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ARTICLE

# Chemical Synthesis of 9(*Z*)-Octadecenamide and Its Hypolipidemic Effect: A Bioactive Agent Found in the Essential Oil of Mountain Celery Seeds

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The unusual hypolipidemic activity of the methanolic fractionate of the essential oil (EOM) obtained from the mountain celery seed was previously reported. The most enriched 9(*Z*)-octadecenamide (oleamide) was speculated to be responsible for the relevant bioactivity. Chemically syntheized oleamide (CSO) yielded 85.1% with a purity of 98.6% when identified by RP-HPLC, FTIR, HREIMS, $^{1}$H NMR, and $^{13}$C NMR. CSO was tested for its antioxidative and hypolipidemic bioactivities. Results indicated CSO was potently hypolipidemic with regard to serum TG, TC, LDL-C, LDL-C/HDL-C, and hepatic TG ($p < 0.05$), but not for serum HDL-C and hepatic TC. In addition, CSO exhibited only poor antioxidative activity, implicating the possibility that the hypolipidemic and antioxidative bioactivity of original EOM was due to another coexisting constituent, probably $\gamma$-selinene. Conclusively, oleamide is a potent hypolipidemic agent as regarding its effects on decreasing serum TG, TC, LDL-C and hepatic TG.

**KEYWORDS: 9(*Z*)-Octadecenamide (oleamide); mountain celery seed; hypolipidemic; antioxidative; $\gamma$-selinene**

## INTRODUCTION

Previously, we identified in the methanol fractionate of the essential oil of mountain celery seeds (MCS) the presence of a huge content of 9(*Z*)-octadecenamide (oleamide), amounting up to 38.6% (*1*). The essential oil of MCS (EOM) had been demonstrated to have a very strong superoxide scavenging activity and hypolipidemic bioactivity, in particular, the elevation effect on the level of high-density lipoprotein-cholesterol (HDL-C) (*1*). We suspected this major bioactivity could be closely related with its high oleamide content. Oleamide is the prototypical long-chain primary fatty acid amide lipid messenger originally discovered in the cerebrospinal fluid of sleep-deprived cats. It was shown to be an endogenous soporific lipid in animal models. Accordingly, oleamide first became known for its potential role acting as the endogenous influencing mechanism mediating the drive to sleep in mammals (*2*,*3*). In addition, oleamide also has profound effects on thermoregulation and acts as an analgesic in several models of experimental pain. Moreover, oleamide has a variety of biochemical activities involving acting as a potential signaling molecule (*4*). Akanmu et al. confirmed the multiplicity of central nervous system receptors and neurotransmitters that oleamide interacts with, hence its numerous and diverse neuropharmacological effects (*5*). Oleamide has an antidepressant-like property (*5*). Psychiatrically, oleamide induced behaviors reminiscent of the actions of endogenous cannabinoids, but the GABAergic and dopaminergic systems, either directly or indirectly, were considered to be crucially involved in the actions of oleamide (*6*).

One of the most potent physiological actions of oleamide is vasodilation, which is mediated by endothelium-derived nitric oxide, by endothelium-dependent hyperpolarization, and also through activation of TRPV1 receptors (*7*, *8*). In addition, oleamide exhibits antiepileptic activity and significantly decreases the degree of pentylenetetrazole-induced seizures (*9*). More attractively, 19-oleamide derivatives are prototypical antimetastasis drugs that act by inhibiting connexin 26 (*10*).

Oleamide induced significant increase of intracellular calcium ion concentration $[Ca^{2+}]_i$ by a phospholipase C-independent release of $Ca^{2+}$ from thapsigargin-sensitive stores and by inducing $Ca^{2+}$ entry (*11*). $[Ca^{2+}]_i$ ions in pheochromocytoma cells, renal tubular cells (*12*), osteoblast-like cells, and bladder cancer cells were increased on stimulation of oleamide, among which the bladder cancer T24 cells responded most sensitively (*11*).

Although many important pharmacologic effects are well established, the biochemical mechanism for the synthesis of oleamide has not yet been defined (*3*), nor the hypolipidemic

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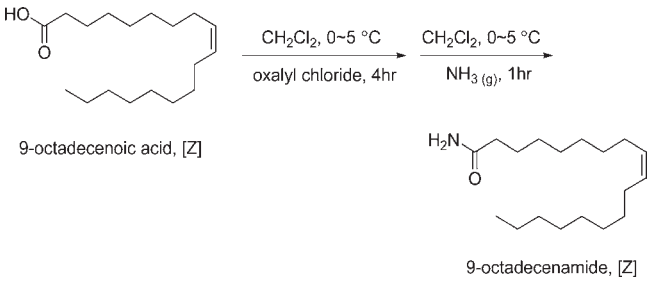


**Figure 1.** Total scheme of chemical synthesis for 9(*Z*)-octadecenamide.

bioactivity of oleamide has been investigated. To investigate whether a pure oleamide could be responsible for the unusual biochemical activity found in EOM (*1*), we performed the chemical synthesis. The synthetic oleamide was identified by a serial instrumental analysis involving reverse phase HPLC (RP-HPLC), Fourier transform IR (FTIR), high-resolution electron ion mass spectrometry (HREIMS), proton nuclear magnetic resonance ($^1$H NMR), and carbon-13 NMR ($^{13}$C NMR). In addition, the relevant antioxidative and hypolipidemic activities of oleamide in animal models were examined.

## MATERIALS AND METHODS

**Chemicals and Reagents.** 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ferrozine, nitroblue tetrazolium (NBT), dihydronicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), ethylenediaminetetraacetate (EDTA) (disodium salt), $H_2O_2$ (30%), peroxidase, phenol red, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid, and citric acid were products of Sigma Aldrich Co. (St. Louis, MO). All chemicals used were of reagent grade.

