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13-Hydroxy-9*Z*,11*E*,15*E*-octadecatrienoic Acid from the Leaves of *Cucurbita moschata*

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A new unsaturated hydroxy fatty acid was isolated from the leaves of *Cucurbita moschata* through repeated silica gel column chromatography and chemical methods. The structure of the new fatty acid was determined as 13-hydroxy-9,11,15-octadecatrienoic acid on the basis of several spectral data including 2D-NMR. The stererostructures of double bonds were determined to be 9*Z*, 11*E* and 15*E* by coupling patterns of related proton signals in the ^{1}H-NMR and NOESY experiments.

Key words: *Cucurbita moschata*, Cucurbitaceae, Unsatuated hydroxy fatty acid, 13-Hydroxy-9*Z*,11*E*,15*E*-octadecatrienoic acid

INTRODUCTION

The leaves of *Cucurbita moschata*, in addition to the fruits, have been favorably ingested in Korean diet. Authors have previously reported the isolation and identification of various fatty acids and a sterol glycoside having a double bond at C-7 and C-8 positions from the leaves of *C. moschata*. (Han *et al.*, 1999) Subsequent work led to the isolation of an unknown unsaturated hydroxy fatty acid. The number, position and stereostructures of such double bonds were revealed to be different from any of the unsaturated fatty acids reported previously (Chisholm & Hopkins, 1964; 1965). This paper describes the isolation and structural determination of the new fatty acid. We also identified the position of the double bonds by using ^{1}H-^{1}H COSY and HMBC techniques. Stereostructural data from NOESY experiment are also presented.

MATERIALS AND METHODS

Matereials

Cucurbita moscahta DUCH. leaves were collected in Kyunggi-Do of Korea, in July 1998, dried in air and deposited at the laboratory of natural products chemistry, Kyunghee University, Suwon, Korea (KH-98-017).

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Instrumentation

Optical rotation: Rudolph Research Autopol III-7214; IR: Perkin-Elmer 599B; UV-Vis: Shimadzu UV-1601; EI-MS: JEOL JMSAX505-WA; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz): JEOL JNM-LA400; Chromatography: silica gel [Kieselgel 60 (70-230 mesh), Merck].

Isolation and purification of a fatty acid

Coarsely powdered leaves (1.5 kg) were extracted overnight in 80% aqueous MeOH (5 l × 2). Upon evaporation of solvent, extracts in water was obtained. Partition of the extracts between H_2O and EtOAc followed by removal of solvent afforded 23 g of EtOAc extracts. 20 g portion of the extracts was subjected to silica gel column chromatography (6 × 65 cm, 410 g)Gradient elution with *n*-hexane-$CHCl_3$-MeOH (2:12:1) → $CHCl_3$-MeOH (13:1) gave nine fractions. Further repeated silica gel column chromatography (5 × 30 cm, 200 g)of the 7th fraction (2.3 g) with varying eluting solvents, such as $CHCl_3$-MeOH (15:2) and *n*-BuOH-EtOAc (1:3), to give a fraction (422 mg). The mixture of the fraction (100 mg), pyridine (5 ml) and acetic anhydride (5 ml) was stirred at room temperature for 15 hr. The reaction mixture was poured

Table I ^{1}H NMR data of **1** and **1a** (400 MHz, $CDCl_3$)[a]

H No.	1	1a	
		δ[b]	HMBC[c]
2	2.35, t, 7.3	2.34, t, 7.4	1, 3, 4
3	1.64, m	1.62, m	1, 2, 4, 5
4, 5, 6, 7	1.25-1.40, m	1.25-1.40, m	
8	2.18, dt, 7.3, 7.3	2.17, dt, 7.3, 6.5	6, 7, 9, 10
9	5.44, m	5.47, m	8, 11
10	5.98, dd, 11.0, 10.7	5.94, dd, 11.0, 11.1	8, 11, 12
11	6.53, dd, 15.3, 11.1	6.51, dd, 15.2, 11.1	9, 10, 13
12	5.69, dd, 15.4, 6.34	5.59, dd, 15.2, 7.4	9, 10, 13, 14, 15
13	4.23, dt, 6.3, 6.6	5.33, m	11, 14, 15, acetyl-C
14a	2.36, dd-like	2.42, ddd, 14.2, 7.2, 6.6	12, 13, 15, 16
14b		2.38, ddd, 14.2, 7.2, 6.8	
15	5.36, m	5.31, m	14, 17
16	5.58	5.49, m	14, 17
17	2.08, dq, 7.4, 7.4	2.05, dq, 7.6, 7.6	15, 16, 18
18	0.97, t, 7.4	0.96, t, 7.6	16, 17
Acety		2.05, s	---

