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# Mass Spectra of Trimethylsilyl Ethers of Dammarane-Type Ginseng-Sapogenins and Their Related Compounds

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Complete trimethylsilylation of 20(*S*)-protopanaxadiol (**11**), 20(*S*)-protopanaxatriol (**12**) (Ginseng-sapogenins) and dammarenediol-II (**13**) was furnished by the action of trimethylsilylimidazole. The mass spectra of the pertrimethylsilyl ethers of **11**, **12**, and **13** (TMSi-**11**, -**12**, and -**13**) exhibited characteristic fragmentations around their C-20, which were diagnostic for structure determination of triterpenes of this type. The mass spectra of TMSi-ethers of the deuterated compounds of **11** (**11**-$d_2$ and -$d_5$) demonstrated that no 1,2-elimination of TMSiOH at C-3 and -12 was involved in the fragmentation.

With reference to the reports[2] of the mass spectrometry of TMSi-oligosaccharides, the mass spectra of Ginseng saponins (ginsenosides-$Rb_1$(**1**), -$Rb_2$(**2**), -Rc(**3**), -Rd(**4**), -Re(**5**), -Rf(**6**), -$Rg_1$(**7**), and -$Rg_2$(**8**) were also studied, the results of which were found to be useful for identification and structure determination of oligoglycosides of this type.

**Keywords**——ginseng-sapogenins and -saponins; dammarane-type triterpenes; mass spectrometry; ginsenosides; trimethylsilylation with trimethylsilylimidazole; *Panax ginseng* C.A. Meyer; *Araliaceae*; anomerous elimination of TMSiOH

The isolation and the structure determination of most of the dammarane-type saponins of Ginseng roots, ginsenoside-$Rb_1$ (**1**), -$Rb_2$ (**2**), -Rc(**3**), -Rd(**4**),[3] -Re(**5**), -Rf(**6**),[4] -$Rg_1$(**7**),[5] -$Rg_2$(**8**),[4] -$Rb_3$(**9**) and 20-gluco-ginsenoside-Rf(**10**)[6] have been established. The common sapogenin of **1**—**4** and **9** is represented by 20(*S*)-protopanaxadiol(**11**) and that of **5**—**8** and **10** is formulated as 20(*S*)-protopanaxatriol(**12**). The studies on mass spectra of dammarane-type triterpenes have been reported[7] and recently, acetylated Ginseng-saponins were subjected to mass spectrometry,[8] the results of which provided appreciable information for identification and structure elucidation of saponins of leaves of *Panax ginseng* C.A. Meyer.[9] It has been known that trimethylsilyl (TMSi) ethers of alcohols are more readily prepared and have higher volatility than other derivatives. In continuation of chemical studies on saponins of *Panax* spp., mass spectra of TMSi-derivatives of the Ginseng sapogenins (**11** and **12**) and their related compounds have been investigated.

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2) N.K. Kochetkov, O.S. Chizhov, and N.V. Molodtshov, *Tetrahedron*, **24**, 5587 (1968); J.P. Kamerling, J.F.G. Vliegenthart, J. Vink, and J.J. De Ridder, *ibid.*, **27**, 4275 (1971).
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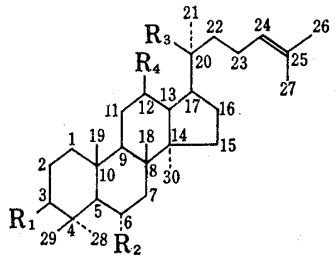
## Results and Discussion

