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Structure Elucidation of Furostanol Glycosides Using Liquid Secondary Ion Mass Spectrometry†

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Abstract : The structures of five genuine furostanol glycosides isolated from *Metanarthecium luteo-viride* MAXIM. (Liliaceae) were determined on the basis of liquid secondary ion mass spectrometric (LSIMS) analysis including liquid secondary ion mass spectrometry/mass spectrometry (LSIMS/MS). These glycosides were elucidated as bisdesmosides of furostanols (i.e. 2-O-acetyl-furometagenin, furometagenin, furonogiragenin, 2-O-acetyl-furometanarthogenin and 2-O-acetyl-3-oxo-furometagenin) as aglycons, which have cyclic hemiacetal moieties, bearing 2,3,4-tri-O-acetyl arabinopyranose at 11-C[1)] and glucopyranose at 26-C. In the LSIMS of these compounds, the protonated molecular ion $[M+H]^+$ was not observed but the fragment ion $[M-OH]^+$ corresponding to the loss of the hydroxyl group at 22-C was observed. By addition of NaCl to the sample matrix, the ion peaks for $[M+Na]^+$ appeared in the spectra, which were used to determine the molecular formulae. Molecular orbital calculation of a model compound indicated that the ions $[M+Na]^+$ were stabilized by formation of a four-membered ring structure bonding two oxygen atoms at the hemiacetal moiety and a sodium ion. Since the energy required for sodium ion addition to the neutral molecule was less than for proton addition, the sodium ion addition is more favorable. In addition, the repulsion energy of the protonated hemiacetal hydroxyl group is only 4.25 kcal/mol, and the potential energy of the fragmentation products ($[M-OH]^+ + H_2O$) is 18.13 kcal/mol less than that of $[M+H]^+$. These data rationalized the easy dehydration from the protonated furostanol glycoside.

INTRODUCTION

Many spirostane-type sapogenols have been elucidated from acidic hydrolysates of the whole glycosidic fraction isolated from *Metanarthecium luteo-viride* MAXIM. (nogiran in Japanese, Liliaceae).[2)] Yosioka et al. determined the structures of three spirostane-type prosapogenols,[2c,3)] four spirostane-type glycosides[4)] and two peracetylated furostane-type glycosides.[5)] However, no genuine furostane-type glycoside has been elucidated. Recently, five new glycosides (designated as FG-1 (**1**) ~ FG-5 (**5**)) were isolated from the subterranean part of this plant without any derivatization, by repeated chromatography.[6)]

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† Dedicated to Professor Dr. Carl Djerassi's seventieth birthday.

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Deceased on 14th April, 1991.

In this paper, we describe the structure of these glycosides, especially FG-2 (**2**), using liquid secondary ion mass spectrometry (LSIMS) and liquid secondary ion mass spectrometry/mass spectrometry (LSIMS/MS).

Since the mass spectrometric behavior observed in LSIMSs of furostanol glycosides was important for structure determination, we elucidated the fragmentation mechanism using molecular orbital (MO) calculations of a model compound.

RESULTS AND DISCUSSION

Five new furostanol glycosides FG-1 (**1**), FG-2 (**2**), FG-3 (**3**), FG-4 (**4**) and FG-5 (**5**) were isolated from the subterranean part of *Metanarthecium luteo-viride* MAXIM., which was collected in Nara prefecture, Japan[6]. Since FG-2 (**2**) was the most polar compound among the five glycosides, we studied mainly the mass spectrometric behavior of **2** for the structure elucidation of these glycosides.

In the LSIMS of **2** shown in Fig. 1, the highest mass-number ion peak appeared at *m/z* 869, as well as fragment ion peaks at *m/z* 707 for [***a***-OH]$^+$, *m/z* 593 for [***b***-OH]$^+$, *m/z* 431 for [***c***-OH]$^+$ and *m/z* 259 for [***d***-OH]$^+$ in the absence of NaCl. A new ion peak appeared at *m/z* 909 by the addition of NaCl to the sample matrix. The abundance of the ion of *m/z* 909 increased and that of the ion of *m/z* 869 decreased as the

