conditions using He as carrier gas at 60 ml/min with a split ratio of 100:1

Esterification. The fatty acid (1 mg) was esterified with 0.5 ml of BF_3–MeOH complex (BF_3; *ca* 14%).

Catalytic hydrogenation. A soln of the fatty acid (1 mg) in MeOH was stirred with 10% Pd-C under H_2 for 1 hr at room temp, filtered and evapd to obtain the sample for GC analysis.

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REFERENCES

1. Metting, B. and Pyne, J. W. (1986) *Enzyme Microb. Technol.* **8**, 386.
2. Ishibashi, M., Moore, R. E., Patterson, G. M. L., Xu, C. and Clardy, J. (1986) *J. Org. Chem.* **51**, 5300.
3. Moore, R. E., Cheuk, C., Yang, X-Q. G., Patterson, G. M. L., Bonjouklian, R., Smitka, T. A., Mynderse, J. S., Foster, R. S., Jones, N. D., Swartzendruber, J. K. and Deeter, J. B. (1987) *J. Org. Chem.* **52**, 1036.
4. Helms, G. L., Moore, R. E., Niemczura, W. P., Patterson, G. M. L., Tomer, K. B. and Gross, M. L. (1988) *J. Org. Chem.* **53**, 1298.
5. Murakami, M., Makabe, K., Yamaguchi, K., Konosu, S. and Wälchli, M. R. (1988) *Tetrahedron Letters* **29**, 1149.
6. Kobayashi, J., Ishibashi, M., Wälchli, M. R., Nakamura, H., Hirata, Y., Sasaki, T. and Ohizumi, Y. (1988) *J. Am. Chem. Soc.* **110**, 490.
7. Nagano, H. and Ikegami, S. (1980) *Seikagaku* **52**, 1208.
8. Kobayashi, A. (1986) *Nippon Nōgeikagaku Kaishi* **60**, 725.
9. Hashimoto, Y. (1979) in *Marine Toxins and Other Bioactive Marine Metabolites* p. 203. Japan. Sci. Soc. Press, Tokyo.
10. Findlay, J. A. and Patil, A. D. (1984) *J. Nat. Prod.* **47**, 815.
11. Gauthier, M. J., Bernard, P. and Aubert, M. (1978) *J. Exp. Mar. Biol. Ecol.* **33**, 37.
12. Kakisawa, H., Asari, F., Kusumi, T., Toma, T., Sakurai, T., Oohusa, T., Hara, Y. and Chihara, M. (1988) *Phytochemistry* **27**, 731.
13. Nishizawa, K. and Chihara, M. (1978) in *Methods in Phycological Studies (Japanese)* p. 754. Kyoritsu Press, Tokyo.

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TWO LACTONIC COMPOUNDS, LANCIFOLIDE AND ISOLANCIFOLIDE, FROM *ACTINODAPHNE LANCIFOLIA*

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Key Word Index—*Actinodaphne lancifolia*; Lauraceae; leaves; wood; C_{15}-lancifolide; lancifolide; isolancifolide; γ-lactone; furan compounds.

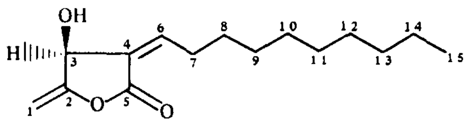
Abstract—Two lactonic compounds, lancifolide and isolancifolide, were isolated from *Actinodaphne lancifolia*. Their structures were elucidated on the basis of spectral and chemical evidence.

INTRODUCTION

Actinodaphne lancifolia (Japanese name 'Kagonoki') is an evergreen tree of the family Lauraceae distributed in the southern part of Japan. So far, there are a few reports [1, 2] on the chemical components of the plant but many terpenes and four furans (sesquirosefuran, longifolin, 5-methyl furfural, and 8-[2'(3'-methyl)furanyl]-2,6-dimethyl-2,6-octadiene-4-one) have been isolated from the essential oil of mesocarps, seeds, roots and leaves. In this paper, we describe the isolation and structural elucidation of two novel lactonic compounds, lancifolide (**1**) and isolancifolide (**2**) from the plant.

