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Studies on the Constituents of Umbelliferae Plants. XV.1) Constituents
of Cnidium officinale: Occurrence of Pregnenolone, Coniferylferulate and Hydroxyphthalides

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Pregnenolone (1), coniferylferulate (2) and nine mono- and dihydroxyphthalide derivatives (5-13) were isolated from the commercial C. officinale rhizome. Their structures were determined from the spectroscopic data and the dihydroxyphthalides senkyunolide-H (11) and -1 (12) and senkyunolide-J (13) were synthesized from the major components ligustilide (14) and senkyunolide-
A (16), respectively. These oxygenated phthalides were absent in the fresh C. officinale rhizome and
they were shown to be derived from the major volatile phthalides during storage of the crude drug. Coniferylferulate (2) also decomposes partly during storage, giving ferulic acid.
Keywords pregnenolone; coniferylferulate; senkyunolide; phthalide; hydroxyphthalide;
Cnidium officinale; Umbelliferae

Senkyu, the dried rhizome of Cnidium officinale MAKINO (syn. Ligusticum officinale KITAGAWA, Umbelliferae) is One of the most frequently occurring drugs in the prescriptions of Chinese traditional medicine used in Japan. C. officinale is a variety of L. wallichii, the original plant of the important crude drug chuan xiong in China, and used in Japan for the same purpose as L. wallichii. It contains a variety of volatile non-polar alkylphthalide derivatives which have been shown to have antifungal and smooth muscle relaxing activ-ities.2,3)In contrast, its more polar and non-volatile components had been less well characterized. During the analysis of commercial C. officinale extract, we found significant amounts of unidentified components in the non-volatile fraction of the mixture. Some of them were absent or present in very minor amounts in the fresh material. Since crude drugs are generally administered after some processing and storage, analysis of these minor components possibly derived from the major original components would be of importance. The present report describes our finding that significant amounts of previously unknown components are contained in commercial C. officinale rhizome.
The dried rhizome of C. officinale was extracted with hexane, ether and methanol and the crude lipids obtained were partitioned with a mixture of hexane-methanol-water (20 : 10 : 2). This simple solvent fractionation was remarkably efficient for the removal of triglycerides, which are often the predominant components of animal and plant tissues. Double application of this fractionation made the methanol layer free from triglycerides and most of the phthalides remained in the methanol layer. The methanol layer contained unidentified polar compounds. Repetitive flash chromatography over a column of silica gel afforded compounds 1-13.

Pregnenolone (1), Coniferyl Ferulate (2), and Falcarindiol (4)
Compound 1, mp 187 188•Ž, C21H32O2, was identified as pregnenolone from its mass
(MS, m/z 316, 298, 283, 273, 255, 231, 213), infrared (IR, 3500, 1680 cm-1) and proton nuclear magnetic resonance [1H-NMR, ƒÂ6 0.63 (3H, s), 1.01 (3H, s), 2.13 (3H, s), 5.35 (1H, m),

