[Chem. Pharm. Bull. 35(4)1427—1433(1987)]

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

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(Received October 6, 1986)

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 -I (**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 activities.[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 °C, $C_{21}H_{32}O_2$, 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 [^{1}H-NMR, δ 0.63 (3H, s), 1.01 (3H, s), 2.13 (3H, s), 5.35 (1H, m),

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 C_{21} steroid in Umbelliferae plants.[4] Although C_{21} 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**, $C_{20}H_{20}O_6$, 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 ^{1}H-NMR [δ 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, J=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 authentic 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 °C 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 methylconiferyl ether because the feruloyl moiety serves as an excellent leaving group in **2**. Heating of **2** in a mixture of dioxane–H_2O (2:1) at 80 °C 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 ^{1}H-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**),[10] and senkyunolide (**16**).[10] To simplify the nomenclature of these new compounds, senkyunolide (**16**) previously isolated by Yamagishi and Kaneshima[10] 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 °C, $C_{12}H_{12}O_3$, was a monohydroxy derivative of butylidenephthalide (**15**) as indicated by the IR (3250, 1730, 1680, 1605 cm^{-1}) and ^{1}H-NMR [butylidene side chain: δ 0.99 (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 (1H, t, J=7.8 Hz)] spectra. Its MS showed the loss of ethyl (m/z 175) and propylene (m/z 162) moieties, as observed in **15**.[10] The signals due to three aromatic protons were found at δ 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 (δ 5.96) was sifted 0.33 ppm to lower field than that of **15** (δ 5.63) and indicated the presence

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 δ 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 δ 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 misinterpretation of the NOE.[1b]

Senkyunolide-C (**6**), mp 95—100 °C, $C_{12}H_{12}O_3$, was also a monohydroxy derivative of **15** as indicated by the IR (3250, 1730, 1680, 1605, 1590 cm^{-1}) and ^{1}H-NMR [δ 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 δ 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 δ 5.59 and the aromatic proton at δ 7.05 established the structure of **6** as 5-hydroxybutylidenephthalide. This compound was independently isolated from the Umbelliferae plant *Ligusticum wallichii*.[11]

Senkyunolide-D (**7**), $C_{12}H_{14}O_4$, 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 (ε, 2600)] and ^{1}H-NMR [δ 6.09 (1H, 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 (ε, 3100); ^{1}H-NMR δ 5.9 (m), 6.19 (br d, J=9 Hz)].[10] Hence the carbonyl group is located at the side chain. In the ^{1}H-NMR, the signals due to protons adjacent to a hydroxyl group were absent. Two double triplet signals at δ 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**), $C_{12}H_{12}O_3$, was a monohydroxy derivative of butylidenephthalide (**15**) like **5** and **6**. Its IR (3400, 1780, 1685, 1610 cm^{-1}), UV [306 nm (ε, 3900), 270 nm (ε, 8600)] and ^{1}H-NMR [four aromatic protons at δ 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 δ 4.87 (dt, J=8.8, 6.8 Hz) was found by a decoupling experiment to be coupled with the C-8 olefinic proton at δ 5.66 (d, J=8.8 Hz). Consequently, senkyunolide-E (**8**) was shown to be 9-hydroxybutylidenephthalide.

Senkyunolide-F (**9**), $C_{12}H_{14}O_3$, was the major component of the hydroxyphthalides found in *C. officinale*. It was a monohydroxy derivative of the major volatile alkylphthalide ligustilide (**14**). Its IR (3400, 1760, 1665 cm^{-1}) and UV [283 nm (ε, 6300), 296 nm (ε, 6100), 323 nm (ε, 7300)] spectra were virtually the same as those of **14** [IR, 1760, 1670 cm^{-1}; UV, 283 nm (ε, 8100), 296 nm (ε, 7600), 324 nm (ε, 9500)].[10] The ^{1}H-NMR chemical shifts of the C-6 and C-7 olefinic protons were observed at δ 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 δ 4.74 (dt, J=8.3, 6.4 Hz) and the C-8 olefinic proton at δ 5.23 (d, J=8.3 Hz). Thus senkyunolide-F (**9**) was shown to be 9-hydroxyligustilide. It is susceptible to autoxidation and during storage in the presence of air it changed gradually into senkyunolide-E (**8**).

