Studies on the Constituents of Umbelliferae Plants. XVI.\(^1\) Isolation and Structures of Three New Ligustilide Derivatives from *Angelica acutiloba*

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Three new ligustilide derivatives (3–5) were isolated from the crude drug toki, the dried roots of the Umbelliferae plant *Angelica acutiloba*, together with a known ligustilide dimer, levistolide A (2), and four known oxygenated ligustilide derivatives, senkyunolide E (6), senkyunolide F (7), senkyunolide H (8) and senkyunolide I (9). Compound 3 was shown to be the angeloyl ester of 7. Compounds 4 and 5, designated tokinolide A and tokinolide B, are dimeric ligustilide derivatives with Diels–Alder-type (5) and cyclobutane-type (4) cyclization systems, respectively. The occurrence of compounds 2 to 9 in a fresh *A. acutiloba* sample was confirmed.

**Keywords**—tokinolide A; tokinolide B; angeloylsenkyunolide F; phthalide; *Angelica acutiloba*; Umbelliferae

Toki is the dried root of an Umbelliferae plant, *Angelica acutiloba*, which is used quite frequently in the prescriptions of Chinese traditional medicine. Its pharmacological actions are not fully established but in many cases it has been used together with senkyu, the rhizome of another Umbelliferae plant, *Cnidium officinale*, for the treatment of obstetrical and gynecological disorders. Volatile alkylphthalide derivatives are common components of *A. acutiloba* and *C. officinale*, and ligustilide (1) is the predominant compound of the phthalide fraction of the two plants. Recently we reported that prolonged storage of *C. officinale* rhizome causes formation and accumulation of a variety of oxygenated ligustilide derivatives.\(^1\) In view of the importance of toki in traditional Chinese medicine, we subsequently studied its phthalide derivatives.

The crude lipid of *A. acutiloba* was subjected to solvent fractionation (hexane–methanol–water) to remove the bulk of the triglycerides. Repeated flash chromatography of the methanol-soluble fraction afforded compounds 2–9, including three new compounds (3–5). All of them were found to be derivatives of ligustilide (1), but were isolated in small amounts. The content of 1 in *A. acutiloba* is known to be less than one-tenth of that in *C. officinale*,\(^2\) whose large phthalide content is rather exceptional in Umbelliferae plants.

Compound 2, mp 113–115 °C, was an optically inactive dimeric derivative of ligustilide (1). Its mass spectrum (MS) showed the molecular ion (M\(^{+}\)) at \(m/z\) 380 but the remaining ions were virtually the same as those of 1.\(^3\) This is a common feature of the ligustilide dimers reported previously from a variety of Umbelliferae plants.\(^4\) Its proton nuclear magnetic resonance (\(^1\)H-NMR) spectrum showed signals of olefinic protons of two butylidene side chains at \(\delta\) 5.00 (1H, t, \(J=7.8\) Hz) and 5.07 (1H, t, \(J=7.8\) Hz) and the signal of an olefinic proton of an \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone at \(\delta\) 7.35 (1H, d, \(J=6.4\) Hz). These and other spectral properties (Experimental) were identical with those reported for a ligustilide dimer, levistolide A, which was isolated from the Umbelliferae plants *Ligusticum wallichii*\(^{40}\) and *Levisticum*
Compound 3, \([\alpha]_D +26^\circ\), C$_{17}$H$_{20}$O$_4$, was a viscous oil which showed, on a thin-layer chromatography (TLC) plate, a bright blue fluorescence under a 254 nm ultraviolet (UV) lamp. This is a characteristic of the cross-conjugated triunsaturated-\(\gamma\)-lactone system found in 1 and senkyunolide F (7), and the presence of the senkyunolide F moiety was indicated by the \(^1\)H-NMR spectrum. It showed the signals of three olefinic protons at \(\delta 6.29\) (dt, \(J=9.8, 1.8\) Hz, 7-H), \(6.06\) (dt, \(J=9.8, 4.0\) Hz, 6-H), and at \(\delta 5.15\) (d, \(J=8.0\) Hz, 10-H). The olefin proton at \(\delta 5.15\) was found to be coupled with a methine at \(\delta 5.77\) (ddd, \(J=8.0, 7.0, 6.2\) Hz), which is assignable to 11-H. Other signals coincided with those of angelic acid (\(\delta 1.91, 3H,\) quint. \(J=1.5\) Hz; \(\delta 2.00, 3H,\) dq, \(J=7.3, 1.5\) Hz; \(\delta 6.09, qq,\) \(J=7.3, 1.5\) Hz). Compound 3 was thus shown to be 11-angeloylsenkyunolide F. It was quite labile to basic or acidic hydrolysis, giving decomposition products, and the attempted determination of the stereochemistry at C-11 was unsuccessful.

