Novel Antitumor Sesquiterpenoids in *Achillea millefolium*

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Three new antitumor sesquiterpenoids, achimillic acids A, B and C, were isolated as methyl esters from *Achillea millefolium* and their structures were determined spectroscopically. The compounds were found to be active against mouse P-388 leukemia cells *in vivo*.

Keywords *Achillea millefolium*.; antitumor activity; achimillic acid; sesquiterpenoid; Compositae; P-388 leukemia

Achillea millefolium LINNAEUS (Compositae, English name: yarrow) is used as an antipyretic and diaphoretic in cases of the common cold and as an emenagogue in Europe,[1] U.S.A.,[2] and Asian countries.[3] Its use against cancer has also been reported.[4] During our search for antitumor components in higher plants, the methanol extract of flowers of *Achillea millefolium* was found to exhibit activity against mouse P-388 leukemia cells *in vivo*. Bioassay-directed fractionation led to the isolation of three new sesquiterpenoids, achimillic acids A, B and C, as methyl esters. We report here the isolation, structural determination and antitumor activity of these compounds.

The methanol (MeOH) extract of the flowers was separated by monitoring the activity as shown in Fig. 1. The active fraction (fraction III), obtained by silica gel chromatography of fraction I, exhibited two spots with *Rf* values of 0.61 and 0.27 in thin-layer chromatography (TLC). The results of preparative TLC indicated that the two spots were interconvertible. The acidic fraction (fraction V), obtained from fraction III, showed a single spot with an *Rf* value of 0.27, but gradually another spot with an *Rf* value of 0.61 appeared on the TLC plate. Treatment of fraction V with methyl iodide and potassium carbonate, followed by chromatographic separation, gave compounds **1**, **2** and **3**, named methyl achimillates A, B and C, respectively. The ^{1}H- and ^{13}C-NMR spectra of these three compounds exhibited signals with splitting patterns and chemical shifts ($\Delta\delta_H \pm 0.7$, $\Delta\delta_C \pm 5$) similar to those of fraction V, except for the incremental methoxy signals. It was presumed that methylation of the achimillic acids occurred without any change in the skeleton. The molecular formula, $C_{16}H_{20}O_5$, of methyl achimillate A was obtained from microanalysis, mass (MS) (M^+, *m/z* 292), ^{1}H-, and ^{13}C-NMR spectra. IR and ^{13}C-NMR spectra suggested the presence of three carbonyl carbons, three double bonds, two methylene, and three methyl carbons. Signals due to methyl groups were observed at δ_H 1.59 (C–CH_3), 2.13 ($COCH_3$), and 3.78 ($COOCH_3$) in the ^{1}H-NMR spectrum. Decoupling experiments with signals at δ_H 4.09 and 6.48 and analysis of the splitting patterns of the signals at δ_H 2.47, 1.87, 1.95, 4.09, and 6.48 suggested the presence of the partial structure –CH_2–CH_2–CH–CH=C for the moiety C-5 to C-9. Decoupling experiments with signals at δ_H 4.09 and 5.71 also revealed the presence of weak ^{1}H–^{1}H spin–spin interaction between these two signals and between these and the signals at δ_H 6.31. This suggested the presence of the partial structure –CH–C=CH_2 of the exomethylene type for C-7, -11, and -13. An α,β-unsaturated ketone for the moiety C-1 to C-3 was suggested by the rather low field resonance of the signal (δ_H 7.36) which showed coupling (J=6 Hz, *Z* geometry) with a signal at δ_H 6.26 and weak coupling with a signal at δ_H 6.48 (J=0.8 Hz, long-range interaction). Assembling the partial structures described above led to the construction of the chemical structure of methyl achimillate A (**1**). The 5*E* geometry was deduced from the long-range coupling constant ($^5J_{3,6}$=0.8 Hz) between signals assignable to H-3 and H-6 with a zig-zag arrangement.[5] This was also supported by the aromatic solvent induced shift (ASIS)[6,7] ($\Delta\delta_H$+0.25) of the H-6 signal produced by benzene. The relative configuration of **1** was confirmed by X-ray crystallographic analysis and

