Lactones from the Leaves of *Litsea japonica* and Their Anti-complement Activity

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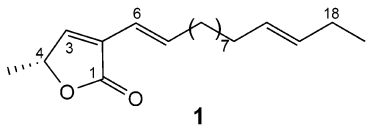
Two new lactones, litsealactone A (**1**) and litsealactone B (**2**), were isolated from the leaves of *Litsea japonica*, together with three known lactones, hamabiwalactone A (**3**), hamabiwalactone B (**4**), and akolactone B (**5**). Hamabiwalactone B (**4**) and akolactone B (**5**) significantly inhibited complement activity in an in vitro anti-complement assay, with IC_{50} values of 149 and 58 μM, respectively.

Complement can be activated by a cascade mechanism of the classical pathway (CP), alternative pathway (AP), or the MBL/MASP (mannan binding lectin/MBL-associated serine protease) pathway.[1] The 30 odd complement fragments that make up the system include proteolytic proenzymes, nonenzymatic components that form functional complexes, cofactors, regulators, and receptors.[2] The proteolytic cascade allows for a considerable amplification, since each proteinase molecule activated at one step can generate multiple copies of an activated enzyme later in the cascade, which in turn cleaves nonenzymatic components, such as C3, C4, and C5. The larger fragments derived from C3, C4, and C5 (i.e., C3b, C4b, and C5b) are involved in biologic effector functions, such as in opsonization, phagocytosis, and immunomodulation. However, the smaller molecules, C3a, C4a, and C5a, called anaphylatoxins, induce the release of mediators from the mast cells and lymphocytes, which causes a variety of inflammatory diseases and can be fatal if occurring after organ transplantation.[2−4] Therefore, modulation of complement activity should be useful in the therapy of inflammatory diseases.

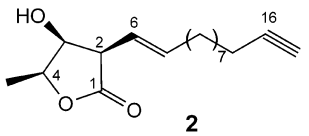
Litsea japonica (Thunb.) Jussieu (Lauraceae) is an evergreen tree, which grows in the Southern areas of Korea and Japan.[5] Lactones[6,7] and essential oils[8] have been isolated from this species. As part of our continuing research to find pharmacologically active compounds from this plant, we isolated five lactones. This paper describes the isolation, structural determination, and the anti-complement activity of these substances, which include two new natural products (**1** and **2**).

The hexane-soluble fraction of the leaves of *L. japonica* was subjected to repeated column chromatography on normal-phase and reversed-phase C_{18} silica gel to give five lactones (**1**−**5**). Three known compounds were identified as hamabiwalactone A (**3**), hamabiawalactone B (**4**), and akolactone B (**5**), respectively, by comparing their spectral data with those previously reported.[7,9]

Litsealactone A (**1**), a colorless oil, was assigned the molecular formula $C_{19}H_{30}O_2$ on the basis of HRFABMS, with a molecular ion at m/z 291.2321 $[M + H]^+$, which indicated five degrees of unsaturation. Its IR spectrum showed an absorption at 1750 cm^{-1}, corresponding to a carbonyl group, and its ^1H NMR exhibited two doublet signals at δ 7.03 (J = 1.5 Hz) and 1.42 (J = 7.2 Hz) and a broad quartet at δ 5.02 (J = 7.2 Hz), which were assigned to an oxygenated allylic methyl moiety on a butanolide moiety, by comparison with that of butenolide **1** isolated from three species of *Hortonia* (Monimiaceae) (Table 1).[10] This observation was further supported by the ^{13}C NMR spectral assignments of **1** (a carbonyl carbon at δ 172.0, an olefinic carbon at δ 146.8, an olefinic quaternary carbon at δ 129.4, a methyl carbon at δ 19.2, and an oxygenated methine carbon at δ 76.9) and by a DEPT experiment. The methyl group (δ 1.42) correlated with the oxygenated methine carbon at δ 76.9 (C-4) and the olefinic carbon at δ 146.8 (C-3) in the HMBC spectrum, indicating the presence of a γ-methyl-α,β-unsaturated butenolide. In addition, the ^1H NMR and $^1H-^1H$ COSY NMR spectra of **1** showed two spin systems that began with olefinic methine (δ 6.09) and methyl (δ 0.96) protons, respectively. The olefinic methine proton coupled to an olefinic proton (δ 6.79) and two methylene protons (δ 2.16). The methyl protons correlated with two methylene protons (δ 2.04), and then the methylene signals were coupled to an olefinic proton (δ 5.36), which was further correlated with a second olefinic proton (δ 5.42) and two methylene protons (δ 1.97). The latter signals also correlated with the overlapping methylenes at δ 1.28, indicating the presence of a long aliphatic chain.



