Pergamon

Tetrahedron, Vol. 52, No. 27, pp. 9017-9024, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00451-6

Coordination of Alkali Metal Cation to Oxygen Functions to Form Adduct Ion in Fast Atom Bombardment Mass Spectrometry

**Naoko Morisaki, Kazuhiko Inoue, Hisayoshi Kobayashi, Ryuichi Shirai,
Masuo Morisaki[‡] and Shigeo Iwasaki***

Institute of Molecular and Cellular Biosciences
The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Kyoritsu College of Pharmacy, Minato-ku, Tokyo 105, Japan[‡]

Abstract: Coordination of an alkali metal cation (A) to two proximate oxygens in a molecule (molecular weight, M) affords a prominent $[M+A]^+$ ion peak in fast atom bombardment mass spectrometry. Relative affinities of various diols and other O-containing compounds for Na^+ were determined from the intensities of the $[M+Na]^+$ ions relative to that of 1,2-hexadecanediol (**1**). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

One of the most important uses of mass spectrometry in organic chemistry is the determination of molecular weight (M). Some organic compounds, however, show only a very weak or no molecular ion peak, even in so-called soft ionizing fast atom bombardment (FAB) mass spectrometry.[1)] In some cases, addition of alkali metal ions induces an adduct ion peak $[M+A]^+$, where A is the atomic weight of the alkali metal cation, and this peak has often been used for molecular weight determination of sugars, peptides and other organic compounds in FAB and other ionization mass spectrometry.[2)]

Formation of stable complexes between polyoxygenated compounds and alkali metal cations has been reported with crown ethers and some other poly ethers,[3)] and sugars.[4)] Other polyfunctional compounds, such as peptides, are also known to form such complexes under FAB conditions. However, the structural requirements for such adduct ion formation as well as the location of the metal remain unclear.[5)]

Recently, we observed that the FAB mass spectrum of fusarielin A (**20**), an antifungal antibiotic from *Fusarium* sp. K432,[6)] showed only a weak pseudo-molecular ion peak $[M-H]^+$ at *m/z* 401 (**Figure 1-A**), but on addition of NaCl, gave an intense $[M+Na]^+$ ion peak at *m/z* 425. **Figure 1-B** shows the FAB mass spectrum of **20** with addition of 0.2 equivalents of NaCl. The intensity of the $[M+Na]^+$ ion peak increased with the amount of NaCl added. Since **20** has a 1,3-diol moiety, we examined the formation of $[M+Na]^+$ ion peak with a variety of diols and related compounds. This report describes the nature of the oxygen functional groups required for stabilization of the $[M+Na]^+$ ion in FAB mass spectrometry.

