[Chem. Pharm. Bull. 36(8)2918—2924(1988)]

Studies on the Constituents of *Polyporus dispansus* and *P. confluens*

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(Received January 28, 1988)

From the lipophilic fraction of fresh *Polyporus dispansus*, three new farnesylphenols (III, IV, V) and a new isopentenylphenol (VII) were isolated along with grifolin (II), *O*-methylgrifolin (I) and grifolic acid (VI). The structures of these new compounds were elucidated as shown in formulas III, IV, V and VII, respectively, on the basis of spectral and chemical evidence. Grifolin (II) and neogrifolin (VIII) were also isolated from *P. confluens*.

Keywords——*Polyporus dispansus*; *Polyporus confluens*; basidiomycetes; farnesylphenol; grifolin; structural determination

A farnesylphenol, grifolin, has been isolated from *Grifola confluens* as an antibiotic constituent[1] and the structure has been established as 2-*trans*,*trans*-farnesyl-5-methylresorcinol (II).[2] Grifolin and an isomer, neogrifolin (VIII), were obtained from *Albatrellus confluens*.[3] Cristatic acid and grifolic acid (VI) were obtained from an European *A. cristatus* and a closely related American *Albatrellus* species.[4] Some other farnesylphenols, LL-Z1272 α, β, γ, δ, ε and ξ, have been isolated from an unclassified *Fusarium* species as inhibitors of the growth of the protozoan, *Tetrahymena pyriformis*, and their structures have been established.[5] Some farnesyl-*O*-phenols have been isolated from a mutant of *Aspergillus rugulosus* I.M.I 84338 and their structures have been established.[6] Grifolin and neogrifolin have been synthesized by the condensation of farnesol (farnesyl bromide) and orcinol (phenyl derivatives),[7] and have been used as the starting materials for obtaining biogenetically related compounds.[8,9]

In the course of our investigation on the constituents of mushrooms (basidiomycetes), new farnesylphenols (III—V) and an isopentenylphenol (VII) were isolated along with grifolin (II), its methyl ether (I) and grifolic acid (VI) from *Polyporus dispansus* LLOYD (Japanese name: kōmori-take) and their structures were established on the basis of physicochemical investigation and by chemical transformations.

The acetone extract was partitioned with an ethyl acetate and water system and the ethyl acetate layer was concentrated *in vacuo* after drying with sodium sulfate, then the residue was chromatographed on Florisil with *n*-hexane containing stepwise increasing concentrations of ethyl acetate and methanol as the elution solvent system. Each fraction was subjected to silica gel column chromatography employing *n*-hexane containing stepwise increasing concentrations of ethyl acetate or chloroform as the eluent. Further purification was performed by high-performance liquid chromatography (HPLC) on a reversed-phase column. This isolation procedure is summarized in Chart 1.

Compound I, $C_{23}H_{34}O_2$, was isolated as a pale yellow oil and compound II, mp 41 °C, $C_{22}H_{32}O_2$, was isolated as colorless needles. As shown in Table II, both compounds (I and II) displayed similar signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectra and the