1



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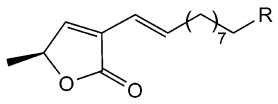

3 R= —C≡CH

4 R= —CH=CH$_2$

5 R= —CH=CH—CH=CH$_2$

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Compounds **1**, **2**, and **3** (in $CDCl_3$)[a]

	1		**2**		**3**	
position	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1		172.0		176.1		172.0
2		129.4	3.39 dd (7.2, 5.1)	50.8		129.3
3	7.03 d (1.5)	146.8	4.30 dd (6.3, 3.0	72.3	7.05 d (1.5)	146.8
4	5.02 br q (7.2)	76.9	4.50 dq (6.3, 3.0)	78.8	5.03 q (6.8)	76.9
5	1.42 d (7.2)	19.2	1.45 d (6.3)	13.7	1.42 d (6.8)	19.1
6	6.09 d (15.9)	118.3	5.49 dd (15.6, 7.2)	119.0	6.09 d (15.9)	118.3
7	6.79 dt (15.9, 7.2)	138.8	5.82 dt (15.6, 6.6)	139.4	6.79 dt (15.9, 7.2)	138.7
8	2.16 q (7.2)	33.4	2.14 q (6.6)	32.5	2.16 q (6.9)	33.3
9	1.28 br s	29.6	1.30 br s	29.2	1.30 br s	29.2
10	1.28 br s	29.4	1.30 br s	29.1	1.30 br s	29.1
11	1.28 br s	29.4	1.30 br s	28.9	1.30 br s	28.9
12	1.28 br s	29.2	1.30 br s	28.9	1.30 br s	28.7
13	1.28 br s	29.1	1.30 br s	28.6	1.30 br s	28.7
14	1.28 br s	28.8	1.30 br s	28.4	1.30 br s	28.4
15	1.97 m	32.5	2.18 td (6.9, 2.7)	18.0	2.18 td (6.9, 2.7)	18.3
16	5.42 m	131.9		84.7		84.7
17	5.36 m	129.3	1.94 t (2.7)	68.1	1.94 t (2.7)	68.0
18	2.04 m	25.6				
19	0.96 t (7.2)	14.0				

[a] δ values in ppm and coupling constants (in parentheses) in Hz.

The ^{1}H NMR resonance at δ 1.28 was assigned to six aliphatic methylenes on the basis of tandem MS data, which showed prominent ions at *m/z* 112 $[C_8H_{16}]^+$ and 167 $[C_{12}H_{23}]^+$. All five sites of unsaturation required by the molecular formula were accounted for by the two double bonds and an α,β-unsaturated lactone ring. In addition, partial structures were linked using the HMBC NMR technique. Long-range correlations between δ 7.03 (H-3) and δ 76.9 (C-4)/118.3 (C-6 in the side chain), together with that between the H-6 methine at δ 6.09 and two quaternary carbons at δ 172.0 (C-1)/129.4 (C-2), confirmed that a C_{14} side chain and a double bond were connected at C-6 and at C-2, respectively. The HMBC correlation observed between H-19 (δ 0.96) and the olefinic carbon (C-17, δ 129.3), together with that between the C-18 methylene at δ 2.04 and C-16 (δ 131.9), confirmed that the position of the other double bond was C-16.

The configuration of the double at C-6 was assigned as *E* on the basis of the vicinal coupling constant (J = 15.9 Hz). Furthermore, irradiation of four methylene protons at δ 1.97 and 2.04 (H2-15 and H2-18) changed the signals of the olefinic protons to two doublets at δ 5.36 and 5.42 (J = 15.6 Hz), allowing the C-16 double bond to be assigned as *E*. The stereochemistry at C-4 was assigned as *R* on the basis of its levorotatory optical activity, compared with that of hamabiwalactone B, which is dextrorotatory ($[\alpha]_D$, +29.4).[11] Therefore, the structure of litsealactone A (**1**) was assigned as (4*S*)-2-(1,11-tetradecadienyl)-4-methylbut-2-enolide.