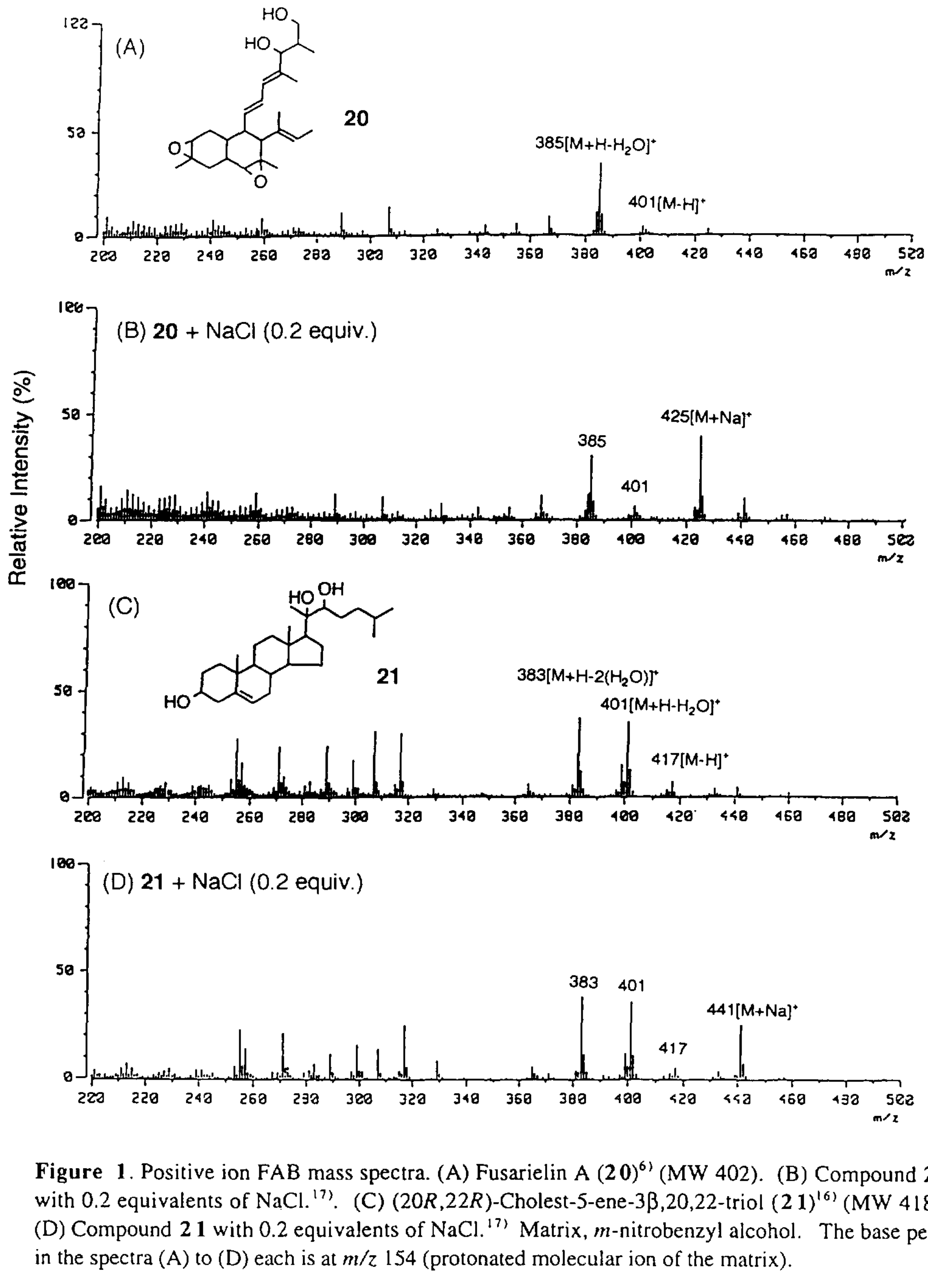


Figure 1. Positive ion FAB mass spectra. (A) Fusarielin A (**20**)[6] (MW 402). (B) Compound **20** with 0.2 equivalents of NaCl.[17]. (C) (20*R*,22*R*)-Cholest-5-ene-3β,20,22-triol (**21**)[16] (MW 418). (D) Compound **21** with 0.2 equivalents of NaCl.[17] Matrix, *m*-nitrobenzyl alcohol. The base peak in the spectra (A) to (D) each is at *m/z* 154 (protonated molecular ion of the matrix).

EXPERIMENTAL

Instrumentation and sample preparation

FAB mass spectra were recorded on a JEOL JMS-HX110 double-focusing mass spectrometer of EBE arrangement with a JMS-DA5000 or DA7000 data system. Ion acceleration voltage was 10 kV, and the fast-atom xenon gas was accelerated at a voltage of 3 kV. Collision-activated dissociation (CAD) spectra were obtained with helium collision gas, which was metered to cause about 20% attenuation of the main beam.7) *m*-Nitrobenzyl alcohol (*m*NBA) was used as the matrix, and LiCl, NaCl and KCl were used as alkali metal cation sources.

Sample solutions for the FAB mass analyses containing 0.2 equivalents of NaCl (**Figure 1-B**, **1-D** and **Figure 2-B**) were prepared by mixing 5 μl each of 0.1 M sample compound in $CHCl_3$-CH_3OH (1:1), 0.02 M NaCl in H_2O-CH_3OH (1:9) and *m*NBA. Solutions for experiments to obtain the relative intensities of sodium adduct ions shown in **Figure 3** were prepared by mixing 5 μl each of 0.1 M sample compound and 0.1 M hexadecane-1,2-diol (**1**), 10 μl of 0.02 M NaCl, and 10 μl of *m*NBA. For the analyses of the relative intensities of Li^+, Na^+ and K^+ adduct ions of the diols listed in **Table 1**, 5 μl each of 0.1 M sample compound, a salt solution prepared by mixing equal volumes of 0.02 M LiCl, NaCl and KCl, and *m*NBA were combined. An aliquot of the mixture was applied to the target tip for FAB mass analyses. FAB mass spectra were obtained by means of a 10 second scan from *m/z* 100 to 1500. The intensity of the Na^+ adduct ion was essentially constant for several minutes. Therefore, three values, at 20, 30 and 40 sec from the start of the analysis, were averaged. Each sample was measured at least twice.