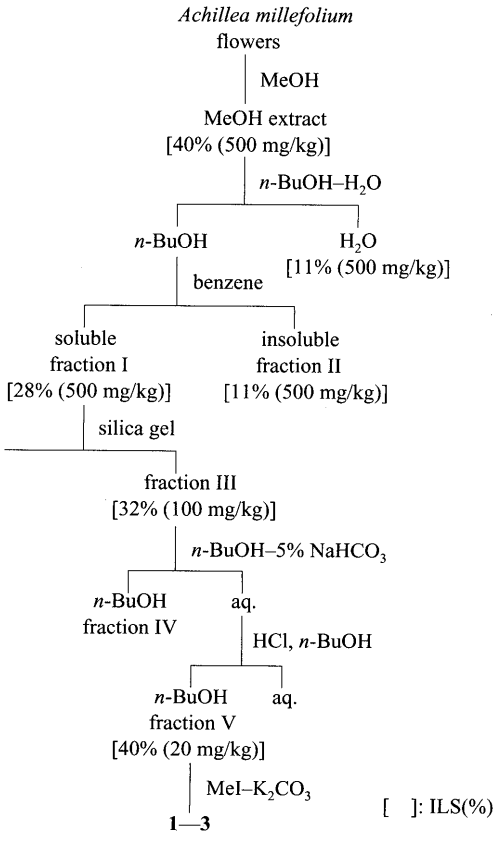
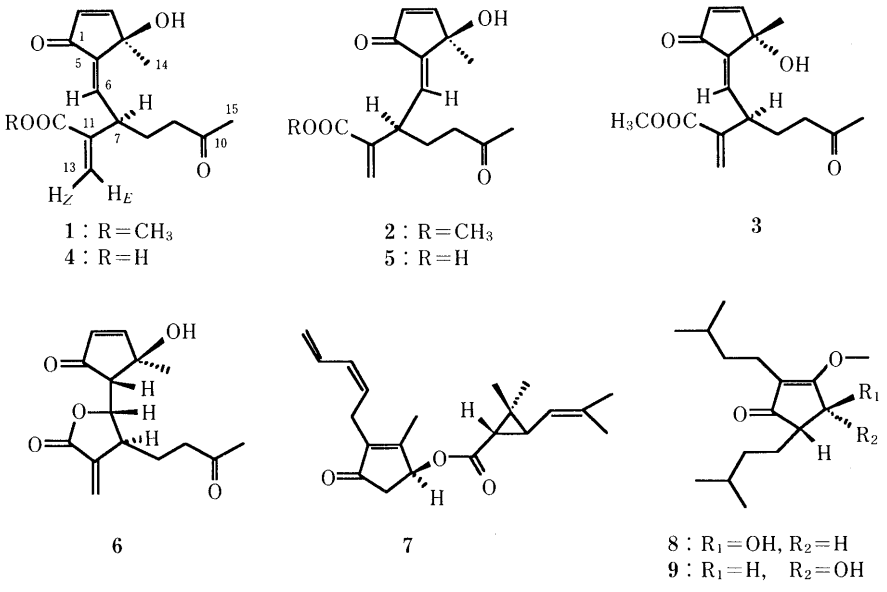


Fig. 1. Fractionation of the Extract of *Achillea millefolium*



1 : R=CH_3
4 : R=H

2 : R=CH_3
5 : R=H

3

6

7

8 : R_1=OH, R_2=H
9 : R_1=H, R_2=OH

Chart 1

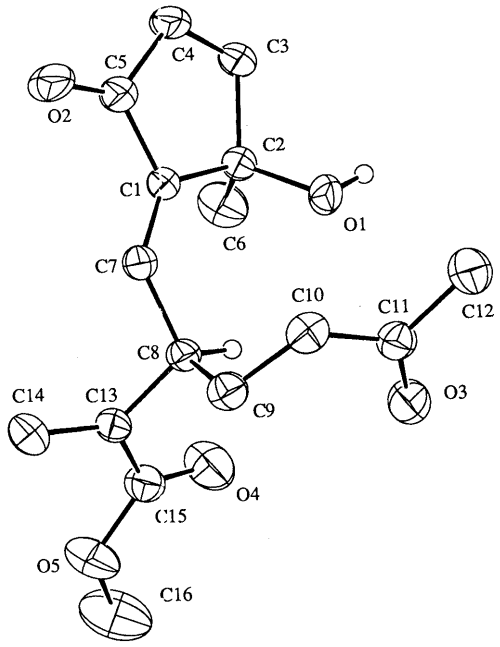


Fig. 2. ORTEP Drawing[15] with Atom Labelings
Hydrogen atoms, except those in the OH group, are omitted for clarity.