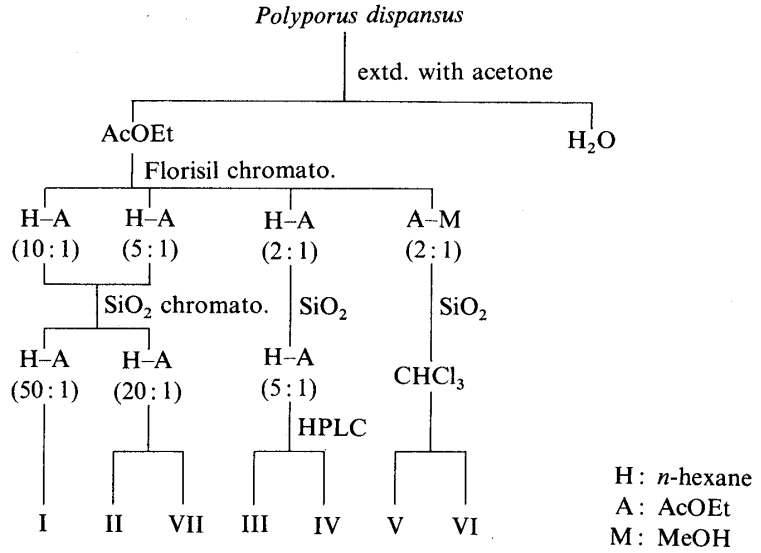

Chart 1

TABLE I. Physicochemical Data for I—VIII

	mp (°C)	Mol. formula	Mol. ions in MS	UV λ_{max}^{MeOH} nm (log ε)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}
I	Oil	$C_{23}H_{34}O_2$	342 (342)	280 (3.93) 273 (3.94)	3450 (OH) 1620, 1598, 1510 (arom.)
II	41	$C_{22}H_{32}O_2$	328.2406 (328.2402)	281 (3.31) 273 (3.34)	3610, 3430 (OH) 1640, 1598, 1520 (arom.)
III	Oil	$C_{22}H_{32}O_3$	344 (344)	281 (3.77) 273 (3.77)	3370 (OH) 1635, 1595, 1518 (arom.)
IV	Oil	$C_{22}H_{32}O_3$	344 (344)	282 (3.47) 274 (3.45)	3370 (OH) 1635, 1615, 1515 (arom.)
V	144—145	$C_{24}H_{34}O_4$	386 (386)	304 (4.04) 265 (4.45)	3400 2800 (OH) 1660 (C=O) 1630, 1575, 1510 (arom.)
VI	93—94	$C_{23}H_{32}O_4$	372 (372)	303 (4.06) 266 (4.43)	3540, 3440, 3300 2800 (OH) 1660 (C=O) 1620, 1582, 1505 (arom.)
VII	76—77	$C_{14}H_{18}O_4$	250.1207 (250.1204)	304 (4.04) 270 (4.48)	3375 (OH) 1725 (C=O) 1645, 1585, 1505 (arom.)
VIII	Oil	$C_{22}H_{32}O_2$	328 (328)	282 (3.61)	3400 (OH) 1610, 1600, 1510 (arom.)

carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra except for an additional singlet (3.77 ppm, 3H) in the ^{1}H-NMR and a quartet (55.7 ppm, C_1) in the ^{13}C-NMR off-resonance spectrum due to a methoxy group in I.

Because II was identified as an antibiotic, grifolin, after comparison of the physicochemical properties (Tables I—III) with the reported data, compound I was supposed to be grifolin monomethyl ether (*O*-methylgrifolin). Compound II yielded its monomethyl ether and dimethyl ether on treatment with diazomethane and the monomethyl ether was identical with I.

Compounds III and IV showed similar chromatographic behavior and their isolation was performed by HPLC on a reversed-phase YMC-Pack A212 Rp 8. They were obtained as oily substances having the same molecular formula, $C_{22}H_{32}O_3$, $[\alpha]_D$ values ($\pm 0°$), and displayed

Table II. ^{1}H-NMR Data for I—VIII

	I	II	III	IV
1-H	s, 6.29	s, 6.20	d, 6.19 (J=3.0)	d, 6.14 (J=3.0)
3-H	s, 6.29	s, 6.20	d, 6.30 (J=3.0)	d, 6.24 (J=3.0)
8-H	s, 2.26	s, 2.18	s, 2.21	s, 2.23
1′-H	d, 3.37 (J=7.0)	d, 3.37 (J=7.0)	dd, 2.65 (J=6.0, 15.7) dd, 2.87 (J=4.8, 15.7)	dd, 3.03 (J=9.5, 15.2) dd, 3.10 (J=7.6, 15.2)
2′-H	t, 5.16 (J=7.0)	t, 5.26 (J=7.0)	dd, 3.89 (J=4.8, 6.0)	dd, 4.66 (J=7.6, 9.5)
4′,5′,8′,9′-H	m, 1.90—2.10	m, 1.90—2.10	m, 1.90—2.10	m, 1.90—2.10
6′,10′-H	m, 5.04	m, 5.06	m, 5.10	m, 5.10
12′-H	s, 1.66	s, 1.66	s, 1.66	s, 1.67
13′-H	s, 1.59	s, 1.57	s, 1.60	s, 1.60
14′-H	s, 1.59	s, 1.57	s, 1.60	s, 1.62
15′-H	s, 1.80	s, 1.80	s, 1.33	s, 1.30
OCH_3	s, 3.77	—	—	—
$COOCH_3$	—	—	—	—

