

# Isolation and characterization of monoacyldiglycerides from bovine udder

Jin-Kyung Limb,\* Young Hwan Kim,<sup>†</sup> So-Yeop Han,\* and Gil-Ja Jhon<sup>1,\*</sup>

Department of Chemistry, Division of Molecular Life Science and Center for Cell Signaling Research,\* Ewha Womans University, Seoul 120-750, Korea, and Mass Spectrometry Group,<sup>†</sup> Korea Basic Science Institute, Taejon 305-600, Korea

**Abstract** Monoacyldiglycerides (MADGs) were isolated from an animal tissue, bovine udder, by solvent extraction and silica gel column chromatography. Monoacyldiglyceride structures were identified using a variety of 1-D and 2-D NMR techniques and collision-induced dissociation (CID) tandem mass spectrometry coupled with fast atom bombardment. CID of sodium-adducted molecular ions ( $[M+Na]^+$ ) generated numerous types of product ions providing information on the double bond position of fatty acyl groups as well as fatty acid compositions. Structural analysis led to the classification of the MADGs isolated from bovine udder as 1-palmitoyl-2-lauroyl-3-acetyl-*rac*-glycerol, 1,2-dimyristoyl-3-acetyl-*rac*-glycerol, 1-palmitoyl-2-myristoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-myristoyl-3-acetyl-*rac*-glycerol, 1-palmitoyl-2-palmitoleoyl-3-acetyl-*rac*-glycerol, 1,2-dipalmitoyl-3-acetyl-*rac*-glycerol, 1-linoleoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-palmitoleoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-stearoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-linoleoyl-3-acetyl-*rac*-glycerol, 1,2-dioleoyl-3-acetyl-*rac*-glycerol, and 1-stearoyl-2-oleoyl-3-acetyl-*rac*-glycerol.—Limb, J.-K., Y. H. Kim, S.-Y. Han, and G.-J. Jhon. Isolation and characterization of monoacyldiglycerides from bovine udder. *J. Lipid Res.* 1999. 40: 2169–2176.

**Supplementary key words** monoacyldiglycerides • bovine udder • 1-D and 2-D NMR • fast atom bombardment • tandem mass spectrometry

All mammalian udders (1, 2) are composed of a specific number of mammary glands (3). Most lipids (4–8) synthesized in mammary glands are in the form of triglycerides and play an important biochemical role as an energy source. According to previous reports (2), the fatty acids that form the triglycerides synthesized in mammary glands are a variety of C4–C18 saturated acids and oleic acid. Even though fatty acids of ruminant milk fat may range in chain length from C2 to C24 (9), there have been no reports on the presence in animal tissues of natural triglycerides containing acetic acid.

The existence of acetic acid as a natural triglyceride component was first found in seed oils of *Balsaminaceae* (10). Later, monoacyldiglycerides were also reported to be present in a variety of plant species, including *Cela-*

*traceae*, *Lardizabalaceae*, *Ranunculaceae*, and others (11–14). However, the structures of these monoacyldiglycerides (MADGs) were investigated only with thin-layer chromatography (TLC), infrared spectroscopy (IR), and gas chromatography (GC) at low resolution (11, 12). The structural analyses of MADGs in seed oils from *Maytenus ilicifolia* (MI), *M. dasyclada* (MD), and *M. cassineformis* (MC) were performed by high resolution GC-mass spectrometry (GC/MS) in negative chemical ionization (NCI) mode (15). Although these reports contained improved structural analyses (10–13), the use of <sup>1</sup>H-nuclear magnetic resonance (NMR), <sup>13</sup>C-NMR, and GC/MS failed to establish the position of attachment of fatty acids to the triglycerides and the position of double bonds within the fatty acid groups.

As an extension of our original study to locate new lipids with interesting biological functions in nature (16), we isolated and structurally characterized a number of naturally occurring monoacyldiglycerides from bovine udder. Structures were characterized by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, distortionless enhancement by polarization transfer (DEPT) 135, <sup>1</sup>H-<sup>1</sup>H COSY NMR, 2-D <sup>1</sup>H-<sup>13</sup>C COSY NMR, FAB-MS, and MS/MS.

Collision-induced dissociation (CID) tandem mass spectrometry (MS/MS) coupled with fast atom bombardment (FAB) is known to be very useful for the structural determination of glycerolipids (17–21). We applied CID-MS/MS coupled with FAB (22) for the structural identification of individual molecular species in a mixture of monoacyldiglycerides isolated from bovine udder. The

Abbreviations: MADGs, monoacyldiglycerides; 1-D, one dimensional; 2-D, two dimensional; NMR, nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; CID, collision-induced dissociation; MS/MS, tandem mass spectrometry; FAB-MS, fast atom bombardment mass spectrometry; COSY, correlation spectrometry; HPTLC, high-performance thin-layer chromatography; PBS, phosphate-buffered saline; HMQC, <sup>1</sup>H-detected heteronuclear multiple-quantum coherence; 3-NBA, 3-nitrobenzyl alcohol;  $[M+Na]^+$ , sodium-adducted molecular ions; MS-1, first mass spectrometer; MS-2, second mass spectrometer; CRF, charge-remote fragmentation.

<sup>1</sup> To whom correspondence should be addressed.

application of these improved techniques led us to better elucidate characteristic fragments of each fatty acid while still attached to its triglyceride backbone, and also locate the position of double bonds in fatty acid chains, unlike previous studies (10–15). We consequently determined that the major components isolated from bovine udder lipid extract were *sn*-3-monoacyldiglycerides (*sn*-3-MADGs).

## MATERIALS AND METHODS

All solvents and reagents used were of the highest commercial quality available. All aqueous solutions were prepared using deionized and distilled water. High performance thin-layer chromatography (HPTLC) and silica gel column chromatography were performed using Merck Kieselgel 60 F<sub>254</sub> and Kieselgel 60, 230–400 mesh ASTM silica gel, respectively. Fresh bovine udder was purchased at a meat market in Seoul, Korea.