Materials

The structures of the compounds tested are listed in **Figure 3**. The diols **1-4** were purchased from Aldrich Chemical Co., Inc. The diols **5** and **6** were obtained by $LiAlH_4$ reduction of β-hydroxydodecanoic acid (a gift from Dr. A. Kawaguchi of The University of Tokyo) and undecanoic γ-lactone (Aldrich Chemical Co., Inc.), respectively. The diol **7**, stearyl alcohol (**12**), 3β-cholestanol (**19**) and *m*NBA were purchased from Tokyo Kasei Kogyo Co., Ltd. Steroidal diols 5α-cholestane-1α,3α-diol (**15**)8), 5α-cholestane-2β,3α-diol (**16**)9) and 5α-cholestane-1α,3β-diol (**17**)8,10) were prepared by the cited methods. ^1H NMR ($CDCl_3$) of **15**: δ 0.63 (3H, s), 0.73 (3H, s), 0.84 (6H, d, J =7.0 Hz), 0.88 (3H, d, J = 7.0 Hz), 3.72 (1H, m), 4.06 (1H, m). **16**: δ 0.64 (3H, s), 0.86 (3H, d, J = 7.0 Hz), 0.87 (3H, d, J = 7.0 Hz), 0.90 (3H, d, J = 7.0 Hz), 0.99 (3H, s), 1.33 (1H, ddd, J = 14.0, 3.0, 3.0 Hz, $H_{4\alpha}$), 1.49 (1H, dd, J = 14.5, 3.5 Hz, H_1), 1.72 (1H, dd, J = 14.5, 2.5 Hz, H_1), 1.88 (1H, ddd, J = 14.0, 14.0, 3.0 Hz, $H_{4\beta}$), 3.85 (1H, m, $H_{3\beta}$), 3.89 (1H, m, $H_{2\alpha}$). Compounds **8**, **9**, and **14** were prepared from the diol **1**, i.e., by treatment of **1** with methylsulfinyl carbanion followed by methylation with methyl iodide;11) by acetylation of the primary hydroxyl group of **1** followed by Swern oxidation,12) then hydrolysis of the acetyl group with K_2CO_3; by reaction of **1** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid. The epoxide **10** was derived from 2-hexadecenoic acid (Tokyo Kasei Kogyo Co., Ltd.) by esterification with ethanol and HCl, followed by diisobutylaluminum hydride reduction, and then oxidation with *m*-chloroperbenzoic acid. The acetate **11** was obtained by acetylation of **12** with acetic anhydride in pyridine. The ether **18** was prepared from **19** by the reaction of 3,4-dihydro-2*H*-pyran in the presence of *p*-toluenesulfonic acid. The ether **13** was prepared by treatment of decanol with octyl iodide in the presence of NaH. Commercial products were used without further purification. All compounds prepared were characterized by ^{1}H NMR (JEOL JNM A-500 NMR spectrometer, 500 MHz), FAB and/or electron impact mass spectrometry.