	V	VI	VII	VIII
1-H	—	—	—	d, 6.23 (J=3.0)
3-H	s, 6.29	s, 6.24	s, 6.20	d, 6.18 (J=3.0)
8-H	s, 2.58	s, 2.52	s, 2.44	s, 2.21
1′-H	d, 3.32 (J=7.0)	d, 3.41 (J=7.0)	d, 3.40 (J=7.0)	d, 3.27 (J=7.0)
2′-H	t, 5.17 (J=7.0)	t, 5.24 (J=7.0)	t, 5.24 (J=7.0)	t, 5.11 (J=7.0)
4′,5′,8′,9′-H	m, 1.90—2.10	m, 1.90—2.10	s, 1.80 (4′-H)	m, 1.84—2.04
6′,10′-H	m, 5.04	m, 5.04	s, 1.74 (5′-H)	m, 5.04
12′-H	s, 1.69	s, 1.66	—	s, 1.66
13′-H	s, 1.57	s, 1.58	—	s, 1.58
14′-H	s, 1.57	s, 1.58	—	s, 1.58
15′-H	s, 1.76	s, 1.81	—	s, 1.78
OCH_3	s, 3.85	—	—	
$COOCH_3$	—	—	s, 3.92	

characteristic physicochemical properties. On comparing the properties with those of grifolin (II), prominent features were found in the aromatic ring proton signals. They appeared as two separated signals, and on irradiation at the aromatic methyl signal, they changed into a pair of doublets (J=3 Hz, *meta*-coupling). These data suggested the deformation of the symmetry of the aromatic ring system found in I and II. Other marked variations from grifolin (II) were the disappearance of one of the vinyl protons and one of the vinyl methyl signals, and the appearance of signals of a carbinyl hydrogen, a methylene group and a tertiary methyl group on the carbon carrying an etheric oxygen (Table II; 1′-H, 2′-H, 15′-H in III and IV).

These characterizations provided the structural formulae III and IV for the compounds. These compounds were racemic, as described later, and the orientation of the hydroxy group on C-2′ was assigned as *trans* to the methyl group on C-3′ for III and *cis* for IV, from the chemical shifts of the methyl groups.

Compounds III and IV were methylated by treatment with diazomethane to provide the methyl ether (IIIm) and (IVm), which were oxidized with Jones reagent, to produce the same