**Plant Material.** Fresh mountain celery (*Cryptotaenia japonica* Hassk) seeds (MCS) were purchased from the local farm of Nan-Tou, a montaineous county located in central Taiwan. These seeds were immediately stored in the dark on shelves maintained at −20 °C.

**Isolation of Essential Oils.** The essential oil of MCS was prepared as previously described (*1*). The yellowish essential oils obtained were combined and subjected to solvent fractionation according to the method of Cheng et al. (*1*). The methanolic fraction (F-4) was stored at −20 °C for further use.

**Mass Preparation of 9(*Z*)-Octadecenamide.** Mass preparative liquid chromatography (MPLC) was used for mass preparation of 9(*Z*)-octadecenamide. The MPLC, having a C-18 column (i.d. × *l* = 1.5 × 24 cm) packed with polygoprep Macherey-Nagel (size 50−60 $\mu$m), was equipped with a Büchi B-684 fraction collector and a Knauer variable-wavelength monitor UV 205. A built-in Eyela-Toricorder Büchi 681 chromatography pump was used to drive the mobile phase. For preparation, 3 g of F-4 was applied into the MPLC and eluted with a mixed mobile phase (acetonitrile/$H_2O$ = 80:20). The elution was controlled at 3 mL/min. The volume of each fraction collected was 25 mL/vial. Finally, the solvent methanol was driven off using the nitrogen blow (residue designated F-4R1). The native oleamide in F-4R1 was confirmed by the following instrumental analyses and comparison with the reference compound.

**Chemical Synthesis of 9(*Z*)-Octadecenamide.** The synthetic method was performed according to that of Xu et al. (*13*) with a slight modification. Briefly, 25 g of 9(*Z*)-octadecenoic acid was accurately weighed and placed into a 500 mL four-mouthed reaction vessel previously dried at 280 °C for 16 h, to which 350 mL of ethylene dichloride ($CH_2Cl_2$) was added. The mixture was cooled to 0−5 °C with constant stirring for 30 min. Twenty-two milliliters of oxalyl chloride was cautiously added in small portions, avoiding the rise of temperature over 5 °C. The reaction mixture was allowed to react at room temperature for 4 h. When the reaction was finished, the mixture was subjected to the vacuum evaporator to drive off the excess $CH_2Cl_2$ and oxalyl chloride. Fresh $CH_2Cl_2$ (250 mL) was replenished into the reaction vessel, and the solution was refrigerated to 0−5 °C. A stream of ammonia gas [$NH_3$(g)] was slowly introduced into the reaction vessel for 1 h to facilitate the amidation. On completion of the reaction, the mixture was subjected to vacuum evaporation to completely eliminate excess $CH_2Cl_2$. Ethyl acetate (500 mL) was added to dissolve the residue. After the solution had been rinsed with 300 mL of distilled water, the ethyl acetate layer was separated and washed sequentially with 200 mL of saturated sodium bicarbonate ($NaHCO_3$) solution and a sufficient amount of distilled water to obtain a neutral pH. The solution was dehydrated with a sufficient amount of anhydrous magnesium sulfate and filtered. The filtrate was vacuum evaporated to completely drive off the ethyl acetate. The crude product was further purified on a C-18 column chromatography column (i.d. × *l* = 1.5 × 24 cm) packed with polygoprep Macherey-Nagel (60−50 $\mu$m) using a mixed mobile phase (acetonitrile/$H_2O$ = 80:20) operated at 3 mL/min. The entire separation process was monitored at 205 nm. The total synthetic scheme is shown in **Figure 1**. The synthetic product oleamide was subjected to the following instrumental analyses against the reference oleamide and was used to test its antioxidative and hypolipidemic bioactivities.

**Table 1.** Ingredients of Experimental Animal Diets[a]

| ingredient | N | H | OM1 | OM2 | OM3 |
|---|---|---|---|---|---|
| casein | 20 | 20 | 20 | 20 | 20 |
| sucrose | 15 | 15 | 15 | 15 | 15 |
| corn starch | 50 | 45 | 45 | 45 | 45 |
| corn oil | 2.5 | 5.0 | 5.0 | 5.0 | 5.0 |
| lard | 2.5 | 5.0 | 5.0 | 5.0 | 5.0 |
| MPx[b] | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| VPx[c] | 1 | 1 | 1 | 1 | 1 |
| choline | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| methionine | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| α-cellulose | 5 | 5 | 5 | 5 | 5 |
| ZOAM | 0 | 0 | 0.05 | 0.10 | 0.30 |

[a] Formulation was based on AIN-76. N, the regular diet for control group; H, high-lipid diet; OM1, diet H plus ZOAM 0.05% w/w; OM2, diet H plus ZOAM 0.10% w/w; OM3, diet H plus ZOAM 0.30% w/w. [b] MPx (mineral premix): $CaHPO_4 \cdot 2H_2O$, NaCl, $K_3C_6H_5O_7$, $K_2SO_4$, $MgO_3$, $MnO_3$, iron citrate, $ZnCO_3$, $CuCO_3$, KI, $NaSeO_3$, $K_2SO_4 \cdot Cr_2(SO_4)_3 \cdot 24H_2O$. [c] VPx (vitamin premix): thiamin hydrochloride, pyridoxine hydrochloride, riboflavin, nicotinic acid, vitamin $B_{12}$, retinyl palmitate, vitamin $D_3$, vitamin E, vitamin K.