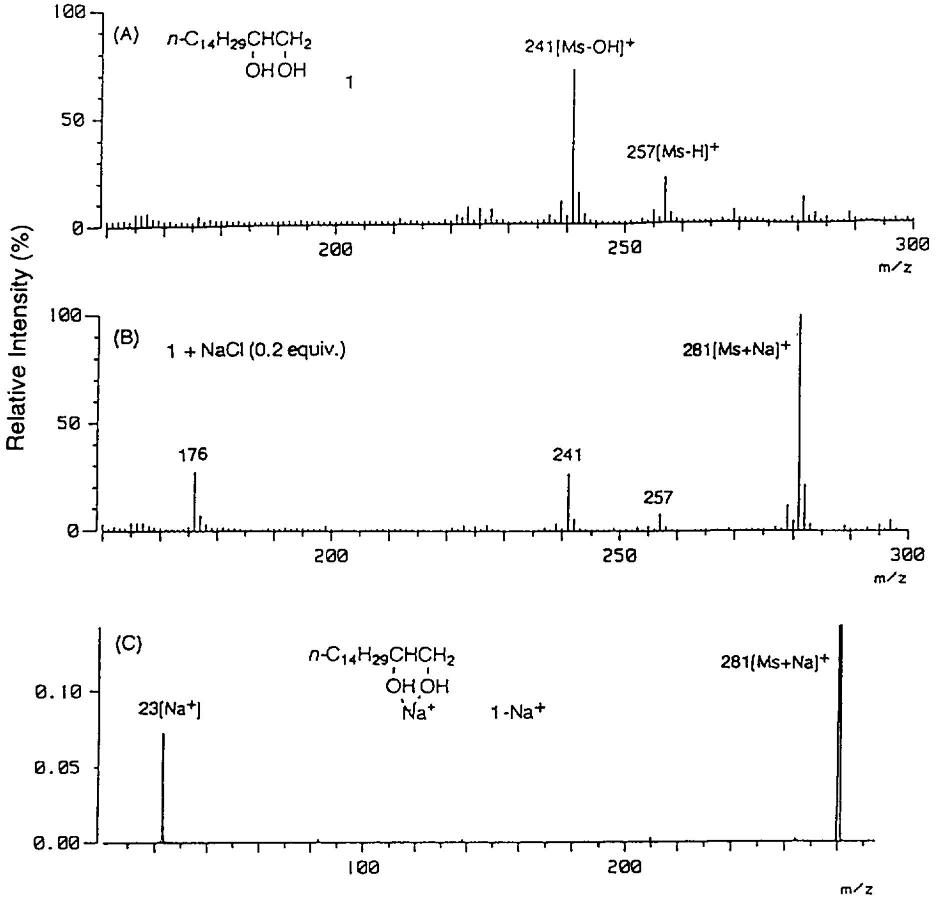


Figure 2. (A) Positive ion FAB mass spectra of 1,2-hexadecanediol (**1**) (MW 258: Ms). Commercial **1** contains about 0.02 equivalents of Na$^+$. The base peak is at *m/z* 154 (Protonated molecular ion of the matrix) (B) Positive ion FAB mass spectrum of **1** with 0.2 equivalents of NaCl.[17] The peak at *m/z* 176 represents the Na$^+$ adduct ion of the matrix. (C) Collision-activated dissociation (CAD) spectrum of the Na$^+$ adduct ion of **1**, *m/z* 281. Matrix, *m*-nitrobenzyl alcohol.

RESULTS AND DISCUSSION

The positive ion FAB mass spectrum of fusarielin A (**20**)[6] (molecular weight 402) showed a dehydroxylated ion peak $[M+H-H_2O]^+$ at *m/z* 385 and a weak pseudo-molecular ion peak $[M-H]^+$ at *m/z* 401 (**Figure 1-A**). On addition of NaCl, an intense sodium adduct ion peak $[M+Na]^+$ appeared at *m/z* 425 (**Figure 1-B**). From these data, the molecular weight of **20** was concluded to be 402. Fusarielin A homologs having a 1,3-dihydroxyl or a 1,3-ketol group also formed intense $[M+Na]^+$ ions in the FAB mass spectra.

Similarly, the FAB mass spectra of cholest-5-ene-2β,3β-diol,(13) (20*R*,22*R*)-cholest-5-ene-3β,20,22-triol (**21**)(14) and 5α-cholestane-3β-benzoyloxy-14α,15α-diol,(15) in which two hydroxy groups are sterically close to each other, each showed a very weak pseudo-molecular ion peak $[M-H]^+$ relative to the dehydroxylated and dehydrated ion peaks, $[M+H-H_2O]^+$ and $[M+H-2(H_2O)]^+$. However, they each exhibited an intense $[M+Na]^+$ peak on addition of NaCl (**Figure 1-C, 1-D**). Other diols such as cholest-5-ene-1α,3β-diol,(16) 5α-cholestane-3β,6α-diol(10) and 5α-cholestane-3β,6β-diol,(16) whose hydroxyl groups are further apart, showed only a weak $[M+Na]^+$ peak. These results suggested that coordination of two oxygen atoms to Na^+ contributes to the stabilization of the $[M+Na]^+$ complex. In order to generalize the structural requirements for the coordination, the relative intensities of the adduct ion peaks of various diols and related O-containing compounds were examined.