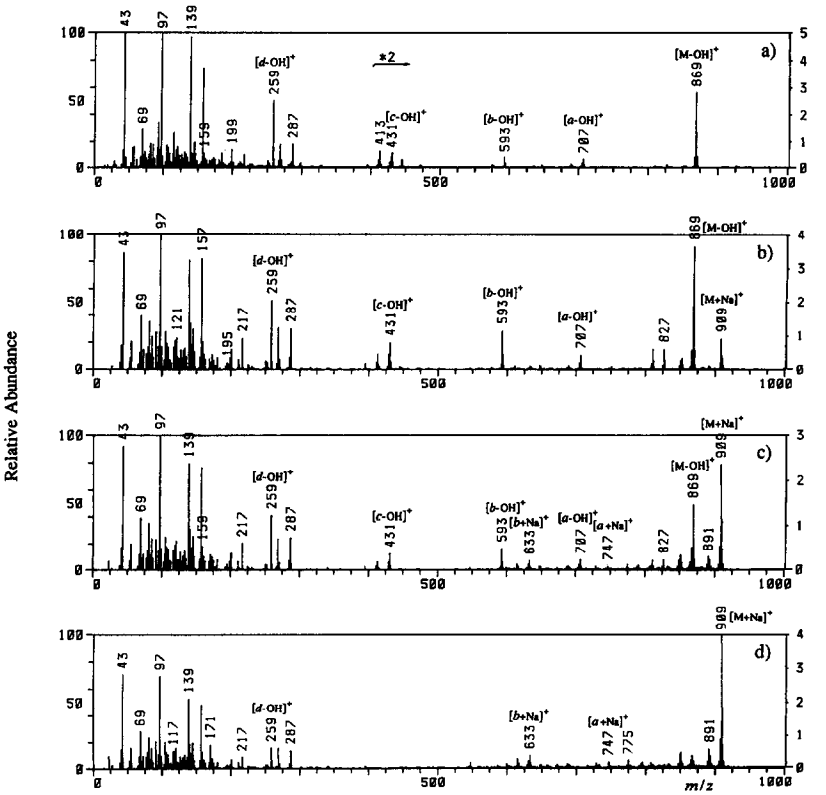


Fig. 1. LSIMS of FG-2 (**2**): a) without NaCl, b) 1 ng NaCl, c) 3 ng NaCl, d) 10 ng NaCl.

amount of NaCl increased, and the ion peak at *m/z* 869 finally disappeared. The accurate mass measurement of these two ion peaks indicated that the elemental compositions of them were $C_{44}H_{69}O_{17}$ and $C_{44}H_{70}O_{18}Na$, and they were assumed to be $[M-OH]^+$ and $[M+Na]^+$. The molecular formula of **2** was determined as $C_{44}H_{70}O_{18}$ (MW 886).

The acid hydrolysis of the prosapogenol (**6**) obtained from FG-2 (**2**) by hydrolysis with β-glucosidase in the NaOAc-HOAc buffer afforded metagenin (**7**, $C_{27}H_{44}O_5$), which was identified by comparison with a authentic sample. In the LSIMS of **6**, the ion peak at *m/z* 707 appeared as the highest mass-number ion, which was the same mass-number as the ion $[a-OH]^+$ in **2** (Fig. 2). Since the LSIMS/MSs of these ions were superimposable as described below, both ions should have the same structure. The addition of NaCl to the sample matrix made 22 mass-number shift, giving an ion peak at *m/z* 729. These ions were assumed to be $[M+H]^+$ and $[M+Na]^+$, the molecular mass of **6** was determined as 706 ($C_{38}H_{58}O_{12}$). The 180 mass-unit difference in molecular masses between **2** and **6** was corresponding to a glycosyl group (162 mass-unit) and a water molecule (18 mass-unit).

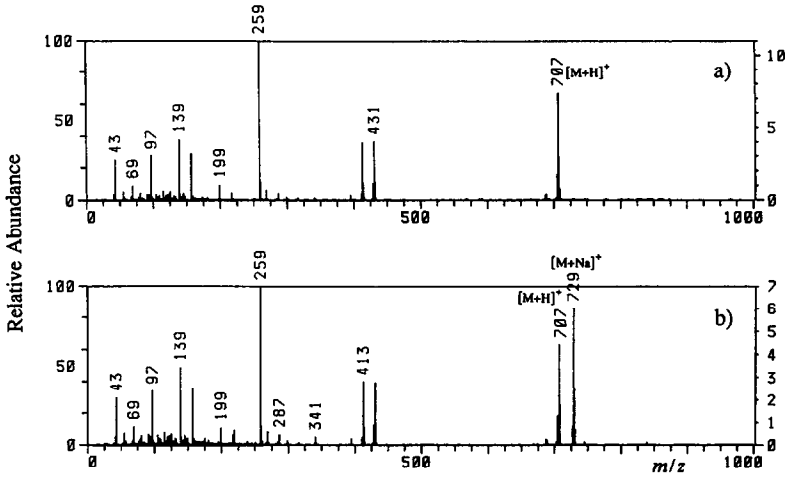


Fig. 2. LSIMS of prosapogenol (**6**): a) without NaCl, b) with 3 ng NaCl.

The molecular weight of **6** was 126 mass-units ($C_6H_6O_3$) larger than that of the known prosapogenol, 11-O-α-L-arabinopyranosyl-metagenin (**8**, $C_{32}H_{52}O_9$, MW. 580)[5]. This indicated the presence of three acetyl groups in **6**. Accurate mass measurement of the fragment ions of *m/z* 431 and 259 in the LSIMS of **6** indicated that the elemental compositions of these ions were $C_{27}H_{43}O_4$ and $C_{11}H_{15}O_7$. The former ion was assumed to be generated by the loss of 2,3,4-tri-O-acetyl-L-arabinopyranose from $[M+H]^+$, and the latter to be the 2,3,4-tri-O-acetyl-L-arabinopyranosyl ion. Consequently, the structure of FG-2 was determined as 11-O-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-26-O-β-D-glucopyranosyl-furometagenin (**2**), which was the genuine glycoside of peracetylated glycoside NF-1 (**9**).[5]

The structures of four other glycosides (FG-1 (**1**), FG-3 (**3**), FG-4 (**4**) and FG-5 (**5**)) were investigated by LSIMS analysis. In the absence of NaCl, the highest mass-number ion peaks appeared at *m/z* 911, 853, 907 and 909, respectively (Fig.3). The highest mass-number ion peaks changed to the ions at *m/z* 951, 893, 947 and 949 by the addition of NaCl, respectively (Fig. 4).