**Table 2.** Percent Yield of Different Fractionates from the Essential Oil of Mountain Celery Seeds Separated on a Silica Gel Column

| fraction | yield[a] (%) |
|---|---|
| native essential oils[b] (EO) | 0.40 ± 0.04 |
| fractionate[c] | |
| F-1 (pentane) | 41.48 ± 2.31 |
| F-2 (ether) | 45.85 ± 1.85 |
| F-3 (acetone) | 4.72 ± 0.08 |
| F-4 (methanol) | 5.97 ± 0.06 |

[a] Obtained from triplicate determinations and expressed as mean ± SD. The percent yields of fractions F-1−F-4 were counted back by setting the original native essential oil (EO) as 100%. [b] Native seed essential oils (EO): obtained from steam distillation of 100 g of pulverized seeds. A silica gel column (i.d. × *l* = 2 cm × 50 cm) packed with 40 g of silica gel (76-230 mesh, Merck) was used. One gram of the product essential oil was transferred onto the silica gel column for eluting separation. [c] F-1, F-2, F-3, and F-4, SEO was successively fractioned by silica gel column using, respectively, solvents pentane, ether, acetone, and methanol, each with 250 mL.

**RP-HPLC Analysis of 9(*Z*)-Octadecenamide.** Sample oleamide (100 mg) was redissolved in 1 mL of the mixed solvent (acetonitrile/$H_2O$ = 80:20). An aliqout of 10 $\mu$L was carefully measured and subjected to the C-18 column in the RP-HPLC (HP1100). The mobile phase comprising acetonitrile/$H_2O$ (80:20) was operated at a flow rate of 1 mL/min and scanned at 205 nm by the UV detector. The presence of oleamide was confirmed by comparison with the reference compound.

**FTIR Analysis of 9(*Z*)-Octadecenamide.** The sample oleamide obtained was mixed thoroughly with KBr powder (IR grade) at a ratio of KBr to oleamide of 200:1. The mixture was macerated to obtain a homogeneous texture and then tabletted. The IR spectrum was taken with the Nicolet Type Protégé 460 IR spectrometer ESP against the KBr blank.

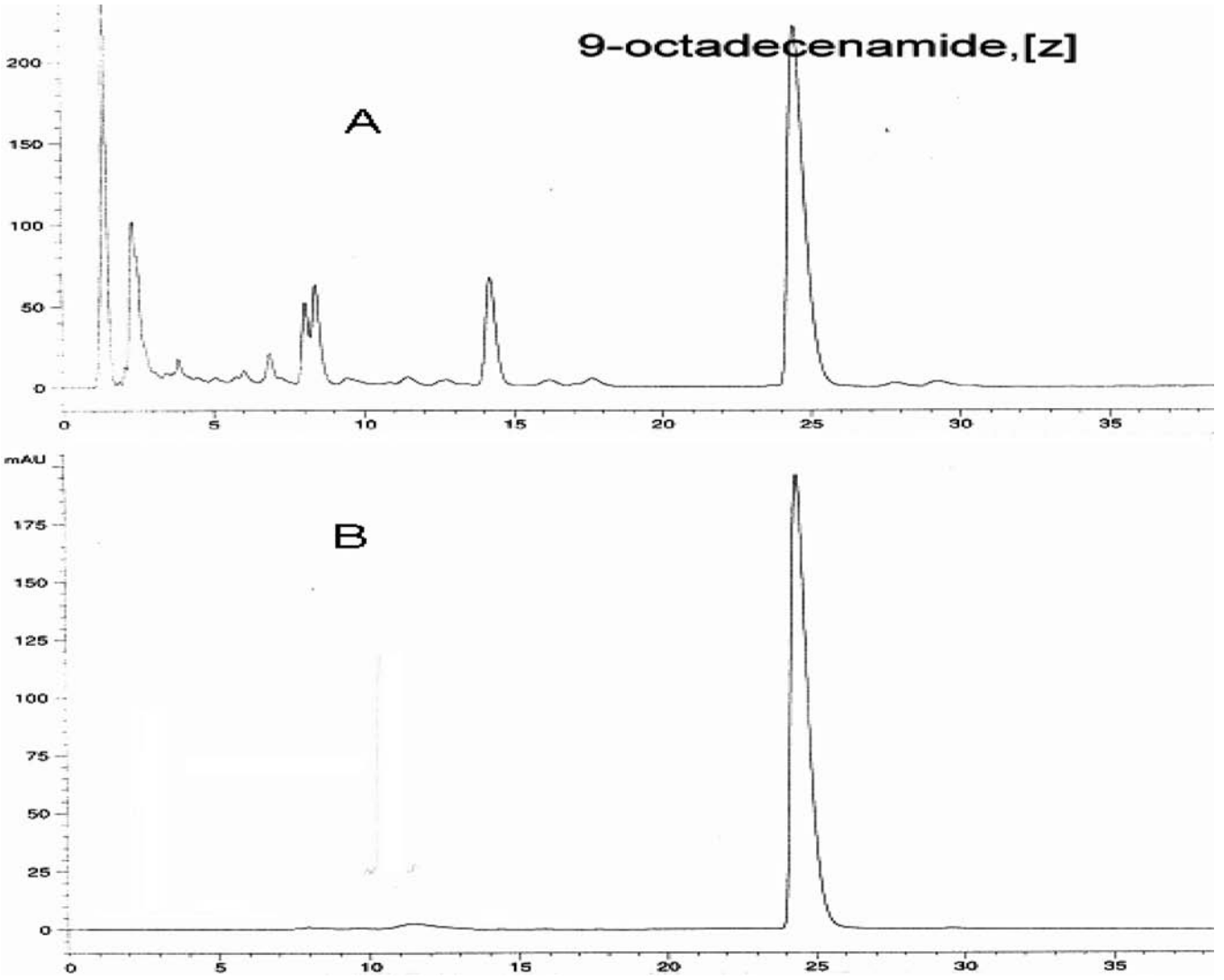


**Figure 2.** RP-HPLC spectrum of 9(*Z*)-octadecenamide: (**A**) methanolic fractionate of the essential oil of mountain celery seeds eluted on silica gel column ($t_R$ = 24.62 min); (**B**) purified 9(*Z*)-octadecenamide ($t_R$ = 24.61 min).