The FAB mass spectrum of hexadecane-1,2-diol (**1**), taken as a standard simple diol, is shown in **Figure 2-A**. The diol **1**, molecular weight 258 (Ms), showed a strong dehydroxylated ion $[Ms+H-H_2O]^+$ peak at *m/z* 241 and a weak $[Ms-H]^+$ ion peak at *m/z* 257. Upon addition of NaCl, **1** formed an intense Na^+ adduct ion $[Ms+Na]^+$ (*m/z* 281: **Figure 2-B**).

The stability of the $[Ms+Na]^+$ ion was shown by the CAD spectrum of $[Ms+Na]^+$ of **1** (**Figure 2-C**). The spectrum gave only the Na^+ ion peak without showing the dehydroxylated ion peak. This implies that Na^+ stabilizes the diol by coordination and prevents fragmentation to the $[Ms+H-H_2O]^+$ ion peak.

The intensity of the $[Ms+Na]^+$ ion peak of the diol **1** linearly increased with the amount of NaCl added, from 0.04 to 1.0 equivalents. Therefore, for the analyses of the intensities of Na^+ adduct ions, the amount of NaCl was determined to be 0.2 equivalents with respect to a sample.(17)

FAB mass spectra of a variety of mono- and dioxygenated compounds were measured in *m*NBA solution containing a sample compound, the standard diol **1** and NaCl in a molar ratio of 1:1:0.4. Relative intensities of Na^+ adduct ion ($[M+Na]^+$) peaks are summarized in **Figure 3**, where the intensity values are given relative to that of the diol **1** for oxygenated *n*-alkanes and the diol **15** for steroids. The relative value of the steroidal diol **15** to the alkyl diol **1** was 0.7. These values are considered to reflect, at least in part, the stability of the Na^+ adducts formed under FAB conditions.

These results indicated that compounds having two neighboring oxygen functions (**1-6**, **8-11**, **15-18**) can form stable Na^+ ion adducts, whereas alcohols and an ether with one oxygen function (**12**, **13**, **19**) did not show stable $[M+Na]^+$ ion peaks.(18) Comparison of the dihydroxyalkanes **1-7** indicated that the 1,2-diols **1-4** and the 1,3-diol **5** formed stable $[M+Na]^+$ ions and the 1,4-diol **6** also gave a stable ion, while the 1,10-diol **7** did not give a stable Na^+ ion adduct, behaving like the monohydroxy compounds **12** and **19**. Steroidal diols **15** and **16**, in which two hydroxy groups are spatially located so that they can coordinate to Na^+, showed $[M+Na]^+$ ion peaks. However, that of **16** was weaker, possibly because only the less stable, and therefore less populated, boat form of the A ring would contribute to coordination. Compound **17**, as mentioned above, gave only a weak peak. No Na^+ adduct ion peak of the dimethylacetal **14** was detected in the FAB mass spectrum upon addition of NaCl. The reason for this remains unknown, but similar results have been obtained with many different compounds having a dimethyl acetal moiety.