### Isolation of monoacyldiglycerides from bovine udder

Bovine udder (2.7 kg) was minced, washed with distilled water, and then repeatedly rinsed with phosphate-buffered saline solution (PBS, pH 7.4), while cooling in ice water. The bovine udder was homogenized in 20 g portions with a mixer for 15 min in CHCl<sub>3</sub>–MeOH 2:1 (v/v) (23). The homogenized bovine udder was extracted overnight at 4°C by stirring with CHCl<sub>3</sub>–MeOH 2:1 (v/v) and then the extract was centrifuged at 4°C and 5,000 rpm (JA-14R) for 30 min. The supernatant in chloroform was concentrated under reduced pressure to afford 63.4 g of a lipid mixture. The resultant residue was subjected to silica gel column chromatography using hexane–diethyl ether 4:1 (v/v) as eluent, collecting a total of eight fractions. NMR analysis showed that the fifth fraction contained monoacyldiglycerides, so this fraction (285 mg) was then chromatographed on a silica gel column (35 cm × 1.3 cm I.D.) using hexane–ethyl acetate 9:1 (v/v) for further purification. The third (55.8 mg) of five fractions collected was further purified by repeated chromatography on a silica gel column (16 cm × 1.0 cm I.D.) eluted with hexane–ethyl acetate 20:1 (v/v), yielding monoacyldiglycerides (6.5 mg); *R<sub>f</sub>* 0.29 (hexane:ethyl acetate, 9:1, v/v). The purity of each band was confirmed by HPTLC and visualization with I<sub>2</sub> vapor. In a comparative HPTLC analysis of these lipid extracts corresponding to the MADGs fraction, weak spots were observed after the first step and the second step purification, but a clear spot appeared as a single band after the third step purification.

### NMR analyses

NMR analyses were conducted at 250 MHz (for <sup>1</sup>H, <sup>1</sup>H–<sup>1</sup>H COSY NMR), 63 MHz (for DEPT 135, <sup>13</sup>C-NMR), and 500 MHz (for HMQC NMR) in CDCl<sub>3</sub> with tetramethylsilane (TMS) as an internal standard. NMR spectra were recorded using the Xwin software package.

### Mass spectrometry

All mass spectrometric analyses were carried out using a JMS-HX110/110A tandem mass spectrometer (JEOL, Tokyo, Japan), a four-sector instrument with an E<sub>1</sub>B<sub>1</sub>E<sub>2</sub>B<sub>2</sub> configuration previously described in detail elsewhere (24, 25). Briefly, an ion source was operated at an accelerating voltage of 10 kV in positive-ion mode with a mass resolution of 1000 (10% valley). Ions were produced by FAB using a cesium ion beam, which was generated from an ion gun and accelerated to 12 keV. Approximately 10 μg of each sample dissolved in chloroform–methanol 1:1 (v/v) was mixed with 1 μl of 3-nitrobenzyl alcohol (3-NBA; Sigma) and NaI

on a FAB probe tip. Using the first mass spectrometer (MS-1) to select sodium-adducted molecules, CID was generated in the collision cell located between B<sub>1</sub> and E<sub>2</sub>, floating at 3.0 kV. Resultant product ions were analyzed by B/E scan method using the second mass spectrometer (MS-2). The collision gas, helium, was introduced into the collision chamber at a pressure sufficient to reduce the precursor ion signal by 70%. Signals were averaged with double scans. The resolution of MS-1 was adjusted such that only the <sup>12</sup>C-species of the precursor ions was transmitted while MS-2 was operated at a resolution of 1000.

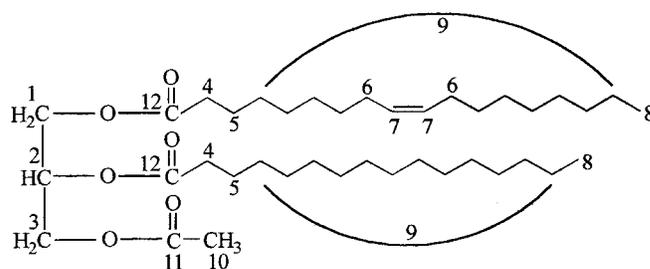
## RESULTS AND DISCUSSION

Extracts from the leaves of *Maytenus ilicifolia* (15), whose major compounds are MADGs, have been utilized internally and externally in a popular medicine. In our study, MADGs were isolated from bovine udder by solvent extraction (23) and silica gel column chromatography. Structural identification of the isolated MADGs by techniques including NMR, FAB-MS, and MS/MS revealed that these MADGs contained an acetate group at the *sn*-3 position of their glycerol backbone. This is the first report that verifies the presence of MADGs in animal tissue, particularly bovine udder.

### Structural identification

Table 1 displays the <sup>1</sup>H and <sup>13</sup>C-NMR spectral data for the lipid extract isolated from bovine udder while DEPT 135 spectral data taken at 63 MHz is shown in Fig. 1. The combination of <sup>13</sup>C and DEPT 135 NMR spectra clearly suggests the presence of the MADG skeleton. For further

TABLE 1. Representative <sup>1</sup>H and <sup>13</sup>C-NMR peak assignments of monoacyldiglycerides isolated from bovine udder

