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Two novel furan derivatives from *Phellinus linteus* with anti-complement activity

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Abstract—Two novel stereoisomers of furan derivatives, phellinusfurans A (**1**) and B (**2**), were isolated from the fruiting body of *Phellinus linteus*. Their structures were elucidated by spectroscopic analysis. Compounds **1** and **2** exhibited significant anti-complement activity with IC_{50} values of 33.6 and 33.7 μM, respectively, in inhibiting the hemolytic activity of human serum against erythrocytes.
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The complement system is a major effector of humoral immunity and is activated by a cascade mechanism via an antigen–antibody-mediated process (classical pathway, CP), an antibody-independent process (alternative pathway, AP), or through mannan binding lectin/MBL-associated serine protease (MBL/MASP).[1,2] The proteolytic cascade allows for a very high amplification rate, which in the next step activates the enzymes later in the cascade. This in turn cleaves the non-enzymes, such as C3, C4, and C5. The pathway converges the C3 convertase step leading to C5 convertase and the self-assembly of the membrane attack complex (MAC). In complement activation, the complement components induce the release of mediators from the mast cells and lymphocytes, causing a variety of diseases (i.e., rheumatoid arthritis, osteoarthritis, atopic dermatitis, lung fluid inflammation, and atherosclerotic lesion), as well as can be fated if occurring after an organ transplantation.[3] This effect is normally beneficial for the host, but can also cause adverse effects depending on the site as extent and duration of complement activation. The modulation of complement activity can be important to the treatment of inflammation.

As parts of a study to develop anti-complementary agents from natural products, the complement-inhibiting properties of the fruiting body of *Phellinus linteus* (Berk & Curt) Aoshima (Hymenochaetaceae), which is commonly referred to as Sangwhang in Korea, have been investigated. This mushroom has been used as a traditional oriental medicine for treating stomachache and arthritis of the knee.[4] A literature survey shows that this species produces a variety of structurally unusual secondary metabolites, such as sphingolipid,[4] hispidine,[5] benzyl hydroflavones,[6] furo[3,2-*c*]pyran-4-ones,[7] pyrano[4,3-*c*][2]benzopyran-1,6-diones,[7,8] and 26-membered macrolide,[9] which have been shown to have biological effects on tyrosinase inhibitor, antioxidant and cytotoxic activity. In this paper, two furan derivatives with an unprecedented carbon skeleton have been isolated[10] and bioassayed for their classical pathway complement inhibitory activity (Fig. 1).

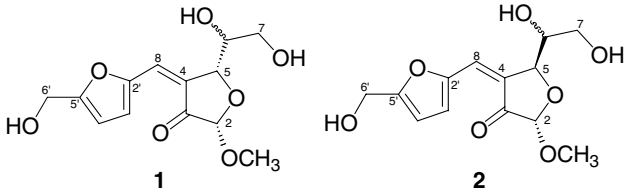

Figure 1. Structures of phellinusfurans A (**1**) and B (**2**).