Senkyunolide-G (**10**), $C_{12}H_{16}O_3$, was a monohydroxy derivative of senkyunolide A (**16**). Its UV [281 nm (ε, 3200)], and ^{1}H-NMR [δ 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 ^{1}H-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

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] $C_{12}H_{16}O_4$, were an isomeric pair of dihydroxyphthalides in which **12** was the major isomer. Both of them showed the signals due to the butylidene side chain in ^{1}H-NMR spectra [δ 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 δ 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 ($C_{10}H_{12}O_3$), 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**), $C_{12}H_{18}O_4$, $[\alpha]_D$-11° ($CHCl_3$), was also found to be a glycol derivative of senkyunolide-A (**16**). It showed the C-3 methine proton at δ 4.86 (dd, J=7.3, 2.9 Hz) and two hydroxymethine protons of the glycol group at δ 3.95 (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 ($C_{10}H_{14}O_3$). Glycolation of **16** by the same procedure as above afforded **13**. Consequently, the structure of senkyunolide-J (**13**) was established as 6,7-*E*-dihydroxysenkyunolide-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.

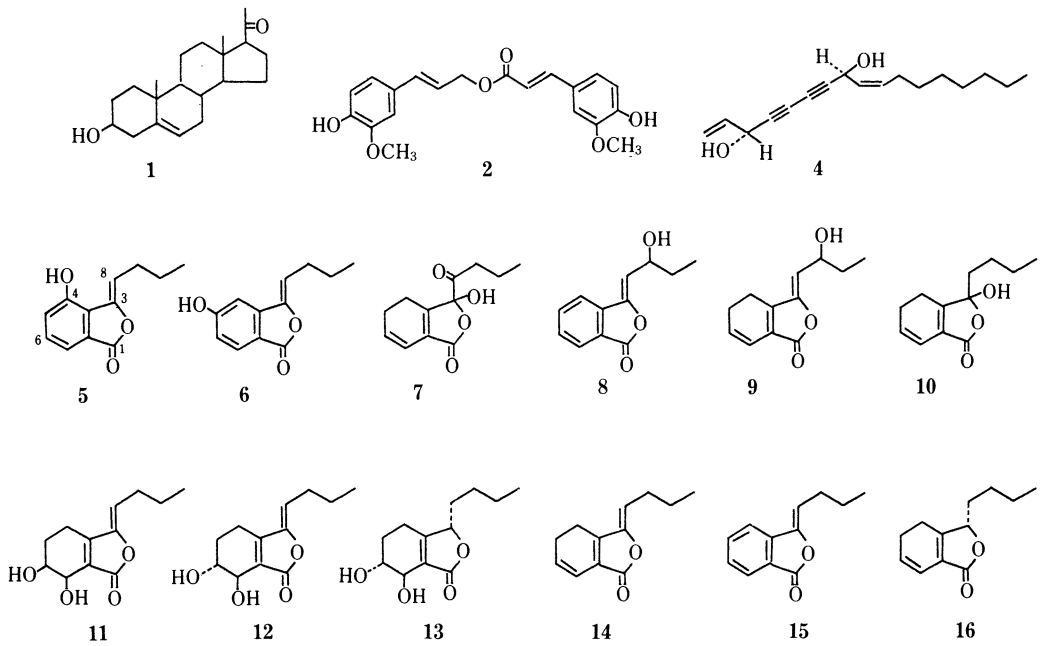

Fig. 1

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. ^{1}H-NMR spectra were determined on a JEOL FX 200 spectrometer at 200 MHz in $CDCl_3$ 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 thoroughly with hexane and ether, then with MeOH until the extract became colorless. The MeOH extract (70 g) was partitioned with a mixture of $CHCl_3$–MeOH–H_2O (8 : 4 : 3, 2 l) and the $CHCl_3$ extract (15 g) was combined with the hexane and ether extract (72 g). It was partitioned with a mixture of hexane–MeOH–H_2O (20 : 10 : 2, 2 l) 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 subfractions 1, 2, and 3 on a column of silica gel by eluting with 2% MeOH in $CHCl_3$. Subfraction 1 contained vanillin and compounds **5** and **7**. Chromatography of the mixture with 5% MeOH in $CHCl_3$ 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 $CHCl_3$, 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 $CHCl_3$ 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 $CHCl_3$.