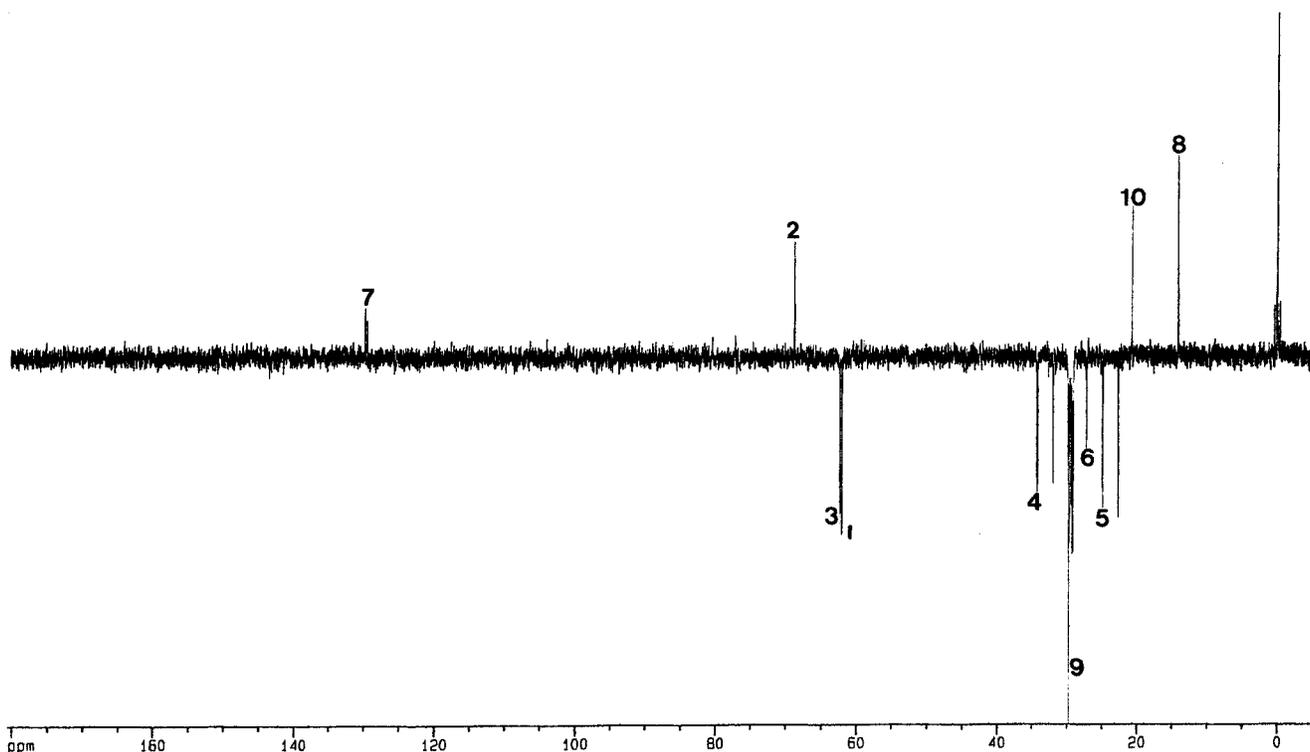


Position	<sup>1</sup> H-NMR <sup>a</sup> δ	<sup>13</sup> C-NMR <sup>b</sup> δ
1	4.30	62.09
2	5.26	68.80
3	4.15	62.39
4	2.33	34.23
5	1.60	22.72
6	2.02	24.89
7	5.32	129.72, 130.04
8	0.92	14.14
9	1.26	22.72, 29.09–29.73, 31.95
10	2.07	20.72
11		170.53
12		172.95, 173.35

Measurements were made in CDCl<sub>3</sub>.

<sup>a</sup> Run at 250 MHz.

<sup>b</sup> Run at 63 MHz.



**Fig. 1.** DEPT 135 spectrum of monoacyldiglycerides isolated from bovine udder (63 MHz). The resonances labeled 1–10 represent the ten kinds of carbon atoms of the monoacyldiglycerides.

structural analysis, we used a variety of homonuclear and heteronuclear 2-D NMR techniques. The 2-D  $^1\text{H}$ - $^1\text{H}$  COSY (250 MHz) and  $^1\text{H}$ - $^{13}\text{C}$  correlation spectrometry (COSY)  $^1\text{H}$ -detected heteronuclear multiple-quantum coherence (HMQC) spectra (500 MHz) of MADGs isolated from bovine udder are presented in **Fig. 2** and **Fig. 3**, respectively. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra (Table 1) of isolated extracts did not give exact signals of the *sn*-1 and *sn*-2 positions of the fatty acid glycerol backbone. However, the 2-D  $^1\text{H}$ - $^1\text{H}$  COSY and HMQC experiments identified almost all of the vicinal protons and proton-bearing carbons in the molecules. In the  $^1\text{H}$ - $^1\text{H}$  COSY NMR experiment, the partial structures of H-1 to H-3, H-4 to H-5, H-6 to H-7, and H-8 to H-9 were unambiguously assigned. In particular,  $^1\text{H}$ - $^1\text{H}$  COSY coupling assisted us in sequencing the carbons at 62.09, 62.39, and 68.80 ppm as the carbon signals of the glycerol backbone. These three signals were associated with  $^1\text{H}$  signals at 4.30, 4.15, and 5.26 ppm based on correlation with the HMQC spectrum of the isolated extracts. The  $^1\text{H}$ -NMR peak at 2.07 ppm for the acetate methyl group (26, 27) was correlated to the  $^{13}\text{C}$  resonance at 20.72 ppm in the HMQC spectrum. Likewise, a double bond signal observed in the  $^1\text{H}$  spectrum at 5.32 ppm was related to the  $^{13}\text{C}$  resonance at 129.72 and 130.04 ppm in the HMQC spectrum. From the above NMR analyses, it was inferred that the extract isolated from bovine udder was a mixture of MADGs. However, the  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT 135,  $^1\text{H}$ - $^1\text{H}$  COSY, and HMQC NMR spectroscopic analyses for the extracts isolated from bovine udder did not serve to decipher the chemical shifts of the mostly over-

lapped methylene protons linked to long-chain fatty acids. Using these spectroscopic techniques, it proved very difficult to identify the composition of acyl groups linked to the glycerol backbone as well as the double bond position in the fatty acyl chains. Although the  $^{13}\text{C}$ -NMR and DEPT 135 NMR peaks were very informative, except for the overlapped methylene carbons, the intensity of double bond peaks at 5.32 ppm in the  $^1\text{H}$ -NMR spectrum could not be explained. To obtain more detailed information on the composition of the MADGs, the isolated fraction of bovine udder was investigated using FAB-MS and MS/MS.

The positive-ion FAB mass spectra of isolated MADGs from bovine udder, shown in **Fig. 4**, displayed very complex spectral patterns due to the presence of numerous molecular species and a variety of fragment ions. FAB of MADGs in a matrix of 3-NBA saturated with NaI produced prominent sodium-adducted molecular ions ( $[\text{M}+\text{Na}]^+$ ) in a high-mass region. These ions differed in chain length and the degree of unsaturation of two long-chain acyl groups. Abundant diglyceride ions ( $[\text{M}+\text{H}-\text{R}_n\text{COOH}]^+$ ) were also observed and were believed caused by the loss of one fatty acyl group as free fatty acid among the two long-chain acyl groups of each molecular species. Hence these ions provided information on the composition of the other long-chain fatty acyl group. The ions of  $m/z$  299, 327, 355, and 381 were found to correspond to diglyceride ions containing lauroyl (C12:0), myristoyl (C14:0), palmitoyl (C16:0), and oleoyl (C18:1) groups, respectively. In addition, other ion species corresponding to acylium ions ( $[\text{R}_n\text{CO}]^+$ ) containing myristoyl, palmitoyl,