Tokinolide A (4) and tokinolide B (5), C$_{24}$H$_{28}$O$_4$, were ligustilide dimers and were optically inactive. Their mass spectra were quite similar to that of levistolide A (2). They showed the molecular ions at \(m/z\) 380 and other ions at \(m/z\) 190, 161 and 148 which were formed by secondary fragmentation of the ligustilide moieties. Unlike 2, tokinolide A is not the Diels–Alder-type dimer, as indicated by the lack of olefin protons due to the \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone moiety. The \(^1\)H-NMR spectrum showed the signals of the olefinic protons of two butylidene side chains at \(\delta 4.66\) (1H, dd, \(J=8.3, 7.3\) Hz) and 5.21 (1H, t, \(J=7.8\) Hz), and two olefinic protons of an isolated double bond at \(\delta 6.00\) (1H, d, \(J=9.8\) Hz) and 6.11 (1H, dt, \(J=9.8, 4.0\) Hz). These signals and the UV absorption of 4 at 276 nm (\(\varepsilon, 18000\)),
which is similar to that of 6,7-dihydroligustilide (273 nm, ε, 17800), indicated that compound 4 is a dimeric ligustilide forming a cyclobutane ring at C-6 and C-7 of one ligustilide molecule with C-8' and C-9' of the other. The geometries of the two butyldiene side chains were shown to be Z, as exemplified by the high-field chemical shifts of the olefin protons of the enol-γ-lactone (δ 4.66) and conjugated enol-γ-lactone moieties (δ 5.21). These protons on the E-side chain suffer significant deshielding from the vicinal oxygen atoms (O-2, O-2'); the signals appears at δ 5.38 in compound 10 (vide infra), and about δ 5.8 in the E-isomers of 8 and 9. Since tokinolide A is optically inactive, it could be formulated as one of the four racemic pairs shown in Chart 2. Of these, the structures A and B are unreasonable since there would be severe steric repulsion between the two bulky cyclohexene rings. The nuclear Overhauser effect (NOE) differential spectrum showed that there was NOE between the protons at δ 6.11 (6'-H) and 3.23 (1H, d, J=9.3 Hz, 7-H). It indicated the proximity of these two protons and narrowed the choice of structure of 4 to C or D. The non-decoupling carbon-13 NMR (13C-NMR) spectrum indicated that the unconjugated lactonic carbonyl carbon (C-1', 175.5 ppm), which appears at lower field than the conjugated lactonic carbonyl (169.5 ppm), is coupled with one proton with a coupling constant of 4.9 Hz. This is a typical value of the long-range coupling constants between two atoms separated by two bonds, or separated by three bonds with an antiparallel system. The long-range coupling constants of those separated by four bonds are generally negligible or less than 1.5 Hz. Irradiation of 7-H (δ 3.23, d, J=9.3 Hz) had no influence on the 1'-carbon signal, but irradiation of 6-H (δ 2.90, ddd, J=9.3, 6.8, 4.4 Hz) changed the 1'-carbon signal into a sharp singlet indicating the presence of three bonds between them. The structure of tokinolide A was thus shown to be 4, a new type of dimeric ligustilide having a cyclobutane ring.

