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COMPONENTS OF *CNIDIUM OFFICINALE* MAKINO: OCCURRENCE OF PREGNENOLONE, CONIFERYL FERULATE, AND HYDROXYPHTHALIDES

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The rhizome of *Cnidium officinale* Makino was found to contain pregnenolone (**1**) and coniferyl ferulate (**2**), and also nine hydroxyphthalide derivatives (**4-12**) which were optically inactive except **12**. Most of them were supposed to be derived secondarily from the major volatile alkylphthalides.

KEYWORDS—pregnenolone; coniferyl ferulate; hydroxyphthalide; *Cnidium officinale* Makino; Umbelliferae

The crude drug senkyu, the dried rhizome of *Cnidium officinale* Makino (synonym, *Ligusticum officinale* Kitagawa, Umbelliferae) contains a variety of volatile alkylphthalide derivatives which have been shown to have antifungal and smooth muscle relaxing activities.[1,2] In contrast, its more polar and non-volatile components had been less well characterized. The present work shows our finding that significant amounts of previously unknown components are contained in *C. officinale*.

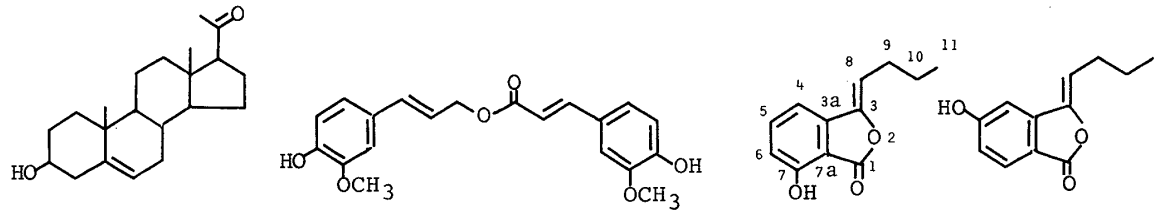
The dried rhizome of *C. officinale* (2 kg) was extracted with hexane, ether and methanol and the combined extracts were partitioned by Folch's method.[3] The lipids obtained were again partitioned with a mixture of hexane-methanol-water (20:10:2) to remove the bulk of triglycerides, which were the major components. The methanol layer contained polar unidentified compounds and most of the known volatile alkylphthalides. It was subjected to repetitive flush chromatography over a column of silica gel and afforded twelve compounds (**1-12**).

Compound **1**, mp 187-188 °C, $C_{21}H_{32}O_2$,[4] was identified with pregnenolone from its mass (MS, *m/z* 316, 298, 283, 273, 255, 231, 213), infrared (IR, 3500, 1680 cm^{-1}) and proton magnetic resonance [^{1}H-NMR, δ 0.63 (3H, s), 1.01 (3H, s), 2.13 (3H, s), 5.35 (1H, m), 3.5 (1H, m)] spectra which were identical with those of the authentic compound. Presence of pregnenolone suggests the simultaneous presence of other C_{21} steroids in *C. officinale* and is of interest in view of the pharmacological activities of senkyu.

Compound **2**, $C_{20}H_{20}O_6$, was obtained in fairly high yield (860 mg). Its IR (3330, 1680, 1615, 1580, 1505 cm^{-1}) and ^{1}H-NMR [δ 3.9 (3H, s), 3.92 (3H, s), 7.66 (1H, d, *J*=15.6Hz), 6.33 (1H, d, *J*=15.6Hz), 6.64 (1H, d, *J*=15.6Hz), 6.21 (1H, dt, *J*=15.6, 6.4Hz), 4.84 (2H, bd, *J*=6.4Hz] spectra were reminiscent of an ester of ferulic acid which is the known component of *C. officinale*. Hydrolysis of **2** in aqueous dioxane at 80°C afforded ferulic acid and coniferyl alcohol which were identified with the authentic samples. Compound **2** was thus shown to be coniferyl ferulate. It is sparingly soluble in water, but by heating **2** in water at 90°C for 30 min, partial hydrolysis was observed.

Compound **3** was identified with vanillin by comparison with the authentic sample.

Compounds **4** to **12** were found to be hydroxy derivatives of the known non-polar alkylphthalides, ligustilide (**13**),[5] butylidenephthalide (**14**),[5] and senkyunolide (**15**).[6]



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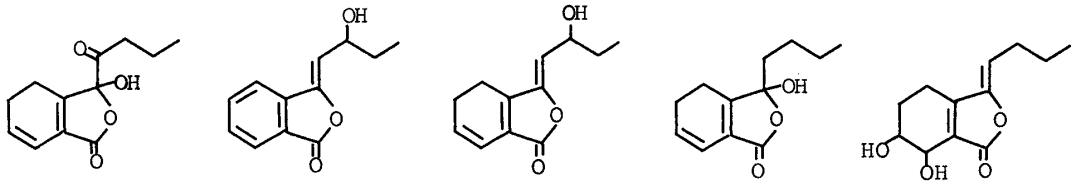
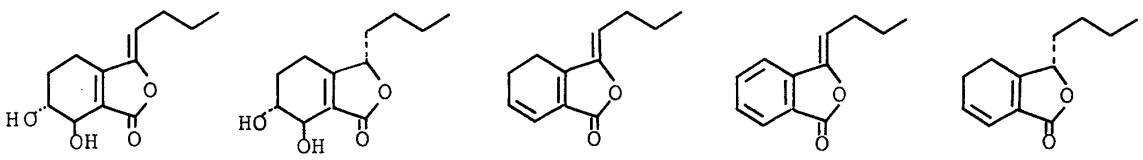