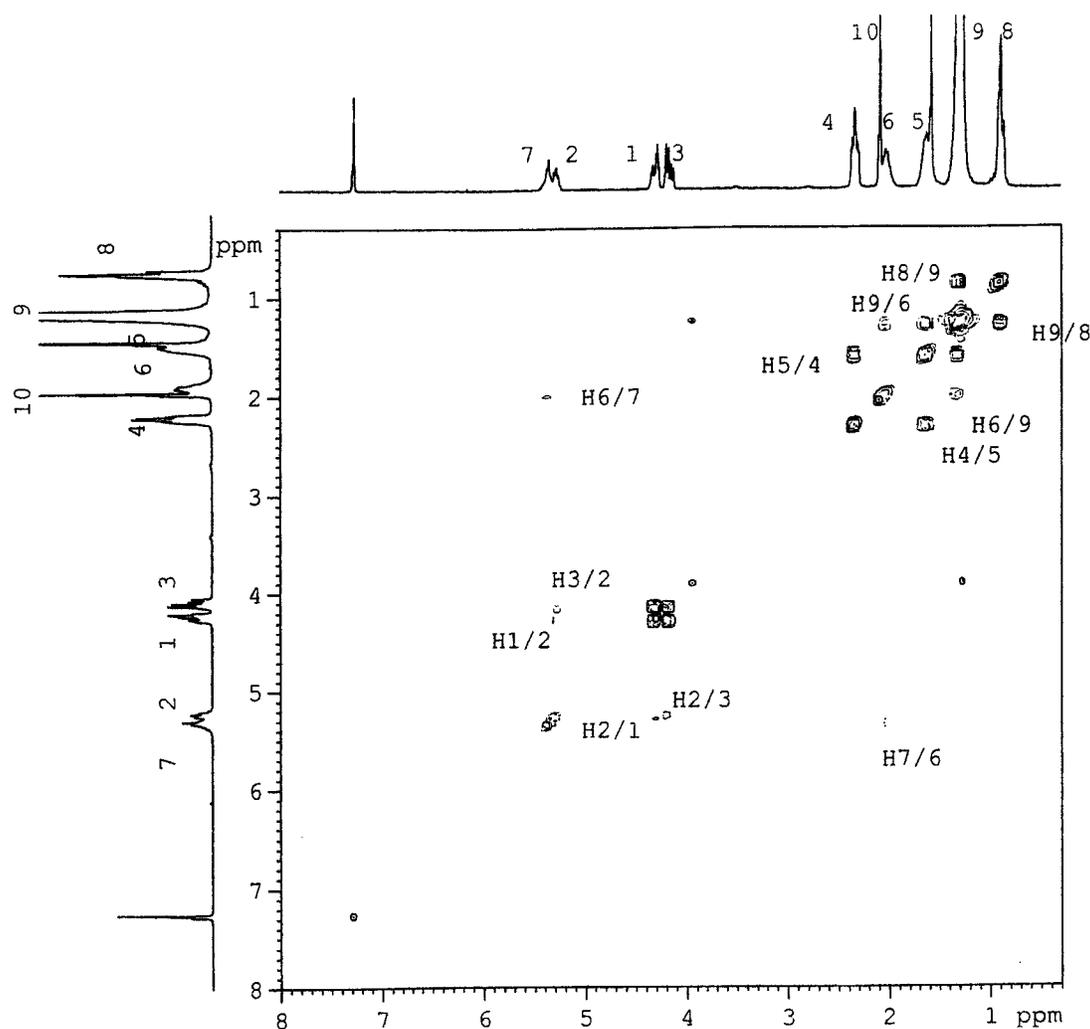


Fig. 2. 2-D  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of monoacyldiglycerides isolated from bovine udder (250 MHz). The resonances labeled 1–10 represent the ten kinds of proton atoms of the monoacyldiglycerides.

and oleoyl groups were observed at  $m/z$  211, 239, and 265, respectively. These fragment ions only provided information on the presence of the fatty acyl groups in the mixture of the molecular species. To acquire more detailed structural information on the sodium-adducted molecular ions observed in the FAB-MS spectrum, we then proceeded to MS/MS. In our previous work, we reported that CID of  $[\text{M}+\text{Na}]^+$  of synthetic MADGs produced ions yielding significant information on the complete structure of the precursor ions (22). From these product ions, the regioselectivity of two long-chain acyl groups at the *sn*-1 and *sn*-2 positions of the glycerol backbone as well as the fatty acid composition and the double bond position in the fatty acyl chains were determined. Thus, this same method was also applicable to the structural identification of each molecular species in a mixture of MADGs isolated from bovine udder. Figure 5 shows the CID spectrum of the major precursor ion at  $m/z$  659. The peak pattern of this spectrum is very similar to that of the CID spectrum of  $[\text{M}+\text{Na}]^+$  of synthetic 1-oleoyl-2-palmitoyl-3-acetyl-*rac*-glycerol (C18:1/C16:0-MADG) reported previously (22). From

two pairs of G and  $^3\text{I}$  ions, this species was found to contain C18:1 (oleoyl) and C16:0 (palmitoyl) groups as two long-chain acyl groups. Charge-remote fragmentation (CRF) along the C18:1 acyl chain yielded a homologous series of ions observed at  $m/z$  643, 629, 615, 601, 587, 573, 559, 545, 533, 519, 505, 491, 477, 463, and 449 ( $^3\text{I}_1$ ) in a high-mass region. This series begins with the product ion of  $m/z$  643 corresponding to the loss of  $\text{CH}_4$  from the alkyl-terminus side and ends with an abundant  $^3\text{I}_1$  ion corresponding to the stable,  $\alpha$ ,  $\beta$ -unsaturated ester. The mass difference between adjacent peaks is 14 u due to a series of  $\text{C}_n\text{H}_{2n+2}$  or  $\text{C}_n\text{H}_{2n}$  fragment losses via 1,4-elimination of charge-remote fragmentation (CRF) (28). The presence of a double bond in the acyl chain reduces the mass difference to 12 u. Thus, the species of  $m/z$  659 contains the oleoyl group. The fragmentation pathways are illustrated in Fig. 5 and results obtained for other species are summarized in Table 2. Each molecular species observed at  $m/z$  577, 631, and 657 was a mixture of two components having different fatty acid compositions.