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3.50 (1H, m)] spectra, which were identical with those of the authentic compound. Compound 1 occurred in the C. officinale rhizome entirely as the free steroid. Acid treatment of the glycoside fraction of the extract only gave a small amount of phytosterol mixture but no trace of 1. This is the first demonstration of the occurrence of C21 steroid in Umbelliferae plants.4) Although C21 steroids have been found in a variety of plants, most of them occur as the oxygenated aglycone of the glycosides. There are a very few examples, such as in some Apocynaceae plants, in which pregnenolone was found.5) Since 1 is the common precursor of all of the hormonal steroids and since C. officinale has traditionally been used in prescriptions for obstetrical and gynecological disorders, the possible significance of 1 in the crude drug is of interest. The amount of 1 found in fresh and commercial C. officinale was about 1 mg in 30 g of dried material. It is known that orally administered pregnenolone is mostly excreted through the enterohepatic circulation in a short period.6) However, the average level of pregnenolone in human plasma is generally less than 10 ng/ml,7) a minute amount compared with the total pregnenolone contained in general prescriptions of senkyu, the dried rhizome of C. officinale.
Compound 2, C20H20O6, was obtained in a fairly high yield (860 mg from 2 kg of the
material). Its IR (3330, 1680, 1615, 1580, 1505 cm-1) and 1H-NMR [6 3.90 (3H, s), 3.92 (3H,
s), 7.66 (1H, d, J= 15.6 Hz), 6.33 (1H, d, J= 15.6 Hz), 6.64 (1H, d, 15.6 Hz), 6.21 (1H, dt,
J= 15.6, 6.4 Hz), 4.84 (2H, br d, J= 6.4 Hz] spectra were reminiscent of those of an ester of
ferulic acid (3), which is a known component of C. officinale.8) Hydrolysis of 2 in aqueous
dioxane afforded 3 and coniferyl alcohol, which were identified by comparison with authen-
tic samples. Compound 2 was thus shown to be coniferylferulate. It is labile to hydrolysis and
the coniferyl alcohol formed decomposes easily. For example, treatment of 2 in 1% HCl in
dioxane or in dilute dioxane at 60•Ž for 10 min causes formation of 3 with a very small
amount of coniferyl alcohol. Treatment under basic conditions gave a similar result. Acid
treatment of 2 in methanol at room temperature resulted in formation of 3 and methylconi-
feryl ether because the feruloyl moiety serves as an excellent leaving group in 2. Heating of 2 in
a mixture of dioxane-H2O (2 : 1) at 80•Ž for 1 h gave 3 and coniferylalcohol without
decomposition. Also, when the pulverized C. officinale was heated in boiling water for 1 h, 2
was entirely converted to 3. Other major components such as phthalides showed little change
of their contents. Since the amount of ferulic acid (3) in the fresh C. officinale is negligible, the
presence of 3 in the commercial C. officinale rhizome is apparently due to accumulation from
2 during storage. In our opinion, determination of the ratio of 2 and 3 affords a simple
method for estimation of the freshness of this crude drug. Compound 4 was identified by 1H-
NMR as falcarindiol, which is a common component in Umbelliferae plants.

Isolation of Hydroxyphthalides (5-13)
Compounds 5 to 13 were found to be the hydroxy derivatives of the major non-polar alkylphthalides, ligustilide (14),9) butylidenephthalide (15),101 and senkyunolide (16).1°1 To simplify the nomenclature of these new compounds, senkyunolide (16) previously isolated by Yamagishi and Kaneshima10)from C. officinale was renamed senkyunolide-A by agreement with them, and the compounds 5 to 13 were designated as senkyunolide-B to senkyunolide-J.
Senkyunolide-B (5), mp 150 153•Ž, C12H12O3, was a monohydroxy derivative of
butylidenephthalide (15) as indicated by the IR (3250, 1730, 1680, 1605 cm -1) and 1H-NMR
[butylidene side chain: 60.99 (3H, t, J= 7 .3 Hz), 1.57 (2H, sext, J= 7 .3 Hz), 2.47 (2H, dt,
J= 7 .8, 7.3 Hz), 5.96 (1 H, t, J= 7.8 Hz)] spectra. Its MS showed the loss of ethyl (m/f 175) and
propylene (mlz., 162) moieties, as observed in 15.10) The signals due to three aromatic protons
were found at 6 7.05 (d, J= 7.8 Hz), 7.34 (t, J= 7.8 Hz), and 7.50 (d, J= 7.8 Hz). Consequently,
the hydroxyl group is attached at C-4 or C-7 of the aromatic ring. The olefinic proton at C-8
(ƒÂ 5.96) was sifted 0.33 ppm to lower field than that of 15 (6 5.63) and indicated the presence