Tokinolide B (5) is a Diels–Alder-type ligustilide dimer like compound 2, and its 1H-NMR spectrum showed the signal of an olefinic proton of a conjugated lactone group at δ 7.53 (1H, d, J=6.6 Hz). Other 1H-NMR signals were quite similar to those of the known compound angeolide (10), which was isolated from an Umbelliferae plant, A. glauca, by Banerjee et al., and indicated that the butyldiene side chain of one ligustilide molecule is included as the dienophile. The major spectral differences between compounds 5 and 10 were the chemical shifts of the olefin proton at C-10' of the enol-γ-lactone moiety and the methine proton at C-10. The chemical shifts of these signals of 10 were reported to be δ 5.38 (dd, J=9.5, 7.0 Hz, 10'-H) and 2.82 (m, 10-H) respectively. In contrast, tokinolide B showed these signals at δ 4.62 (dd, J=8.8, 6.8 Hz) and 1.66 (m) respectively. This indicates that the side chain of 5 takes the Z-form, unlike that of 10, and the C-10' proton is unaffected by the neighboring lactonic oxygen atom (O-2'). The unusually deshielded nature of 10-H (δ 2.82) of 10 is due to the deshielding effect of the lactonic oxygen (O-2) of the other ligustilide moiety. The marked up-field shift of this signal in tokinolide B shows the absence of this effect on 10-H. These observations indicate that tokinolide B (5) is a C-10 epimer of angeolide (10), and the geometry of its side chain is 3'(10')-Z. From the two-dimensional 1H-NMR spectrum, the signal of one of the methylene protons at C-4 was found to appear at δ 1.30, which is markedly high for an allylic proton. This is due to the influence of the 1' carbonyl group, which a Dreiding model indicated to be sterically very close, including C-4 in its shielding region. From these results, tokinolide B was shown to be the dimeric compound 5, which is composed of two 3(10)-Z ligustilide molecules, in contrast to angeolide (10) which is composed of two 3(10)-E ligustilide molecules. Compounds 6 to 9 were identified as senkyunolide E, senkunolide F, senkyunolide H and senkunolide I, respectively, by direct comparison of their infrared (IR), UV, MS and 1H-NMR data with those of authentic samples previously isolated from C. officinale. Except for 3, the compounds found in the present study were optically inactive and are considered to be racemic mixtures. However, high-performance liquid chromatographic
(HPLC) examination indicated the presence of these compounds in the fresh A. acutiloba extract. Their amounts were mostly less than ten mg in 1 kg of sample but there were no marked differences between the fresh samples and samples which has been dried and kept for two months, except that compounds 6 and 7, whose contents were negligible in the fresh samples, increased to 3—5 mg/kg (6) and 15—30 mg/kg (7) in the stored samples. The contents of angoeloxy senkyunolide F (3) and senkyunolide I (9) were relatively high; 397 mg of 3 and 124 mg of 9 were isolated from 4.5 kg of A. acutiloba. This high content of optically active angoeloxy senkyunolide F (3), compared with other optically inactive hydroxyphthalides (6—9), suggests that the formation process of 3 in A. acutiloba is different from that of 6 to 9. Also, our previous assumption that the hydroxyphthalides are derived entirely by autoxidation during the preparation and storage of the crude drug, might not be correct.

The contents of the ligustilide dimers (2, 4, 5) were found to be almost the same between the fresh and the dried and aged materials, and these compounds were considered to be original components of the plant. This is not unusual, since the hitherto known ligustilide dimers found in Umbelliferae plants are invariably racemic and in some cases are quite abundant. Neither the heating of 1 in benzene, toluene, or xylene nor the prolonged storage of 1 (six months) at room temperature caused the formation of these ligustilide dimers. Most of the ligustilide fraction remained unchanged while some was partly oxidized, giving butylidenephthalide. Only the formation of a small amount of dialdehyde (11) by autoxidation was confirmed when 1 was refluxed in benzene, toluene, or xylene for 3 h.

**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-NMR spectra were determined on a JEOL JNM GX-270 spectrometer at 270 MHz in CDCl3 solution with TMS as an internal standard. MS were determined on a JEOL JMS-D300 (EI-MS) and JEOL JMS-O150G-2 (FD-MS) spectrometers. IR spectra were taken on a JASCO A-102 spectrometer. Column chromatography was carried out by the flash chromatography method. HPLC was carried out using Senshu Pak silica 1151N (150 × 4.6 mm i.d.) with 5% ethyl acetate in hexane.