In our previous work, it was observed that the relative intensity ratio ( $G_2/G_1$ ) of the two G ions in the CID spectra of

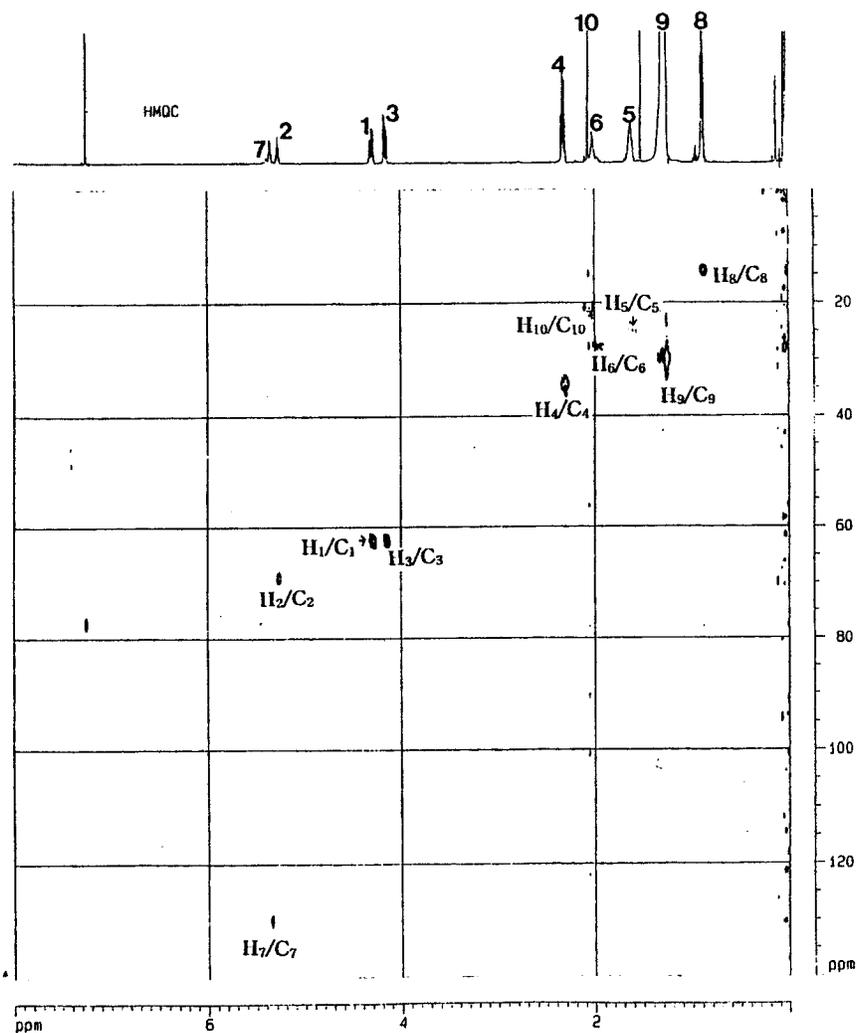


Fig. 3. 2-D  $^1\text{H}$ - $^{13}\text{C}$  COSY HMQC spectrum of monoacetyldiglycerides isolated from bovine udder (500 MHz). The resonances labeled 1–10 represent the ten kinds of proton and carbon atoms of the monoacetyldiglycerides.

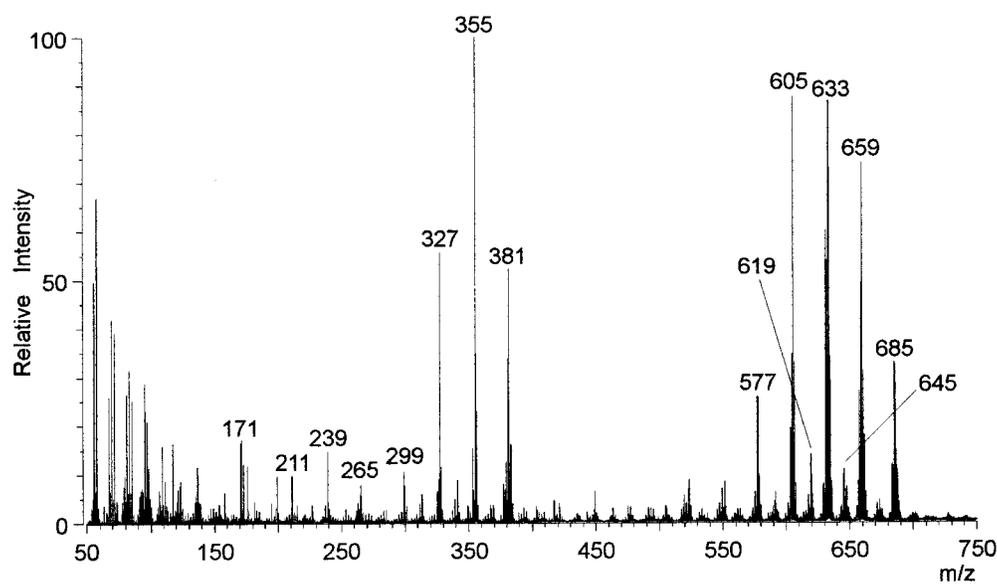
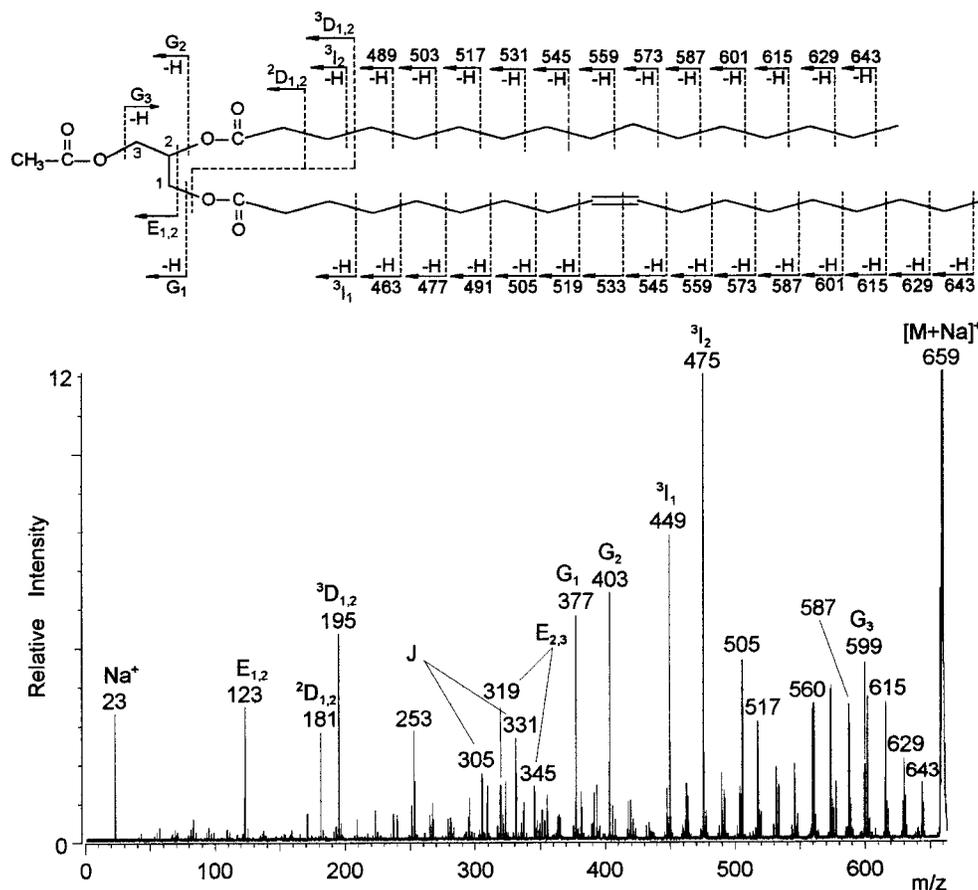