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of a deshielding effect due to a nearby hydroxyl group. It is also known that the butylidene side chain in 14 and 15 takes Z-form and the corresponding E-form is quite unstable (vide infra). The hydroxyl proton appeared at (5 5.97, overlapped with the C-8 proton. Simultaneous irradiation of the C-8 proton and hydroxyl proton caused nuclear Overhauser effect (NOE) at the doublet at (5 7.05, but it was not observed after deuterium exchange. These results indicate that senkyunolide-B (5) is 4-hydroxybutylidenephthalide. It was erroneously described as 7- hydroxybutylidenephthalide in our preliminary communication as a result of misinterpre-tation of the NOE.1b)
Senkyunolide-C (6), mp 95 100•Ž, C,21-1,203, was also a monohydroxy derivative of 15
as indicated by the IR (3250, 1730, 1680, 1605, 1590 cm-1) and 1H-NMR [ƒÂ 0.98 (3H, t,
J= 7.3 Hz), 1.54 (2H, sext, J=7.3 Hz), 2.43 (2H, dt, J= 7.8, 7.3 Hz), 5.59 (1H, t, J= 7.8 Hz)]
spectra. Its MS showed strong ions at m/z 175 and 162, as in 5. Three aromatic proton signals
were found at 6 7.00 (dd, J= 2.4, 8.3 Hz), 7.05 (d, J= 2.4 Hz), and at 7.76 (d, J= 8.3 Hz). From
this coupling pattern it is evident that the hydroxyl group is attached at C-5 or C-6. The
presence of NOE between the olefinic proton at 6 5.59 and the aromatic proton at (5 7.05
established the structure of 6 as 5-hydroxybutylidenephthalide. This compound was inde-
pendently isolated frOm the Umbelliferae plant Ligusticum wallichii.11)
Senkyunolide-D (7), Ci2H,404, was a monooxo monohydroxyderivative of 16 (IR, 3250,
1765, 1725, 1680 cm-1). The presence of a cross-conjugated diene was indicated by the

ultraviolet [UV, 287 nm (r, 2600)] and 1H-NMR [6 6.09 (IH, dt, J = 9.8, 4.0 Hz), 6.27 (1H, dt,
J =9 .8, 2.0 Hz)] spectra, which were similar to those of 16 [UV, 277 nm (r, 3100); H-NMR
6 5.9 (m), 6.19 (br d, J= Hz)].10) Hence the carbonyl group is located at the side chain. In the
H-NMR, the signals due to protons adjacent to a hydroxyl group were absent

. Two double

triplet signals at 6 2.28 (J = 18.1, 7.3 Hz) and 2.65 (J= 18.1, 6.8 Hz) due to the protons
adjacent to the carbonyl group indicated the presence of a propyl ketone side chain. This was
confirmed by the MS, which showed strong peaks at m/z 151 and 71 due to cleavage of the
ketol group. Thus, senkyunolide D (7) was shown to be 3-hydroxy-8-oxosenkyunolide-A .
Senkyunolide-E (8), C12H,203, was a monohydroxy derivative of butylidenephthalide
(15) like 5 and 6. Its IR (3400, 1780, 1685, 1610 cm-1), UV [306 nm (ƒÃ, 3900), 270 nm (ƒÃ, 8600)]
and H-NMR [four aromatic protons at 6 7.52 7.76 (3H, m), 7.91 (1H, d, J= 7.3 Hz)] spectra
were virtually the same as those of 15.10) The side chain bears a hydroxyl group and the

hydroxymethine proton at (54.87 (dt, J= 8.8, 6.8 Hz) was found by a decoupling experiment to

be coupled with the C-8 olefinic proton at 6 5.66 (d, J =8.8 Hz). Consequently, senkyunolide-E (8) was shown to be 9-hydroxybutylidenephthalide.
Senkyunolide-F (9), C12F14O3, was the major component of the hydroxyphthalides found in C. officinak. It was a monohydroxy derivative of the major volatile alkylphthalide ligustilide (14). Its IR (3400, 1760, 1665 cm-1) and UV [283 nm (e, 6300), 296 nm (r, 6100)

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323 nm (c, 7300)] spectra were virtually the same as those of 14 [IR, 1760, l670 cm-1; UV 283 nm (c, 8100), 296 nm (c, 7600), 324 nm (r, 9500)].10) The 1H-NMR chemical shifts of the C-6 and C-7 olefinic protons were observed at 6 6.06 (dt, J =9.8, 3.9 Hz) and 6.29 (dt, J =9 .8, 2.0 Hz). The hydroxyl group was shown to be attached at C-9, by decoupling of the signals of the hydroxymethine proton at 6 4.74 (dt, J= 8.3, 6.4 Hz) and the C-8 olefinic proton at 6 5.23 (d, J= 8.3 Hz). Thus senkyunolide-F (9) was shown to be 9-hydroxvli2ustilide. It is suscentible to autoxidation and during storage in the presence of air it changed gradually into senkyunolide-E (8).
Senkyunolide-G (10), Ci2H1603, was a monohydroxy derivative of senkyunolide A (16).
Its UV [281 nm (r, 3200)], and 1H-NMR [6 5.97 (1H, dt, J= 9.8, 3.0 Hz), 6.17 (1H, br d, J =9.8 Hz)] spectra were similar to those of senkyunolide-A (16)