RESULTS AND DISCUSSION

Lancifolide (**1**), colourless oil, $C_{15}H_{24}O_3$, exhibited OH (3400 cm^{-1}) and α,β-unsaturated-γ-lactone (1680 and 1780 cm^{-1}) bands in its IR spectrum. From analysis of ^1H NMR and UV spectra, **1** has the same β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone structure as that of obtusilactone (**3**) [3]. The structure of γ-lactone segment was also determined as follows. On selective hydrogenation using $Rh(Ph_3P)_3Cl$ in benzene, **1** afforded a mixture of **4a** and **4b**, which, without further separation, was treated with acetic anhydride in pyridine to yield the unstable compound **5**. On the other hand, when **1** was



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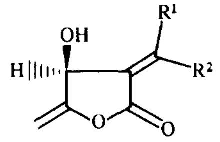
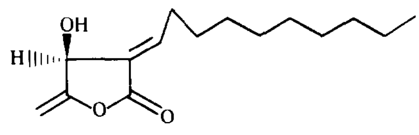


3 R^1 = H, R^2 = CH_2—$(CH_2)_8$—CH═CH_2
7 R^1 = CH_2—$(CH_2)_8$—CH═CH_2, R^2 = H



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hydrogenated over 5% Pd–$BaSO_4$ in ethanol, it gave **6** as the sole product; the stereostructure of **6** remains unsettled. Next, the remaining structure of **1**, except for the β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone skeleton, was clarified from observation of ^{13}C NMR which indicated the presence of a long methylene chain, a *n*-nonyl group consisting of eight methylene groups and one methyl group. The geometry of the trisubstituted double bond conjugated to a lactone carbonyl group is discussed below.

Isolancifolide (**2**) had the same molecular formula ($C_{15}H_{24}O_3$) and also possessed the same β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone structure as that of **1**, as deduced from analysis of the spectral data (UV, IR, ^{1}H and ^{13}C NMR). On hydrogenation with 5% Pd–$BaSO_4$, **2** provided the tetrahydro-derivative which was identical to **6**, also derived from **1**, in all respects (mp, mmp, $[\alpha]_D$, IR, ^{1}H NMR, and chromatographic properties). Hence, **2** is the geometrical isomer of the trisubstituted double bond in **1**. Comparison of the ^{1}H NMR chemical shifts of the olefinic β-protons in **1** (δ 6.69) and **2** (δ 7.09) indicated that **1** had a β-*trans* proton (cisoid enone system) and 2 had a β-*cis* proton (transoid enone system) [4].

Consequently, the structure of lancifolide is represented as formula **1** and isolancifolide as **2**. The lancifolides (**1**, **2** and **6**) are all laevorotatory and hence possess the (3*S*)-configuration, being analogous to the obtusilactones with respect to absolute configuration [5].

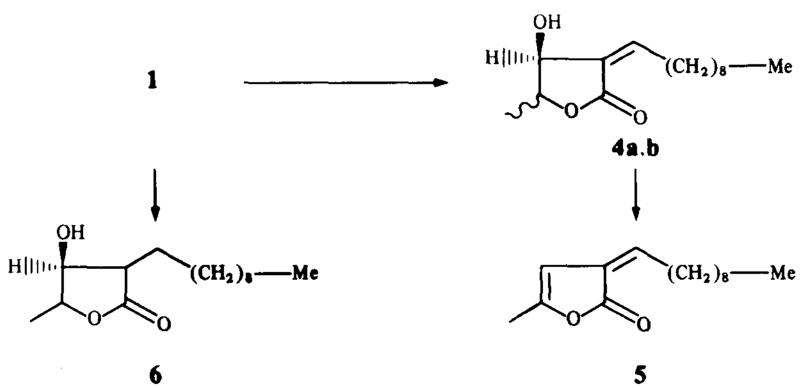
This is the first time that C_{15}-γ-lactones have been isolated from a natural source. However, C_{17}-, C_{19}-, C_{21}- and C_{23}-compounds of this type have already been reported from other members of the Lauraceae [3, 5–7].

