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14. For a detailed discussion on the chemical shift of the 18-CH_3 group of (*E*)-20 (22)-dehydro compounds, see reference **7b** and **7c**.
15. Spectral data for **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82 (t, *J*=7.45 Hz, 1H), 4.14 (q, *J*=7.14 Hz, 2H), 2.32 (m, 4H), 2.24 (s, 3H), 1.27 (t, *J*=7.16 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.48, 129.98, 120.78, 60.48, 33.39, 31.54, 23.27, 14.16; IR (neat, cm^{-1}): 2982, 1740. **9**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82 (t, *J*=7.45 Hz, 1H), 2.12 (m, 2H), 1.55 (m, 2H), 1.23 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.48, 129.98, 120.78, 60.48, 33.39, 31.54, 23.27, 14.16; IR (neat, cm^{-1}): 3386, 2971, 1653. **4**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.51 (t, *J*=6.59 Hz, 1H), 2.19 (m, 2H), 1.85 (s, 3H) 1.24 (s, 6H), 0.83-1.64 (series of m, 29H); IR (neat, cm^{-1}): 3385, 2965, 1653. **2**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.58 (m, 2H), 5.38 (d, *J*=4.17 Hz, 1H), 4.52 (m, 1H), 2.33-1.23 (series of m, 21H), 1.79 (s, 3H), 1.25 (s, 6H), 1.18 (s, 9H), 1.06 (s, 3H), 0.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.03, 156.22, 140.09, 131.00, 126.21, 125.24, 122.29, 73.47, 71.07, 57.54, 50.19, 46.69, 43.50, 38.60, 38.00, 36.84, 36.71, 36.03, 31.52, 30.94, 30.27, 29.26, 27.62, 27.14, 23.44, 21.02, 19.26, 16.28, 15.31; IR (KBr, cm^{-1}): 3366, 2967, 1724, 1684, 1654, 1479, 1458. **1**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.37 (m, 1H), 5.21 (m, 1H), 4.56 (m, 1H), 2.31-1.18 (series of m, 24H), 1.65 (s, 3H), 1.24 (s, 6H), 1.18 (s, 9H), 1.02 (s, 3H), 0.545 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.04, 139.85, 134.89, 125.16, 122.41, 73.51, 71.13, 58.80, 56.18, 50.22, 43.73, 43.47, 38.61, 38.00, 37.00, 36.67, 32.14, 31.84, 29.24, 27.65, 27.15, 24.68, 24.27, 23.11, 21.01, 19.39, 17.86, 12.91.

Structural Confirmation of Ginsenosides By Fragmentation Pattern Using Tandem Mass Spectrometry

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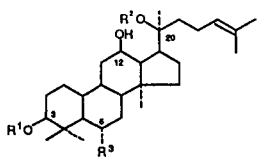
Ginseng has long been used as a tonic, health promoter in Asian countries including Korea and China.[1,2] Its pharmacological effect as an anti-cancer drug has been known to the general public since 1987.[3] In recent years, many scientific studies have reported that the biological activities of ginseng are due to its active components, saponins.[4,5] Korean ginseng (*Panax ginseng*) contains a series of ginseng saponins called ginsenosides containing a large quantity of steroid glycosides.[6] Novel analytical techniques revealed the structures of major ginsenosides, Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg (Figure 1).[7,8] Fast-growing market of ginseng products and their well-accepted pharmacological effects have greatly prompted us to pursue research on the isolation and structure determination of ginsenosides.

There are several analytical methods applicable for the identification of ginsenosides in ginseng extracts.[9] Fast atom bombardment mass spectrometry (FAB-MS) has been generally used for structural analysis of steroid glycosides containing two or more sugar units.[10] In addition, electrospray ionization mass spectrometry (ESI-MS) has been rapidly become an alternative method for the analysis of biomolecules.[11] Tandem mass spectrometric technique has especially been useful in the structure determination of biomolecules.[12] An important point of tandem mass spectrometry (MS/MS) is the ability to produce fragment ions from selected MS-1 ions.[13] Herein, we report our result on the first study of the fragmentation patterns of ginsenosides by tandem mass spectrometry. These fragmentation patterns can help us explain not only the structure of the biomolecule, but also its elemental composition.[14]

Ginsenosides were extracted from commercially available Korean Ginseng by the Folch-Suzuki partition method.[15,16] The compositions and structures of ginsenosides in ginseng have been investigated by means of high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), nuclear magnetic resonance



	R1	R2	R3
Rb_1	-Glc[2→1]Glc	-Glc[6→1]Glc	-H
Rb_2	-Glc[2→1]Glc	-Glc[6→1]Ara(p)	-H
Rc	-Glc[2→1]Glc	-Glc[6→1]Ara(f)	-H
Rd	-Glc[2→1]Glc	-Glc	-H
Re	-H	-Glc	-O-Glc[2→1]
Rf	-H	-H	Rha
Rg	-H	-Glc	-O-Glc[6→1]

*Glc: Glucose. *ara(p): α-L-Arablnopyranose. *ara(f): α-L-Aarablnofuranose. *Rha: Rhamnose

Figure 1. The structure of ginsenosides.