Table III. ^{13}C-NMR Data for I—VIII

	I	II	III	IV	V	VI	VII	VIII
C-1	109.6 (d)	109.3 (d)	107.9 (d)	102.7 (d)	104.3 (s)	103.8 (s)	105.3 (s)	109.9 (d)
C-2	137.2 (s)	137.2 (s)	137.8 (s)	139.4 (s)	142.5 (s)	142.6 (s)	140.8 (s)	154.1 (s)
C-3	104.3 (d)	109.3 (d)	110.1 (d)	108.7 (d)	106.6 (d)	112.0 (d)	111.3 (d)	101.2 (d)
C-4	157.8 (s)	154.6 (s)	153.5 (s)	152.2 (s)	162.1 (s)	160.4 (s)	159.2 (s)	155.2 (s)
C-5	112.3 (s)	111.0 (s)	103.6 (s)	109.7 (s)	115.0 (s)	111.6 (s)	111.5 (s)	118.4 (s)
C-6	155.3 (s)	154.6 (s)	154.3 (s)	161.2 (s)	162.8 (s)	163.6 (s)	162.7 (s)	138.7 (s)
C-7	—	—	—	—	176.7 (s)	176.6 (s)	172.7 (s)	—
C-8	21.5 (q)	21.0 (q)	21.2 (q)	21.5 (q)	24.8 (q)	24.2 (q)	24.1 (q)	20.2 (q)
C-1′	22.0 (t)	22.3 (t)	21.7 (t)	22.0 (t)	21.8 (t)	22.0 (t)	22.1 (t)	25.2 (t)
C-2′	122.3 (d)	122.0 (d)	68.0 (d)	89.3 (d)	122.1 (d)	121.2 (d)	121.6 (d)	122.3 (d)
C-3′	137.6 (s)	138.4 (s)	78.3 (s)	77.0 (s)	135.0 (s)	139.0 (s)	135.1 (s)	137.3 (s)
C-4′	39.7 (t)	39.7 (t)	36.9 (t)	36.8 (t)	39.8 (t)	39.7 (t)	25.8 (q)	39.8 (t)
C-5′	26.5 (t)	26.7 (t)	25.7 (t)	27.3 (t)	26.6 (t)	26.4 (t)	17.9 (q)	26.6 (t)
C-6′	123.9 (d)	123.7 (d)	123.8 (d)	124.0 (d)	124.3 (d)	123.6 (d)	—	123.9 (d)
C-7′	135.4 (s)	135.4 (s)	135.6 (s)	135.6 (s)	134.7 (s)	135.6 (s)	—	135.4 (s)
C-8′	39.8 (t)	39.7 (t)	39.6 (t)	39.7 (t)	39.8 (s)	39.7 (t)	—	39.8 (t)
C-9′	26.8 (t)	26.7 (t)	26.7 (t)	26.7 (t)	26.8 (t)	26.7 (t)	—	26.8 (t)
C-10′	124.5 (d)	124.5 (d)	124.2 (d)	124.2 (d)	124.4 (d)	124.3 (d)	—	124.5 (d)
C-11′	131.2 (s)	131.0 (s)	131.3 (s)	131.4 (s)	131.1 (s)	131.2 (s)	—	131.4 (s)
C-12′	25.7 (q)	25.7 (q)	25.7 (q)	25.7 (q)	25.6 (q)	25.7 (q)	—	25.8 (q)
C-13′	17.7 (q)	17.7 (q)	17.7 (q)	17.7 (q)	17.7 (q)	17.7 (q)	—	17.8 (q)
C-14′	16.2 (q)	16.1 (q)	16.0 (q)	16.0 (q)	16.1 (q)	16.3 (q)	—	16.4 (q)
C-15′	16.0 (q)	16.0 (q)	19.4 (q)	22.8 (q)	16.1 (q)	16.0 (q)	—	16.2 (q)
OMe	55.7 (q)	—	—	—	55.5 (q)	—	—	—
COOMe	—	—	—	—	—	—	51.7 (q)	—

keto derivative. The identification was confirmed by measurements of melting point, infrared (IR), ^{1}H-NMR and circular dichroism (CD) spectra. This keto compound showed a plane line in the CD spectrum, suggesting it to be a racemic compound. Therefore, the starting compounds (III and IV) were also racemic.

Finally, grifolin was epoxidized with *m*-chloroperbenzoic acid to provide a mono-epoxide (IIo), $C_{22}H_{32}O_3$, mass spectrum (MS) *m/z*: 344, ^{1}H-NMR: 1.47 ppm (3H, s, 15′-H), 2.67 (1H, t, J=4.8 Hz, 2′-H) along with other mono-epoxides.

The mono-epoxide (IIo) was treated with *p*-toluenesulfonic acid in dioxane to produce III and IV. It is of interest that two stereoisomers, *trans* (III) and *cis* (IV), were obtained from a homogeneous mono-epoxide (IIo). The yields of III and IV varied depending on the experimental conditions (temperature, dilution and so on), suggesting an initial cleavage of the C-2′–O bond or the C-3′–O bond to produce two stereoisomers.

Compound V, mp 144—145 °C, $C_{24}H_{34}O_4$, was obtained as colorless needles after recrystallization from *n*-hexane. Compound VI, mp 93—94 °C, $C_{23}H_{32}O_4$, was also obtained as colorless needles after recrystallization from *n*-hexane. Both compounds were similar in IR, ultraviolet (UV) (Table I), ^{1}H-NMR (Table II) and ^{13}C-NMR spectra (Table III), except for the presence of an additional methyl group (MS *m/z*: 386, 3.92 ppm in ^{1}H-NMR and 55.5 ppm in ^{13}C-NMR) in V. Therefore, V was supposed to be a methyl ester or a methyl ether of VI. Because methylation of VI with diazomethane provided a methyl ester of V, the structure of V was concluded to be as shown in Chart 2, considering that a carboxy group and *ortho* phenolic group would show resistance to methylation with diazomethane owing to strong hydrogen bonding between them. Compound VI was converted to grifolin (II) by refluxing in alkaline solution. This compound (VI) was identified as grifolic acid, because these properties were the