Fig. 4. Positive-ion FAB mass spectrum of MADGs isolated from bovine udder. A matrix of 3-nitrobenzyl alcohol saturated with NaI was used.



**Fig. 5.** FAB tandem mass spectrum of  $[M+Na]^+$  observed at  $m/z$  659. The fragmentation pathways are shown above the spectrum. The nomenclatures used are taken from refs. 22 and 25. The subscript in the symbol represents the relative position (*sn*-1, *sn*-2 or *sn*-3) of the cleavage in the fatty acyl chain and the superscript shows the cleaved bond position relative to the carbonyl carbon of the fatty acyl group.

$[M+Na]^+$  of standard MADGs was always larger than two (22). However, in the case of the species at  $m/z$  659, the ratio was 1.07. The small difference in the relative abun-

**TABLE 2.** Structural identification of monoacyldiglycerides isolated from bovine udder

MW	$[M+Na]^+$	Structure of Two Fatty Acyl Groups <sup>a</sup>
554.4	577.4	C16:0/C12:0 + C14:0/C14:0
582.4	605.4	C16:0/C14:0
608.4	631.4	C18:1/C14:0 + C16:0/C16:1
610.4	633.4	C16:0/C16:0
634.5	657.5	C18:2/C16:0 + C18:1/C16:1
636.5	659.5	C18:1/C16:0
638.5	661.5	C18:0/C16:0
660.5	683.5	C18:1/C18:2
662.5	685.5	C18:1/C18:1
664.5	687.5	C18:0/C18:1

<sup>a</sup> Individual molecular species of MADG are designated with the acyl groups at *sn*-1 and *sn*-2 positions listed in order, respectively (i.e., C16:0/C14:0 = *sn*-1/*sn*-2). The fatty acyl groups are symbolized by the convention, carbon number:double bond number (i.e., C12:0 (lauroyl), C14:0 (myristoyl), C16:0 (palmitoyl), C18:1 (oleoyl), C18:2 (linoleoyl)). The regioispecificity of fatty acyl linkages is determined from the intensity ratio of  $G_1$  and  $G_2$  ions observed in the CID spectrum of  $[M+Na]^+$  of each molecular species.

dance of the two G ions is due to the fact that the species is a mixture of two regioisomers which may exchange their fatty acyl groups at the *sn*-1 and *sn*-2 positions. This result was confirmed by the presence of two pairs of  $E_{2,3}$  and J ions corresponding to the sodiated propenyl ester with a *sn*-1 acyl group and vinyl ester ions with a *sn*-2 acyl group, respectively. The  $E_{2,3}$  and J ions produced from the regioisomer of C18:1/C16:0-MADG were observed at  $m/z$  345 and 305, respectively, while the same ions from the regioisomer of C16:0/C18:1-MADG appeared at  $m/z$  319 and 331, respectively. Similarly, it was proved that other species with different fatty acyl groups in Table 2 were a mixture of two regioisomers. On the other hand, based on the  $G_2/G_1$  ratios of synthetic standard C18:1/C16:0-, C16:0/C18:1-MADGs, and the molecular species observed at  $m/z$  659, each regioisomer in the mixture of the species could be relatively quantified. From three replicate experiments performed under the same measurement conditions, the  $G_2/G_1$  ratio of the standard C18:1/C16:0-MADG was measured as  $2.11 \pm 0.08$ , while that of C16:0/C18:1-MADG was  $2.48 \pm 0.05$ . As a result, the relative quantity of regioisomer C18:1/C16:0-MADG in the mixture was  $61 \pm 3\%$ . As shown in Table 2, complete interpretations of the MS/MS spectra led us to conclude that the MADGs extracted from bovine

udder were 1-palmitoyl-2-lauroyl-3-acetyl-*rac*-glycerol, 1,2-dimyristoyl-3-acetyl-*rac*-glycerol, 1-palmitoyl-2-myristoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-myristoyl-3-acetyl-*rac*-glycerol, 1-palmitoyl-2-palmitoleoyl-3-acetyl-*rac*-glycerol, 1,2-dipalmitoyl-3-acetyl-*rac*-glycerol, 1-linoleoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-palmitoleoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-stearoyl-2-palmitoyl-3-acetyl-*rac*-glycerol, 1-oleoyl-2-linoleoyl-3-acetyl-*rac*-glycerol, 1,2-dioleoyl-3-acetyl-*rac*-glycerol, and 1-stearoyl-2-oleoyl-3-acetyl-*rac*-glycerol.