6* 7* 8* 9* 10*



11* 12** 13 14 15

* racemic mixture
** 6,7-trans but the absolute configuration unknown

To simplify the nomenclature of these new compounds, senkyunolide (**15**) isolated by Yamagishi et al.[6] from C. officinale was renamed senkyunolide A and the compounds **4** to **12** were designated as senkyunolide B to J.

Senkyunolide B (**4**), mp 150-153°C, $C_{12}H_{12}O_3$, was a monohydroxy derivative of butylidenephthalide (**14**) as indicated by IR (3250, 1730, 1680, 1605 cm^{-1}) and ^{1}H-NMR [butylidene side chain: δ 0.99 (3H, t, J=7.3Hz), 1.57 (2H, sext, J=7.3Hz), 2.47 (2H, dt, J=7.8, 7.3Hz), 5.96 (1H, t, J=7.8Hz)]spectra. Its mass spectrum showed the loss of ethyl (m/z 175) and propylene (m/z 162) as observed in **14**.[6] The signals due to three aromatic protons were found at δ 7.05 (d, J=7.8Hz), 7.34 (t, J=7.8Hz), and 7.50 (d, J=7.8Hz). Consequently, the hydroxyl group is attached at C-4 or C-7 on the aromatic ring. Presence of nulear Overhauser effect (NOE) between the olefinic proton at δ 5.96 and the doublet at δ 7.05 indicates that senkyunolide B (**4**) is 7-hydroxybutylidenephthalide.

Senkyunolide C (**5**), mp 95-100°C, $C_{12}H_{12}O_3$, was also a monohydroxy derivative of **14** as indicated by IR (3250, 1730, 1680, 1605, 1590 cm^{-1}) and ^{1}H-NMR [δ 0.98 (3H, t, J=7.3Hz), 1.54 (2H, sext, J=7.3Hz), 2.43 (2H, dt, J=7.8, 7.3Hz), 5.59 (1H, t, J=7.8Hz)] spectra. Its mass spectrum showed the strong ions at m/z 175 and 162 as in **4**. Three aromatic proton signals were found at δ 7.0 (dd, J=2, 8.3Hz), 7.05 (d, J=2Hz), 7.76 (d, J=8.3Hz)]. From this coupling pattern it is evident that the hydroxyl group is attached at C-5 or C-6. Presence of NOE between the olefinic proton at δ 5.59 and the aromatic proton at δ 7.05 established the structure of **5** as 5-hydroxybutylidenephthalide .

Senkyunolide D (**6**), $C_{12}H_{14}O_4$, was a monooxo monohydroxyderivative of **15** (IR, 3250, 1765, 1725, 1680 cm^{-1}). Presence of a cross-conjugated diene was shown from the

ultraviolet [UV, 287 nm (ε, 2600)] and ^{1}H-NMR [δ 6.09 (1H, dt, J=9.8, 4Hz), 6.27 (1H, dt, J=9.8, 2Hz)] spectra which were similar to those of **15** [UV, 277 nm (ε, 3100); ^{1}H-NMR, δ 5.9 (m), 6.2 (bd, J=9Hz)]. Hence the carbonyl group is located at the side chain but in ^{1}H-NMR, the signals due to the protons adjacent to hydroxyl group were absent. Two double triplet signals at δ 2.28 (J=18.1, 7.3Hz) and 2.65 (J=18.1, 6.8Hz) due to the protons adjacent to carbonyl group indicated the presence of propyl ketone side chain. This was confirmed by the mass spectrum which showed strong peaks at m/z 151 and 71 due to the cleavage of ketol group. Thus senkyunolide D (**6**) was shown to be 3-hydroxy 8-oxo senkyunolide A.

Senkyunolide E (**7**), $C_{12}H_{12}O_3$, was a monohydroxy derivative of butylidenephthalide (**14**) as **4** and **5**. 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=7.3Hz)] spectra were virtually the same with those of **14**.[6] The side chain bears a hydroxyl group and the hydroxymethine proton at δ 4.87 (dt, J=8.8, 6.8Hz) was found by decoupling experiment to be coupled with the C-8 olefinic proton at δ 5.66 (d, J=8.8Hz). Consequently, senkyunolide E (**7**) was shown to be 9-hydroxybutylidenephthalide.