**Fractionation of A. acutiloba Extract**—Commercial roots of A. acutiloba were used as the source material. The dried and pulverized material (4.5 kg) was extracted thoroughly with hexane–Et2O (2:1) and CHCl3–MeOH (1:1). The CHCl3–MeOH extract (450 g) was partitioned with a mixture of CHCl3–MeOH–H2O (8:4:3) and separated into upper (327 g) and lower (96 g) extracts. The lower extract was combined with the hexane–Et2O extract (58 g), partitioned with a mixture of hexane–MeOH–H2O (20:10:2), and separated into upper (110.6 g) and lower (327 g) extracts. The lower extract was combined with the hexane–Et2O extract (58 g), partitioned with a solvent mixture of hexane–MeOH–H2O (20:10:2), and separated into upper (327 g) and lower (96 g) extracts. The lower extract was submitted to flash chromatography over a column of silica gel and eluted with mixtures of EtOAc–hexane (2:1, fraction 1, 13.2 g), EtOAc–hexane (1:1, fraction 2, 7.0 g) and MeOH–CHCl3 (1:9) and MeOH (fraction 3, 20.2 g). Repeated flash chromatography of fractions 1 and 2 with Et2O–CHCl3 and EtOAc–hexane gave 3 (397 mg), 4 (27 mg) and 5 (31 mg) from fraction 1, and 2 (53 mg), 6 (9 mg) and 7 (35 mg) from fraction 2. The Rf’s of 2—7 on silica gel TLC plate with EtOAc–hexane (3:7) were 0.67 (3), 0.64 (4), 0.59 (5), 0.37 (2), 0.35 (8) and 0.27 (9). Flash chromatography of fraction 3 with 3%, MeOH in CHCl3 afforded 8 (less than 1 mg) and 9 (124 mg). The Rf’s of 8 and 9 on silica gel TLC plate with 5% MeOH in CHCl3 were 0.35 (8) and 0.27 (9).

**Levistolide A (2)—** mp 113—115 °C, [α]D +2.0° (c = 0.4, CHCl3). 1H-NMR δ: 0.92 (6H, t, J = 7.3 Hz), 2.55 (1H, br, J = 8.3, 7.3 Hz), 2.98 (1H, m), 3.25 (1H, d, J = 8.8 Hz), 5.00 (1H, t, J = 7.8 Hz), 5.07 (1H, t, J = 7.8 Hz), 7.35 (1H, d, J = 6.4 Hz). UV λmax nm (ε): 275 (12000), 227 (8000).IR νmax cm−1: 1730, 1630. MS m/z: 288, 231, 205, 189. High-resolution MS: Found C17H20O4 (M+) 288.1361. Calcd for C17H20O4 (M+) 288.1361.

**Angeloxy senkyunolide F (3)—** Oil, [α]D +26° (c = 0.70, CHCl3). 1H-NMR δ: 0.98 (3H, t, J = 7.3 Hz, 13-H3), 2.55 (1H, br, J = 8.3, 7.3 Hz), 5.00 (1H, t, J = 7.8 Hz), 5.07 (1H, t, J = 7.8 Hz), 7.35 (1H, d, J = 6.4 Hz). UV λmax nm (ε): 275 (12000), 227 (8000).IR νmax cm−1: 1730, 1630. MS m/z: 380, 320 (9900), 284 (9000), 270 (10700), 261 (9900), 215 (21700). IR νmax cm−1: 1770, 1715. MS m/z: 288, 231, 205, 189. High-resolution MS: Found 288.1367. Calcd for C24H28O4 (M+): 288.1361.

**Tokinolide B (5)—** Oil, [α]D +20° (c = 0.6, CHCl3). 1H-NMR δ: 0.82 (3H, t, J = 7.3 Hz, 7-H3), 2.55 (1H, br, J = 8.3, 7.3 Hz), 5.00 (1H, t, J = 7.8 Hz), 5.07 (1H, t, J = 7.8 Hz), 7.35 (1H, d, J = 6.4 Hz). UV λmax nm (ε): 275 (12000), 227 (8000).IR νmax cm−1: 1730, 1630. MS m/z: 380, 320 (9900), 284 (9000), 270 (10700), 261 (9900), 215 (21700). IR νmax cm−1: 1770, 1715. MS m/z: 288, 231, 205, 189. High-resolution MS: Found 288.1367. Calcd for C24H28O4 (M+): 288.1361.