Litsealactone B (**2**) was isolated as colorless oil and found to have the molecular formula $C_{17}H_{26}O_3$ by HRFABMS. Its IR spectrum showed the absorption of a lactone carbonyl group (1740 cm^{-1}).[12] The ^{1}H NMR spectrum of **2** exhibited signals for two oxygenated protons at δ 4.30 (dd, J = 6.3, 3.0 Hz) and 4.50 (dq, J = 6.3, 3.0 Hz), a methine at δ 3.39 (dd, J = 7.2, 5.1 Hz), and a methyl at δ 1.45 (d, J = 6.3 Hz). Its ^{13}C NMR spectrum contained 17 resonance peaks, including a lactone carbonyl carbon at δ 176.1, two oxygenated methine carbons at δ 72.3 and 78.8, and a methyl group at δ 13.7. These spectral features confirmed the presence of a 3-hydroxy-4-methyl-δ-lactone moiety in **2**, by comparison with those of the acetogenins isolated from *Porcelia macrocarpa* (Annonaceae).[13] In addition, the signals of two olefinic protons at δ 5.49 (dd, J = 15.6, 7.2 Hz) and 5.82 (dt, J = 15.6, 6.6 Hz), methylene protons at δ 2.14 (q, J = 6.6 Hz) and 2.18 (td, J = 6.9, 2.7 Hz), a broad singlet at δ 1.30, and an ethynyl group at δ 1.94 (t, J = 2.7 Hz) were observed in the ^{1}H NMR spectrum, indicating that a long-chain acetylene was present, by comparison with that of hamabiwalacton A (**3**). The presence of the acetylene group was further supported by the absorption of IR spectrum at 2125 cm^{-1}. In the ^{1}H−^{1}H COSY spectrum, the olefinic proton (δ 5.49) coupled to its vicinal partner (δ 5.82), which in turn, correlated with two methylene protons (δ 2.14). The latter signal also correlated with overlapped methylenes (δ 1.30). The ethynyl proton (δ 1.94) coupled to two methylene protons (δ 2.18), which were further coupled to overlapping methylenes (δ 1.30). The eight continuous methylenes were confirmed by ^{13}C NMR and tandem MS data, which showed a prominent ion at *m/z* 111 $[C_8H_{16}-H]^+$. Analysis of all data obtained above showed that the aliphatic chain of **1** contains 12 carbon atoms and that it is unbranched. The HMBC spectrum also showed a correlation between the signal at δ 3.39 (H-2) and δ 119.0 (C-6)/139.4 (C-7), together with a correlation between the H-6 methine at δ 5.49 and δ 176.1 (C-1)/72.3 (C-3), and established that the C_{12} side chain is connected to C-2. The geometry of the C-6 double bond was determined to be in the *E* configuration on the basis of the vicinal coupling constant (J = 15.6 Hz). The absolute configurations at C-2, C-3, and C-4 were assigned as 2*R*, 3*S*, 4*S* on the basis of the ^{1}H NMR chemical shifts of H-3−H-5 and from the optical rotation ($[\alpha]_D$, −2.9°) of **2**; these results resemble those of δ-lactones isolated from *Trichilia claussenii* (Meliaceae).[14] This all-*cis* stereochemistry of the lactone ring substituents of **2** was further supported by NOE interactions observed between H-2 and H-3/H-4 in the NOESY spectrum, and the ^{13}C NMR chemical shift of the C-5 methyl (δ 13.7) indicated that this group is *cis* to the C-3 hydroxyl group, because the *trans* configuration of that had approximately δ 18 ppm.[14] Accordingly, the structure of litsealactone B (**2**) was assigned as (2*R*,3*S*,4*S*)-2-(1-dodecyl-11-yn)-3-hydroxy-4-methylbutanolide.