Keywords: *Phellinus linteus*; Furan derivatives; Structural determination; Anti-complement activity.
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Compound **1** was obtained as a brownish yellow powder. FABMS evidenced a quasi-molecular ion peak at *m/z* 307 $[M+Na]^+$, with the molecular formula $C_{13}H_{16}O_7$, and this was verified by the ^{13}C NMR spectrum.[11] Compound **1** also manifested two doublet olefinic signals at δ 6.54 (d, $J = 3.6$ Hz) and δ 7.20 (d, $J = 3.6$ Hz), which were attributed to the presence of a furan ring by virtue of their coupling pattern on the ^1H NMR spectrum, as compared with that of the dihydroxerulin isolated from *Xerula melanotricha*.[12] This observation was bolstered further by the ^{13}C NMR spectral assignments (two olefinic methine carbons at δ 110.3 and δ 121.7, and two oxygenated olefinic quaternary carbons at δ 149.3 and δ 160.6) coupled with the results of the DEPT, HMQC, and HMBC experiments. Also, the ^1H NMR spectrum evidenced a hydroxymethyl signal at δ 4.48 (2H, br s), which was directly correlated with the olefinic carbon signals (δ 121.7 and δ 160.6) in the HMBC spectrum, and suggested the presence of a hydroxymethyl-furan ring in compound **1**. Furthermore, the ^1H NMR spectrum of compound **1** revealed signals for two oxymethine protons at δ 4.91 (s) and δ 5.33 (dd, $J = 4.8$, 2.1 Hz). The oxymethine signal (δ 5.33) was correlated with the olefinic proton at δ 7.21 (d, $J = 2.1$ Hz) and the other oxymethine proton at δ 3.77 (m), and the latter proton was further coupled to the hydroxymethyl protons at δ 3.49 (2H, br s) in the ^1H–^1H COSY spectrum. The ^{13}C NMR spectrum, in combination with the results of the DEPT and HMQC experiments, also revealed signals for three oxymethine carbons (δ 74.4; δ 80.8; δ 99.1), two olefinic carbons (δ 121.4; δ 126.7), a carbonyl carbon (δ 196.8), and a hydroxymethyl carbon (δ 62.6), and the connectivity of these carbons was established by the results of the HMBC experiment. In the HMBC spectrum, the correlations from δ_H 4.91/5.33 to δ_C 126.7/196.8 and δ_H 5.33 to δ_C 62.6/74.4/121.4 suggested the presence of the 5-(1,2-dihydroxyethyl)-4-ylmethylene-dihydrofuran-3-one moiety. In addition, the ^1H NMR spectrum revealed a methoxy group (δ_H 3.41), which was correlated with the oxygenated carbon at δ_C 99.1, and confirmed that compound **1** harbors a structural moiety of 5-(1,2-dihydroxyethyl)-4-ylmethylene-2-methoxy-dihydrofuran-3-one. This result was verified further by the detection of the fragment ions *m/z* 61 $[C_2H_5O_2]^+$ and 175 $[C_7H_{11}O_5]^+$ in the EIMS spectrum. The two partial structures were linked via the HMBC experiment. The long-range correlations between δ_H 7.21 (H-8) and δ_C 110.3 (C-3′)/149.3 (C-2′), and δ_H 6.54 (H-3′) and δ_C 121.4 (C-8) confirmed that the hydroxymethyl-furan ring was linked to the 5-(1,2-dihydroxyethyl)-4-ylmethylene-2-methoxy-dihydrofuran-3-one by C-8. The results of NOESY measurement confirmed the indicated structure and also proved the relative stereochemistry at C-2, C-5, and C-8. The proton signal at δ 7.21 (H-8) was correlated with the signal at δ 5.33 (H-5), whereas that of H-8 exerted no influence on the signal at δ 3.77 (H-6) in the NOESY spectrum, which demonstrated the *Z*-configuration of the double bond at C-8 and the α-orientation of the dihydroxyethyl group at C-5. However, the proton signal at δ 5.33 could not be correlated with the signal at δ 3.41 (OCH_3) in the NOESY spectrum, which bolstered the theorized α-orientation of the methoxy group at C-2. However, the absolute configuration at C-6 remained unidentified. Thus, the structure of phellinusfuran A (**1**) was confirmed to be 5α-(6,7-dihydroxyethyl)-4-(5′-hydroxymethyl-furan-2- ylmethylene)-2α-methoxy-dihydrofuran-3-one.

Compound **2** appeared as a brownish-yellow powder. The molecular formula was identified as $C_{13}H_{16}O_7$, identical to that of **1**, according to the results of FABMS and ^{13}C NMR.[13] In addition, the NMR spectral data of compound **2** were also almost completely identical to those of **1**. However, careful examination of the NOESY spectra obtained for both compounds revealed significant differences in the NOE correlation peaks of the protons. The NOE correlations between δ 7.11 (H-8) and δ 5.46 (H-5)/3.93 (H-6), and δ 5.46 (H-5) and δ 3.28 (OCH_3) revealed that the configuration of the dihydroxyethyl group was a β-orientation. In consequence, the structure of phellinusfuran B (**2**) was identified as 5β-(6,7-dihydroxyethyl)-4-(5′-hydroxymethyl-furan-2-ylmethylene)-2α-methoxy-dihydrofuran-3-one.

Compounds **1** and **2** were bioassayed with regard to their classical pathway complement inhibitory activity via an in vitro assay technique.[14] Both compounds exhibited significant inhibitory effects on the CP of the complement system, evidencing IC_{50} values of 33.6 and 33.7 μM, respectively, compared with a positive control, rosmarinic acid (IC_{50} 180 μM). This showed that the two furan derivative stereoisomers exhibited comparable inhibitory effects on the complement system.