EXPERIMENTAL

Mps: uncorr. CC was run on Merck silica gel 60 (230–400 mesh) and Florisil (100–200 mesh). TLC was performed on precoated Kieselgel 60 F_{254} plates. Chemical shifts are given in ppm. HPLC was conducted on a Develosil pack (ODS-10) column (20 × 250 mm) using a UV detector (254 nm).

Extraction and separation of compounds. A. lancifolia (Sieb. et Zucc.) Meissn. was collected at Toyota, Aichi prefecture, in April 1987. Leaves (3.3 kg) and wood (20.1 kg) were collected. The MeOH extract of leaves was divided into the *n*-hexane sol. (57.87 g) and $CHCl_3$ sol. frs (57.78 g). The *n*-hexane sol. fr. was chromatographed on Florisil with C_6H_6 as an eluent to give a dark brown oil (12 g), a part (1.2 g) of which was sepd by CC on silica gel (C_6H_6–EtOAc, 9:1) followed by prep. TLC using 5% $AgNO_3$–Kieselgel 60 F_{254} (toluene–EtOAc, 5:1) to afford lancifolide (**1**) (98 mg) and isolancifolide (**2**) (32 mg). The $CHCl_3$ sol. fr. was chromatographed on Florisil (C_6H_6) to afford an oil (7 g), a portion of which (1.2 g) was subjected to CC on 5% $AgNO_3$–Kieselgel 60 F_{254} (CH_2Cl_2–EtOAc, 9:1) and subsequently to HPLC (MeOH–H_2O, 3:1; flow rate 9 ml) providing isoobtusilactone (**7**) (8 mg) R_t 77 min and obtusilactone (**3**) (10 mg) at R_t 83 min.

The MeOH extract of the wood was similarly divided into *n*-hexane and $CHCl_3$ sol. fractions. The *n*-hexane sol. fr. (86.26 g) was chromatographed on a Florisil column. Elution with *n*-hexane gave an oil (20.7 g), a part of which (2 g) was sepd by CC on silica gel (*n*-hexane) following by prep. TLC (*n*-hexane–EtOAc, 9:1) yielding sesquirosefuran (1.4 g) and longifolin (9 mg). Elution with *n*-hexane–Et_2O (9:1) provided an oil (22 g), a portion of which (0.3 g) was treated in the same manner as that described for the *n*-hexane sol. fr. of leaves to yield **1** (14.3 mg) and **2** (8 mg). The $CHCl_3$ sol. fr. (50 g) was chromato-



graphed on silica gel ($CHCl_3$) to give an oil (4 g), a portion of which (200 mg) was treated in the same way as the above fr. eluting with *n*-hexane–Et_2O (9:1) to yield **1** (5 mg) and **2** (3 mg).

Lancifolide (**1**). Colourless oil. $[\alpha]_D$ −49.0° ($CHCl_3$; *c* 0.58). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3400, 1780, 1680. UV λ_{max}^{EtOH} nm: 224. CIMS (*iso*-C_4H_9) *m/z*: 253 $[M+1]^+$. FAB-HRMS *m/z*: 253.1774 ($[M+1]^+$, calcd for $C_{15}H_{25}O_3$: 253.1802). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.27 (14H, *br s*), 2.31 (1H, *br d*, *J* = 8.4 Hz, OH), 2.77 (2H, *m*, 7-H), 4.68 (1H, *dd*, *J* = 2.7, 1.7 Hz, 1-H), 4.89 (1H, *dd*, *J* = 2.7, 2.0 Hz, 1-H), 5.11 (1H, *ddd*, *J* = 8.4, 2.0, 1.7 Hz, 3-H), 6.69 (1H, *td*, *J* = 7.7, 2.0 Hz, 6-H). ^{13}C NMR ($CDCl_3$): δ 165.4 (*s*, C-5), 157.6 (*s*, C-2), 151.4 (*d*, C-6), 126.9 (*s*, C-4), 90.3 (*t*, C-1), 68.9 (*d*, C-3), 31.9 (*t*, C-7), 29.7 (*t*), 29.4 (*t*), 29.3 (*t*), 29.3 (*t*), 28.8 (*t*), 28.4 (*t*), 22.7 (*t*, C-14), 14.2 (*q*, C-15).