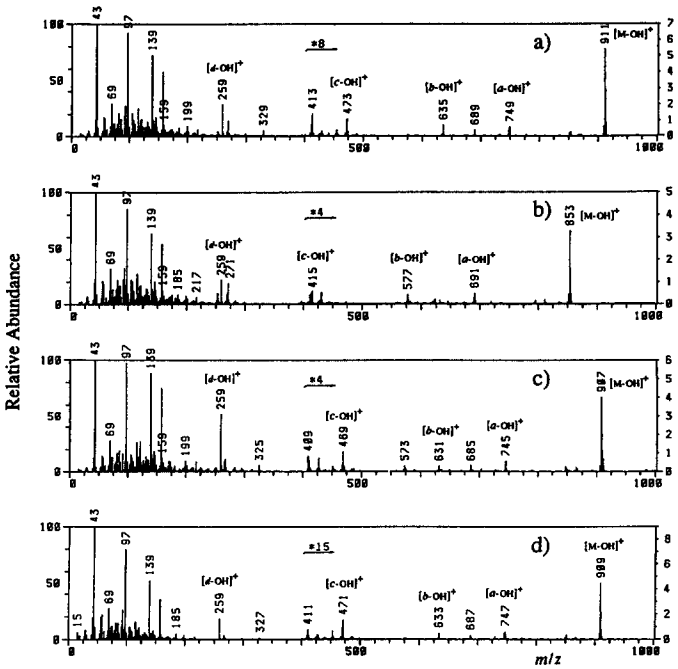


Fig. 3. LSIMS of four other glycosides without NaCl: a) FG-1 (**1**), b) FG-3 (**3**), c) FG-4 (**4**), d) FG-5 (**5**).

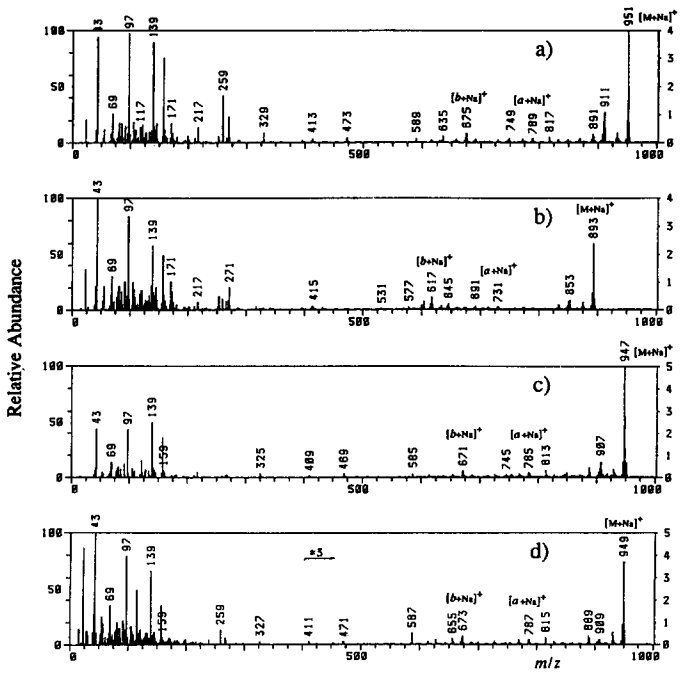
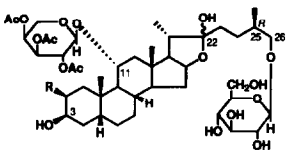


Fig. 4. LSIMS of four other glycosides with NaCl: a) FG-1 (**1**), b) FG-3 (**3**), c) FG-4 (**4**), d) FG-5 (**5**).

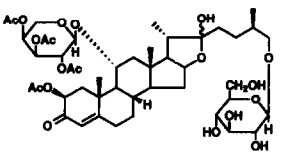
The four former ions were assumed to be $[M-OH]^+$ and the four latter ions to be $[M+Na]^+$, and the molecular formulae of these compounds were determined as $C_{46}H_{72}O_{19}$, $C_{44}H_{70}O_{17}$, $C_{46}H_{68}O_{19}$ and $C_{46}H_{70}O_{19}$, respectively. The fragment ion peaks for $[a-OH]^+$, $[b-OH]^+$ and $[c-OH]^+$ of these compounds appeared at the mass-number (m/z) with the mass-unit reductions (162, 276 and 438, respectively) from the ion $[M-OH]^+$. These reduction were observed in the LSIMS of **2**. This fact indicated that these ions were generated by the loss of the same structure portion. In addition, 2,3,4-tri-O-acetyl-L-arabinopyrosyl ion for $[d-OH]^+$ was observed at the same m/z 259 in each LSIMS of four glycosides.