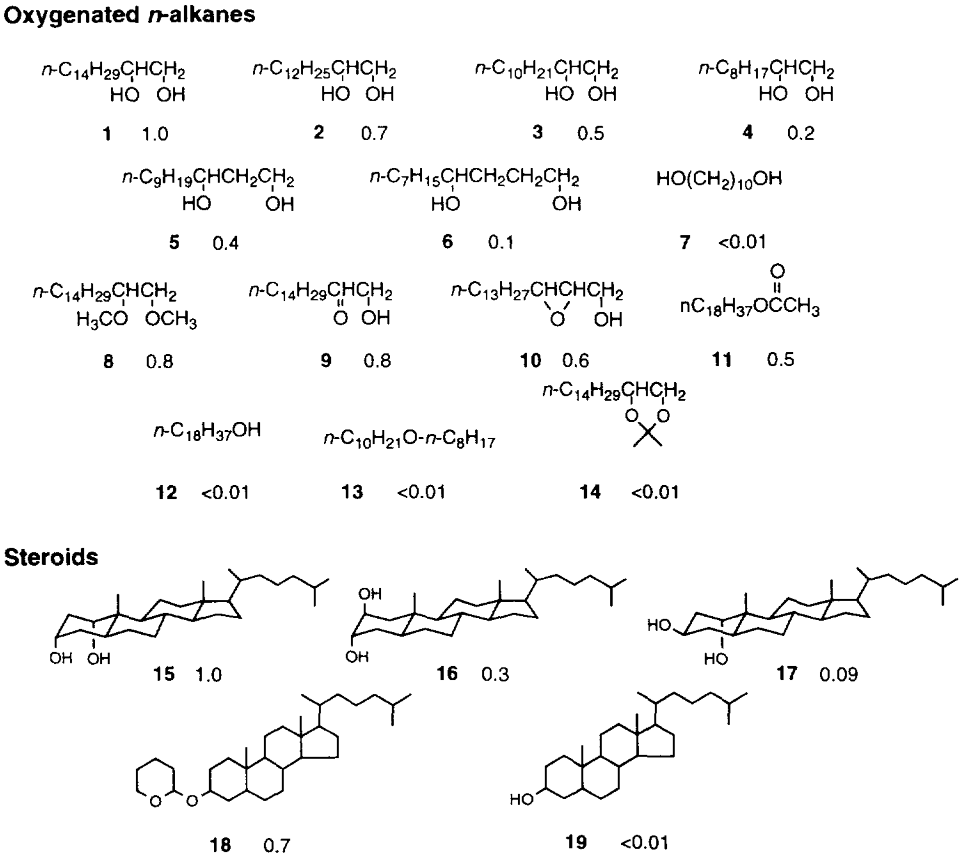
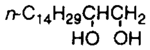
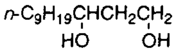
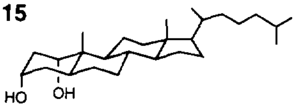
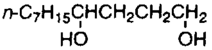


Figure 3. Relative intensities of sodium adduct ions in FAB mass spectrometry. The intensity values are given relative to that of the diol **1** for oxygenated *n*-alkanes and that of the diol **15** for steroids. Relative intensity ($[M+Na]^+/[Ms+Na]^+$) was obtained by the analysis of a mixture of a compound (molecular weight: M), the diol **1** (molecular weight: Ms) and NaCl (1:1:0.4). Matrix: *m*-nitrobenzyl alcohol. The relative value of **15** to **1** is 0.7. For details of sample preparation and measurement, see **EXPERIMENTAL**.

Binding abilities of alkali metal cations to sugars,[19,20)] peptides,[5b)] cyclic ethers[3c)] and other organic compounds[21,22)] have been extensively investigated. In general, the affinity of alkali metal cations decreases in the order $Li^+ > Na^+ > K^+$,[22-24)] except for cyclic poly ethers, whose binding selectivity depends on the ring size.[3a,c,g,i)] To examine the relative binding abilities of diols to Li^+, Na^+ and K^+, FAB mass spectra of diols were taken in *m*NBA solution containing the diol, LiCl, NaCl and KCl in a molar ratio of 15:1:1:1, and the relative intensities of $[M+Li]^+$, $[M+Na]^+$ and $[M+K]^+$ were compared. The results are listed in **Table 1**. As expected, the order of affinity was, in general, $Li^+ > Na^+ > K^+$. It was found that the smaller Li^+ ion coordinates more favorably to the aliphatic 1,3-diol **5** and the steroidal 1,3-diaxial diol **15** than to the 1,2-diol **1** and the 1,4-diol **6**, whereas larger K^+ binds more favorably to the 1,4-diol **6** than to the 1,2- and the 1,3-diols (**1**, **5** and **15**).

Leary and Pedersen have reported on Li^+ adduct ions of vicinal diols in FAB mass spectrometry.[25)] We have shown that not just vicinal diols, but in principle all compounds with two appropriately arranged oxygen functions form $[M+A]^+$ ions.

