was dissolved into a mixture of glycerol and methanol (3/1 v/v) (1.0 ml; 0.07 M). The above three solutions were mixed: 10 μL of the solution (1) +10 μL of the solution (2) +20 μL of the solution (3). After mixing well with a vibrater, 1.5 μL of the resulted solution was deposited on a probe-tip as a sample solution for quantitative measurements on positive ion FABMS. This corresponded the molar concentration ratio of each monosaccharide (M) to the internal standard (R) to be 1:1.

In the case of octylammonium ion series, octylammonium chloride (0.12 g) was dissolved into methanol (10 mL), (0.07 M), and it was used as the solution (2) in the above series mentioned.

The probe-tip was placed in the ion source and ca. 2 min were allowed for the source vacuum to settle (5×10^{-6} Torr, 1 Torr=133.322 Pa), and the Ar atom gun was turned on. After a further 1 min, FAB spectra were recorded. At an accelerating voltage of 3 kV, the mass range m/z 200—400 was scanned in 3 s scan cycle time. Thirty successive spectra of each sample solution were acquired and the 10 scans between 10 and 20 were summed to get the quantitative spectra for the family **I** or the 10 scans between 15 to 25 for the family **II**. The relative abundance at the required m/z values was measured and averaged over the 3 runs, and

compared with the data of other monosaccharides after normalization to the constant abundance of the internal standard (R).[20]

MNDO Calculations. Calculations of geometries and energies for the $(M+A)^+$ ion composed of $\mathbf{I_h}$ (or $\mathbf{I_g}$, $\mathbf{I_f}$) and Li^+ were carried out on a FACOM S3500 superminicomputer (ANCHOR in TASMAC system)[43] by using MNDO molecular orbital methods.[49] The geometries and energies were obtained from the standard DFP optimization procedures. For example, for obtaining an initial geometry of $\mathbf{I_h}$, crystal structure data of underivatized α-D-talose were employed.[50]

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