the perspective view of the molecule is shown in Fig. 2. It has been reported that pyrethrin I (**7**) exhibits a positive Cotton effect in its circular dichroism (CD) spectra.[8] (4*S*,5*S*)- and (4*R*,5*S*)-dihydrodeoxohumulinic acid methyl enol ethers (**8** and **9**, respectively) have been reported to exhibit similar CD spectra except that one is the opposite of the other.[9] Therefore, the C-4 epimer of **7** was believed to exhibit a negative Cotton effect. The positive Cotton effect exhibited by **1** suggests that methyl achimillate A possesses a 4*R* configuration.

The ^{1}H- and ^{13}C-NMR, IR and MS spectra of methyl achimillates B (**2**) and C (**3**) were similar to those of **1** and indicated that both compounds were stereoisomers of **1**. The 5*Z* geometry of **2** was suggested by the long-range coupling (^5J=0.5 Hz) between signals due to H-2 and H-6 with a zig-zag arrangement, supplemented by the ASIS ($\Delta\delta_H$+0.30) on the H-7 signal and the positive, but smaller ASIS effect ($\Delta\delta_H$+0.09), on the H-6 signal of **2** compared with that of **1**. As observed on TLC, fraction V, which was thought to consist mainly of the carboxylic acids (**4** and **5**) of **1** and **2**, gradually underwent a change to give a lactone (**6**). Compound **6** was identified as secotanapartholide A[10,11] following detailed analysis of the IR, MS, ^{1}H- and ^{13}C-NMR spectral data. The ^{1}H- and ^{13}C-NMR spectral data of **6** were in good agreement with those reported elsewhere.[12] Treatment of **6** with $NaHCO_3$ resulted in a mixture of **4** and **5** (3 : 1 from the ^{1}H-NMR spectrum). Methylation of this mixture resulted in **1** and **2**. That mixture reached an equilibrium with **6** in $CDCl_3$ or CD_3OH solution. Compound **6** remained unchanged in $CDCl_3$ solution, but reached a similar equilibrium in CD_3OH solution. Easy interconversion of **4**, **5**, and **6** suggested that the configuration at the C-7 of **2** was the same as that of **1**. The positive Cotton effect exhibited by **2** indicated that **1** and **2** possess the same absolute configuration at C-4.

The long-range coupling constant ($^5J_{3,6}$=0.8 Hz) between signals due to H-3 and H-6 and the ASIS effect ($\Delta\delta_H$+0.25) suggested the 5*E* geometry of methyl achimillate C (**3**). A negative Cotton effect observed in the CD spectrum of **3** demonstrated that **1** and **3** were epimeric at C-4. The assignments of the ^{1}H- and ^{13}C-NMR signals of **1**, **2**, **3** and **6** are shown in Tables I and II.

The antitumor activities of these compounds were tested. As shown in Table III, compounds **1**, **3** and **6** exhibited activity against mouse P-388 leukemia cells *in vivo*. Compounds **1** and **6** were inactive against L-1210 leukemia cells.

Many antitumor sesquiterpenoids have been reported and their antitumor activity has been attributed to the α-methylene-γ-lactone structure.[13] The achimillic acids we obtained are antitumor sesquiterpenoids of unusual structure, lacking the α-methylene-γ-lactone moiety. Their

TABLE I. ^{1}H-NMR Spectral Data [δ_H (ppm), Multiplicities,[a] Number of Protons, and $J_{H,H}$ (Hz)[b] in $CDCl_3$]