In the LSIMS of each prosapogenol given by the enzymatic hydrolysis of four glycosides (**1**, **3**, **4**, and **5**), the protonated molecular ion $[M+H]^+$ appeared at the same mass-number as the ion peak for $[a-OH]^+$ of each glycoside. The fragment ion peaks for $[c-OH]^+$ and $[d-OH]^+$ also observed.

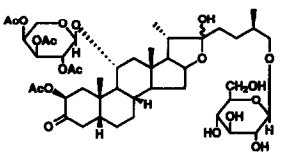
Glycosides FG-1 (**1**), FG-3 (**3**), FG-4 (**4**), and FG-5 (**5**) had the same sugar moiety bonded at the same position, but differed in aglycon structure. Considering the sapogenols obtained by acid hydrolysis of the glycosides, we concluded that their structures were bisdesmosides, in which 2,3,4-tri-O-acetyl-α-L-arabinopyranose was substituted at 11-C and β-D-glucopyranose was substituted at 26-C of the aglycons (2-O-acetyl-furometagenin for **1**, furonogiragenin for **3**, 2-O-acetyl-furometanarthogenin for **4** and 2-O-acetyl-3-oxo-furometagenin for **5**) (Fig. 5).



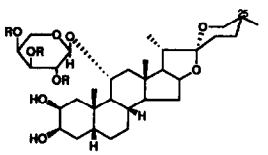
FG-1 (1) : R=OAc $C_{46}H_{72}O_{19}$ (928)
FG-2 (2) : R=OH $C_{44}H_{70}O_{18}$ (886)
FG-3 (3) : R=H $C_{44}H_{70}O_{17}$ (870)



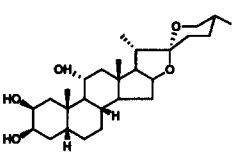
FG-4 (4) : $C_{46}H_{68}O_{19}$ (924)



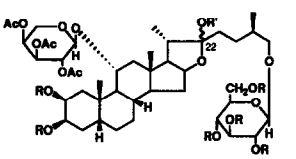
FG-5 (5) : $C_{46}H_{70}O_{19}$ (926)



6 : R=Ac $C_{38}H_{58}O_{12}$ (706)
8 : R=H $C_{32}H_{52}O_9$ (580)



7 : $C_{27}H_{44}O_5$ (448)



9 : R=Ac, R'=Me $C_{57}H_{84}O_{24}$ (1152)
10 : R=H , R'=Me $C_{45}H_{72}O_{18}$ (900)
11 : R=Ac, R'=H $C_{56}H_{82}O_{24}$ (1138)

Fig. 5. Structures of five genuine furostanol glycosides (**1**~**5**) and the related compounds (**6**~**11**).

Mass spectral fragmentation of genuine glycosides

Whereas fragment ions $[M-OH]^+$ were observed as the highest mass-number ion peaks in the LSIMS of five furostanol glycosides, prominent ions $[M+Na]^+$ appeared in the spectra of these compounds with NaCl in the matrix. In the mass spectra of their spirostane-type sapogenols and prosapogenols without hemiacetal hydroxyl groups, protonated molecular ions $[M+H]^+$ were evident. Thus the furostanol glycosides were assumed to give $[M-OH]^+$ by the loss of hydroxyl group from the hemiacetal moiety.

As shown in the TLC/LSIMS[7] (Fig. 6a) of an equilibrium mixture of 22-O-methylated FG-2 (**10**) and FG-2 (**2**) which was obtained by treating **2** with methanol, **10** gave the ion peak corresponding to $[M-OMe]^+$ at m/z 869, and that corresponding to $[M+Na]^+$ at m/z 923 in the presence of NaCl. In the TLC/

LSIMS of 22-O-desmethyl derivative of NF-1 (**11**) obtained by treating **9** with water as an equilibrium mixture, the ion peaks appeared at *m/z* 1121 and 1161, corresponding to $[M-OH]^+$ and $[M+Na]^+$, respectively (Fig. 6b). Thus, we confirmed that a hydroxyl group (methoxyl group in **10**) was eliminated from the 22-hemiacetal moiety.

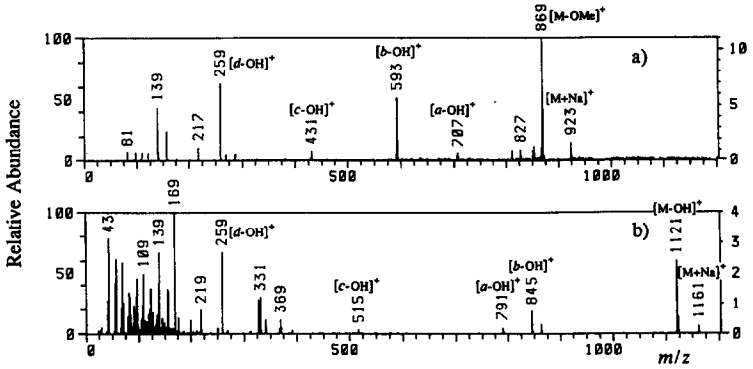


Fig. 6. TLC/LSIMS : a) 22-O-methylated FG-2 (**10**), b) 22-O-desmethylated NF-1 (**11**).