Trimethylsilylation of **11**, **12** and dammarenediol-II(**13**) was carried out with trimethylsilylimidazole, which is the powerful on-column TMSi-reagent.[10] The IR spectra of the resulted TMSi-derivatives, TMSi-**11**, -**12** and -**13** exhibited no OH absorption, indicating that the above reagent can readily trimethylsilylate even the hindered 20-*tert*-OH of **11**—**13** which is known to remain unacetylated or unmethylated under the usual reaction conditions. This complete trimethylsilylation gave rise to the characteristic fragmentation mode in the mass spectra; a series of prominent fragment ions ($A_n$) associated with fission between C-20—22 and a strong fragment ion (C) (base peak) attributable to cleavage between C-17—20. Although these fragmentations were observed also in the spectra of **12** and **13** as well as their acetates, their relative intensities are so lower than those of the TMSi-derivatives that they are not diagnostic for identification and structure determination. Appearance of these ions with high intensities in the spectra of TMSi-derivatives would be explained in term of stability of the cation, $TMSiO\overset{+}{=}C<$, being promising in the structure studies of dammarane-type triterpenes.

The elemental composition of ion C, which is common in the spectra of TMSi-**11**—**13**, was secured by high resolution mass spectrometry, found *m/e* 199.1518, calcd. for $C_{11}H_{23}OSi$ 199.1510. The structure of this ion was further substantiated by the fact that the mass spectrum of TMSi-20(*S*)-dihydroprotopanaxadiol (TMSi-2H-**11**) exhibited no strong ion at *m/e* 199 but showed its base peak at *m/e* 201 (=199+2H).

Formulation of ions $A_n$ was established by the following evidences. Ion $A_1$ of TMSi-**11** (found *m/e* 593.4242, calcd. for $C_{33}H_{65}O_3Si_3$ 593.4237) which is accompanied by ions $A_2$: *m/e* 503 (593-TMSiOH) and $A_3$ *m/e* 413 (593—2×TMSiOH), is less by *m/e* 88 (TMSiO in place of H) than the corresponding ion $A_1$ of TMSi-**12** (found *m/e* 681.4586, calcd. for $C_{36}H_{73}O_4Si_4$



dammarenediol-II (**13**)
$R_1$=–OH, $R_2$=–H, $R_3$=–OH, $R_4$=–H
20(*S*)-protopanaxadiol (**11**)
$R_1$=–OH, $R_2$=–H, $R_3$=–OH, $R_4$=–OH
20(*S*)-protopanaxatriol (**12**)
$R_1$=–OH, $R_2$=–OH, $R_3$=–OH, $R_4$=–OH

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Ginsenoside-$Rb_1$ (**1**) | –O–Glc$^2$—Glc | –H | –O–Glc$^6$—Glc | –OH |
| Ginsenoside-$Rb_2$ (**2**) | –O–Glc$^2$—Glc | –H | –O–Glc$^6$—Ara(pyr.) | –OH |
| Ginsenoside-Rc (**3**) | –O–Glc$^2$—Glc | –H | –O–Glc$^6$—Ara(fur.) | –OH |
| Ginsenoside-Rd (**4**) | –O–Glc$^2$—Glc | –H | –O–Glc | –OH |
| Ginsenoside-Re (**5**) | –OH | –O–Glc$^2$—Rha | –O–Glc | –OH |
| Ginsenoside-Rf (**6**) | –OH | –O–Glc$^2$—Glc | –OH | –OH |
| Ginsenoside-$Rg_1$ (**7**) | –OH | –O–Glc | –O–Glc | –OH |
| Ginsenoside-$Rg_2$ (**8**) | –OH | –O–Glc$^2$—Rha | –OH | –OH |
| Ginsenoside-$Rb_3$ (**9**) | –O–Glc$^2$—Glc | –H | –O–Glc$^6$—Xyl | –OH |
| 20-Gluco-ginsenoside-Rf (**10**) | –OH | –O–Glc$^2$—Glc | –O–Glc | –OH |

Glc: β-D-glucopyranose, Rha: α-L-rhamnopyranose, Ara: α-L-arabinose, Xyl: β-D-xylopyranose.

Fig. 1

10) E.C. Horning, M.G. Horning, N. Ikekawa, E.M. Chambaz, P.I. Jaakonmaki, and C.J.W. Brooks, *J. Gas Chromatog.*, **5**, 283 (1967); Y. Shoda, K. Hashimoto, T. Inoue, and T. Sawada, *Yakugaku Zasshi*, **89**, 734 (1969).