	1	2	3	4
H-2	6.26 br d 1H	6.23 br d 1H	6.25 br d 1H	6.07 br d 1H
H-3	7.36 dd 1H	7.29 br d 1H	7.42 dd 1H	7.49 dd 1H
H-5	—	—	—	2.70 br d 1H
H-6	6.48 br d 1H	6.30 br d 1H	6.19 dd 1H	4.47 br dd 1H
H-7	4.09 br dt 1H	4.70 m 1H	3.95 br dt 1H	3.46 m 1H
H-8	1.87 m 1H 1.95 m 1H	1.95 m 2H	1.97 m 2H	1.94 m 2H
H-9	2.47 br t 2H	2.43 m 2H	2.48 br t 2H 2.66 m 1H	2.56 m 1H
H-13$_E$	5.71 br s 1H	5.75 br s 1H	5.84 br d 1H	5.78 br d 1H
H-13$_Z$	6.31 br s 1H	6.23 br d 1H	6.43 br s 1H	6.35 br d 1H
H-14	1.59 s 3H	1.51 s 3H	1.68 s 3H	1.58 br s 3H
H-15	2.13 s 3H	2.11 s 3H	2.13 s 3H	2.21 s 3H
OCH_3	3.78 s 3H	3.73 s 3H	3.76 s 3H	—
$^3J_{2,3}$	6.0	6.0	6.0	6.0
$^4J_{2,5}$	—	—	—	<0.2
$^4J_{3,5}$	—	—	—	0.4
$^5J_{2,6}$	<0.2	0.5	<0.2	<0.2
$^5J_{3,6}$	0.8	<0.2	0.8	0.4
$^4J_{5,14}$	—	—	—	0.3
$^3J_{5,6}$	—	—	—	10.3
$^3J_{6,7}$	11.3	10.1	11.4	2.2
$^3J_{7,8}$	7.2	c)	7.2	c)
$^3J_{8,9}$	7.0	c)	7.0	c)
$^4J_{7,13E}$	0.5	0.5	0.8	2.0
$^4J_{7,13Z}$	<0.2	<0.2	<0.2	2.0
$^2J_{13E,13Z}$	<0.2	0.8	<0.2	<0.2

a) Abbreviations for signal multiplicities are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad signal. b) Long-range ^{1}H–^{1}H spin–spin coupling constants ($J_{H,H}$) were determined in the resolution enhanced spectra and/or in the decoupled spectra. c) Not determined.

TABLE II. ^{13}C-NMR Spectral Data [δ_C (ppm) and Multiplicities[a] in $CDCl_3$]

	1	2	3	4
C-1	195.3 s	195.7 s	195.2 s	202.8 s
C-2	132.9 d	134.8 d	132.3 d	130.9 d
C-3	164.2 d	161.5 d	164.6 d	167.0 d
C-4	76.1 s	76.2 s	75.7 s	78.7 s
C-5	141.3 s	141.5 s	140.4 s	62.6 d
C-6	136.0 d	139.6 d	135.9 d	80.5 d
C-7	38.3 d	38.1 d	38.2 d	42.1 d
C-8	29.0 t	29.0 t	26.6 t	28.6 t
C-9	41.1 t	41.3 t	40.7 t	39.7 t
C-10	209.7 s	208.3 s	207.3 s	208.8 s
C-11	141.5 s	141.7 s	141.2 s	137.7 s
C-12	166.7 s	166.9 s	167.9 s	170.2 s
C-13	126.2 t	126.6 t	126.8 t	124.9 t
C-14	26.0 q	26.6 q	27.0 q	25.0 q
C-15	29.7 q	30.0 q	29.9 q	30.0 q
OCH_3	52.1 q	51.9 q	52.6 q	—

a) Abbreviations for signal multiplicities of single-frequency off-resonance decoupling (SFORD) spectra are as follows: s, singlet; d, doublet; t, triplet; q, quartet.

activity is thought to be due to their α,β-unsaturated ketone or ester groups.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrophotometer and IR spectra were recorded on a JASCO DS 403-G infrared spectrometer. The 200.058 MHz ^{1}H- and 50.309 MHz ^{13}C-NMR spectra were recorded using a Varian XL-200 spectrometer in $CDCl_3$ and C_6D_6 solution. Chemical shifts were expressed as δ (ppm downfield from the internal tetramethylsilane signal). The accuracies of δ_H, δ_C, and $J_{H,H}$ are $\pm$0.01 ppm, $\pm$0.1 ppm, and $\pm$0.2 Hz, respectively. Electron impact-mass spectra (EIMS) and high resolution liquid secondary ion MS (HR LSIMS) were recorded using Hitachi M-68 and M-90 mass spectrometers, respectively. CD spectra were recorded on a JASCO J-40C spectropolarimeter. Optical rotation was determined on a Perkin Elmer 241 polarimeter. TLC and preparative TLC were carried out on precoated Silica gel 60 F-254 plates (0.2 mm, Merck) using $CHCl_3$–MeOH–H_2O (8:2:0.2) as a developing solvent. Spots were detected under UV light.