, as found with 7.
Hydroxymethine signals were absent in the 1H-NMR spectrum. The MS showed cleavage of the butyl group to give a base peak at m/z 151. Treatment of 10 with p-toluenesulfonic acid

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afforded 14, while on heating of 14 in dilute sulfuric acid, formation of 10 was observed. From
these results senkyunolide G (10) was shown to be 3-hydroxysenkyunolide-A.
Senkyunolide-H and -I (11 and 12),12) CH -12-16 - 4, were an isomeric pair of dihydroxyph-
thalides in which 12 was the major isomer. Both of them showed the signals due to the
butylidene side chain in 1H-NMR spectra [(5 0.95 (3H, t, J=7.3 Hz), 1.50 (2H, sext,
J=7.3 Hz), 2.36 (2H, dt, J=7.8, 7.3 Hz). They have a glycol group in the cyclohexene ring
and their vicinal hydroxymethine protons appeared at ƒÂ 4.05 (ddd, J= 7.8, 3.9, 2.4 Hz) and
4.62 (d, J= 3.9 Hz) in 11 and at 3.95 (ddd, J=9.9, 6.4, 3.4 Hz) and 4.50 (d, J=6.4 Hz) in 12.
The MS of 11 and 12 showed the base peak at m/z 180 (C10F112O3), due to retro-Diels-Alder
cleavage at the cyclohexene ring and established the position of the glycol group at C-6 and C-
7. Glycolation of ligustilide (14) with m-chloroperbenzoic acid in methylene chloride followed
by hydrolysis afforded compounds 11 and 12, of which 12 was the major product. The
preferential formation of the E-isomer in the ordinary hydrolysis of epoxides indicates that
the major product 12 is the E-isomer and the minor product 11 is the Z-isomer. The ratio of
11 and 12 obtained was about 1: 6 which is similar to the ratio of 11 and 12 (1 : 5) isolated
from C. officinale.

Senkyunolide-J (13), C12H1804, [7]D-11 (CHCl3), was also found to be a glycol derivative of senkyunolide-A (16). It showed the C-3 methine proton at 64.86 (dd, J= 7.3, 2.9 Hz) and two hydroxymethine protons of the glycol group at 63.95 (ddd, J= 8.8, 5.9, 2.9 Hz) and 4.41 (br d, J= 5.9 Hz). The retro-Diels-Alder cleavage ion was observed at m/z 182 (C10H14O3). Glycolation of 16 by the same procedure as above afforded 13. Consequently, the structure of senkyunolide-J (13) was established as 6, 7-E-dihydroxy-senkyunolide-A, though the absolute configuration is unknown.
Thus, nine hydroxylated phthalides were isolated from the commercial C. officinale rhizome. The geometry of the butylidene side chain in compounds 5, 6, 8, 9, 11, and 12 was shown to be Z. This was indicated by the chemical shifts of the C-8 olefinic protons and from the formation of trace amounts of less stable E-isomers on storage of the purified samples.

Fig. 1

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Banerjee et al. showed that the chemical shift of the olefinic proton at C-8 of the E-isomer of 14 was shifted 0.51 ppm downfield from that of the stable Z-isomer.13) In the present study, the downfield shifts observed in the unstable E-isomers were 0.44 ppm in 9, 0.52 ppm in 11, and 12, and 0.25 ppm in 6 and 8. In contrast, compound 5 was stable and no isomerization product was found.
Except for 13, all the hydroxyphthalides isolated were optically inactive and compounds 7 to 12 were supposed to be racemic mixtures. Hydroxyphthalides obtained in the present study from the commercial material (2 kg) amounted to 1.95 g which is about one sixth of the total phthalide fraction. Of these, the major products were senkyunolide-F (9) and senkyunolide-I (12). Since the fresh C. officinale rhizome did not contain any of these hydroxylated phthalides (Experimental), they were supposed to be derived from the major phthalides ligustilide (14) or senkyunolide-A (16) during storage. Thus fresh and stored samples of this crude drug show marked differences in the contents of major constituent phthalides, ferulic acid and coniferylferulate. The effects of this on the quality and pharmacological evaluation are unknown, and are currently under investigation.