The LSIMS/MSs described below afforded information about many characteristic fragmentation pathways of sugar group elimination. The fragmentation pathways from the ions $[M+Na]^+$ and $[M-OH]^+$ of **2** were investigated by LSIMS/MS measurements as shown in Fig 7.

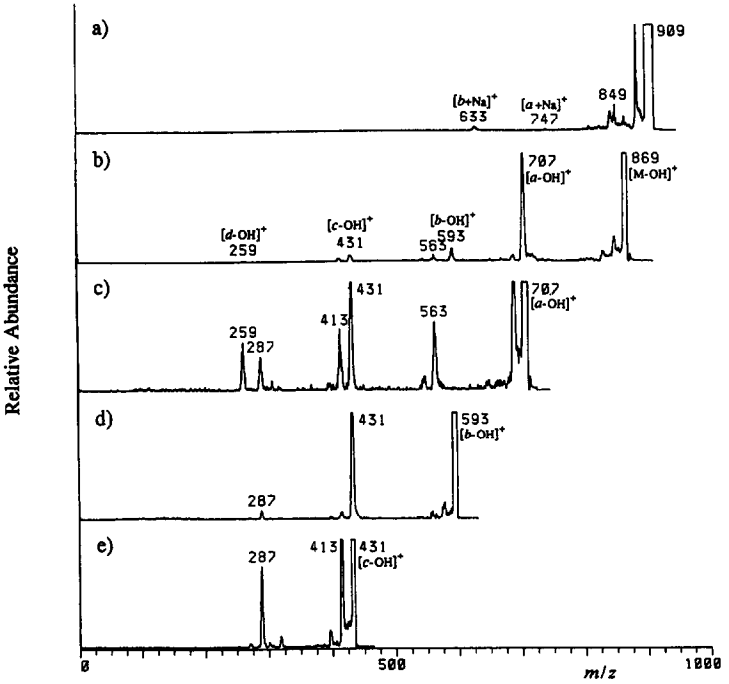


Fig. 7. LSIMS/MS of **2**: a) $[M+Na]^+$, b) $[M-OH]^+$, c) $[a\text{-}OH]^+$, d) $[b\text{-}OH]^+$, e) $[c\text{-}OH]^+$.

In the LSIMS/MS of $[M+Na]^+$, the fragment ions $[a+Na]^+$ and $[b+Na]^+$ appeared as small peaks which indicated that $[M+Na]^+$ was stable and that the fragmentation pathways giving these ions were less favorable. The ion $[a\text{-}OH]^+$ generated by elimination of glucosyl group, the ion $[b\text{-}OH]^+$ generated by the loss of acetyl arabinose, the ion $[c\text{-}OH]^+$ generated by elimination of glucosyl group and acetyl arabinose from $[M\text{-}OH]^+$ and the acetyl arabinosyl ion $[d\text{-}OH]^+$, which were observed in the LSIMS/MS of $[M\text{-}OH]^+$. The ions $[c\text{-}OH]^+$ and $[d\text{-}OH]^+$ appeared in the LSIMS/MS of the ion $[a\text{-}OH]^+$, and a pathway of the ion $[c\text{-}OH]^+$ from the ion $[b\text{-}OH]^+$ was observed. The mass difference of 276 between the ion of *m/z* 563 in LSIMS/MS of ions $[M\text{-}OH]^+$ and $[a\text{-}OH]^+$ and the ion of *m/z* 287 in those of ions $[b\text{-}OH]^+$ and $[c\text{-}OH]^+$, corresponded to acetyl arabinose. These ions were assignable to the ions generated by the fission in the ring E of the aglycon moiety.

As shown in Fig. 8, both LSIMS/MSs of the ion $[M\text{-}OH]^+$ of **2** and the ion $[M\text{-}OMe]^+$ of **10** were

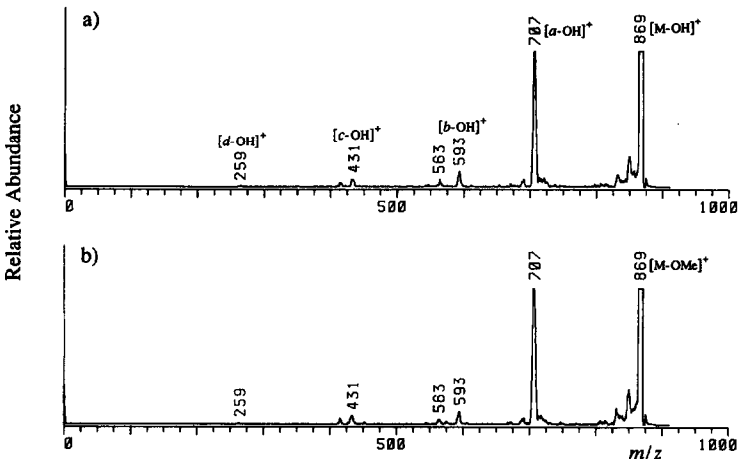


Fig. 8. Identification of ion structures by LSIMS/MS: a) $[M\text{-}OH]^+$ of **2**, b) $[M\text{-}OMe]^+$ of **10**.