In the molecular ion region shown in Fig. 4, minor peaks that cannot be assigned to MADG species were observed at  $m/z$  619 and 645. To determine structural differ-

ences between these species and MADGs, these ions were investigated by FAB-CID-MS/MS. Fig. 6(a) shows the CID spectrum of  $[M+Na]^+$  observed at  $m/z$  619. For comparison with this spectrum, the CID spectrum of  $[M+Na]^+$  ( $m/z$  633) of MADG with two palmitoyl groups is presented in Fig. 6(b). Differences between the two spectra appear in the high-mass region where the product ions generated by fragmentation along the long hydrocarbon chains are observed. The peaks of  $m/z$  377, 393, 407, and 421 observed in Fig. 6(a) are believed due to the CRFs along the hexadecyl-chain ether linked to the glycerol backbone. Hence, this species was identified as 1-hexadecyl-2-palmitoyl-3-acetyl-*rac*-glycerol. Similarly, the species of

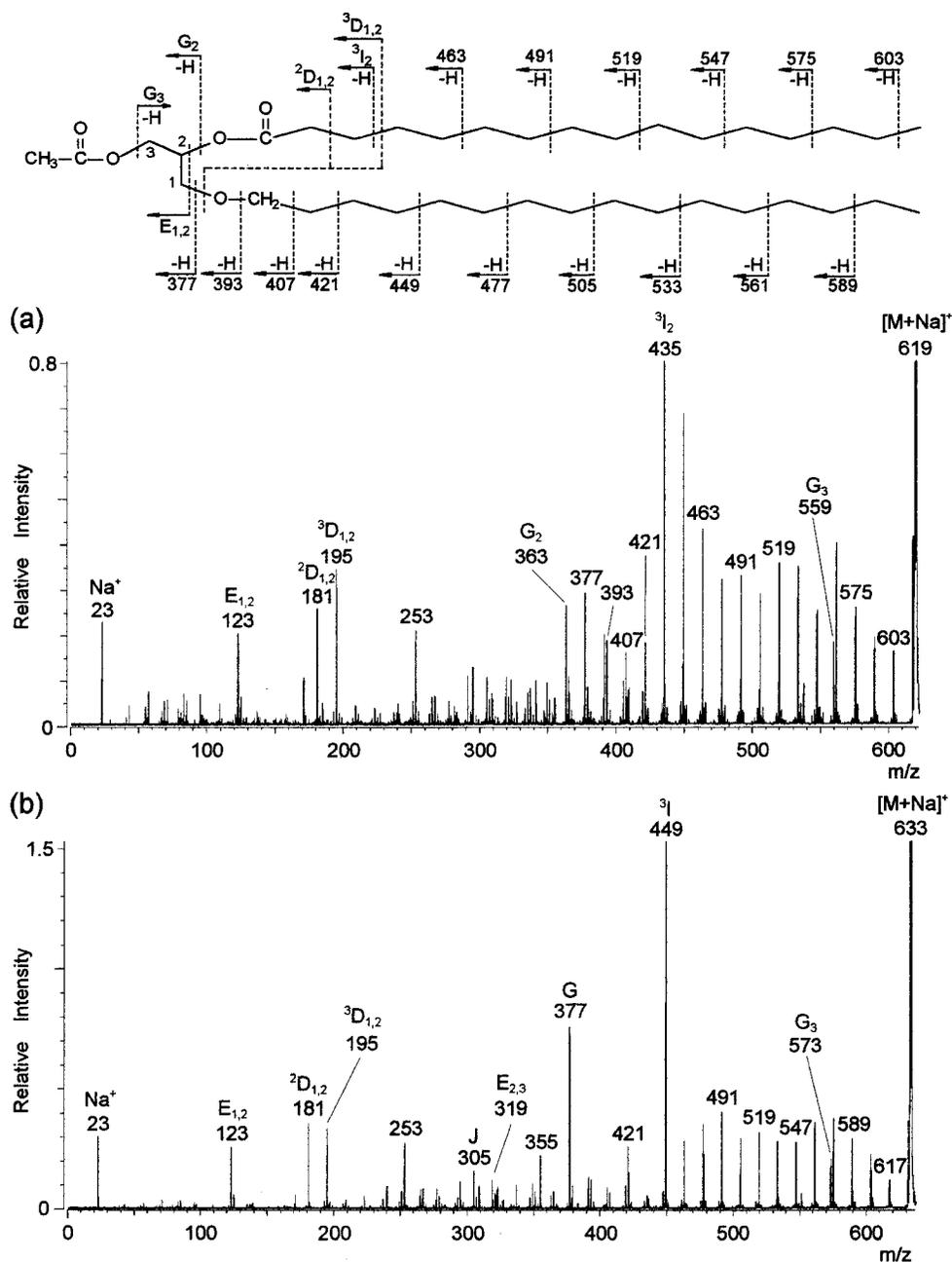


Fig. 6. FAB tandem mass spectra of  $[M+Na]^+$  observed at  $m/z$  (a) 619 and (b) 633. The fragmentation pathways for the product ions observed in Fig. 6(a) are also shown.

*m/z* 645 was identified as a mixture of 1-hexadecyl-2-oleoyl-3-acetyl-*rac*-glycerol and 1-octadecenyl-2-palmitoyl-3-acetyl-*rac*-glycerol.

## Conclusions

To the best of our knowledge, our isolation of MADGs from bovine udder is the first study showing that acetic acid can be a natural triglyceride component of animal tissue. Previous studies (11–15) identified neither the fatty acid position at the glycerol backbone nor the double bond position of the fatty acid chain attached to the glycerol backbone of MADGs. Using <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT 135 NMR, <sup>1</sup>H-<sup>1</sup>H COSY NMR, <sup>1</sup>H-<sup>13</sup>C COSY NMR, FAB-MS, and MS/MS, we were able to determine complete information on the position of acyl groups, double bond position in the fatty acyl groups as well as fatty acid compositions of MADGs. From our NMR experiments, the major compounds of the isolated natural products were found to be MADGs, where the presence of *sn*-3-acetyl was clearly confirmed. The relative intensity of *sp*<sup>2</sup> C-Hs at 5.32 ppm in the <sup>1</sup>H-NMR spectra showed that the MADG acyl groups were composed of both saturated and unsaturated fatty acids. Further, FAB-MS and MS-MS analyses provided complete structural determination of the MADG species. The FAB-MS and MS/MS experiments also showed that trace amounts of compounds (*m/z* 619, 645) had *sn*-1-ether linkages in their glycerol skeleton. The search for a new lipase of *sn*-3-MADGs which may exist in bovine udder is currently underway. ■

The financial supports for this work from the Korea Research Foundation (1998-015-D00198, 1998-015-D00233), KOSEF through the Center for Cell Signaling Research (CCSR), and most through the Women's University Research Fund (1998) are greatly acknowledged. The authors thank Ms. Y. J. Kim for helping with isolation of MADGs, Ms. H. J. Cho for assisting in NMR experiments, and Ms. S-H. Cho at Ewha Womans University and Dr. Y. C. Yoon at Konkuk University for helpful discussion.