Isolancifolide (**2**). Colourless oil. $[\alpha]_D$ −59.0° ($CHCl_3$; *c* 0.50). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1780, 1685, 1670. UV λ_{max}^{EtOH} nm: 218. FABMS *m/z*: 253 $[M+1]^+$. FAB-HRMS *m/z*: 253.1769 ($[M+1]^+$, calcd for $C_{15}H_{25}O_3$: 253.1802). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.27 (14H, *br s*), 2.10 (1H, *br d*, *J* = 6.4 Hz, OH), 2.48 (2H, *m*, 7-H), 4.73 (1H, *dd*, *J* = 2.7, 1.3 Hz, 1-H), 4.96 (1H, *dd*, *J* = 2.7, 1.7 Hz, 1-H), 5.26 (1H, *br d*, *J* = 6.4 Hz, 3-H), 7.09 (1H, *td*, *J* = 7.7, 2.0 Hz, 6-H). ^{13}C NMR ($CDCl_3$): δ 166.8 (*s*, C-5), 157.8 (*s*, C-2), 150.2 (*d*, C-6), 127.5 (*s*, C-4), 91.4 (*t*, C-1), 66.6 (*d*, C-3), 31.9 (*t*, C-7), 29.8 (*t*), 29.5 (*t*), 29.5 (*t*), 29.4 (*t*), 29.4 (*t*), 28.4 (*t*), 22.8 (*t*, C-14), 14.2 (*q*, C-15).

Selective hydrogenation of **1**. **1** (46.4 mg) was hydrogenated over $Rh(Ph_3P)_3Cl$ (25 mg) in dry C_6H_6 (8 ml) at room temp. for 30 min and the solvent evapd to give an oil. The oil was chromatographed on silica gel ($CHCl_3$–Me_2CO, 19:1) followed by chromatography on silica gel (C_6H_6–EtOAc, 3:1) affording a mixture of **4a** and **4b** (40 mg). A part (9.4 mg) of the mixt. was sepd by HPLC (MeOH–H_2O, 2:1; flow rate 9 ml) yielding **4a** (2.8 mg) R_t 100 min and **4b** (3.6 mg) R_t 112 min. Compound (**4a**), colourless oil. $[\alpha]_D$ −62.4° ($CHCl_3$; *c* 0.17). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1755, 1675. MS *m/z*: 254 $[M]^+$, 237, 236, 210, 207, 197, 179. FAB-HRMS *m/z*: 255.1939 ($[M+1]^+$, calcd for $C_{15}H_{27}O_3$: 255.1959). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.26 (14H, *br s*), 1.40 (3H, *d*, *J* = 6.4 Hz, 1-H), 2.74 (2H, *m*, 7-H), 4.55 (1H, *dq*, *J* = 6.4, 5.4 Hz, 2-H), 4.66 (1H, *br d*, *J* = 5.4 Hz, 3-H), 6.56 (1H, *td*, *J* = 7.7, 1.4 Hz, 6-H). Compound (**4b**), colourless oil. $[\alpha]_D$ −8.8° ($CHCl_3$, *c* 0.32). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1755, 1675. MS *m/z*: 254 $[M]^+$, 237, 236, 210, 207, 197, 179. FAB-HRMS *m/z*: 255.1943 ($[M+1]^+$, calcd for $C_{15}H_{27}O_3$: 255.1959). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.26 (14H, *br s*), 1.39 (3H, *d*, *J* = 6.4 Hz, 1-H), 2.75 (2H, *m*, 7-H), 4.35 (1H, *dq*, *J* = 6.1, 3.7 Hz, 2-H), 4.36 (1H, *m*, 3-H), 6.54 (1H, *td*, *J* = 7.7, 1.4 Hz, 6-H).