**Tokinolide A (4)—** Oil, [α]D +20° (c = 0.6, CHCl3). 1H-NMR δ: 0.80 (6H, t, J = 7.3 Hz), 0.94 (3H, t, J = 7.3 Hz), 2.39 (1H, ddd, J = 6.2, 3.5 Hz, 4-H3), 2.56 (1H, ddd, J = 6.2, 3.5 Hz, 4-H3), 2.90 (1H, ddd, J = 9.3, 6.8, 4.4 Hz, 6-H3), 3.23 (1H, d, J = 9.3 Hz, 7-H3), 4.66 (1H, dd, J = 8.3, 7.3 Hz, 10-H3), 5.21 (1H, t, J = 7.8 Hz, 10-H3), 6.00 (1H, br, d, J = 9.8 Hz, 7-H3), 6.11 (1H, d, J = 9.8 Hz, 7-H3). UV λmax nm (ε): 276 (18000).IR νmax cm−1: 1760, 1700. MS m/z: 380, 191, 190, 161, 148. High-resolution MS: Found 380.996. Calcd for C24H28O4 380.987.
4.62 (1H, dd, J = 8.8, 6.8 Hz, 10'-H), 5.92 (1H, dt, J = 9.5, 4.0 Hz, 6-H), 6.17 (1H, d, J = 6.6 Hz, 7-H), 7.53 (1H, d, J = 6.6 Hz, 7'-H). UV λ<sub>max</sub> nm (ε): 280 (6100). IR ν<sub>NNujol</sub> cm<sup>-1</sup>: 1775, 1755, 1695. MS m/z: 380, 191, 190, 161. High-resolution MS: Found 380.1990. Calcd for C<sub>24</sub>H<sub>28</sub>O<sub>4</sub> 380.1987.

**Senkyunolide E (6)**—Oil, [α]<sub>D</sub>, 0 ºK (c = 0.4, CHCl<sub>3</sub>). 1H-NMR: 1.00 (3H, t, J = 7.1 Hz), 4.87 (1H, dt, J = 8.3, 6.5 Hz), 5.74 (1H, d, J = 8.3 Hz), 7.50-8.00 (4H, m). UV λ<sub>max</sub> nm (ε): 235 (11000), 259 (11000), 268 (9000). IR ν<sub>NNujol</sub> cm<sup>-1</sup>: 3400, 1785, 1685, 1610. MS m/z: 204 (M<sup>+</sup>), 186, 175, 147, 129.

**Senkyunolide F (7)**—Oil, [α]<sub>D</sub>, 0 ºK (c = 0.6, CHCl<sub>3</sub>). 1H-NMR: 0.97 (3H, t, J = 7.3 Hz), 4.74 (1H, dt, J = 8.3, 6.5 Hz), 5.22 (1H, d, J = 8.3 Hz), 6.06 (1H, dt, J = 9.8, 3.9 Hz), 6.30 (1H, d, J = 9.8 Hz). UV λ<sub>max</sub> nm (ε): 270 (4500), 282 (4300), 296 (sh, 4000), 321 (4400). IR ν<sub>NNujol</sub> cm<sup>-1</sup>: 3400, 1770, 1610. MS m/z: 206 (M<sup>+</sup>), 188, 177, 149.

**Senkyunolide H**—Oil. MS m/z: 224 (M<sup>+</sup>), 180, 175, 151.

**Senkyunolide I**—Oil, [α]<sub>D</sub>, 0 ºK (c = 1.1, CHCl<sub>3</sub>). 1H-NMR: 0.95 (3H, t, J = 6.8 Hz), 3.39 (1H, ddd, J = 9.5, 6.5, 3.5 Hz), 4.50 (1H, d, J = 6.5 Hz), 5.29 (1H, t, J = 7.8 Hz). UV λ<sub>max</sub> nm (ε): 274 (14600). IR ν<sub>NNujol</sub> cm<sup>-1</sup>: 3400, 1740, 1685, 1640. MS m/z: 224 (M<sup>+</sup>), 206, 180, 165, 151, 138.

**Pyrolysis of Ligustilide**—A solution of 300 mg of I in 2 ml of xylene was refluxed for 3 h. TLC (ethyl acetate:hexane = 3:17) examination of the mixture showed the formation of small amounts of more polar products which were different from the ligustilide dimers 2, 4, and 5. Heating of I in benzene and toluene gave similar results. The mixture obtained by heating in xylene was worked up as usual and separated over a column of silica gel with ethyl acetate-hexane (3:17). The most polar product (11, 27 mg) was purified. Oil. 1H-NMR: 0.99 (3H, t, J = 7.3 Hz, 13-H<sub>3</sub>), 1.57 (2H, dt, J = 7.8, 7.1 Hz, 12-H<sub>2</sub>), 2.49 (2H, dt, J = 7.8, 7.3 Hz, 11-H<sub>2</sub>), 2.84 (2H, m), 5.99 (1H, t, J = 8.1 Hz, 10-H), 9.80 (1H, brs, 6-H), 10.0 (1H, s, 7-H). MS m/z: 222 (M<sup>+</sup>). IR ν<sub>NNujol</sub> cm<sup>-1</sup>: 2710, 1760, 1720, 1680, 1650, 1580.

**References**