681.4570) which is followed by ions $A_2$ *m/e* 591 (681-TMSiOH), $A_3$ *m/e* 501 (681−2×TMSiOH), and $A_4$ *m/e* 411 (681−3×TMSiOH). The spectra of TMSi-**13** showed its $A_1$ and $A_2$ ions at *m/e* 505 and 415 (505-TMSiOH), respectively, supporting this assignment. Further, the mass spectrum of TMSi-2H-**11** exhibited the same series of fragment ions as that of TMSi-**11**.

Being similar to the mass spectra of the acetate,[8] the molecular ion ($M^+$) could be hardly detected in the case of the TMSi-derivatives. Instead, a series of ion $B_n$, $M^+-(TMSiOH)_n$ are observed as indicated in Fig. 2.

For the purpose of the assignments of $^{13}C$ NMR signals of Ginseng sapogenins and their related dammarane-type triterpenes, deuterated compounds, 20(*S*)-protopanaxadiol-2,2-$^2H_2$

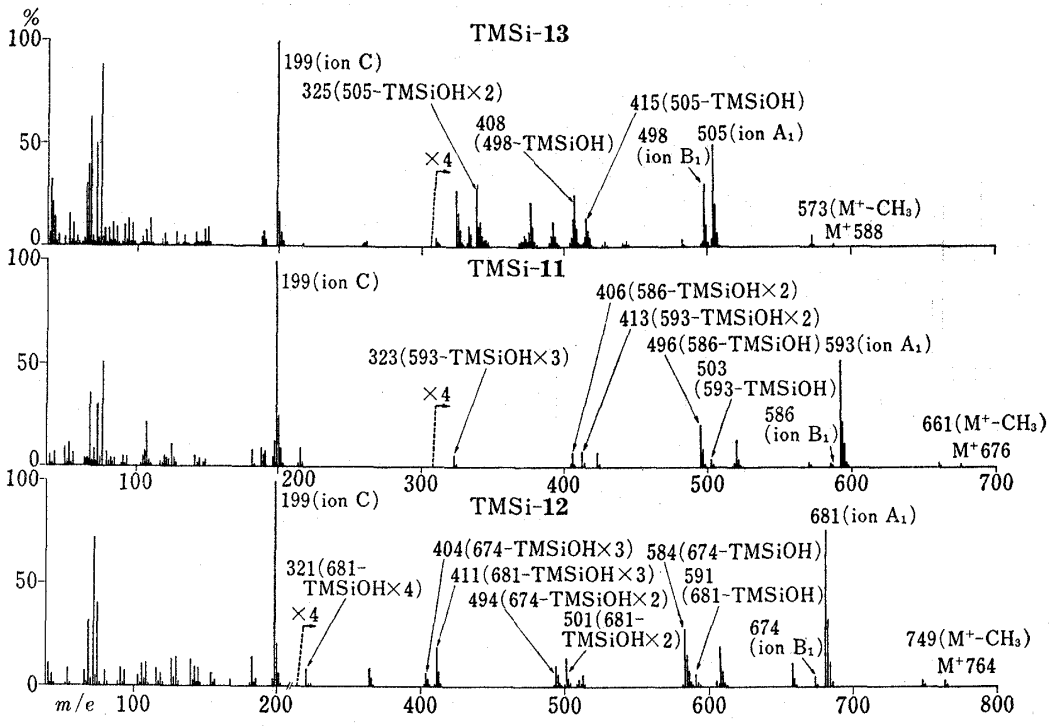
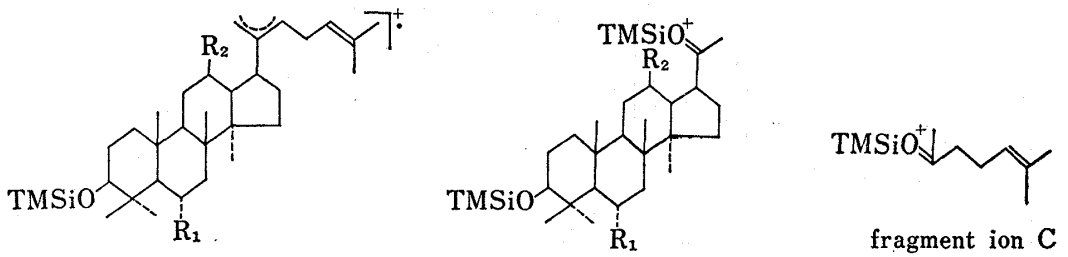