4: Colorless oil. $[\alpha]_D$ +180° (c=1.1, $CHCl_3$). ^{1}H-NMR (δ): 5.26 (1H, br d, J=10.3 Hz, 1-H_a), 5.47 (1H, br d, J=17.1 Hz, 1-H_b), 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, J=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-H_3).

5: mp 150—153 °C. $[\alpha]_D$ 0° (c=0.22, $CHCl_3$). ^{1}H-NMR (δ): 0.99 (3H, t, J=7.3 Hz, 11-H_3), 1.57 (2H, sext, J=7.3 Hz, 10-H_2), 2.47 (2H, dt, J=7.8, 7.3 Hz, 9-H_2), 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 λ_{max}^{EtOH} nm (ε): 223 (12900), 256 (6700), 330 (4200). IR ν_{max}^{Nujol} 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 $C_{12}H_{12}O_3$ (M^+)=204.0786.

6: mp 95—100 °C. $[\alpha]_D$ 0° (c=0.67, $CHCl_3$). ^{1}H-NMR (δ): 0.98 (3H, t, J=7.3 Hz, 11-H_3), 1.54 (2H, sext, J=7.3 Hz, 10-H_2), 2.43 (2H, dt, J=7.8, 7.3 Hz, 9-H_2), 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 λ_{max}^{EtOH} nm (ε): 228 (9100), 256 (19300). IR ν_{max}^{Nujol} cm^{-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 $C_{12}H_{12}O_3$(M^+)=204.0785.

7: Colorless oil. $[\alpha]_D$ 0° ($c=1.43$, $CHCl_3$). ^{1}H-NMR (δ): 0.92 (3H, t, $J=7.3$ Hz, 11-H_3), 1.67 (2H, sext, $J=7.3$ Hz, 10-H_2), 2.28 (1H, dt, $J=18.1$, 7.3 Hz, 9-H_a), 2.65 (1H, dt, $J=18.1$, 6.8 Hz, 9-H_b), 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 λ_{max}^{EtOH} nm (ε): 287 (2600), 326 (2250, shoulder). IR ν_{max}^{Nujol} cm^{-1}: 3250, 1765, 1725, 1680, 1600, 1575. MS *m/z* (%): 222 (1.3, M^+), 204 (1.6), 134 (100), 106 (42), 79 (38), 77 (34), 71 (73). High-resolution MS: Found 222.0872. Calcd for $C_{12}H_{14}O_4$ (M^+) = 222.0890.

10: Colorless oil. $[\alpha]_D$ 0° ($c=1.25$, $CHCl_3$). ^{1}H-NMR (δ): 0.90 (3H, t, $J=6.8$ Hz, 11-H_3), 5.97 (1H, dt, $J=9.8$, 3.0 Hz, 6-H), 6.17 (1H, br d, $J=9.8$ Hz, 7-H). UV λ_{max}^{EtOH} nm (ε): 281 (3200). IR ν_{max}^{Nujol} cm^{-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 $C_{12}H_{16}O_3$ (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 $CHCl_3$ 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—188 °C. ^{1}H-NMR (δ): 0.63 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 2.13 (3H, s, 21-H_3), 5.35 (1H, m, 6-H), 3.50 (1H, m, 3α-H). MS *m/z* (%): 316 (42, M^+), 298 (34), 283 (29), 273 (4), 255 (12), 231 (32), 213 (20). IR ν_{max}^{Nujol} cm^{-1}: 1680, 950, 795.

2: Colorless oil. $[\alpha]_D$ 0° ($c=2.27$, $CHCl_3$). ^{1}H-NMR (δ): 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 λ_{max}^{EtOH} nm (ε): 272 (11900), 298 (12800), 320 (13300). IR ν_{max}^{Nujol} cm^{-1}: 3330, 1680, 1615, 1580, 1505. MS *m/z* (%): 356 (14, M^+), 194 (92), 179 (25), 177 (100), 162 (30), 145 (19), 131 (67). High-resolution MS: Found 356.1284. Calcd for $C_{20}H_{20}O_6$ (M^+) = 356.1261.

3: mp 169—171 °C. MS *m/z* (%): 194 (M^+).