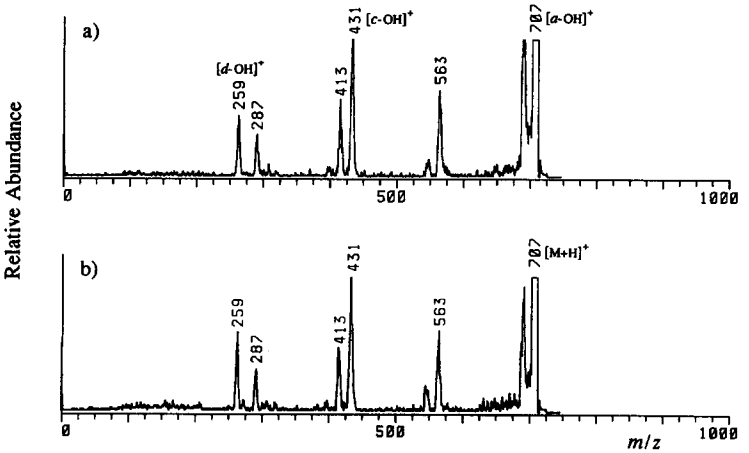
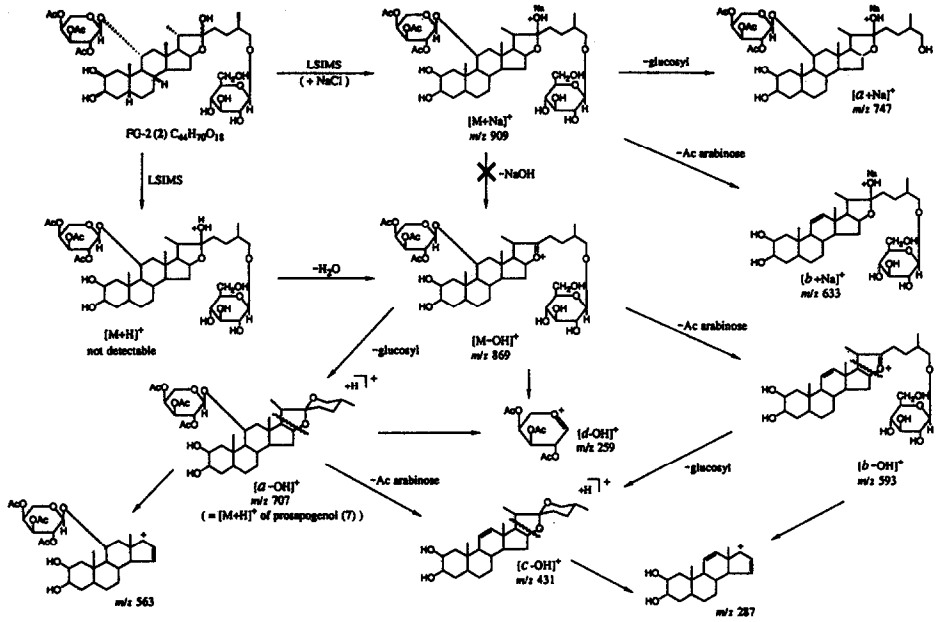


Fig. 9. Identification of ion structures by LSIMS/MS: a) $[a\text{-}OH]^+$ of **2**, b) $[M+H]^+$ of **6**.

superimposable, indicating that these ions have the same structure. The structure of the ion $[a\text{-}OH]^+$ of **2** was also determined by comparing the LSIMS/MS of the ion $[a\text{-}OH]^+$ with that of the ion $[M+H]^+$ of **6** (Fig. 9). On the basis of the LSIMS/MS shown above, the fragmentation pathway of the furostanol glycosides was determined as Scheme 1.



Scheme 1.

MO calculation for the fragmentation of $[M\text{-}OH]^+$

Since the LSIMS behavior, especially the presence of the ion $[M\text{-}OH]^+$ instead of $[M+H]^+$ was interesting and important for structure determination, we elucidated the fragmentation mechanism.

The hemiacetal hydroxyl on the ring E played an important role in this behavior. The molecular orbital (MO) calculations of a model compound[8] shown in Fig. 10 related to rings D and E of the furostanol glycosides were carried out. The optimized geometry of each molecular species was calculated by the modified neglect of diatomic overlap (MNDO) calculation[9], a semiempirical MO calculation.

The heat of formation of the optimized geometry of the neutral molecule was -116.06 kcal/mol, and net atomic charges localized at 5-O and 17-O were -0.3414 and -0.3366, respectively. The bond length of 6-C~17-O (1.4028 Å) was slightly smaller than those of 4-C~5-O (1.4052Å) and 5-O~6-C (1.4173 Å).