Chart 1




| | Fragment ion $B_1$ | | | Fragment ion $A_1$ | | |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | | $R_1$ | $R_2$ | |
| TMSi-11 | H | OTMSi | TMSi-11 | H | OTMSi | |
| TMSi-12 | OTMSi | OTMSi | TMSi-12 | OTMSi | OTMSi | |
| TMSi-13 | H | H | TMSi-13 | H | H | |
| fragment ion $B_n$: ion $B_1$-$(TMSiOH)_{n-1}$ | | | fragment ion $A_n$: ion $A_1$-$(TMSiOH)_{n-1}$ | | | |

Fig. 2

(**11**-$d_2$) and -2,2,11,11,13-$^2H_5$ (**11**-$d_5$) were prepared.[11] The determination of $^2H$ contents in these compounds was performed by means of mass spectrometry of their TMSi-derivatives in reference to the present findings. Unexpected rearrangements (1,3-, 1,4-, or 1,5-elimina-

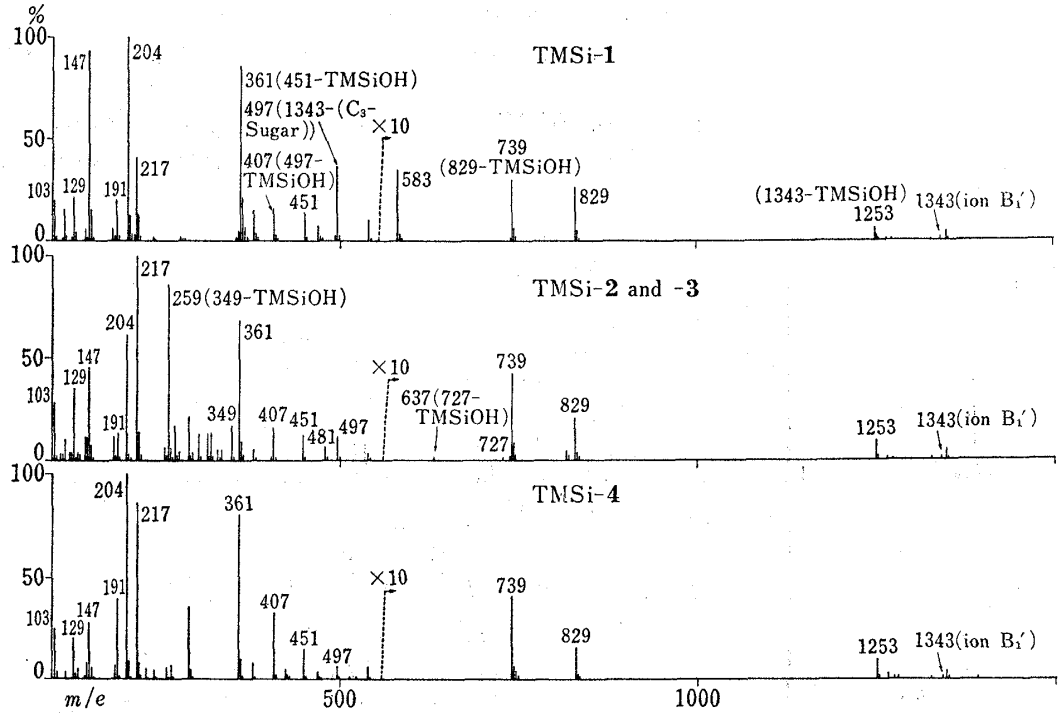



Chart 2

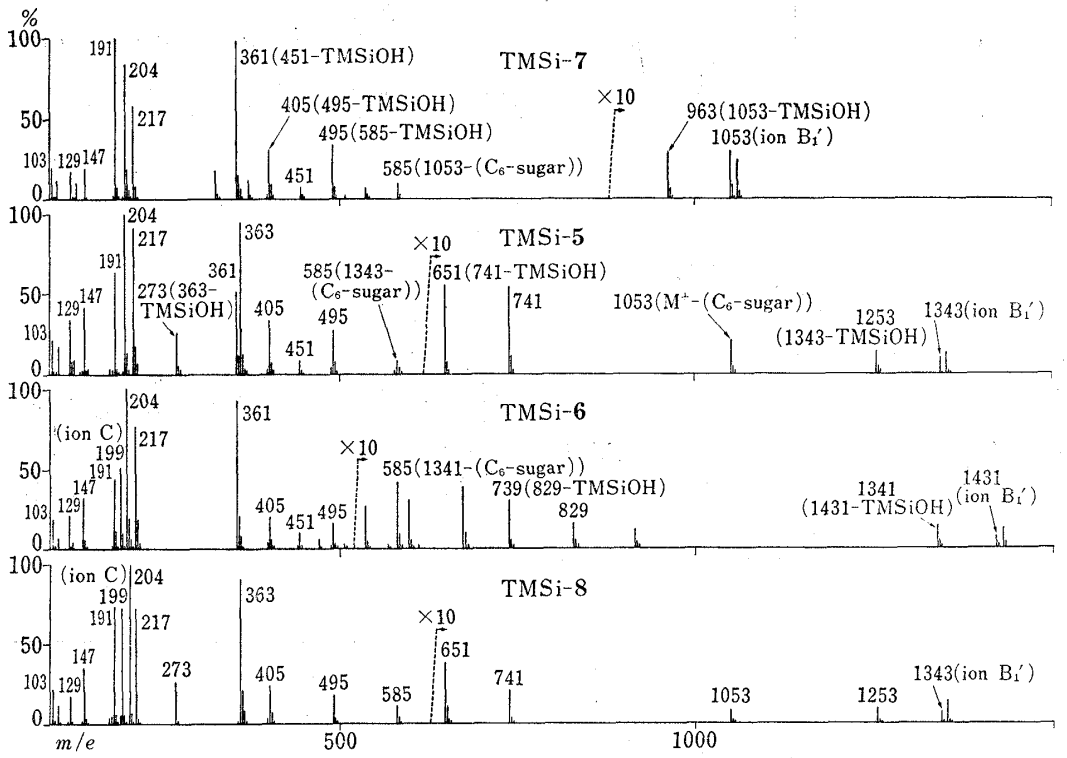
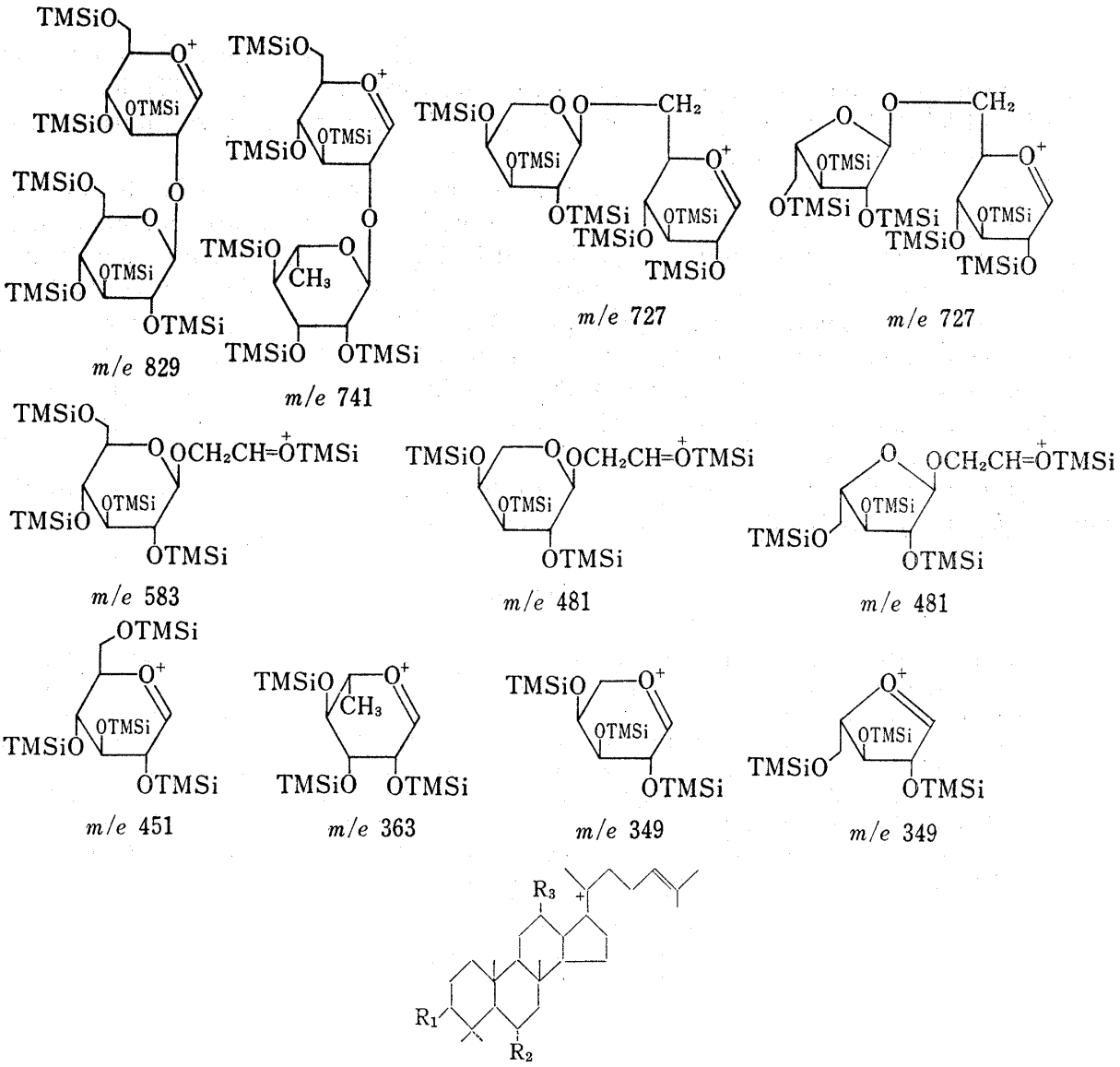