**HREIMS Analysis of 9(*Z*)-Octadecenamide.** Five milligrams of the sample 9(*Z*)-octadecenamide was dissolved in methanol to make a concentration of 1 mg/mL. An aliquot of 20 $\mu$L was injected into the sample injection port of the GC-HREI-MS (GC HP 6890), which was installed with the detector HP 5973 MSD and the analyzer capillary type DB-1 (i.d. × *l* = 0.25 mm × 60 m, membrane thickness = 0.25 $\mu$m). Helium was used as the carrier gas operated at 1 mL/min. The ionization voltage was operated at 70 eV, and the ion source temperature was set at 230 °C. The split ratio was 50:1. The temperature was initially 40 °C, which was held for 10 min and then programed at an elevation rate of 2 °C/min to 240 °C and held for 20 min.

**$^1$H NMR Analysis of 9(*Z*)-Octadecenamide.** The $^1$H NMR spectrum was taken with the Bruker 400 MHz spectrometer. In brief, 10 mg of sample was dissolved in $CDCl_3$ and analyzed with the $^1$H NMR spectrometer. The chemical shift of oleamide was determined (in ppm) against the authentic 9(*Z*)-octadecenamide with respect to that of the internal standard tetramethylsilane (TMS).

**$^{13}$C NMR Analysis of 9(*Z*)-Octadecenamide.** Similar to the above, the sample was analyzed with $^{13}$C NMR ($CDCl_3$, 100 MHz).

**Antioxidative Capability of 9(*Z*)-Octadecenamide.** *Scavenging Capability for DPPH Free Radicals*. The method described by Shimada et al. (*14*) was adopted. Briefly, to 1 mL of oleamide (2 mg/mL) were added 4 mL of methanol and 1 mL of DPPH (0.2 mM, Merck). The mixture was thoroughly agitated and left to stand in the dark for 30 min. The absorbance was measured at 517 nm using a Hitachi U-2001 spectrophotometer. BHA and L-ascorbic acid were used as the positive controls, whereas citric acid served as the negative control.

*Scavenging Capability for $H_2O_2$*. The testing procedures were carried out following the method of Rinkus and Taylor (*15*) with a slight modification. In brief, to 1 mL of aliquots of oleamide (2 mg/mL) was added 0.4 mL of $H_2O_2$ (4 mM). The mixture was left to stand at ambient temperature for 20 min ($S_m$). In parallel, the horseradish peroxidase (HRPase) and phenol red (PR) were freshly prepared in the potassium phosphate solution (0.2 M, pH 6.2) to a final concentration of 0.5 mg/mL (HRPase) or 7.5 mM (PR), respectively. On mixing, the ratio HRPase/PR was adjusted to 1:2 (HPR). To 1 mL of $S_m$ was added 0.6 mL of HRP. The mixture was left to stand for 10 min and ice cooled for another 10 min at ambient temperature. The optical density was measured at 610 nm. L-Ascorbic acid was used as the reference control.

*Scavenging Capability for Superoxide Anions*. The testing method was modified slightly from that of Gülcin et al. (*16*). Briefly, the sample oleamide was dissolved in a mixed solvent composed of MeOH/ether (6:4) to a concentration of 2 mg/mL. To 1 mL of the aliquot were added 1 mL of NBT (50 $\mu$M NBT in 16 mM Tris-HCl buffer, pH 8.0) and 1 mL of NADH (78 $\mu$M NADH in 16 mM Tris-HCl buffer, pH 8.0). After thorough mixing, 1 mL of PMS (10 $\mu$M PMS in 16 mM Tris-HCl buffer, pH 8.0) was added and agitated thoroughly. After incubation at 25 °C for 5 min, the absorbance was measured at 560 nm. BHA (2 mg/mL) was used as the positive control.

*Chelating Capability for Ferrous Ions*. According to Dinis et al. (*17*), 1 mL of oleamide aliquots (2 mg/mL) was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM $FeCl_3 \cdot 4H_2O$. After thorough agitation, the reaction mixture was left to stand for 30 s. To the mixture was added 0.1 mL of