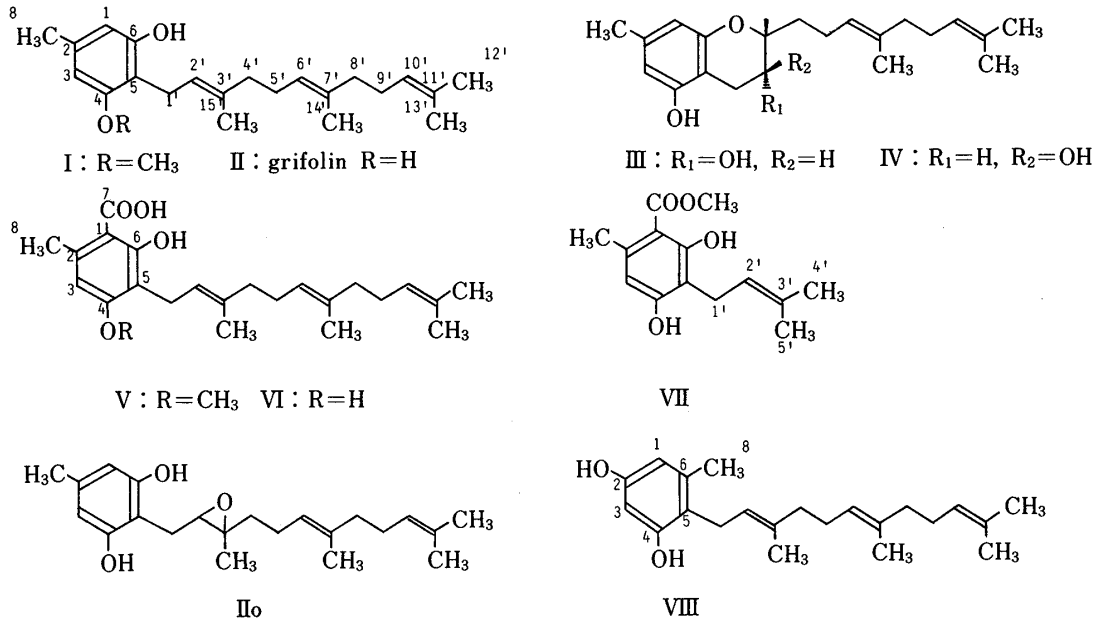

Chart 2. Structures of Compound I—VIII and a Related Derivative (IIo)

same as the reported data.[4]

Compound VII, mp 76—77 °C, $C_{14}H_{18}O_4$, was obtained as colorless needles after recrystallization from *n*-hexane, and was proved to be a methyl ester, because the diacetate was obtained on treatment with acetic anhydride and pyridine. As depicted in Tables II and III, signals due to the aromatic moiety of VII were similar to those of VI, and the side chain was assigned as an isopentenyl group.

Grifolin (II) and neogrifolin (VIII) were obtained from *Polyporus confluens* (ALB.*et* SCHW.) FR. (Japanese name: ningyotake) collected in Miyagi prefecture as described in the experimental section. These compounds have been obtained from *Albatrellus confluens* (ALB. *et* SCHW. *ex* FR.) KOTL. *et* POUZ.[3] According to the Hamlyn Book of Mushrooms and Fungi,[10] and the Encyclopedia of Mushrooms,[11] *A. confluens* may be a synonym of *Polyporus confluens*, although this relationship has not yet been established.

Experimental[12]

Isolation Procedure——Fruiting bodies (1.6 kg) of *Polyporus dispansus* were collected in early autumn in the neighborhood of our university campus and the mountainous area of Marumori, Miyagi prefecture. After air-drying for a couple of days, the materials were extracted with acetone (10 l, three times). An example of the isolation procedures is given in the following section.