Table 1. Relative intensities of alkali metal adduct ions of diols in FAB mass spectrometry[a]

Compound	Li^+ adduct ion $[M+Li]^+/[M+Na]^+$	Na^+ adduct ion	K^+ adduct ion $[M+K]^+/[M+Na]^+$
1  $n\text{-}C_{14}H_{29}\underset{HO}{CH}\underset{OH}{CH_2}$	1.7	1.0	0.13
5  $n\text{-}C_9H_{19}\underset{HO}{CH}CH_2\underset{OH}{CH_2}$	6.4	1.0	0.11
15 	5.4	1.0	0.10
6  $n\text{-}C_7H_{15}\underset{HO}{CH}CH_2CH_2\underset{OH}{CH_2}$	2.8	1.0	0.21

a. Each value was obtained by the analysis of a mixture of a diol (molecular weight: M), LiCl, NaCl and KCl (15:1:1:1). Matrix: *m*-nitrobenzyl alcohol. Intensities of the adduct ions are listed relative to the value of Na^+ adduct ions. For details of sample preparation and measurement, see **EXPERIMENTAL**.

In conclusion, the following results were obtained.

1. Not only poly oxygen compounds such as sugars and polyethers, but also compounds having two proximate oxygens efficiently coordinate to an alkali metal cation (A^+) and afford the adduct ion peak ($[M+A]^+$) in the FAB mass spectrum.
2. The intensity of the $[M+A]^+$ ion peak decreases as the two oxygens are further apart.
3. Affinity of Li^+, Na^+ and K^+ ions for diols is in the order of $Li^+>Na^+>K^+$.

ACKNOWLEDGEMENTS

We are grateful to Dr. Masami Sawada of Osaka University for useful discussions, and also to Dr. Akihiko Kawaguchi of The University of Tokyo for a sample of β-hydroxydodecanoic acid.

REFERENCES AND NOTES

1. (a) Barber, M.; Bordoli, R. S.; Sedgwick, R.D.; Tyler, A. N. *J. Chem. Soc. Chem. Comn.* **1981**, 325-327. (b) Williams, D. H.; Bradley, C.; Bojesen, G.; Santikarn, S.; Taylor, L. C. E. *J. Am. Chem. Soc.* **1981**, *103*, 5700-5704. (c) Pauw, E. D. *Methods in Enzymology, Vol. 193, Mass Spectrometry*; McCloskey, J. A., Ed.; Academic Press, Inc.: San Diego, **1990**; pp 201-214.
2. (a) Rinehart, K. L. Jr.; Cook, J. C. Jr.; Meng, H.; Olson, K. L.; Pandey, R. C. *Nature*, **1977**, *269*, 823-833. (b) Pramanik, B. N.; Das, P. R.; Bose, A. K. *J. Nat. Prod.*, **1989**, *52*, 534-546. (c) Siegel, M. M.; Tsao, R.; Oppenheimer, S.; Chang, T. T. *Anal. Chem.* **1990**, *62*, 322-327.
3. (a) Maleknia, S.; Brodbelt, J. *J. Am. Chem. Soc.* **1992**, *114*, 4295-4298. (b) Zhang, H.; Chu, I-H.; Leming, S; Dearden, D. V. *ibid.*, **1991**, *113*, 7415-7417. (c) Johnstone, R. A. W.; Rose, M. E. *J. Chem. Soc., Chem. Comun.* **1983**, 1268-1270. (d) Johnstone, R. A. W.; Lewis, I. A. S.; Rose, M. E.

Tetrahedron, **1983**, *39*, 1597-1603. (e) Takahashi, T.; Uchiyama, A.; Yamada, K.; Lynn, B. C.; Gokel, G. W. *Tetrahedron Letters*, **1992**, *33*, 3825-3828. (f) Lattimer, R. P. *J. Am. Soc. Mass Spectrom.* 1992, 3, 225-234. (g) Takayama, M.; Fukai, T.; Nomura, T.; Yamauchi, T. *Org. Mass Spectrom.* **1991**, *26*, 655-659. (h) Zhang, H.; Dearden, D. V. *J. Am. Chem. Soc.*, **1992**, *114*, 2754-2755. (i) Chu, I-H.; Zhang, H.; Dearden, D. V. *ibid.*, **1993**, *115*, 5736-5744.