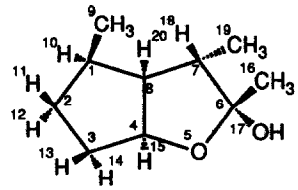


Fig. 10. Structure of model compound used for MO calculation.

For the MO calculation of the ions generated by Cs^+ bombardment, we hypothetically set the molecular ion $[M]^{+\cdot}$, the protonated molecular ion $[M+H]^+$ and the dehydroxy molecular ion $[M-OH]^+$. The heats of formation of the optimized geometries of these three ions were discussed. Since the proton was could be located at 5-O or 17-O in the protonated molecular ion $[M+H]^+$, and there were some stereoisomers with regard to an orientation of the proton, the most energetically stable isomer was explored.

The heats of formation for the ions $M^{+\cdot}$, $[M+H_{(5\text{-}O)}]^+$ [10], $[M+H_{(17\text{-}O)}]^+$ and $[M-OH]^+$ were 110.18, 62.85, 64.83 and 112.94 kcal/mol, respectively. Consequently, it was assumed that the ions $M^{+\cdot}$ and $[M-OH]^+$ were not directly generated by Cs^+ bombardment. Although the ion $[M+H_{(5\text{-}O)}]^+$ was energetically more stable than the other, the difference was only 1.98 kcal/mol and the dehydration from the ion $[M+H_{(5\text{-}O)}]^+$ was more unfavorable as described below. The ion $[M+H_{(17\text{-}O)}]^+$ would be more likely to participate.

The bond lengths of 4-C~5-O, 5-O~6-C and 6-C~17-O in the ion $[M+H_{(5\text{-}O)}]^+$ were 1.4606, 1.5204 and 1.3715 Å, respectively. The two former values were larger and the latter was smaller than in the neutral molecule, thus bond cleavage of the 6-C~17-O would be unfavorable. The ion $[M+H_{(5\text{-}O)}]^+$ would not be a precursor ion of the ion $[M-OH]^+$. On the other hand, the bond lengths for the ion $[M+H_{(17\text{-}O)}]^+$ were 1.4272, 1.3681, and 1.5384 Å, respectively, thus the bond length 6-C~17-O possessed a larger value than the other, and hence the simple cleavage of the bond 6-C~17-O that occurred in the ion $[M+H_{(17\text{-}O)}]^+$ would generate the ion $[M-OH]^+$. The variation in the potential energy for the elimination of H_2O from the ion $[M+H_{(17\text{-}O)}]^+$ was calculated using the length of the bond 6-C~17-O as the reaction coordinate.

As shown in Fig. 11, the activation energy for the bond cleavage was only 4.25 kcal/mol, and the potential energy of the fragmentation products ($[M-OH]^+ + H_2O$) was 18.13 kcal/mol less than that of the ion $[M+H_{(17\text{-}O)}]^+$. Thus, the ion $[M-OH]^+$ was clearly shown to be the stable ion. From this result, the generation of the ion $[M-OH]^+$ by eliminating a water molecule from the ion $[M+H]^+$ could proceed using the excess internal energy which had been accumulated by Cs^+ bombardment. From the above description, the ion $[M+H]^+$ should not be observed in the LSIMS of the glycosides.

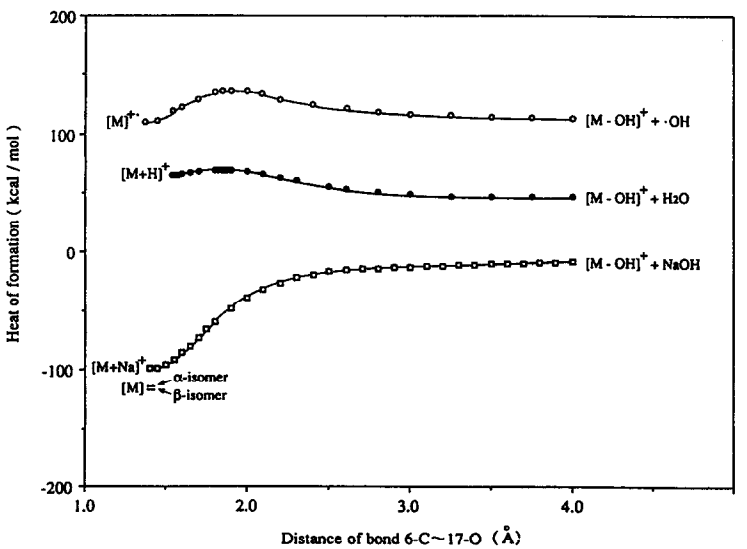


Fig. 11. Calculated potential energy curves for $[M-OH]^+$ formation.