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on
a JASCO DIP-4 digital polarimeter. 1H-N MR spectra were determined on a JEOL FX 200 spectrometer at 200 MHz in CDCl3 solution with tetramethylsilane as an internal standard. MS were determined on JEOL JMS D-300 (EI-MS) and JEOL JMS 01SG-2 (FD-MS) spectrometers. IR spectra were taken on a JASCO A-102 spectrometer. Column chromatography was carried out by the flash chromatography method.14)
Fractionation of C. officinale Extract Commercial rhizomes of C. officinale, cultivated in Kitami, Hokkaido, were used. The dried and pulverized material (2 kg) was extracted thoroghly with hexane and ether, then with MeOH until the extract became colorless. The MeOH extract (70 g) was partitioned with a mixture of CHCl3-MeOH-H2O (8 : 4 3, 21) and the CHCl3 extract (15 g) was combined with the hexane and ether extract (72 g). It was partitioned with a mixture of hexane-MeOH-H2O (20 : 10 : 2, 21) and the upper layer and lower layer were separated. The upper layer was washed with the lower layer of the same solvent mixture as above. Similarly, the lower layer of the extract was washed with the upper layer of the solvent mixture. Evaporation of the combined two upper extracts gave 41 g of non-polar extract containing mainly glycerides. Also, evaporation of the combined two lower layer extracts gave 44 g of more polar components containing phthalides and polar lipids. A portion of this (21 g) was charged on a column of silica gel (500 g) and eluted with mixtures of hexane-ethyl acetate (1 : 4, frs. 1-5), hexane-ethyl acetate (3.5 : 6.5, frs. 6-14), and 5% MeOH in hexane-ethyl acetate (3.5 : 6.5, frs. 15-19). The eluates (each 500 ml) were collected. Fractions 2-4 (10.5 g) were composed predominantly of volatile alkylphthalides with smaller amounts of fatty acids.

Isolation of Compounds 4-7 and 10 Fractions 5-8 were combined (500 mg) and separated into subtractions
1, 2, and 3 on a column of silica gel by eluting with 2% MeOH in CHCl3. Subfraction 1 contained vanillin and
compounds 5 and 7. Chromatography of the mixture with 5% MeOH in CHCl3 gave 5 (60 mg) and a mixture containing vanillin and 7. A portion of the mixture (20 mg) was submitted to preparative thin layer chromatography
(TLC) with 2% MeOH in CHCl3, giving vanillin and 14.3 mg of 7. Vanillin was identified by comparison with an
authentic sample (TLC and MS). Chromatography of subfraction 2 (220 mg) with 15% MeOH in CHCl3 gave
compounds 6 (40 mg) and 10 (160 mg). Subfraction 3 (130 mg) contained mostly 4 and it was purified by
chromatography with 15% ether in CHCl3.
4: Colorless oil. [ƒ¿]D+ 180•‹(c = 1.1, CHCl3). (ƒÂ): 5.26 (1H, brd, J= 10.3 Hz, 1-H.), 5.47 (1H, brd,
J= 17.1 Hz, 1-H,), 5.94 (1H, ddd, J= 17.1, 10.3, 5.4 Hz, 2-H), 4.94 (1H, d, J= 5.4 Hz, 3-H), 5.21 (1H, d, 8.3 Hz, 8- H), 5.51 (1H, dd, J=8.3, 10.3 Hz, 9-H), 5.62 (1H, dt, J= 10.3, 7.3 Hz, 10-H), 0.88 (3H, t, J-= 7.3 Hz, 17-H3).
5: mp 150-153•Ž. [ƒ¿]D0•‹(c=0.22, CHCl3). 1H-NMR (ƒÂ): 0.99 (3H, t, J= 7.3 Hz, 11-H3), 1.57 (2H, sext,
J=7.3 Hz, 10-H2), 2.47 (2H, dt, J =7 .8, 7.3 Hz, 9-H2), 5.96 (1H, t, J=7.8 Hz, 8-H), 7.05 (1H, d, J=7.8 Hz), 7.34 (1H,
t, J= 7.8 Hz), 7.50 (1H, d, J= 7.8 Hz). UV ƒÉETOHmaxnm (e): 223 (12900), 256 (6700), 330 (4200). IR vNujolmax cm-1: 3200,
1730, 1680, 1605, 1495. MS m/z (%): 204 (24, M +), 175 (100), 162 (25), 147 (27), 134 (7), 119 (11), 91 (13), 65 (8).
High-resolution MS: Found 204.0791. Calcd for C,2F1,203)= 204.0786.
6: mp 95-100•Ž. MD (c = 0.67, CHCl3). 1H-NMR (ƒÂ): 0.98 (3H, t, J= 7.3 Hz, 11-H3), 1.54 (2H, sext,
J= 7.3 Hz, 10-H2), 2.43 (2H, dt, J=7.8, 7.3 Hz, 9-H2), 5.59 (1H, t, J= 7.8 Hz), 7.00 (1H, dd, J= 8.3, 2.4 Hz), 7.05 (1H,
d, J=2.4 Hz), 7.76 (1H, d, J= 8.3 Hz). UV ƒÉEtOHmax nm (ƒÃ): 228 (9100), 256 (19300). IR vNujolmaxcm-1 3250, 1730, 1680,
1605, 1590. MS m/z (%): 204 (24, M +), 175 (100), 162 (73), 147 (43), 119 (21), 91 (16). High resolution MS: Found
204.0773. Calcd for C12H12O3(M +)= 204.0785.