Senkyunolide F (**8**), $C_{12}H_{14}O_3$, was the major component (400 mg) of the hydroxyphthalides found in C. officinale. It was the monohydroxy derivative of the major volatile alkylphthalide ligustilide (**13**). 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 with those of **13** [IR, 1760, 1670, cm^{-1}); UV, 283 nm (ε, 8100), 296 nm (ε, 7600), 324 nm (ε, 9500)].[6] The ^{1}H-NMR chemical shifts of 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, 2Hz). 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, 6.4Hz) and the C-8 olefinic proton at δ 5.23 (d, J=8.3Hz). Thus senkyunolide F (**8**) was shown to be 9-hydroxyligustilide.

Senkyunolide G (**9**), $C_{12}H_{16}O_3$, was a monohydroxy derivative of senkyunolide A (**15**). Its UV [281 nm (ε, 3200)], and ^{1}H-NMR [δ 5.97 (1H, dt, J=9.8, 3Hz), 6.17 (1H, bd, J=9.8 Hz)] spectra were similar to those of senkyunolide A (**15**) as found in compound **6**. Hydroxymethine signals were absent in ^{1}H-NMR spectrum. The mass spectrum showed the cleavage of butyl group to give base peak at m/z 151. Treatment of **9** with p-toluenesulfonyl chloride afforded **13**, while by heating **13** in dilute sulfuric acid, formation of **9** was observed. From these results senkyunolide G (**9**) was shown to be 3-hydroxysenkyunolide A.

Senkyunolide H and I (**10** and **11**),[7] $C_{12}H_{16}O_4$, were an isomeric pair of dihydroxyphthalides in which **11** 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 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 **10** and at 3.95 (ddd, J=9.9, 6.4, 3.4 Hz) and 4.50 (d, J=6.4 Hz) in **11**. The mass spectra of **10** and **11** showed the base peak at m/z 180 ($C_{10}H_{12}O_3$), which was formed by retro-Diels-Alder cleavage at the cyclohexene ring and established the position of glycol group at C-6 and C-7. Glycolation of ligustilide (**13**) with m-chloroperbenzoic acid in methylenechloride followed by hydrolysis afforded compounds **10** and **11** in which **11** was the major product. The preferential formation of trans-isomer in the ordinary hydrolysis of epoxides indicates that the major product **11** is trans-isomer and the minor product **10** is cis-isomer. The ratio of the products was about 5:1 which was similar to the ratio of

11 and **10** isolated from C. officinale.

Senkyunolide J (**12**), $C_{12}H_{18}O_4$, $[\alpha]_D$ -11° ($CHCl_3$), was also found to be a glycol derivative of senkyunolide A (**15**). It showed C-3 methine proton at δ 4.86 (dd, J=7.3, 3 Hz) and two hydroxymethine protons of glycol group at δ 3.95 (ddd, J=8.8, 5.9, 3 Hz) and 4.41 (bd, J=5.9 Hz). The retro-Diels-Alder cleavage ion was observed at m/z 182 ($C_{10}H_{14}O_3$). Glycolation of **15** in the same procedure as above afforded **12** quantitatively so that the structure of senkyunolide J (**12**) was established as 6,7-trans-dihydroxysenkyunolide A.

Except for senkyunolide J (**12**), all the hydroxyphthalides found in the present work were optically inactive. Senkyunolide D to I (**6** to **11**) were supposed to be racemic mixtures which were derived secondarily from ligustilide (**13**) during the storage.[9] Also, senkyunolid J (**12**) is believed to be derived from senkyunolide A (**15**). Occurrence of such hydroxyphthalides is of interest in view of the pharmacological and pharmacognostic evaluation of senkyu, which is one of the most frequently prescribed crude drugs in Chinese herb medicines.

REFERENCES AND NOTES

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7) Compound **11** was reported by Yamagishi et al. at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1977; T. Yamagishi and S. Homma, Abstract Papers, 97th Meeting, II, p.238, and was designated as senkyunolide I by Dr. Yamagishi's permission. Recently Kaouadji et al., apparently unaware of Yamagishi's work, isolated the same compound from Ligusticum wallichii, and designated it ligustidiol.[8]
8) M. Kaouadji, M. Puech-Baronnat and A. Mariotte, Tetrahedron Letters, 24, 4675 (1983).
9) The geometry of the butylidene side chain in compounds **4**, **5**, **7**, **8**, **10**, and **11** was shown to be Z. This was indicated by the presence of NOE in **4** and **5**, and from the formation of less stable E-isomers on storage of the purified samples. E-Isomers showed chemical shifts of the olefinic proton at C-8 about 0.3 ppm lower field in **8**, **10** and **11**, and about 0.25 ppm lower field in **5** and **7**, than the corresponding stable Z-isomers.[10]
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ADDED IN PROOF (August 23, 1984) Since the submission of this manuscript, an account of the isolation of compound **5** from L. wallichii by Puech-Baronnat et al., has appeared (M. Puech-Baronnat, M. Kaouadji, and A. M. Mariotte, Planta Med., 50, 105 (1984).

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