The energy variation in the pathway of the elimination of the hydroxyl radical from the ion $[M]^{+\bullet}$ with a bond length of 6-C~17-O to give the ion $[M-OH]^+$ was also investigated. This indicated that the required activation energy was large enough at 25.86 kcal/mol, and the potential energy for the fragmentation products ($[M-OH]^+ + OH^\bullet$) was also high (13.00 kcal/mol) which is larger than that for $[M]^{+\bullet}$. Thus, we rejected the notion of the generation of the ion $[M-OH]^+$ from the ion $[M]^{+\bullet}$.

Consequently, we concluded from the MNDO calculation using a model compound, in LSIMS of the glycosides, that the protonated molecular ion $[M+H]^+$ in which a proton attached at the oxygen atom of the hemiacetal hydroxyl group was generated by Cs^+ bombardment and a water molecule was immediately eliminated.

The next feature we have to discuss was the formation of the ion $[M+Na]^+$. Two fragment ions $[a+Na]^+$ and $[b+Na]^+$ generated by elimination of a glucosyl group at 26-C, and of an acetyl arabinose at 11-C were found in LSIMSs of the glycosides with NaCl and LSIMS/MS of $[M+Na]^+$. These two fragment ions contained a sodium ion which was thought to be located at an aglycon portion. We investigated the physical features of a model compound which had a sodium ion at 5-O or 17-O as we did for the protonated molecular ion.

The ion radius of a sodium ion and the van der Waals radius of an oxygen atom are 1.16[11] and 1.52 Å,[12] respectively. The sodium ion is located at a distance of 2.68 Å from 5-O or 17-O in the hypothetical geometry of $[M+Na]^+$. In both instances, the optimized geometries indicated that the four-membered ring structure in which a sodium ion was located at a distance of ca. 3.4 Å from both of 5-O and 17-O was most energetically stable. The heat of formation of the optimized geometry was -99.05 kcal/mol, which was only 17.01 kcal/mol larger than that of the neutral molecule. The bond lengths of 4-C~5-O, 5-O~6-C and 6-C~17-O, being 1.4113, 1.4155 and 1.4138 Å, were almost same as those of the neutral molecule and indicated that sodium ion addition did not induce any structure change. A localized charge of each atom also did not yield any difference from that of the neutral molecule.

The energy value increased with the bond length of 6-C~17-O, and the heat of formation for the fragmentation products ($[M-OH]^+ + NaOH$) was 90.66 kcal/mol larger than that of $[M+Na]^+$. The hemiacetal hydroxyl group with a sodium ion was not eliminated from the ion $[M+Na]^+$ which reasonably explained why $[M-OH]^+$ did not appear in the LSIMS and LSIMS/MS of the ion $[M+Na]^+$ of the glycoside. That the structure and the charge distribution of the ion $[M+Na]^+$ of the model compound did not show much difference from those of the neutral molecule indicated that fragmentation of the ion $[M+Na]^+$ hardly took place.

CONCLUSION

The structure of five new genuine furostanol glycosides (FG-1 (**1**) ~FG-5 (**5**)) isolated from "Nogiran" were determined by LSIMS and LSIMS/MS as being bisdesmosides in which 2,3,4-tri-O-acetyl-α-L-arabinopyranose was substituted at 11-C and β-D-glucopyranose was substituted at 26-C of aglycons (2-O-acetyl-furometagenin for **1**, furometagenin for **2**, 2-O-acetyl-furonogiragenin for **3**, 2-O-acetyl-3-oxo-furometagenin for **4** and 2-O-acetyl-furometanarthogenin for **5**). A remarkable feature was revealed by LSIMS of the glycosides which have a hemiacetal hydroxyl group; they do not give protonated molecular ions $[M+H]^+$. but $[M-OH]^+$. This was theoretically investigated by MO calculations. It was clarified that

the protonated molecular ion $[M+H]^+$ had a proton on the oxygen atom of the hemiacetal hydroxyl group, and the dehydration from this ion did not require much energy. The sodium atom of the ion $[M+Na]^+$ was located between two oxygen atoms of the hemiacetal moiety and formed the four-membered ring for stabilization. Thus, the reason why the ion $[M+Na]^+$ did not generate fragment ions was explained. Hence, it is necessary to have LSIMS-information about the genuine glycoside molecules which have cyclic hemiacetal moieties to add NaCl to the sample matrix.

EXPERIMENTAL

Isolation and physicochemical data of all furostanol glycosides explained here as well as the related compounds will be reported elsewhere[6].

LSIMS, HRLSIMS (accurate mass measurement) and LSIMS/MS were obtained with Hitachi M-90 triple analyzer mass spectrometer (BEE geometry) equipped with a Cs-ion gun. A few micrograms of a sample in ca. 2 μl of matrix on a silver plate was bombarded with Cs ions accelerated at 16 kV.

As the internal standard for accurate mass measurement, various molecular weights of polyethylene glycol (PEG) were used depending on the sample, and NaI or KI were added as necessary.

For the LSIMS/MS, a precursor ion selected at MS-I (BE) was activated by collision with He and fragmented to give daughter ions, which were analyzed by MS-II (electric sector).

MNDO calculations were carried out with a VAX 6320 computer (DEC) and the MOPAC ver. 6.0 package.

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REFERENCES AND NOTES

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