8: Colorless oil. $[\alpha]_D$ 0° ($c=1.1$, $CHCl_3$). ^{1}H-NMR (δ): 1.00 (3H, t, $J=7.3$ Hz, 11-H_3), 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 λ_{max}^{EtOH} nm (ε): 216 (9500), 235 (10000), 260 (10500), 270 (8600), 306 (3900). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1780, 1685, 1610. MS *m/z* (%): 204 (10, M^+), 186 (5), 175 (100), 147 (90), 133 (11), 129 (23), 115 (5), 105 (18), 95 (26). High-resolution MS: Found 204.0809. Calcd for $C_{12}H_{12}O_3$ (M^+) = 204.0787.

9: Colorless oil. $[\alpha]_D$ 0° ($c=1.89$, $CHCl_3$). ^{1}H-NMR (δ): 0.97 (3H, t, $J=7.3$ Hz, 11-H_3), 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_{max}^{EtOH} nm (ε): 283 (6300), 296 (6100), 323 (7300). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1760, 1665, 1620. MS *m/z* (%): 206 (18, M^+), 188 (9.6), 177 (100), 150 (62), 147 (16), 132 (28), 149 (73). High-resolution MS: Found 206.0928. Calcd for $C_{12}H_{14}O_3$ (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 $CHCl_3$ 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–$CHCl_3$ (1 : 1) gave **13** (50 mg).

11: Colorless oil. $[\alpha]_D$ 0° ($c=1.66$, $CHCl_3$). ^{1}H-NMR (δ): 0.95 (3H, t, $J=7.3$ Hz, 11-H_3), 1.50 (2H, sext, $J=7.3$ Hz, 10-H_2), 2.36 (2H, dt, $J=7.8$, 7.3 Hz, 9-H_2), 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 λ_{max}^{EtOH} nm (ε): 274 (12100). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1755, 1675, 1635. MS *m/z* (%): 224 (27, M^+), 206 (4), 180 (100), 165 (17), 151 (42), 138 (14), 123 (12), 95 (18), 55 (42). High-resolution MS: Found 224.1067. Calcd for $C_{12}H_{16}O_4$ (M^+) = 224.1049.

12: Colorless oil. $[\alpha]_D$ 0° ($c=0.79$, $CHCl_3$). ^{1}H-NMR (δ): 0.95 (3H, t, $J=7.3$ Hz, 11-H_3), 1.50 (2H, sext, $J=7.3$ Hz, 10-H_2), 2.36 (2H, dt, $J=7.8$, 7.3 Hz, 9-H_2), 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 λ_{max}^{EtOH} nm (ε): 273 (15200). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1740, 1665, 1625. MS *m/z* (%): 224 (26, M^+), 206 (3), 180 (100), 165 (17), 151 (41), 138 (15), 123 (12), 95 (20), 55 (38). High-resolution MS: Found 224.1033. Calcd for $C_{12}H_{16}O_4$ (M^+) = 224.1049.

13: Colorless oil. $[\alpha]_D$ −11° ($c=1.45$, $CHCl_3$). ^{1}H-NMR (δ): 0.91 (3H, t, $J=6.8$ Hz, 11-H_3), 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 λ_{max}^{EtOH} nm (ε): 214 (9000). IR ν_{max}^{Nujol} cm^{-1}: 3360, 1730, 1670. MS *m/z* (%): 226 (1, M^+), 208 (1.5), 182 (79), 139 (50), 126 (100). High-resolution MS: Found 226.1205. Calcd for $C_{12}H_{18}O_4$ (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 $NaHCO_3$ soln., H_2O, and saturated NaCl soln. The solvent was evaporated off and the residue was dissolved in a mixture of tetrahydrofuran–H_2O (6 : 1, 7 ml). Addition of a drop of 78% perchloric acid caused facile hydrolysis of the oxide and the mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$ soln, H_2O, and saturated NaCl 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 $CHCl_3$ and 10% MeOH in hexane–$CHCl_3$ (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

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 $CHCl_3$ (80 ml), MeOH (40 ml) ad H_2O (30 ml) and the extract was separated by suction filtration. The upper layer gave 6.3 g of H_2O-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 H_2O (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–$CHCl_3$ (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 ($CHCl_3$–MeOH–H_2O (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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