Acetylation **4a** *and* **4b**. The mixt (40 mg) was treated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. for 4 hr. Ice was added to the reaction mixt which was then extracted with EtOAc. The EtOAc layer was dried (Na_2SO_4) and evapd. The residue was purified by CC on silica gel (*n*-hexane–C_6H_6, 1:1) to afford a pale yellow oil (**5**) (3.8 mg, 10% yield). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780. MS *m/z*: 236 ($[M]^+$, $C_{15}H_{24}O_2$), 193, 179. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.26 (14H, *br s*), 2.11 (3H, *s*, 1-H), 2.28 (2H, *q*, *J* = 7.7 Hz, 7-H), 5.80 (1H, *s*, 3-H), 6.57 (1H, *t*, *J* = 7.7 Hz, 6-H).

Hydrogenation of **1**. **1** (20 mg) was hydrogenated over 5% Pd–$BaSO_4$ (20 mg) in EtOH (5 ml). After absorption of H_2 had ceased, the reaction mixt. was filtered. The filtrate was evapd to give a residue, which was purified by CC on silica gel (EtOAc). The resulting crude solid was recrystallized from C_6H_6 to afford colourless prisms (**6**) (19 mg, 94% yield), mp 82–83°. $[\alpha]_D$ 40.0° ($CHCl_3$; *c* 0.15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 3450, 1770. MS *m/z*: 256 $[M]^+$, 239, 200, 185, 167. HRMS *m/z*: 256.2055 ($[M]^+$, calcd for $C_{15}H_{28}O_3$: 256.2037). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, *t*, *J* = 6.7 Hz, 15-H), 1.27 (18H, *br s*), 1.44 (3H, *d*, *J* = 6.4 Hz, 1-H), 2.58 (1H, *dt*, *J* = 4.7, 10.1 Hz, 4-H), 4.32 (1H, *ddd*, *J* = 4.7, 3.9, 3.0 Hz, 3-H), 4.46 (1H, *dq*, *J* = 3.0, 6.4 Hz, 2-H).

Hydrogenation of **2**. **2** (30.9 mg) was hydrogenated over 5% Pd–$BaSO_4$ (30.9 mg) in EtOH (7 ml). After absorption of H_2 had ceased, the reaction mixt. was worked-up as described above for **1** to provide colourless prisms (**6**) (30 mg, 96% yield), mp 82–83°. This compound was identical in all respects with the product, derived from **1**.

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REFERENCES

1. Nii, H., Furukawa, K., Iwakiri, M. and Kubota, T. (1982) *Nippon Nogeikagaku Kaishi* **56**, 261.
2. Nii, H., Hanafusa, M., Furukawa, K., Iwakiri, M. and Kubota, T. (1983) *Yukagaku* **32**, 277.
3. Niwa, M., Iguchi, M. and Yamamura, S. (1975) *Tetrahedron Letters* 1539.
4. Jackman, L. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* 2nd Edn, p. 222. Pergamon Press, Oxford.
5. Martinez, V., J. C., Yoshida, M. and Gottlieb, O. R. (1981) *Phytochemistry* **20**, 459.
6. Niwa, M., Iguchi, M. and Yamamura, S. (1975) *Chemistry Letters* 655.
7. Takeda, K., Sakurawi, K. and Ishii, H. (1972) *Tetrahedron* **28**, 3757.