Manuscript received 24 February 1999, in revised form 12 July 1999, and in re-revised form 16 August 1999.

## REFERENCES

- Schmidt, G. H., L. D. Van Vleck, and M. F. Hutjens. 1988. Principles of Dairy Science. TKM Productions; Simon & Schuster, Cliffs, NJ. 32–45.
- Schmidt, G. H. 1971. Biology of Lactation. W. H. Freeman & Co., editors. San Francisco. 6–11.
- Turner, C. W. 1952. The Mammary Gland 1. The Anatomy of the Udder of Cattle and Domestic Animals. Lucas Brothers, editors. Columbia.
- Smith, S. 1980. Mechanism of chain length determination in biosynthesis of milk fatty acids. *J. Dairy Sci.* **63**: 337–352.
- Hansen, H. O., I. Grunnet, and J. Knudsen. 1984. Triacylglycerol synthesis in goat mammary gland: the effect of ATP, Mg<sup>2+</sup> and glycerol 3-phosphate on the esterification of fatty acids synthesized de novo. *Biochem. J.* **220**: 513–519.
- Hansen, H. O., I. Grunnet, and J. Knudsen. 1984. Triacylglycerol synthesis in goat mammary gland: factors influencing the esterification of fatty acids synthesized de novo. *Biochem. J.* **220**: 521–527.
- Dils, R. R. 1986. Comparative aspects of milk fat synthesis. *J. Dairy Sci.* **69**: 904–910.
- Hansen, H. O., and J. Knudsen. 1987. Effect of exogenous long-chain fatty acids in lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: esterification of long-chain exogenous fatty acids. *J. Dairy Sci.* **70**: 1344–1349.
- Myher, J. J., A. Kuksis, and L. Marai. 1988. Identification of the more complex triacylglycerols in bovine milk fat by gas chromatography-mass spectrometry using polar capillary columns. *J. Chromatogr.* **452**: 93–118.
- Kaufmann, H. P., and M. Keller. 1948. Fats. CXVI. Significance of conjugate-unsaturated aliphatic acids in the chemistry of the fats. 4. The occurrence of parinaric acid and acetic acid in the seed fats of the Balsaminaceae. *Chem. Ber.* **81**: 152.
- Kleiman, R., R. W. Miller, F. R. Earle, and L. A. Wolff. 1966. Optically active aceto-triglycerides of oil from *Euonymus verrucosus* seed. *Lipids.* **1**: 286–287.
- Kleiman, R., R. W. Miller, F. R. Earle, and L. A. Wolff. 1967. (S)-1,2-Diacyl-3-acetins: optically active triglycerides from *Euonymus verrucosus* seed oil. *Lipids.* **2**: 473–478.
- Bedi, K. L., C. A. Atal, and K. T. Achaya. 1971. Indian seed oils. VII. Four oils with unusual constituents. *J. Sci. Fd Agric.* **22**: 140.
- Bagby, M. O., and C. R. Smith, Jr. 1967. Asymmetric triglycerides from *Impatiens edgeworthii* seed oil. *Biochim. Biophys. Acta.* **137**: 475–477.
- Spitzer, V., and R. Aichholz. 1996. Analysis of naturally occurring -acetotriacylglycerides by gas chromatography—Chemical ionization mass spectrometry. *J. High Resol Chromatogr.* **19**: 497–502.
- Jhon, G.-J., S.-Y. Park, S.-Y. Han, S. Lee, Y. Kim, and Y.-S. Chang. 1999. Studies of the chemical structure of gangliosides in deer antler, *Cervus nippon*. *Chem. Pharm. Bull.* **47**: 123–127.
- Jensen, N. J., K. B. Tomer, and M. L. Gross. 1986. Fast atom bombardment and tandem mass spectrometry of phosphatidylserine and phosphatidylcholine. *Lipids.* **21**: 580–588.
- Jensen, N. J., K. B. Tomer, and M. L. Gross. 1987. FAB MS/MS for phosphatidylinositol-, glycerol-, ethanalamine and other complex phospholipids. *Lipids.* **22**: 480–489.
- Bryant, D. K., R. C. Orlando, C. Fenselau, R. C. Sowder, and L. E. Henderson. 1991. Four-sector tandem mass spectrometric analysis of complex mixtures of phosphatidylcholines present in a human immunodeficiency virus preparation. *Anal. Chem.* **63**: 1110–1114.
- Kayganich, K. A., and R. C. Murphy. 1992. Fast atom bombardment tandem mass spectrometric identification of diacyl, alkylacyl, and alk-1-enylacyl molecular species of glycerophosphoethanolamine in human polymorphonuclear leukocytes. *Anal. Chem.* **64**: 2965–2971.
- Gage, D. A., Z. H. Huang, and C. Benning. 1992. Comparison of sulfoquinovosyl diacylglycerol from spinach and the purple bacterium *Rhodobacter sphaeroides* by fast atom bombardment tandem mass spectrometry. *Lipids.* **27**: 632–636.
- Kim, Y. H., S.-Y. Han, S.-H. Cho, J. S. Yoo, and G.-J. Jhon. 1999. Structural determination of synthetic monoacyldiglycerides by tandem mass spectrometry of sodium-adducted molecules. *Rapid Commun. Mass Spectrom.* **13**: 481–487.
- Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**: 497–509.
- Kim, Y. H., J. S. Yoo, C. H. Lee, Y. M. Goo, and M. S. Kim. 1996. Application of fast atom bombardment combined with tandem mass spectrometry to the structural elucidation of *O*-demethylabierixin and related polyether antibiotics. *J. Mass Spectrom.* **31**: 855–860.
- Kim, Y. H., J. S. Yoo, and M. S. Kim. 1997. Structural characterization of sulfoquinovosyl, monogalactosyl and digalactosyl diacylglycerols by FAB-CID-MS/MS. *J. Mass Spectrom.* **32**: 969–977.
- Sengupta, A., and H. N. Bhargava. 1970. Chemical investigation of the seed fat of *Celastrus paniculatus*. *J. Sci. Fd Agric.* **21**: 628–631.
- Gunstone, F. D. 1995. New Trends in Lipid and Lipoprotein Analysis. J. L. Sebedio and E. G. Perkins, editors. AOCS Press, Champaign, IL. 250.
- Jensen, N. J., K. B. Tomer, and M. L. Gross. 1985. Gas-phase ion decompositions occurring remote to a charge site. *J. Am. Chem. Soc.* **107**: 1863–1868.