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7: Colorless oil. [ƒ¿], 0•‹ (c= 1.43, CHCl3). 1H-NMR (ƒÂ): 0.92 (3H, t, J = 7.3 Hz, 11-H3), 1.67 (2H, sext, J = 7 .3 Hz,
10-H2), 2.28 (1H, dt, J= 18.1, 7.3 Hz, 9-H.), 2.65 (1H, dt, J= 18.1, 6.8 Hz, 9-Hb), 6.09 (1H, dt, J=9.8, 4.0 Hz, 6-H),
6.27 (1H, dt, J = 9 . 8 , 2.0 Hz, 7-H). UV ƒÉEtOHmaxnm (ƒÃ): 287 (2600), 326 (2250, shoulder). IR vEtOHmaxcm-1: 3250, 1765, 1725,
1680, 1600, 1575. MS m/z (%): 222 (1.3, -Mt), 204 (1.6), .134 (100), 106 (42), 79 (38), 77 (34), 71 (73). High-resolution
MS: Found 222.0872. Calcd for C12FH404 (M +)= 222.0890.
10: Colorless oil. [ƒ¿], 0 (c= 1.25, CHCl3). 11-1-NMR (6): 0.90 (3H, t, J = 6.8 Hz, 11-H3), 5.97 (1H, dt, J =9 .8 ,
3.0 Hz, 6-H), 6.17 (1H, brd, J = 9 .8 Hz, 7-H). UV ƒÉEtOHmaxnm (E): 281 (3200). IR vEtOHmaxcm-1: 3350, 1760 (shoulder),
1735, 1600. MS m/z (%): 208 (3, M), 190 (7), 180 (11), 161 (5), 151 (100), 133 (4), 124 (26), 123 (74), 105 (14), 79 (42),
77 (33). High resolution MS: Found 208.1102. Calcd for C12H1603 (M)= 208.1101:
Isolation of Compounds 1-3, 8 and 9 Fractions 9-14 (1.98 g) were combined. Chromatography of the
mixture with 2% MeOH in CHCl3 gave 2 (860 mg), a mixture (500 mg) containing compounds 1, 8 and 9, and 3
(500 mg), in order of elution. The mixture was separated on a silica gel column with 40% ethyl acetate in hexane to
give 1 (19.6 mg), 8 (21.1 mg), and 9 (320 mg).
1: mp 187-188C. H-NMR (6): 0.63 (3H, s, 18-H3), 1.01 (3H, s, 19-H3), 2.13 (3H, s, 21-H3), 5.35 (1H, m, 6-H),
3.50 (1H, m, 3a-H). MS m z (%): 316 (42, M t), 298 (34), 283 (29), 273 (4), 255 (12), 231 (32), 213 (20). IR cm -1:
1680, 950, 795.
2: Colorless oil. [ƒ¿]D 0•‹ (c = 2.27, CHCl3). 11-I-NMR (6): 6.84-6.94 (4H, m), 7.03 (1H, d, J = 1.5 Hz), 7.08
(1H, dd, J = 8 .3, 1.5 Hz), 6.64 (1H, d, J = 15 .6 Hz), 6.21 (1H, dt, J = 15 .6, 6.4 Hz), 4.84 (2H, br d, J = 6 .4 Hz), 3.90 (3H, s), 3.92 (3H, s), 6.33 (1H, d, J= 15.6 Hz), 7.66 (1H, d, J= 15.6 Hz). UV >IT= nm (c): 272 (11900), 298 (12800).
320 (13300). IRVUmjolmax cm -1: 3330, 1680, 1615, 1580, 1505. MS m/z (%): 356 (14, M t), 194 (92), 179 (25), 177 (100),
162 (30), 145 (19), 131 .(67). High-resolution MS: Found 356.1284. Calcd for C20H2006 (M)= 356.1261.
3: mp 169-171 C. MS m/z (%): 194 (M +).
8: Colorless oil. [a], 0 (c= 1.1, CHCl3). 1H-NMR (6): 1.00 (3H, t, J=7.3 Hz, 11-H3), 4.87 (1H, dt, J=8.8,
6.8 Hz, 9-H), 5.66 (1H, d, J = 8.8 Hz, 8-H), 7.52-7.76 (3H, m), 7.91 (1H, d, J =7 .3 Hz). UV 21 nm (E): 216 (9500),
235 (10000), 260 (10500), 270 (8600), 306 (3900). IR vNujolmax cm-1: 3400, 1780, 1685, 1610. MS m/z (%): 204 (10, M t),
186 (5), 175 (100), 147 (90), 133 (11), 129 (23), 115 (5), 105 (18), 95 (26). High-resolution MS: Found 204.0809. Calcd
for Cl2H12O3 (M -4-)= 204.0787.
9: Colorless oil. [a], 0 (c = 1.89, CHCl3). 11-1-NMR (6): 0.97 (3H, t, J = 7 .3 Hz, 11-H3), 4.74 (1H, dt, J = 8 .3 ,
6.4 Hz, 9-H), 5.23 (1H, d, J=8.3 Hz, 8-H), 6.06 (1H, dt, J=9.8, 3.9 Hz, 6-H), 6.29 (1H, dt, J=9.8, 2.0 Hz, 7-H). UV
λ