X-Ray Crystallographic Analysis Colorless prismatic crystals of **1** were grown from a MeOH solution. A crystal with dimensions 0.3 × 0.3 × 0.1 mm was used for data collection. All measurements were done on a Rigaku AFC5R diffractometer using graphite monochromated CuK_α radiation (λ=1.54178 Å). The crystal data are as follows: $C_{16}H_{20}O_5$, M_r=292.33, triclinic, space group P_1, a=7.620(1) Å, b=9.151(1) Å, c=6.546(2) Å, α=108.10(1)°, β=107.97(2)°, γ=89.72(1)°, V= 410.6(1) Å^3, Z=1, D_{cal}=1.18 g/cm^3.

Of the 1657 reflections in the range $2\theta<140°$ which were collected, 1511 were unique (R_{int}=0.004). The data were corrected for Lorentz and polarization effects, but not for absorption effects. The structure was solved by a direct method.[14] The non-hydrogen atoms were refined anisotropically. A hydrogen atom in the OH group was refined isotropically, while the rest were included in fixed positions. The final cycle of full-matrix least-squares refinement was based on 1482 observed reflections [$I>1.0\sigma(I)$] and 192 variable parameters. The final R value was 0.037. The final atomic parameters are listed in Table IV and bond lengths and bond angles are listed in Table V. A perspective view of the molecule is presented in Fig. 2.[15] The maximum and minimum peaks on the final difference Fourier map corresponded to 0.24 and $-0.37e^{-3}$, respectively. Neutral atom scattering factors were taken from Cromer and Waber.[16] All calculations were performed using the teXsan[17] crystallographic software package from the Molecular Structure Corporation and a Vax station 3100 computer.

Bioassay BDF1 male mice, 6 weeks of age, were used. Seven to ten animals for each test or control group, were inoculated intraperitoneally with 10^6 P-388 cells in 0.1 ml saline. Test compounds were suspended in vehicle (NaCl, 0.9%; polysorbate 80, 0.4%; CM-cellulose, 0.5%; benzyl alcohol, 0.9% in 100 ml of water) and administered as a single intraperitoneal injection on the day following the tumor inoculation. Antitumor activity was evaluated by the increase in life span (ILS) compared with controls. The antitumor activity against L 1210 (5×10^5 cells) was tested in a similar fashion to P-388.

TABLE III. Antitumor Activity against P-388 Leukemia Cells

Dose (mg/kg)	ILS (%)			
	1	2	3	6
1	30			
2		17	18	38
5	34	15	39	35
20	−32	−7	−23	−68
50	Toxic	−87	Toxic	Toxic

Extraction and Isolation The air-dried flowers (1.6 kg) of *Achillea millefolium* (collected at Ohji, Nara Prefecture) were extracted twice with MeOH (total volume 22 l) and the extract concentrated *in vacuo*. The residue (185 g) was partitioned between *n*-butanol (*n*-BuOH) and H_2O to give fractions soluble in *n*-BuOH (135 g) and H_2O (41.0 g). The *n*-BuOH-soluble fraction was triturated with benzene to yield benzene-soluble (fraction I, 97.6 g) and -insoluble (fraction II, 36.9 g) fractions. Fraction I (5 g) was chromatographed on silica gel (Silica gel 60, 70—230 mesh, Merck). After elution with $CHCl_3$, $CHCl_3$–Me_2CO, and $CHCl_3$–MeOH–H_2O, an active fraction (fraction III, 480 mg) was eluted with $CHCl_3$–MeOH–H_2O (30:10:1) and then MeOH. Fraction III (200 mg) was dissolved in *n*-BuOH (30 ml) and extracted with 5% $NaHCO_3$ (3 × 10 ml). The aqueous phase was acidified with dilute HCl and extracted with *n*-BuOH. The *n*-BuOH phase was washed with H_2O and evaporated to give fraction V (65 mg).