Acetone was evaporated under reduced pressure and the concentrated extract (*ca.* 500 ml) was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with water and dried over Na_2SO_4. One part of the residue (50 g) after the evaporation of the solvent was chromatographed on Florisil (100 × 3.5 cm i.d.). Fractions eluted with *n*-hexane–AcOEt (10 : 1) were chromatographed on SiO_2 (50 × 2.5 cm i.d.). Elution with *n*-hexane–AcOEt (50 : 1) provided I as a colorless oil (25 mg). Gas chromatography (GC) of I gave one peak of t_R 3.5 min under the following conditions: column, OV-1 1.5% (1.5 m × 3 mm i.d.), carrier gas, N_2/ at 40 ml/min, 1.0 kg/cm^2; column temperature, 210°C. MS *m/z*: 342. IR, ^{1}H-NMR and ^{13}C-NMR data are given in Tables I—III. Grifolin (II) was treated with diazomethane at room temperature to provide the di-*O*-methyl ether and mono-*O*-methyl ether, the latter of which was identical with the isolated compound (I) in IR, NMR and GC comparisons.

Elution with *n*-hexane–AcOEt (20 : 1) provided grifolin (II) as colorless needles (6.6 g), after recrystallization from *n*-hexane in an ice stocker. Its high-resolution MS showed the molecular ion at *m/z* 328.2406. (*Anal.* Calcd for $C_{22}H_{32}O_2$: 328.2402). IR, ^{1}H-NMR and ^{13}C-NMR data (Tables I—III) were identical with those reported for grifolin.

Compound VII was obtained from the eluate with *n*-hexane–AcOEt (20 : 1) as colorless needles from *n*-hexane,

mp 76—77 °C. The high-resolution MS showed the molecular ion at *m/z*: 250.1207. (*Anal.* Calcd for $C_{14}H_{18}O_4$: 250.1204). IR, ^{1}H-NMR and ^{13}C-NMR data are given in Tables I—III. Elution with *n*-hexane–AcOEt (5 : 1) gave two hydroxyisogrifolins (III and IV) as a mixture, which was further separated by HPLC (column, YMC-Pack A-212 RP_8 (150 × 6 mm i.d.); solvent, MeOH–H_2O (5 : 1)) to yield III (15.1 mg) and IV (16.2 mg), both as oils, $[\alpha]_D \pm 0°$ (c=0.80, MeOH in III; c=0.61, MeOH in IV). The electron impact mass spectra (EIMS) of both compounds showed *m/z*: 344 (M^+), suggesting they were isomeric. IR, ^{1}H-NMR and ^{13}C-NMR data for III and IV are given in Tables I—III. The eluate with AcOEt–MeOH (2 : 1) from the Florisil column was rechromatographed on silica gel using $CHCl_3$ as an eluent to yield V (976 mg) and VI (829 mg).

Compound V was obtained as colorless needles after recrystallization from *n*-hexane, mp 144—145 °C, EIMS *m/z*: 386 (M^+). IR, ^{1}H-NMR and ^{13}C-NMR data are given in Tables I—III.

Compound VI was obtained as colorless needles from *n*-hexane, mp 93—94 °C. EIMS *m/z*: 372 (M^+). IR, ^{1}H-NMR and ^{13}C-NMR data are given in Tables I—III. These data were the same as the reported data for grifolic acid.[4]

Isolation Procedure——The fruiting bodies (95 g) of *Polyporus confluens*, collected in Miyagi prefecture in 1986, were extracted twice with MeOH at room temperature (first, two weeks; second, one day). After removal of the solvent under reduced pressure, the residue (30 ml) was extracted twice with AcOEt (50 ml). The combined AcOEt extracts were washed with water, dried over Na_2SO_4, and concentrated to give a syrup (2.25 g). The residual extract was chromatographed repeatedly on silica gel (15 × 2.0 cm i.d.) using *n*-hexane–AcOEt as the eluant to afford grifolin (II, 628.6 mg), and neogrifolin (VIII, 410.8 mg).

Grifolin (II) was obtained as colorless needles by recrystallization from *n*-hexane and identified by direct comparison (IR, UV, and ^{1}H-NMR and ^{13}C-NMR spectra) with an authentic specimen. Neogrifolin (VIII) was obtained as an oil; the MS, UV, ^{1}H-NMR and ^{13}C-NMR data are summarized in Tables I—III.