[a]Chemical shifts (δ) expressed relative to TMS.
[b]Chemical shift, multiplicity, coupling constants (*J*) in Hz.
[c]Carbon atom showing correlation with corresponding proton in the HMBC spectrum.

into ice water (250 ml) and extracted with EtOAc (250 ml × 2). The organic layer was washed with 5% aq. HCl, satd. $NaHCO_3$ and saline, successively, followed by drying over $MgSO_4$ anhydrous. Removal of solvent and CC (3 × 25 cm, 120 g) on silica gel eluting with *n*-hexane-EtOAc (5:10) afforded a pure compound **1a** (124 mg).

Compound 1a (13-O-acetyl-9*Z*,11*E*,15*E*-octadecatrienoic acid). Colorless oil; $[\alpha]_D$: +5.3°($CHCl_3$, c 0.8); UV (MeOH, log ε) λ_{max} : 205 (3.72), 241 (3.99) nm; IR ($CHCl_3$) ν_{max} : 3050, 2936, 1722, 1674, 1655, 985, 965 cm^{-1}; EIMS (*m/z*): 336 [M^+], 294, 290, 267, 225, 221, 207, 189, 161, 147, 133, 57; HREIMS: Found: 336.2300, Calcd for $C_{20}H_{32}O_4$: 336.2302; ^{1}H-NMR (400 MHz, $CDCl_3$, δ): see Table I; ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C): see Table II.

Deacetylation of 1a Deacetylation of 1a: 30 mg portion of 1a was dissolved in MeOH containing 1% aq. KOH (4 ml) and stirred at room temperature for 1 hr. Neutralization of the reaction mixture by adding Dowex 50W × 8 (H^+ form) and silica gel CC (3 × 15 cm, 70 g) eluting with $CHCl_3$-MeOH (20:1) afforded compound 1.

Compound 1 (13-hydroxy-9*Z*,11*E*,15*E*-octadecatrienoic acid) White powder ($CHCl_3$-MeOH); $[\alpha]_D$: +6.9°($CHCl_3$, c 0.7) UV (MeOH, log ε) λ_{max}: 201 (3.64), 236 (3.93) nm; IR ($CHCl_3$) ν_{max}: 3625, 3025, 2940, 1720, 1675, 1655, 984, 964 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ): see Table I; ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C): see Table II.

RESULTS AND DISCUSSION

Compound **1a**, obtained as colorless oil in $CHCl_3$-MeOH, showed the absorbance band at 1674, 1722 cm^{-1} in the IR spectrum ($CHCl_3$) due to double and ester bond, respectively. The ^{13}C-NMR spectrum of **1a** exhibited one carboxyl (δ 179.71), one ester (δ 170.41), six olefinic methines (δ 122.86, 127.41, 128.10, 130.21, 134.57, 135.40), one oxygenated methine (δ 74.25), nine methylenes (δ 20.61, 24.59, 27.64, 28.88 (×2), 28.96, 29.35, 32.30, 33.98) and two methyl (δ 14.50, 21.24) carbon signals, indicating **1a** to be a C_{18} fatty acid containing three double bonds and an acetylated-hydroxyl group. This result was confirmed by its molecular weight, 336, based on molecular ion peak (M^+, *m/z* 336) in the EI/MS spectrum. In addition, in the ^{1}H-NMR spectrum of **1a**, one terminal methyl at δ 0.96 (3H, t, *J*=7.6 Hz), one acetyl-methyl at δ 2.05 (3H, s), six olefinic methines at δ 5.31, 5.47, 5.49, 5.59, 5.94, 6.51 (each 1H), one oxygenated methine with ester bond at 5.33 (1H) and five methylenes at δ 1.62 (2H), 1.25-1.40 (8H) along with four allyl methylenes at δ 2.05 (2H, dq), 2.17 (2H, dt), 2.34 (2H, dd), 2.38, 2.42 (each 1H, both ddd) proton signals were observed. ^{1}H-^{1}H connectivities from H-7 through H-18 were observed in the ^{1}H-^{1}H COSY and further clearly confirmed by proton decoupling experiments; such informations revealed the position of three double bonds and one hydroxyl to be C-9, C-11, C-15 and C-13, respectively. All proton signals except those due to four