4. (a) Sawada, M.; Ouyang, L.; Takai, Y.; Yamada, H.; Hanafusa, T. *Chemistry Letters*, **1989**, 1743-1746. (b) Zhou, Z.; Ogden, S.; Leary, J. A. *J. Org. Chem.* **1990**, *55*, 5444-5446. (c) Orlando, R.; Bush, C. A.; Fenselau, C. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 747-754. (d) Sawada, M.; Ouyang, L.; Takai, Y.; Yamada, H.; Hanafusa, T.; Kinoshita, T.; Mochizuki, T. *ChemistryLetters*, **1990**, 1361-1363. (e) Sawada, M.; Shizuma, M.; Takai, Y.; Yamada, H.; Tanaka, T.; Okumura, Y.; Hidaka, Y.; Hanafusa, T.; Takahashi, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*,1275-1279.
5. (a) Teesch, L. M.; Adams, J. *Org. Mass Spectrom.* **1992**, *27*, 931-943. (b) Teesch, L. M.; Orlando, R. C.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 3668-3675. (c) Tang, X.; Ens, W.; Standing, K.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791-1799. (d) Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835-2842. (e) Grese, R. P.; Gross, M. L. *ibid.* **1990**, *112*, 5098-5104. (f) Hofmeister, G. E.; Zhou, Z.; Leay, J. A. *ibid.* **1991**, *113*, 5964-5970. (g) Staempfli, A.; Zhou, Z.; Leary, J. A. *J. Org. Chem.* **1992**, *57*, 3590-3594. (h) Leary, J. A.; Zhou, Z.; Ogden, S. A.; Williams, T. D. *Am. Soc. Mass. Spectrom.*, **1990**, *1*, 473-480. (i) Summerfield, S. G.; Dale, V. C. M.; Despeyroux, D.; Jennings, K. R. *J. Am. Chem. Soc.* **1995**, *117*, 10093-10097.
6. Kobayashi, H.; Sunaga, R.; Furihata, K.; Morisaki, N.; Iwasaki, S. *J. Antibiot.* **1995**, *48*, 42-52.
7. Kinoshita, T.; Nakamura, T.; Nagaki, H. *Nippon Kagaku Kaishi* **1986**, 1665-1670.
8. Shoppee, C. W.; Roy, S. K.; Goodrich, B. S. *J. Chem. Soc.*, **1961**, 1583-1590.
9. Parrilli, M.; Adinolfi, M.; Barone, G.; Laonigro, G.; Mangoni, L. *Gazzetta Chem. Ital.* **1975**, *25*, 1301-1305.
10. Morisaki, M.; Saika, A.; Bannai, K.; Sawamura, M; Rubio-Lightbourn, J.; Ikekawa, N. *Chem. Pharm. Bull.* **1975**, *23*, 3272-3278.
11. Hakomori, S. *J. Biochem.* **1964**, *55*, 205-208.
12. Mancuso, A. J.; Huang, S-L.; Swern, D. *J. Org. Chem.*, **1978**, *43*, 2480-2482.
13. Morisaki, M.; Bannai, K.; Ikekawa, N. *Chem. Pharm. Bull.* **1976**, *24*, 1948-1949.
14. Morisaki, M.; Sato, S.; Ikekawa, N. *Chem. Pharm. Bull.* **1977**, *25*, 2576-2583.
15. Fujita, N.; Morisaki, M. unpublished data. mp. 165-168°.
16. Morisaki, M.; Bannai, K.; Ikekawa, N. *Chem. Pharm. Bull.* **1973**, *21*, 1853-1854.
17. Because *m*NBA used as the matrix also binds to Na^+, the amount of Na^+ bound to the sample compound would be smaller than the equivalents indicated. Keough, T.; *Anal. Chem.* **1985**, *57*, 2027-2034.
18. Relative intensity value of Na^+ addut ion of 5α-cholestan-3-one to that of **1** ($[M+Na]^+/[Ms+Na]^+$) was 0.9. Therefore, some contribution of a carbonyl group might be expected to the value of the ketol **9** and the ester **11**.
19. Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113* 812-820.
20. Puzo, G.; Promé, J. C. *Org. Mass Spectrom.* **1985**, *20*, 288-291.
21. Adams, J.; Gross, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 6915-6921.
22. Wong, P. S. H.; Antonio, B. J.; Dearden, D. V. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 632-637.
23. Bursey, M. M.; Marbury, G. D.; Hass, J. R. *Biomed. Mass Spectrom.* **1984**, *11*, 522-527.
24. Eichmann, E. S.; Alvarez, E.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 535-542.
25. Leary, J. A. ; Pedersen, S. F. *J. Org. Chem.* **1989**, *54*, 5650-5651.

(*Received in Japan* 21 *March* 1996; *accepted* 10 *May* 1996)