Fraction V (200 mg) was refluxed with methyl iodide (1 ml) and K_2CO_3

TABLE IV. Fractional Atomic Parameters and Equivalent Isotropic Thermal Parameters with Their Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq}
O1	0.9248	0.8823	0.2944	4.74 (9)
O2	0.959 (1)	0.7102 (4)	0.883 (1)	8.1 (1)
O3	0.7890 (5)	1.2384 (4)	0.299 (1)	6.5 (1)
O4	1.3097 (5)	1.3206 (4)	0.714 (1)	7.1 (1)
O5	1.3882 (5)	1.4798 (4)	1.068 (1)	7.0 (1)
C1	1.018 (1)	0.8687 (4)	0.670 (1)	3.8 (1)
C2	1.059 (1)	0.8252 (4)	0.449 (1)	3.8 (1)
C3	1.033 (1)	0.6515 (4)	0.372 (1)	4.8 (1)
C4	0.997 (1)	0.5961 (4)	0.520 (1)	5.8 (1)
C5	0.987 (1)	0.7227 (4)	0.715 (1)	5.5 (1)
C6	1.254 (1)	0.8811 (5)	0.471 (1)	6.0 (1)
C7	0.999 (1)	1.0064 (4)	0.803 (1)	4.1 (1)
C8	1.015 (1)	1.1593 (4)	0.767 (1)	3.5 (1)
C9	0.828 (1)	1.2314 (4)	0.738 (1)	4.4 (1)
C10	0.672 (1)	1.1391 (4)	0.529 (1)	4.7 (1)
C11	0.683 (1)	1.1450 (5)	0.308 (1)	4.7 (1)
C12	0.557 (1)	1.0309 (5)	0.097 (1)	6.6 (2)
C13	1.166 (1)	1.2708 (4)	0.963 (1)	3.8 (1)
C14	1.190 (1)	1.2910 (5)	1.178 (1)	5.8 (1)
C15	1.293 (1)	1.3573 (4)	0.898 (1)	4.6 (1)
C16	1.521 (1)	1.564 (1)	1.022 (1)	10.9 (3)

TABLE V. Intramolecular Distances (Å) and Intramolecular Bond Angles (°)

Distance							
O1	C2	1.425 (4)		C3	C4	1.317 (7)	
O2	C5	1.218 (6)		C4	C5	1.458 (5)	
O3	C11	1.208 (6)		C7	C8	1.501 (6)	
O4	C15	1.192 (6)		C8	C9	1.548 (6)	
O5	C15	1.326 (4)		C8	C13	1.519 (4)	
O5	C16	1.433 (8)		C9	C10	1.513 (4)	
C1	C2	1.507 (6)		C10	C11	1.491 (7)	
C1	C5	1.491 (6)		C11	C12	1.488 (5)	
C1	C7	1.327 (5)		C13	C14	1.319 (6)	
C2	C3	1.504 (5)		C13	C15	1.482 (7)	
C2	C6	1.519 (6)					
Bond angle							
C15	O5	C16	116.0 (4)	C1	C7	C8	127.5 (4)
C2	C1	C5	107.4 (3)	C7	C8	C9	110.6 (3)
C2	C1	C7	129.8 (4)	C7	C8	C13	111.3 (3)
C5	C1	C7	122.7 (4)	C9	C8	C13	109.6 (3)
O1	C2	C1	108.5 (3)	C8	C9	C10	114.0 (3)
O1	C2	C3	110.4 (2)	C9	C10	C11	115.9 (4)
O1	C2	C6	110.4 (3)	O3	C11	C10	121.8 (3)
C1	C2	C3	102.2 (3)	O3	C11	C12	120.6 (4)
C1	C2	C6	114.5 (3)	C10	C11	C12	117.6 (4)
C3	C2	C6	110.7 (3)	C8	C13	C14	123.7 (4)
C2	C3	C4	113.6 (3)	C8	C13	C15	115.7 (3)
C3	C4	C5	109.9 (4)	C14	C13	C15	120.6 (3)
O2	C5	C1	127.1 (3)	O4	C15	O5	123.0 (5)
O2	C5	C4	126.2 (4)	O4	C15	C13	124.1 (3)
C1	C5	C4	106.7 (4)	O5	C15	C13	112.9 (4)

Estimated standard deviations are given in parentheses.

(160 mg) in dry acetone (10 ml) for 3 h, with methyl iodide (1 ml) being added after 1 and 2 h. After addition of H_2O, the reaction mixture was extracted with AcOEt and the organic phase was then washed with H_2O and concentrated *in vacuo*. The residue obtained (131 mg) was chromatographed on silica gel (30 g) to give three fractions (fractions V-1, V-2, and V-3). Fraction V-3 (54 mg) was crystallized from diethyl ether–pentane and recrystallized from benzene to give **1** (35 mg). Fraction V-2 (28 mg) was rechromatographed on silica gel to give **2** (12 mg). Fraction V-3 (30 mg) was treated in the same way to give **3** (19 mg).