Methylation of III——III (10 mg) was dissolved in ether (0.5 ml) and treated with CH_2N_2 in the usual manner. The product was purified by SiO_2 chromatography. Elution with *n*-hexane–AcOEt (10 : 1) provided the methyl ether (IIIm) as an oil (8 mg). EIMS *m/z*: 358 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1621, 1590, 1500 (arom.). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, s, 15′-H), 1.60 (6H, s, 13′, 14′-H), 1.65 (3H, s, 12′-H), 1.90—2.10 (8H, m, 4′,5′,8′,9′-H), 2.25 (3H, s, 8-H), 2.68 (1H, dd, J=6.0, 15.0 Hz, 1′-H), 2.77 (1H, dd, J=5.0, 15.0 Hz, 1′-H), 3.75 (3H, s, OCH_3), 3.90 (1H, dd, J=5.0, 6.0 Hz, 2′-H), 5.07 (2H, m, 6′, 10′-H), 6.18 (1H, s, 1-H), 6.25 (1H, s, 3-H).

Methylation of IV——IV (10 mg) was dissolved in ether (0.5 ml) and treated with CH_2N_2 in the usual manner. The product was purified by SiO_2 chromatography. Elution with *n*-hexane–AcOEt (10 : 1) provided the methyl ether (IVm) as an oil (8 mg). EIMS *m/z*: 358 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1622, 1610, 1509 (arom.). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, s, 15′-H), 1.60 (6H, s, 13′,14′-H), 1.65 (3H, s, 12′-H), 1.90—2.10 (8H, m, 4′,5′,8′,9′-H), 2.27 (3H, s, 8-H), 2.95 (1H, dd, J=9.0, 15.0 Hz, 1′-H), 3.10 (1H, dd, J=8.0, 15.0 Hz, 1′-H), 3.77 (3H, s, OCH_3), 4.61 (1H, dd, J=8.0, 9.0 Hz, 2′-H), 5.10 (2H, m, 6′, 10′-H), 6.18 (1H, s, 1-H), 6.23 (1H, s, 3-H).

Oxidation of IIIm and IVm——IIIm (8 mg) was dissolved in acetone (2 ml) and Jones' reagent (0.5 ml) was added. The reaction mixture was stirred for 2 h under ice cooling, then extracted with ether. The organic layer was washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and then H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the product was purified by SiO_2 chromatography. Elution with *n*-hexane–AcOEt (20 : 1) provided the keto derivative as a colorless oil (7 mg). Thin layer chromatography (TLC): *n*-hexane–AcOEt (5 : 1; *Rf* 0.62). EIMS *m/z*: 356 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (C=O), 1622, 1598, 1505 (arom.). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, s, 15′-H), 1.55 (6H, s, 13′, 14′-H), 1.65 (3H, s, 12′-H), 1.90—2.10 (8H, m, 4′, 5′,8′,9′-H), 2.32 (3H, s, 8-H), 3.42 (2H, s, 1′-H), 3.76 (3H, s, OCH_3), 5.02 (2H, m, 6′, 10′-H), 6.32 (1H, s, 1-H), 6.41 (1H, s, 3-H). IVm (8 mg) was treated similarly, and the keto derivative was obtained as a colorless oil (5 mg). The product from IVm was identical with the keto derivative of IIIm on direct comparison in (TLC [*n*-hexane–AcOEt (5 : 1; *Rf* 0.51)], EIMS, IR and ^{1}H-NMR).

Synthesis of III and IV——Grifolin (II) (1 g) was dissolved in $CHCl_3$ (70 ml) at −30 °C, *m*-chloroperbenzoic acid (660 mg) was added, and the mixture was stirred for 3 h. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with 5% $NaHSO_3$, 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was chromatographed on SiO_2 with benzene–AcOEt as the elution solvent system. Elution with benzene–AcOEt (50 : 1) provided the mono-epoxide (IIo) (219 mg) as an oil. EIMS *m/z*: 344 (M^+), 326, 207, 137. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (3H, s, 15′-H), 1.57 (6H, s, 13′, 14′-H), 1.64 (3H, s, 12′-H), 1.90—2.10 (8 H, m, 4′,5′,8′,9′-H), 2.17 (3H, s, 8-H), 2.67 (1H, t, J=4.8 Hz, 2′-H), 3.32 (2H, d, J=7.0 Hz, 1′-H), 5.00 (2H, m, 6′, 10′-H), 6.17 (2H, s, 1,3-H). IIo was dissolved in 1,4-dioxane (19 ml) and *p*-toluenesulfonic acid monohydrate (190 mg) was added. The reaction mixture was stirred for 3 h at room temperature. The reaction mixture was extracted with $CHCl_3$, and the organic layer was washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent. IV (25 mg) was eluted first, followed by III (131 mg). These synthetic III and IV were identical with the natural compounds III and IV, respectively in terms of MS, IR and ^{1}H-NMR behavior.