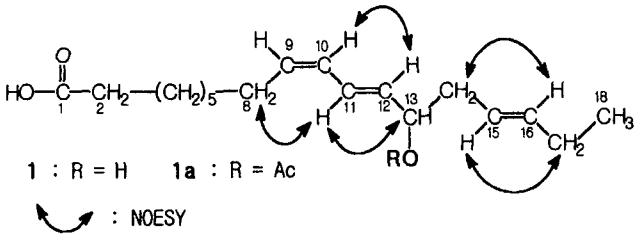

Fig. 1. Chemical structures of **1** and **1a**

Table II. ^{13}C NMR data of **1** and **1a** (100 MHz, $CDCl_3$) [a]

C No.	1	1a
1	178.39	179.71
2	35.25	33.98
3	24.62	24.59
4, 5, 6, 7	29.31	29.35
	28.80	28.96
	28.80	28.88
	28.80	28.88
8	27.57	27.64
9	134.87	134.57
10	125.88	127.41
11	127.78	128.10
12	132.93	130.21
13	72.14	74.25
14	33.75	32.30
15	123.69	122.86
16	135.33	135.40
17	20.76	20.61
18	14.21	14.50
Acetyl		170.41, 21.24

[a]Chemical shifts (δ) expressed relative to TMS.

methylene groups of aliphatic region in the ^1H-NMR were assigned unambiguously on the basis of NOESY and HMBC (Table I) experiments as well as above results. The assignment of carbon signals was reinforced by the observation of cross peaks occurring between carbon and corresponding proton signals in the HMQC spectrum. The stereochemical configuration of C-9 double bond was suggested to be *Z* from coupling constant (*J*=11.0 Hz) between H-9 and H-10. It was further confirmed from the fact that in the NOESY spectrum; the correlation peaks between H-8 and H-10 and between H-9 and H-11 were not observed, but the ones between H-7 and H-9 and between H-8 and H-11 were observed. And then the coupling constant (*J*=15.2 Hz) between H-11 and H-12 led to determination of the stereochemical configuration of C-11 double bond to be *E*, which was further confirmed by the presence of cross peaks between H-10 and H-12 and between H-11 and H-13, in addition to the absence of cross peaks between H-10 and H-13 in the NOESY spectrum. The stereochemical configuration of C-15 double bond was also revealed to be *E* by the analysis of NOESY experiment in which NOE between H-14 and H-17 was not observed, but the ones between H-14 and H-16 and between H-15 and H-17 were observed. Altogether, the chemical structure of 1a was determined to be 13-*O*-acetyl-9*Z*,11*E*,15*E*- octadecatrienoic acid. (Fig. 1)

In order to obtain the pure genuine component, compound **1a** (30 mg) was deacetylated with alkaline treatment and then purified through silica gel column chromatography eluting with $CHCl_3$-MeOH (20:1) to afford compound **1** (24 mg). Compound **1** was identified as 13-hydroxy-9*Z*,11*E*,15*E*-octadecatrienoic acid on the basis of several spectral evidences. The identity of compound **1** was confirmed by proving the presence of compound **1** in EtOAc fraction through direct comparison of TLC. Currently, we are studying the absolute configuraion of C-13.

α-Linolenic acid, the most abundant octadecatrienoic acid in the nature, has carbon-carbon double bonds at C-9, C-12 and C-15 (Chisholm & Hopkins, 1965). As a relevant monohydroxyoctadecatrienoic acids from natural source, 2-hydroxy-9,12,15-triene, 2-hydroxylinolenic acid, from the seed oil of *Thymus vulgaris* (Smith &Wolff, 1968) and 18-hydroxy-9,11,13-triene, kamlolenic acid, from the seeds of *Mallotus discolor* (Hopkins *et al.*, 1968) derivatives have been reported thus far. To our knowledge, this is the first report of the occurrence of compound **1**.

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