Fraction V was dissolved in $CHCl_3$ and kept at room temperature for 2 d. After removing the solvent, the residue was chromatographed on microcrystalline cellulose (Avicel, Asahi Chemical Industry Co.) using *n*-hexane–$CHCl_3$ (9 : 1) as the eluent. The fraction with an *Rf* value of 0.61 on TLC was repeatedly chromatographed on silanized silica gel (Silica gel 60 silanized, 70—230 mesh, Merck) to give **6**.

Methyl Achimillate A (1) Colorless needles, mp 76—77 °C. $[\alpha]_D$ −23.6° (c=1.0, $CHCl_3$). EIMS *m/z*: 292 (M^+), 274, 260, 215, 199, 171, 105, 91, 77, 69, 55, 43. *Anal.* Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.71; H, 6.71. UV λ_{max}^{EtOH} nm (ε): 245 (8600, sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1718, 1663, 1627, 1370, 1265, 1220, 1140, 950. CD (c=0.082, MeOH) [θ] (nm): 0 (405), 1390 (380, sh), 5340 (360, sh), 6770 (349, positive maximum), 720 (300, sh), 0 (293), −41700 (257.5, negative maximum), 0 (233.5), 14700 (215), 0 (195), −2300 (190), 0 (185).

Methyl Achimillate B (2) Colorless oil. $[\alpha]_D$ −78.2° (c=1.0, $CHCl_3$). EIMS *m/z*: 292 (M^+), 274, 260, 215, 199, 171, 105, 91, 77, 69, 55, 43. HR LSIMS *m/z*: Calcd for $C_{16}H_{20}O_5$+H: 293.1383. Found: 293.1388 (M+H)$^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1725, 1707, 1655, 1628, 1370, 1265, 1200, 1142, 950. CD (c=0.0683, MeOH) [θ] (nm): 0 (420), 2300 (390, sh), 5390 (370, sh), 5890 (316, positive maximum), 0 (298), −28100 (260, negative maximum), −12300 (232, sh), 0 (215.5), 6500 (205, positive maximum), 0 (197), −3200 (191), 0 (185).

Methyl Achimillate C (3) Colorless oil. $[\alpha]_D$ −87.3° (c=1.0, $CHCl_3$). EIMS *m/z*: 292 (M^+), 274, 260, 215, 199, 171, 105, 91, 77, 69, 55, 43. HR LSIMS *m/z*: Calcd for $C_{16}H_{20}O_5$+H: 293.1383. Found: 293.1388 (M+H)$^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1717, 1662, 1626, 1365, 1255, 1220, 1145, 955. CD (c=0.0624, MeOH) [θ] (nm): 0 (410), −1210 (380, sh), −4610 (360, sh), −5690 (350, negative maximum), 0 (300), 31500 (255, positive maximum), 0 (235.5), −27400 (220, negative maximum), 0 (190).

Compound 6 Colorless oil. $[\alpha]_D$ 6.1° (c=1.1, $CHCl_3$). EIMS *m/z*: 278 (M^+), 260, 163, 111, 98, 94, 67, 55, 43. HR LSIMS *m/z*: Calcd for $C_{15}H_{18}O_5$+H: 279.1227. Found: 279.1232 (M+H)$^+$. UV λ_{max}^{EtOH} nm (ε): 211 (14000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1765, 1710, 1660, 1593, 1370. CD (c=0.119, MeOH) [θ] (nm): 0 (375), 4720 (323, positive maximum), 720 (283), 1310 (265, positive maximum), 0 (255), −21400 (220, negative maximum), 0 (206), 13800 (200).

Treatment of 6 with $NaHCO_3$ Compound **6** (150 mg) was stirred with 5% aqueous $NaHCO_3$ (30 ml) for 1 h. The mixture was then acidified with dilute HCl and extracted with AcOEt. Evaporation of the solvent produced a yellowish oil (130 mg), which was methylated with methyl iodide and K_2CO_3 as described above. The methylated product was separated by chromatography on silica gel to give **1** (61 mg) and **2** (18 mg). These two compounds were identical with samples obtained above.

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