(NMR) spectroscopy and mass spectrometry (MS). Steroid glycosides purified by preparative thin layer chromatography (PREP-TLC) were separated into seven bands (O-1 to O-7) (Figure 2). The components of O-5 and O-7 were further purified several times by silica gel column chromatography with various eluents and then analyzed by ESI-MS and FAB-MS.[17]

The mass spectra showed that the molecular weights of components O-5 and O-7 were suspected to be 946 and 766, respectively. The results of mass spectrometric analysis for each components are summarized in Table 1. The negative ESI mass spectrum shows a pseudomolecular ion at m/z 981 $(M+Cl)^-$ and the negative FAB at m/z 945 $(M-H)^-$ and m/z 981 $(M+Cl)^-$. The negative ESI and FAB mass spectra of O-7 also show pseudomolecular ions at m/z 801 $(M+Cl)^-$ and at m/z 765 $(M-H)^-$, respectively. From our previous experiences we suspect ginsenoside-Re is the component of O-5 and ginsenoside-Rf is the component of O-7. This is further confirmed by MS/MS experiments.

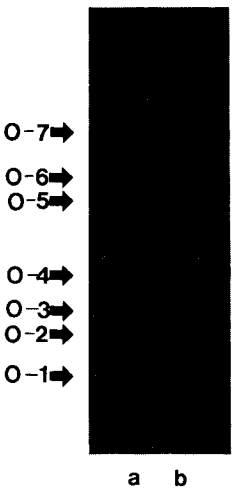


Figure 2. Preparative thin layer chromatogram of whole lipid extracts from ginseng. The plate was developed with C/M/2.5 M NH_4OH (65 : 50 : 8, v/v/v) and bands were detected by heating at 120 °C with orcinol-ferric chloride-sulfuric acid. (a), (b) whole extracts from ginseng (1.30 mg, 1.63 mg).

Table 1. Mass spectrometric components of O- 5 and O-7

	O-5	O-7
ESI-MS (m/z)	981 $(M+Cl)^-$	801 $(M+Cl)^-$
FAB-MS (m/z)	945 $(M-H)^-$, 981 $(M+Cl)^-$	765 $(M-H)^-$

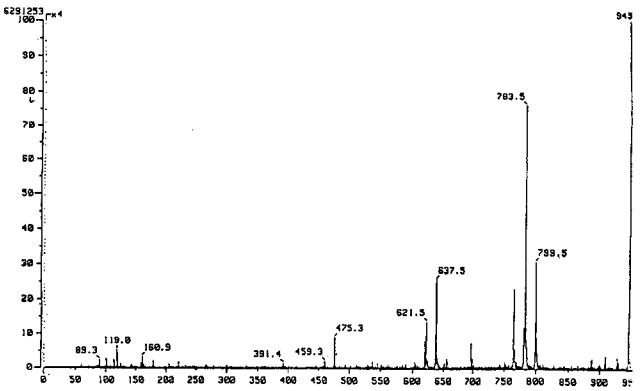


Figure 3. Negative FAB MS/MS spectrum of ginsenoside-Re.

FAB-MS/MS spectra were taken with a four sector mass spectrometer (JMS-SX/SX 102A tandem MS, JEOL, Japan). The ion source was operated at +/− 8.0 kV accelerating voltage in the positive and negative ion mode, respectively, with the mass resolution of 2000. A m-nitrobenzyl alcohol (NBA)/glycerol (1 : 1) matrix was used. Negative ion mode FAB MS/MS spectrum of O-5 shown in Figure 3 displays the fragment ions. However, the positive FAB MS/MS results were not as good as those in the negative ion mode because the intensity of precursor ions of positive mode was very weak.

All major fragment ions appeared to arise from the loss of sugar groups due to cleavage of the glycosidic linkage.[18] Cleavage patterns of ginsenosides are as follows. Cleavage of the glycosidic bond appears to occur on both sides of the connecting oxygen atom. When the cleavage occurs at the bond between the oxygen and the sugar, additional hydrogen can be lost, producing an even electron ion.