EtOH

max nm (s): 283 (6300), 296 (6100), 323 (7300). IR vNujolmaxr= cm-1: 3400, 1760, 1665, 1620. MS m/z (X): 206 (18, M),
188 (9.6), 177 (100), 150 (62), 147 (16), 132 (28), 149 (73). High-resolution MS: Found 206.0928. Calcd for C12H14O3
(M +)= 206.0943.
Isolation of Compounds 11-13 Fractions 15-17 (920 mg) contained a mixture of 11 and 12.
Chromatography of the mixture with 6% MeOH in CHCl3 gave 11 (45.3 mg) and 12 (239 mg). Purification of fraction
18 by column chromatography with 10% MeOH in hexane-ethyl acetate (1 : 1), 10% MeOH in hexane-ether (1 : 1)
and 8% MeOH in hexane-CHCl3 (1 : 1) gave 13 (50 mg).
11: Colorless oil. [ƒ¿D 0•‹(c = 1.66, CHCl3). 1H-NMR (ƒÂ): 0.95 (3H, t, J= 7.3 Hz, 11-H3), 1.50 (2H, sext,
J= 7.3 Hz, 10-H2), 2.36 (2H, dt, J= 7.8, 7.3 Hz, 9-H2), 5.31 (1H, t, J= 7.8 Hz, 8-H), 4.05 (1H, ddd, J= 7.8, 3.9, 2.4 Hz,
6-H), 4.62 (1H, d, J== 3.9 Hz, 7-H). UV iliE2,11 nm (s): 274 (12100). IR vNujolmaxcm-1: 3400, 1755, 1675, 1635. MS m/: (%):
224 (27, M t), 206 (4), 180 (100), 165 (17), 151 (42), 138 (14), 123 (12), 95 (18), 55 (42). High-resolution MS: Found
224.1067. Calcd for C12H16O4 (M) = 224.1049.
12: Colorless oil. [a],)0 (c=0.79, CHCl3). H-NMR (6): 0.95 (3H, t, J= 7.3 Hz, 11-H3), 1.50 (2H, sext,
J =7 .3 Hz, 10-H2), 2.36 (2H, dt, J = 7 .8, 7.3 Hz, 9-H2), 5.29 (1H, t, J =7 .8 Hz, 8-H), 3.95 (1H, ddd, J = 9 .9, 6.4, 3.4 Hz,
6-H), 4.50 (1H, br d, J = 6.4 Hz, 7-H). UV nm (s): 273 (15200). IR vNujolmaxcm-1: 3400, 1740, 1665, 1625. MS m/z
(%): 224 (26, M t), 206 (3), 180 (100), 165 (17), 151 (41), 138 (15), 123 (12), 95 (20), 55 (38). High-resolution MS:
Found 224.1033. Calcd for C, (M+)= 224.1049.
13: Colorless oil. [ƒ¿]D - 11•‹ (c= 1.45, CHCl3). 1H-NMR (6): 0.91 (3H, t, J= 6.8 Hz, 11-H3), 4.86 (1H, dd, J= 7.3,
2.9 Hz, 3-H), 3.95 (1H, ddd, J= 8.8, 5.9, 2.9 Hz, 6-H), 4.41 (1H, br d, J= 5.9 Hz, 7-H). UV ƒÉNujolmax nm (ƒÃ): 214 (9000). IR