Compounds **1**−**5** were tested for their anti-complement activity upon the complement system as total hemolytic activity. The results (IC_{50} values) are summarized in Table 2. Hamabiawalactone B (**4**) and akolactone B (**5**) proved to have strong anti-complement activity, with IC_{50} values of 149 and 58 μM, compared to rosmarinic acid (IC_{50}, 180 μM), which was used as a positive control.[15] On the other hand,

Table 2. Inhibitory Effects of Compounds **1**−**5** on the Complement System in Vitro

compound	IC_{50} (μM)
litsealactone A (**1**)	787 ± 53
litsealactone B (**2**)	N.E.[a]
hamabiwalactone A (**3**)	714 ± 32
hamabiwalactone B (**4**)	149 ± 9
akolactone B (**5**)	58 ± 4
rosmarinic acid[b]	180 ± 13

[a] No effect. [b] Positive control.

compounds **1**−**3** were inactive in this assay system. In addition, actinolides A and B isolated from *Actinodaphne lancifolia* (Lauraceae), which are long carbon chain α,β-unsaturated methyl-butanolides, were devoid of any significant anti-complement activity.[16] These results support the finding that the terminal double bond of long-chain butanolides might play an important role in anti-complement activity, and hence *L. japonica* is a candidate as a new medicinal plant with anti-complement activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in $CHCl_3$. UV spectra were recorded on a UV-2000 spectrometer. FT-IR spectra were obtained on a JASCO-40 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian UNITY 500 spectrometer, with chemical shifts being represented in ppm and tetramethylsilane used as an internal standard. HR-FABMS and tandem MS were measured on a JMS-HX 110/110A spectrometer (JEOL).

Plant Material. The leaves of *L. japonica* (5.5 kg) were collected at Jeju (Korea) in August 2000 and dried at room temperature. A voucher specimen (PB-2916) is deposited at the Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

Extraction and Isolation. The air-dried leaves (5.5 kg) of *L. japonica* were extracted with MeOH. The MeOH (655 g) extract was suspended in H_2O and extracted with hexane and EtOAc, successively, to give the hexane- (110 g) and EtOAc-soluble fractions (69 g), respectively. The hexane-soluble fraction was chromatographed on a column of reversed-phase (RP) C_{18} silica gel eluted with 50% aqueous MeOH to give three fractions (A−C; 9, 37, and 49 g). Repeated column chromatography of fraction B on silica gel (hexane−acetone) and RP C_{18} (30% aqueous MeOH), followed by preparative TLC (hexane−acetone), afforded **1** (22.8 mg), **2** (94.2 mg), **3** (32.9 mg), **4** (801.2 mg), and **5** (19.9 mg).

Litsealactone A (1): colorless oil; $[\alpha]_D^{20}$ −47.5° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 206 (3.82), 213 (3.71) nm; IR (CCl_4) ν_{max} 2920, 1750, 1460, 1380, 1210, 1060 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; tandem MS *m/z* 291 $[M + H]^+$, 193 $[C_{14}H_{25}]^+$, 167 $[C_{12}H_{23}]^+$, 112 $[C_8H_{16}]^+$, 97 $[C_5H_5O_2]^+$; HRFABMS *m/z* 291.2321 (calcd for $C_{19}H_{31}O_2$, 291.2324).

Litsealactone B (2): colorless oil; $[\alpha]_D^{20}$ −2.9° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 202 (4.17), 205 (3.58) nm; IR (CCl_4) ν_{max} 3440, 2910, 2125, 1740, 1460, 1390, 1190, 1135, 1050 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; tandem MS *m/z* 301 $[M + Na]^+$, 163 $[C_{12}H_{19}]^+$, 138 $[C_{10}H_{17} + H]^+$, 111 $[C_8H_{16} - H]^+$; HRFABMS *m/z* 301.1779 (calcd for $C_{17}H_{26}O_3Na$, 301.1780).

Anti-complement Assay. A diluted solution of normal human serum (complement serum, 80 μL) was mixed with a gelatin veronal buffer (GVB^{2+}, 80 μL) with or without sample. Each sample was dissolved in DMSO, and it was used as a negative control. The mixture was preincubated at 37 °C for 30 min, after which sensitized erythrocytes (sheep red blood cells, 40 μL) were added. After incubation under the same conditions, the mixture was centrifuged (4 °C, 1500 rpm) and the optical density of the supernatant (100 μL) was measured at 450 nm.[17] Rosmarinic acid was used as a positive control. The purities of compounds used for the assay were above 95% and were checked by HPLC. Anti-complement activity was determined by means of triplicate measurements and expressed as the 50% inhibitory concentration (IC_{50} value) from complement-dependent hemolysis of the control.[18]

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