Chart 3

11) 11) J. Asakawa, R. Kasai, K. Yamasaki, and O. Tanaka, *Tetrahedron*, 33, 1935 (1977).

tion) involving the loss of $H_2O$, ROH or TMSiOH in the mass fragmentation of cycloalcanol derivatives have been reported.[12] It is notable that ions, $A_3$ and $B_3$ in the spectra of TMSi-**11**-$d_2$ and -$d_5$ retained all of their $^2H$, demonstrating that no 1,2-elimination of TMSiOH is involved also in the fragmentation of TMSi-triterpenes of this type.

In application of the above results, the Ginseng saponins, **1**—**8** were trimethylsilylated with the same reagent and the resulted TMSi-saponins were subjected to mass spectrometry. The appearance of the strong fragment ion C (*m/e* 199) which is limited to the spectra of TMSi-




fragment ion $B_1'$

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| TMSi-**1**—**4** | TMSi-sugar | H | OTMSi |
| TMSi-**5**—**8** | OTMSi | TMSi-sugar | OTMSi |

fragment ion $B_n'$: ion $B_1'$-$(TMSiOH)_{n-1}$

Fig. 3

12) Review: D.G.I. Kingston, B.W. Hobrock, M.M. Bursey, and J.T. Bursey, *Chem. Rev.*, **75**, 693 (1975).

saponins having no glycosyl linkage at C-20-OH (TMSi-**6** and -**8**) is useful for elucidation of the location of glycosyl linkage and the structure of the side-chain.

In contrast to the case of TMSi-**11**—**13**, the series of ions, $A_n$ could not be observed even in the spectra of TMSi-**6** and -**8**. Another striking difference between TMSi-saponins and -sapogenins is that the spectra of the former exhibited a series of ions, *m/e* $B_{n+1}$($B'_n$ ions) instead of $B_n$ ions of the latter. The structure of $B'_n$ ions can be formulated as illustrated in Fig. 3. These significant differences in the fragmentation mode between the spectra of TMSi-saponins and -sapogenins would be due to difference of the sample temperature required for determination of the spectra: 160—200° for TMSi-saponins and 120° for TMSi-sapogenins.

Since TMSi-O- and TMSi-glycosyl-O- linkages are readily cleaved, the spectra of the sets of TMSi-saponins having the same structures except for C-20 substituents, "TMSi-**1**—**4**" and "TMSi-**5** and **8**" showed the similar patterns of ions with regard to the fragmentation associated with the aglycone moieties.

The mass spectrometry of TMSi-oligosaccharides has been elaborated by Kochetkov *et al.* and Kamerling, *et al.*[2] With reference to their observations, the peaks attributable to sugar units of TMSi-saponins were assigned as indicated in Fig. 3.[13] The fragment ions characteristic of the 1,6-linked biose-unit were observed in the spectra of TMSi-**1** (*m/e* 583 due to "TMSi-hexose$^{6}_{-1}$TMSi-hexose") and TMSi-**2** and -**3** (*m/e* 481 due to "TMSi-hexose$^{6}_{-1}$TMSi-pentose"), being useful for micro-detection of contamination of **1** in a sample of **2** or **3** and *vice versa*. It is notable that TMSi-**2** and -**3** which are different in the ring size of arabinoside unit, showed the almost same fragmentation patterns, being impossible to be distinguished from each other by mass spectrometry.

The present findings were recently applied to the structure elucidation of leaves-saponins of *Panax japonicus* C.A.Meyer, which will be reported elsewhere.[14]

## Experimental

Isolation and purification of the free saponins were reported previously.[3-5] The trimethylsilyl derivatives were synthesized as follows. A solution of a sample (1—3 mg) in trimethylsilylimidazole (three drops) in a stoppered micro-tube was heated at 90° for 1 hr. After dilution with a few drops of $H_2O$, the reaction mixture was extracted with *n*-$C_6H_{14}$. After washing with $H_2O$ several times, *n*-$C_6H_{14}$ layer was concentrated to dryness and subjected to determination of mass spectrum. High resolution mass spectra were measured on a JEOL JMS-01SG-2 (Mattauch-Herzog type) double focusing mass spectrometer with direct insertion of the probe into the ion source. In measurement by the photoplate method, Ilford type $Q_2$ thin glass was used as a dry-plate and perfluorokerosene was employed as an internal calibration standard. Conditions were as follows: ionizing voltage 75 eV; ionizing current 200 μA; ion accelerating voltage 10 kV and 4 kV.

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13) Recently gas chromatographic and mass spectrometric analysis of TMSi-Ginseng saponins was reported: E. Bombardelli, A. Bonati, B. Gabetta, E.M. Martinelli, and G. Mustich, *Fitoterapis*, **3**, 99 (1976).
14) S. Yahara, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2041 (1977).