Methylation of VI——VI (5 mg) was dissolved in ether (0.5 ml)] and treated with CH_2N_2 in the usual manner. After usual work-up, the product was obtained as a colorless oil (5 mg). EIMS *m/z*: 400 (M^+), 368, 209, 177. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650 (C=O), 1615, 1505 (arom.). ^{1}H-NMR ($CDCl_3$) δ: 1.58 (6H, s, 13′, 14′-H), 1.68 (3H, s, 12′-H),

1.78 (3H, s, 15′-H), 1.90—2.10 (8H, m, 4′, 5′, 8′, 9′-H), 2.50 (3H, s, 8-H), 3.32 (2H, d, J=7.0 Hz, 1′-H), 3.82 (3H, s, OCH_3), 3.90 (3H, s, $COOCH_3$), 5.06 (2H, m, 6′, 10′-H), 5.24 (1H, t, J=7.0 Hz, 2′-H), 6.18 (1H, s, 3-H).

Methylation of V——V (5 mg) was treated as described for VI to yield the methyl ester of V (5 mg) as an oil. The methyl ester of V was identical with that of VI on direct comparison (TLC [*n*-hexane–AcOEt (5 : 1; *Rf* 0.62)], MS, IR and ^{1}H-NMR).

Decarboxylation of VI——VI (7 mg) was dissolved in 1% NaOH–MeOH (10 ml) and the solution was stirred at 60°C for 3 h. After cooling to room temperature, the reaction mixture was extracted with ether. The organic layer was washed with 1% HCl and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was chromatographed on SiO_2 with *n*-hexane–AcOEt as the elution solvent system. Elution with *n*-hexane–AcOEt (20 : 1) provided II as an oil. The product was identified as grifolin (II) by comparison (TLC [*n*-hexane–AcOEt (5 : 1; *Rf* 0.38)], MS and ^{1}H-NMR) with an authentic specimen of the isolated grifolin.

Acetylation of VII——Acetic anhydride (1 ml) was added to a solution of VII (5 mg) in pyridine (0.5 ml). The reaction mixture was kept at room temperature for 24 h, and then extracted with AcOEt. The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on SiO_2 to give the diacetate as a colorless powder. EIMS *m/z*: 334 (M^+), 292, 260, 218. ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, s, 5′-H), 1.65 (3H, s, 4′-H), 2.23 (6H, s, $OCOCH_3$), 2.33 (3H, s, 8-H), 3.10 (2H, *d*, J=7.0 Hz, 17-H), 3.80 (3H, s, $COOCH_3$), 5.27 (1H, t, J=7.0 Hz, 2′-H), 6.77 (1H, s, 3-H).

References and Notes

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12) Melting points were measured on a Yanagimoto micro hot plate and are uncorrected. IR spectra were measured with a Shimadzu IR-27G infrared spectrometer. ^{1}H-NMR spectra were taken with JEOL JMN-PMX 60 (at 60 MHz) and JEOL JMN-FX-100 (at 100 MHz) spectrometers using tetramethylsilane as an internal standard. ^{13}C-NMR spectra were measured with a JEOL JMN-FX 100 spectrometer operating at 25.05 MHz. The coupling patterns are indicated as follows: singlet=s, doublet=d, multiplet=m, and broad=br. MS were measured with Hitachi M-52 and JEOL JMS-01SG-2 mass spectrometers. $[\alpha]_D$ values were measured on a JACS DIP-340 polarimeter. UV spectra were measured on a Hitachi U-3200 spectrometer. TLC was carried out with pre-coated Silica gel $60F_{254}$ plates (Merck) the indicated solvent systems.