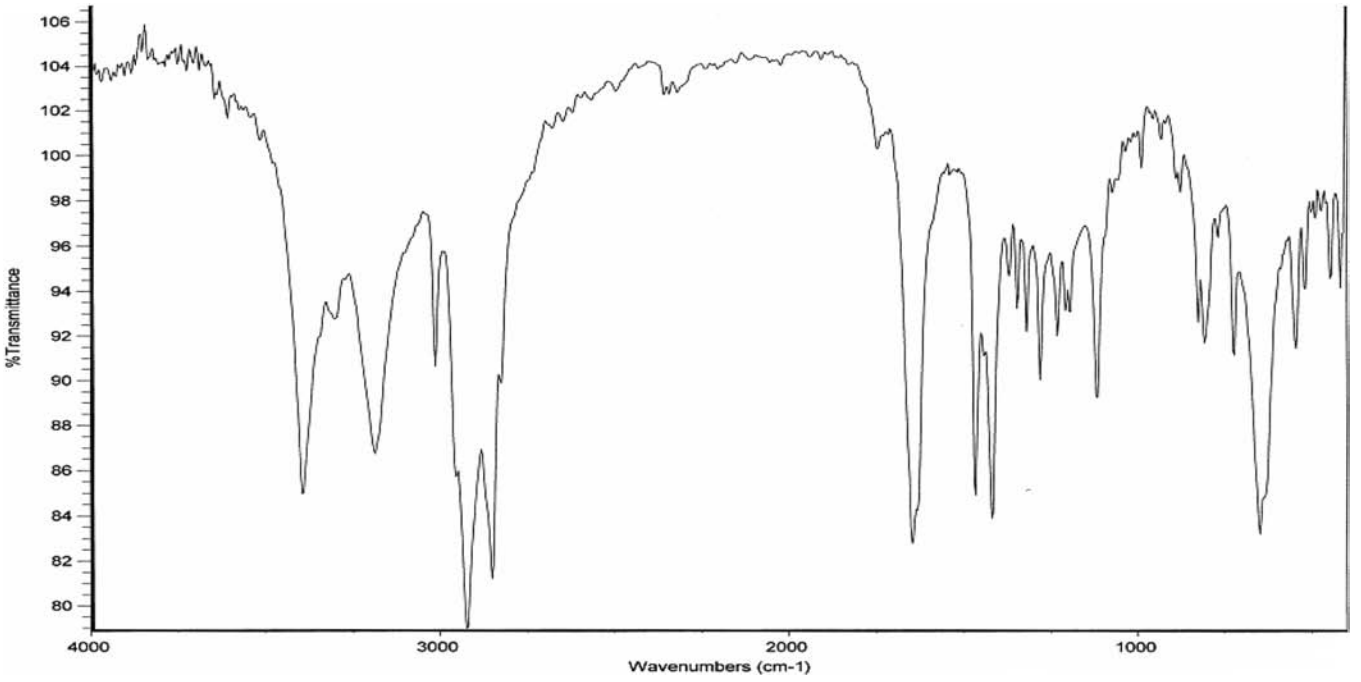


**Figure 3.** FTIR spectrum of purified 9(*Z*)-octadecenamide. The KBr tabletting technique was used. The ratio of IR grade KBr/ZOAM was 200:1. The IR spectra clearly revealed the amide-$NH_2$ absorption bands at 3393 and 3186 $cm^{-1}$ and an amide C═O absorption band at 1646 $cm^{-1}$.

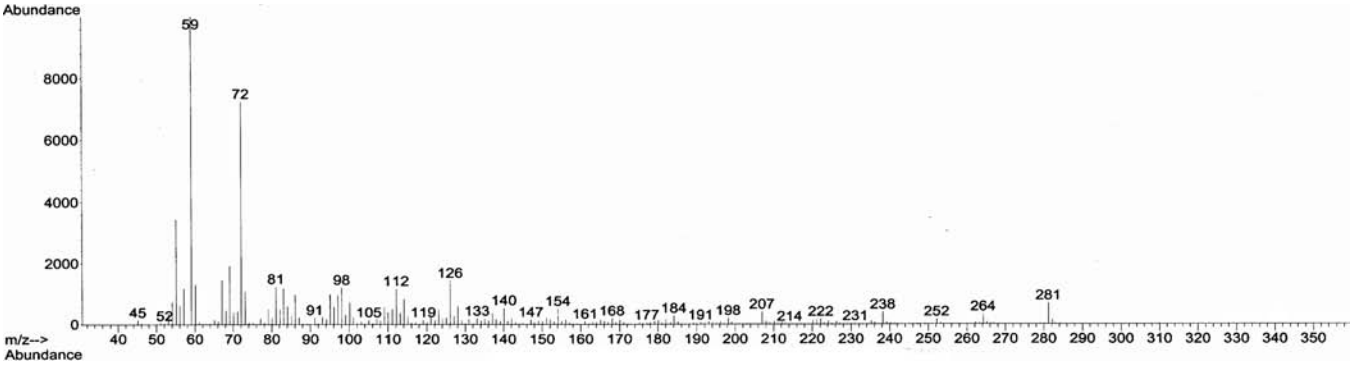


**Figure 4.** HREIMS analysis of 9(*Z*)-octadecenamide. The spectrum obtained from the HREIMS analysis was HREIMS (*m*/*z* $[M]^+$, 281.2719; calcd, 281.2725). The corresponding *m*/*z* peaks in HREIMS sequentially were molecular ion 281 $[M]^+$, 264 (3), 238 (3), 126 (14), 112 (11), 98 (12), 83 (12), 81 (12), 72 (74), and 59 (100) (**Figure 3**). Elemental analysis revealed its chemical formula to be $C_{18}H_{35}NO$, mp at 73.5−74.5 °C.

ferrozine (5 mM, Merck). After vigorous mixing, the mixture was left to stand at ambient temperature for 10 min. The absorbance was measured at 562 nm. EDTA and citric acid were used as the positive controls.

**Hamster Diets, Serum, and Hepatic Lipid Extracts.** Forty male Syrian hamsters, aged 5−6 weeks, were purchased from the National Laboratory Animal Centre. All studies performed with the hamster model were approved by the Hungkuang University Ethics Committee of Human and Anmial Experiments in accordance with the Helsinki Declaration of 1975. For the first 2 weeks, the hamsters were acclimated by supplying only the regular diet [Fu-Sow "Longevity" Brand in Chinese]. Then the hamsters were randomly grouped by body weight into five groups, eight per group and two in each cage. Two groups were separately used as different controls: one on the regular diet (group N) and the other fed a high-lipid diet (group H) that contained 5% corn oil and 5% lard (**Table 1**). The remaining three groups were fed diets OM1, OM2, and OM3, which were prepared by mixing 0.05, 0.10, and 0.30% w/w of oleamide, respectively, with diet H (**Table 1**).

Each diet was thoroughly mixed to ensure homogeneous composition, and the finished diets were stored at 4 °C for use. The animal room was conditioned at 24 ± 1 °C and 40−60% relative humidity, with light cycle changed every 12 h. Water and diet were accessed ad libitum. The body weight and diet uptake were recorded every 2 days until the end of experiment. After 10 weeks of feeding, the hamsters were first fasted for 12 h and then $CO_2$-euthanized to bleed from the abdominal artery. The collected blood was centrifuged at 1800*g* for 10 min at 4 °C. The sera were separated and stored at −70 °C for lipid determination (SL). The livers and kidneys were dissected immediately after euthanization and washed twice with 150 mM ice-cold saline. The adhering water was wiped off and their weights were taken. The livers were transferred into a zipper-sealing bag and stored at −70 °C for analysis of the hepatic lipid content. The protocol for determination of the serum and hepatic lipids in majority was adopted from "*The Manual of Combined Agents*" provided by Randox Laboratories Ltd. with slight modification (*1*).