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max cm-13360, 1730, 1670. MS m/z (%): 226 (1, M t), 208 (1.5), 182 (79), 139 (50), 126 (100). High-resolution MS:
Found 226.1205. Calcd for C12H18O4 (M)= 226.1205.
Glycolation of Ligustilide (14) and Senkyunolide-A (16) (a) A solution of ligustilide (14, 200 mg) in 5 ml of methylene chloride was treated with 100 mg of m-chloroperbenzoic acid and the mixture was refluxed for 2 min. After cooling, the mixture was washed with saturated NaHCO3 soln., H2O, and saturated NaCI soln. The solvent was evaporated off and the residue was dissolved in a mixture of tetrahydrofuran—H2O (6 : 1, 7 ml). Addition of a drop of 78 perchloric acid caused facile hydrolysis of the oxide and the mixture was diluted with CHCl3 and washed with saturated NaHCO3 soln, H2O, and saturated NaCI soln. Evaporation of the solvent gave a mixture containing an isomeric mixture of diols. It was separated by chromatography as described for the separation of 11 and 12. The major product (132 mg) and the less polar minor product (22 mg) were identified as 12 and 11, respectively, by TLC (8% MeOH in CHCl3 and 10% MeOH in hexane—CHCl3 (1: 1)) and MS comparisons with authentic samples. (b) Senkyunolide-A (16, 40 mg) was treated in exactly the same manner as described above. The product obtained

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was virtually a single diol. It was purified by the same procedure as described for 13 and identified by TLC examination as described in (a) and by IR spectral comparison with natural 13.
General Procedure for the Small Scale Extraction and Separation of C. officinale Samples -Dried and pulverized rhizome C. officinale (30 g) was stirred for 30 min in a mixture of CHCl (80 ml), MeOH (40 ml) ad H2O (30 ml) and the extract was separated by suction filtration. The upper layer gave 6.3 g of H2O-soluble compounds after evaporation of the solvent. Similarly, the lower layer gave 1.25 g of total lipids. It was stirred in a mixture of hexane (50 ml), MeOH (25 ml) and H2O (5 ml) for 10 min. The upper layer gave triglycerides and other less polar lipids (0.79 g). The lower layer gave 0.48 g of polar lipid fraction. It was charged on a column of silica gel (Wako gel C-300, 2.2•~ 13.5 cm) and eluted with ethyl acetate-hexane (1 : 9, 300 ml), ethyl acetate-hexane (1 : 3, 400 ml), MeOH-CHCl3 (1 : 9, 150 ml) and MeOH (100 ml), and the fractions (16 ml each) were collected. The components eluted were as follows: non-polar alkylphthalides (frs. 2-14). 4-7 and 10 (frs. 22-30). 1 (frs. 31-34). 8 (frs. 36-40). 9 (frs. 40-46). 2 (frs. 48-55). 3 (frs. 52-56). 11-13 (frs. 56-58). This procedure was also applied to a fresh C. officinale sample collected in Kitami, Hokkaido. The material (100 g) was homogenized directly in the solvent mixture (CHCl3- MeOH-H2O (8 : 4 : 3, 150 ml)) and the extract was submitted to the same procedure as described above. Comparison of the column chromatographic fractions from fresh and commercial samples showed virtually the same elution pattern except that the amounts of ferulic acid (3) and hydroxyphthalides (5-13) obtained from the fresh material were negligible.

References and Notes

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