In the case of ginsenoside, the ion of m/z 799 corresponds to ions formed by loss of the terminal rhamnose (− 146 amu) and a concerting H transfer from this part to the remaining ion. The ion of m/z 783 in the FAB mass spectrum was resulted by a loss of oxygen (− 16 amu). The following losses occurred: -glucose, -O, -glucose and -O. As the results of these fragmentations, several characteristic mass peaks (m/z 637, 621, 475, and 459) are formed (Figure 3).

ESI-MS spectra also were taken with a high resolution mass spectrometer (JMS-700 MS, JEOL, Japan). The ion source was operated at 5 kV accelerating voltage in the positive and negative ion modes. The mobile phase used was ethanol and the flow rate was 50 μL/min. In these mass spectra, sodium and chlorinated adduct ions were abundant under normal ESI conditions.[19] However, in this O-5 sample, fragmentation mass spectra could be obtained by increasing ring potential between first and second skimmers in the ESI source (Figure 4). The ions at m/z 783 and m/z 637 in the ESI mass spectrum resulted from losses of rhamnose (− 146 amu) and oxygen (− 16 amu) followed by a loss of glucose

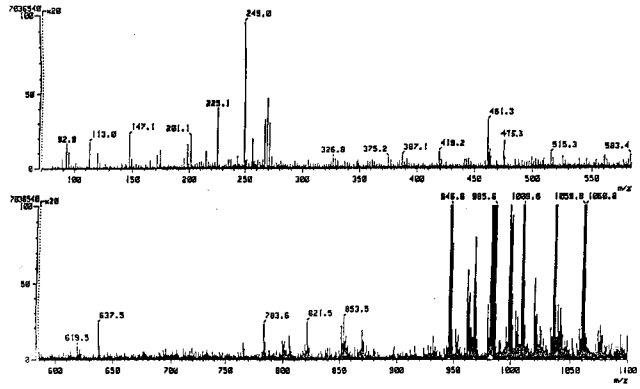


Figure 4. Negative ESI-MS spectrum of ginsenoside-Re.

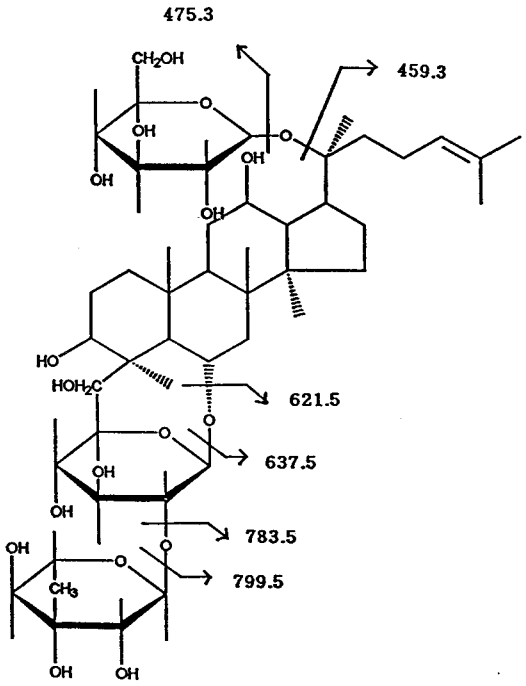


Figure 5. Fragmentation patterns of ginsenoside-Re.

which was separated by an oxygen (− 146 amu). The ion at m/z 475 in the ESI mass spectrum was also formed from cleavage of glucose (− 162 amu). The ions observed above m/z 982 in this ring potential MS/MS spectrum could not be identified.

In conclusion, our study focused on the fragmentation patterns of sugar rings of ginsenosides, using FAB-MS, ESI-MS and tandem-MS. Observation of these fragmentations allowed us to identify the ginsenoside structure in the isolated biological fluid (Figure 5). The results presented in this study also demonstrated the usefulness of MS/MS for the structural determination of ginsenosides. Therefore, the high sensitivity of the FAB-MS and ESI-MS techniques, together with the fragmentation pattern generated by MS/MS, will be useful for the identification of unknown biomolecules. Further study on the optimized fragmentation conditions and quantification of a series of glycosides by using stable isotopes is now being carried out.

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19. The Mass data; (a) negative ESI-MS m/z 981 $(M+Cl)^-$, (b) negative FAB-MS m/z 945 $(M-H)^-$ and m/z 981 $(M+Cl)^-$, (c) negative ESI-MS m/z 801 $(M+Cl)^-$, (d) negative FAB-MS m/z 765 $(M-H)^-$.