**Statistical Analysis.** Data obtained in the same group were analyzed by Student's *t* test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL). Statistical Analysis System (2000) software was used to analyze the variances, and Duncan's multiple-range tests were used to test the significance of difference between paired means. The significance of difference was judged by a confidence level of $p < 0.05$.

## RESULTS AND DISCUSSION

**Four Fractions Were Obtained.** On fractionation of the mountain celery seed essential oils, four fractions were obtained. The percent yield of each fraction was 41.48, 45.85, 4.72, and 5.97%

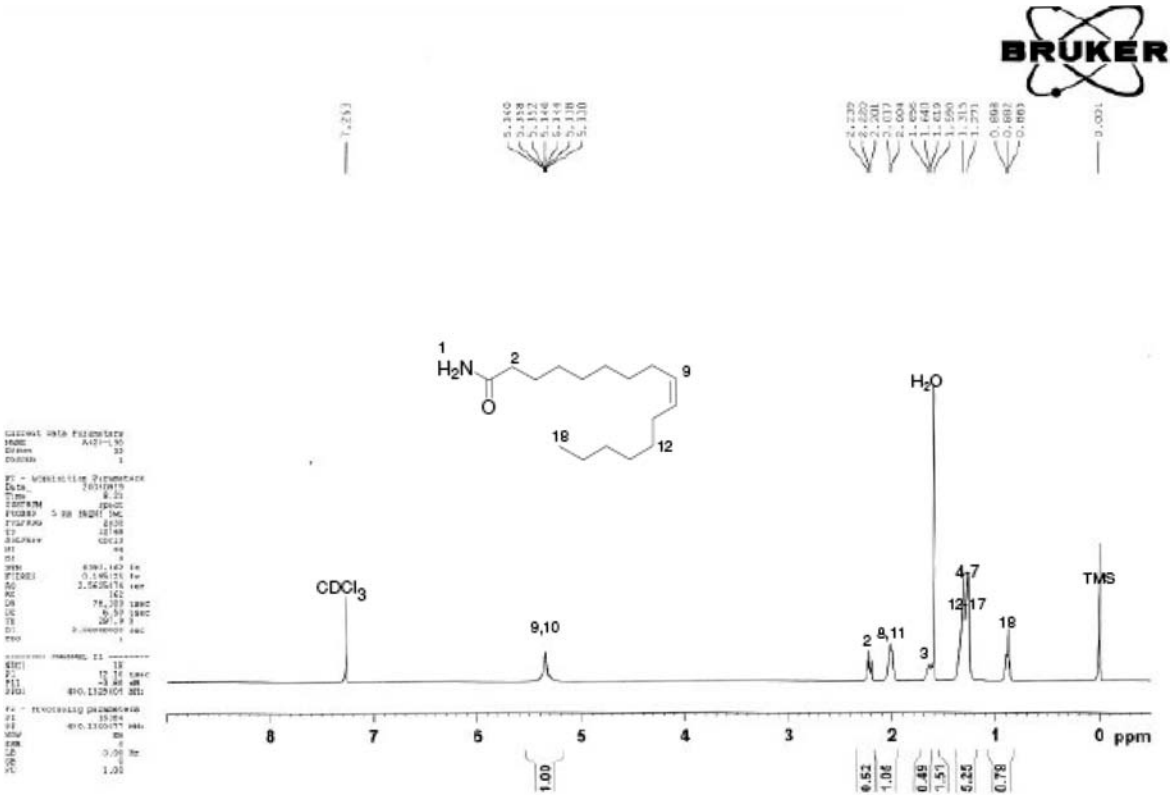


**Figure 5.** $^{1}$H NMR ($CDCl_3$, 400 MHz) spectrum of 9(*Z*)-octadecenamide. In the $^{1}$H NMR ($CDCl_3$, 400 MHz) spectrum, the characteristic absorptions appeared at $\delta$ 5.35 (2H, m, CH═CH), 2.22 (2H, t, $J$ = 7.6 Hz, $CH_2CONH_2$), 2.01 (4H, m), 1.65 (2H, m), 1.32−1.27 (20H, m), and 0.88 (3H, t, $J$ = 6.6 Hz, $CH_3$).

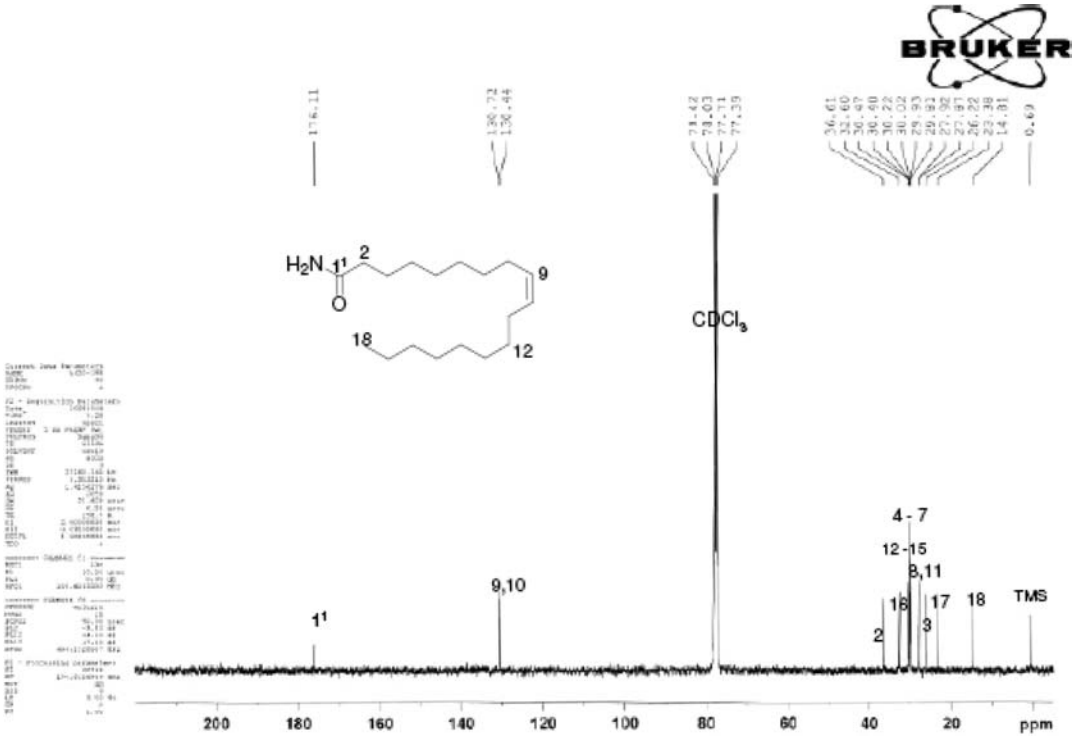


**Figure 6.** $^{13}$C NMR ($CDCl_3$, 100 MHz) spectrum of 9(*Z*)-octadecenamide. The $^{13}$C NMR ($CDCl_3$, 100 MHz) spectrum showed values of $\delta$ 176.11, 130.72, 130.44, 36.61, 32.60, 30.47, 30.40, 30.22, 30.02, 29.93, 29.81, 29.81, 27.92, 27.87, 26.22, 23.38, and 14.81, characteristic of an aliphatic chain.

obtained from pentane, ether, acetone, and methanol elution, respectively (**Table 2**).

**High Yield and High Purity of Synthetic 9(*Z*)-Octadecenamide Were Reached.** The synthetic 9(*Z*)-octadecenamide was a whitish powder. Its yield was 21.2 g, corresponding to a percent yield of 85.1%. Instrumental analysis showed its purity attained 98.6%. In addition, elemental analysis revealed the chemical formula to be $C_{18}H_{35}NO$ (data not shown) with a melting point of 73.5−74.5 °C.

**Identification of 9(*Z*)-Octadecenamide.** *RP-HPLC Analysis.* The RP-HPLC patterns taken for products before and after

prurificaion are shown in **Figure 2**. The retention times ($t_R$) of the sample oleamide obtained from the methanolic fraction and the purified 9(*Z*)-octadecenamide were 24.62 and 24.61 min, respectively.

*FTIR Analysis*. The synthesized 9(*Z*)-octadecenamide was a whitish powder. The IR spectra clearly revealed the characteristic absorption bands of the amide-$NH_2$ at 3393 and 3186 $cm^{-1}$ and the amide C=O band at 1646 $cm^{-1}$, respectively (**Figure 3**).

*HREIMS Analysis*. The HREIMS spectra rvealed the molecular ion peak to be $m/z$ $[M]^+$ = 281.2719 (calcd, 281.2725). The other $m/z$ peaks appearing in the HREIMS spetra were 264 (3), 238 (3), 126 (14), 112 (11), 98 (12), 83 (12), 81 (12), 72 (74), and 59 (100) (**Figure 4**).

*$^1H$ NMR ($CDCl_3$, 400 MHz) Spectral Analysis*. In the $^1H$ NMR ($CDCl_3$, 400 MHz) spectra, the characteristic absorptions appeared at $\delta$ 5.35 (2H, m, CH=CH), 2.22 (2H, t, $J$ = 7.6 Hz, $CH_2CONH_2$), 2.01 (4H,m,), 1.65 (2H, m,), 1.32−1.27 (20H, m,), and 0.88 (3H, t, $J$ = 6.6 Hz, $CH_3$) (**Figure 5**).

*$^{13}C$ NMR ($CDCl_3$, 100 MHz) Spectral Analysis*. The $^{13}C$ NMR ($CDCl_3$, 100 MHz) spectra showed sequentially the chemical shifts appearing at $\delta$ 176.11, 130.72, 130.44, 36.61, 32.60, 30.47, 30.40, 30.22, 30.02, 29.93, 29.81, 29.81, 27.92, 27.87, 26.22, 23.38, and 14.81 (**Figure 6**) (*13*).

**Antioxidative Capability.** ZOAM exhibited only moderate scavenging capability toward DPPH free radicals, $H_2O_2$, and the superoxide anion $^{\bullet}O_2^-$ and, as a contrast, a rather inferior metal chelation power (**Table 3**). These results implicated the main role of oleamide in vivo was not responsible for the antioxidative and free radical scavenging as often cited in much of the literature elsewhere.

**Table 3.** Comparison of the Antioxidative and Metal-Chelating Capabilities among 9(*Z*)-Octadecenamide and Other Reference Compounds Conventionally Used[a]

| | scavenging capability (%) | | | |
|---|---|---|---|---|
| compound | DPPH | $H_2O_2$ | $^{\bullet}O_2^-$ | metal chelating (%) |
| BHA | 95.3 ± 3.6 | | 91.5 ± 3.5 | |
| BHT | | | 70.4 ± 4.6 | |
| EDTA | | | | 95 ± 3.9 |
| citric acid | | | | 17.2 ± 3.4 |
| L-ascorbic acid | 68.0 ± 3.2 | 90.3 ± 4.3 | | |
| ZOAM[b] | 25.0 ± 3.9 | 0.0 | 48.6 ± 6.4 | 11.2 ± 2.6 |

[a] The concentration for all compounds tested was 2 mg/mL. [b] ZOAM, 9(*Z*)-octadecenamide.

**Hypolipidemic Bioactivity.** Oleamide showed very potent hypolipidemic bioactivity. Levels of serum TG, TC, LDL-C, and hepatic TG were significantly reduced in a dose-responsive manner compared with the high-fat−high-cholesterol group H (**Table 4**); nonetheless, no effect was found for serum HDL-C and hepatic TC. Astonishingly, although oleamide did not show any HDL-C elevating effect, the ratio LDL-C/HDL-C was suppressed from 2.6 (group H) to 2.3, 2.2, and 1.8, respectively, by OM1, OM2, and OM3 (**Table 4**), suggesting that another coexisting constituent, probably $\gamma$-selinene, the second enriched principle (12.59%) in the methanolic fraction of EOM (*1*), contributed to the HDL-C elevation effect. Selinenes were demonstrated to be very effective antiatherogenics (*18*).

Xu et al. demonstrated that oleamide acted like an acyl-CoA: cholesterol acyltransferse inhibitor, effectively suppressing rat liver microsomal ACAT, hACAT-1, and hACAT-2 (*13*). Recently, Jones et al. demonstrated that compared with sunflower oil rich in linoleic acid (18:2n-6) and flaxseed oil rich in linolenic acid (18:3n-3), diets rich in oleic acid (18:1n-9) derived from olive oil may offer increased oxidation, translating into increased energy expenditure postprandially (*19*), which alternatively explains clearly the hypolipidemic effect of oleamide. Moreover, if needed, oleamide and oleic acid are intertransformable biochemically in vivo. The de novo synthesis of oleamide probably involves the synthesis of oleoylglycine with subsequent conversion to oleamide by peptidylglycine α-amidating monooxygenase (PAM). Conversely, the breakdown of oleamide is affected by the fatty acid amide hydrolase (FAAH) (*7*). FAAH is an integral membrane enzyme within the amidase-signature family that terminates the action of several endogenous lipid messengers, including oleamide and the endocannabinoid anandamide. The hydrolysis of such messengers leads to molecules devoid of biological activity and, therefore, modulates a number of neurobehavioral processes in mammals, including pain, sleep, feeding, and locomotor activity (*20*).

On the other hand, the hydrolyzed product oleate (C18:1) is besides palmitate (C16:0), the most abundant fatty acid in the human diet, and recently its involvement in the development of insulin resistance has been broadly discussed (*7*). More recently, in human hepatocyte HepG2 cells, Vock et al. identified 14 oleate-dependent genes (*4*). However, even until present, the influence of

**Table 4.** Hypolipidemic Effect of 9(*Z*)-Octadecenamide in Male Hamsters[a]

| growth parameter | N | H | OM1 | OM2 | OM3 |
|---|---|---|---|---|---|
| food intake | 7.5 ± 0.1 | 7.5 ± 0.2 | 7.4 ± 0.1 | 7.4 ± 0.2 | 7.4 ± 0.2 |
| body wt gain (g) | 13.1 ± 3.1 | 13.0 ± 2.3 | 12.5 ± 2.0 | 12.9 ± 2.5 | 12.7 ± 2.0 |
| feed efficiency[b] | 1.7 ± 0.2 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 |
| organ wt gain[c] | | | | | |
| liver | 3.3 ± 0.5 | 3.4 ± 0.6 | 3.2 ± 0.2 | 3.4 ± 0.7 | 3.2 ± 0.5 |
| kidney | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.2 |
| serum[d] (mg/dL) | | | | | |
| TG | 142 ± 8 b | 195 ± 16 a | 161 ± 5 b | 156 ± 5 b | 135 ± 6 b |
| TC | 147 ± 5 b | 175 ± 19 a | 162 ± 7 ab | 155 ± 11 ab | 144 ± 18 b |
| LDL-C | 84 ± 5 c | 1 125 ± 17 a | 110 ± 4 ab | 106 ± 8 ab | 89 ± 3 bc |
| HDL-C | 49 ± 2 | 51 ± 4 | 49 ± 5 | 49 ± 5 | 51 ± 7 |
| LDL-C/HDL-C | 1.8 ± 0.2 b | 2.6 ± 0.6 a | 2.3 ± 0.2 ab | 2.2 ± 0.14 ab | 1.8 ± 0.3 b |
| liver (mg/g) | | | | | |
| TG | 30 ± 1 b | 34 ± 1 a | 31 ± 0 b | 30 ± 0 b | 30 ± 1 b |
| TC | 27 ± 1 | 29 ± 2 | 27 ± 1 | 27 ± 1 | 27 ± 1 |

[a] N, the regular diet for control group; H, high-lipid diet; OM1, diet H plus ZOAM 0.05% w/w; OM2, diet H plus ZOAM 0.10% w/w; OM3, diet H plus ZOAM 0.30% w/w. [b] Feed efficiency = [wt gain (g)/total diet intake (g)] × 100; fed for 8 weeks. [c] Relative organ weight = [organ wt (g)/ body wt (g)] × 100. [d] TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C/HDL-C, ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol. Values in the same row with different letters are significantly different ($p < 0.05$).

oleamide on gene expression in mammalian cells is still poorly understood. Speculatively, the soporific effect (*2*, *5*) and the gene-modualting bioactivity of oleamide (*4*) other than the hypolipidemic effect will become the main targets for further study.

In summary, the chemical synthesis of oleamide was shown to be successful as confirmed by RP-HPLC (**Figure 2**), FTIR (**Figure 3**), HREIMS (**Figure 4**), $^{1}$H NMR (**Figure 5**), and $^{13}$C NMR (**Figure 6**) in parallel to the elemental analysis (data not shown). The product oleamide was identified to be an efficient hypolipidemic but a poor antioxidative agent.

## ABBREVIATIONS USED

MCS, mountain celery seeds; EOM, methanolic fractionate of the mountain celery seed essential oil; ZOAM, 9(*Z*)-oleamide, 9(*Z*)-octadecenamide, *cis*-9,10-octadecenoamide; FTIR, Fourier transform infrared spectral analysis; $^{13}$C NMR, carbon-13 nuclear magnetic resonance spectrometer; $^{1}$H NMR, proton nuclear magnetic resonance spectrometer; HREIMS, high-resolution electron ion mass spectrometer; MPLC, mass preparative liquid chromatography; RP-HPLC, reverse phase high-performance chromatography.

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