In summary, the novel furan derivatives, designated as phellinusfurans A (**1**) and B (**2**) in this study, were isolated from the fruiting bodies of *P. linteus*. These two compounds both exhibited significant anti-complement effects. However, the two stereoisomers evidenced no differences with regard to their inhibitory activities on the complement system.

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10. The lyophilized fruiting body of *P. linteus* (4.5 kg) was powdered and refluxed with MeOH (3× 8 L). The extract (838 g) was suspended in H_2O and partitioned sequentially with CH_2Cl_2 (101.5 g), EtOAc (36.7 g), *n*-BuOH (366 g), and H_2O (244 g). The EtOAc-soluble fraction (36 g) was chromatographed over a Si gel column (Silica gel 60, Merck, 1.2 kg) eluted with CH_2Cl_2/MeOH (9:1), yielding 12 subfractions (F01–F12). Fraction 7 (F07, 4.33 g) was then chromatographed on a Sephadex LH-20 column with MeOH, yielding nine subfractions (F0701–F0709). Fraction 4 (F0704, 2.2 g) was subjected to column chromatography over a Si gel column with CH_2Cl_2/MeOH (20:1 to MeOH), yielding seven fractions (F0704-1–F0704-7). Fraction 5 (F0704-5, 368.5 mg) was successively fractionated using Si gel with CH_2Cl_2/MeOH (20:1 to MeOH) to ten subfractions (F0704-5-1 to F0704-5-1). Phellinusfuran A (**1**, 13.4 mg) and phellinusfuran B (**2**, 13.3 mg) were purified by RP-18 column chromatography (70% MeOH) of fraction 2 (F0704-5-2, 27 mg) and fraction 3 (F0704-5-3, 22 mg), respectively, followed by a Si gel column chromatography with CH_2Cl_2/MeOH (20:1).
11. Analytical data for **1**; brownish yellow powder; UV (MeOH) λ_{max} (log ε): 357 nm (4.88); [α] −1.9 (*c* 0.012, MeOH); EIMS *m*/*z* (rel int): 61 (12), 77 (41), 109 (11), 175 (15), 206 (100), 223 (61), 284 (0.63); LR-FABMS: 307 $[M+Na]^+$; HR-FABMS *m*/*z*: 307.0667 $[M+Na]^+$ (calcd for $C_{13}H_{16}O_7Na$: 307.0671); ^1H NMR (400 MHz, DMSO-d_6): δ 7.21 (1H, d, J = 2.1 Hz, H-8), 7.20 (1H, d, J = 3.6 Hz, H-4′), 6.54 (1H, d, J = 3.6 Hz, H-3′), 5.33 (1H, dd, J = 4.8, 2.1 Hz, H-5), 4.91 (1H, s, H-2), 4.48 (2H, br s, H-6′), 3.77 (1H, m, H-6), 3.49 (2H, br s, H-7), 3.41 (3H, s, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.8 (C-3), 160.6 (C-5′), 149.3 (C-2′), 126.7 (C-4), 121.7 (C-4′), 121.4 (C-8), 110.3 (C-3′), 99.1 (C-2), 80.8 (C-5), 74.4 (C-6), 62.6 (C-7), 56.0 (C-6′), 55.6 (OCH_3).
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13. Analytical data for **2**; brownish yellow powder; UV (MeOH) λ_{max} (log ε): 352 nm (4.55); [α] +2.6 (*c* 0.012, MeOH); EIMS *m*/*z* (rel int): 61 (22), 77 (57), 109 (12), 175 (38), 206 (100), 223 (88), 284 (0.86); LR-FABMS: 307 $[M+Na]^+$; HR-FABMS *m*/*z*: 307.0664 $[M+Na]^+$ (calcd for $C_{13}H_{16}O_7Na$: 307.0671); ^1H NMR (400 MHz, DMSO-d_6): δ 7.11 (1H, d, J = 2.1 Hz, H-8), 7.09 (1H, d, J = 3.4 Hz, H-4′), 6.55 (1H, d, J = 3.4 Hz, H-3′), 5.46 (1H, br s, H-5), 5.05 (1H, s, H-2), 4.50 (2H, br d, H-6′), 3.93 (1H, m, H-6), 3.33 (2H, br s, H-7), 3.28 (3H, s, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.0 (C-3), 160.4 (C-5′), 149.1 (C-2′), 126.7 (C-4), 120.9 (C-4′), 119.3 (C-8), 110.3 (C-3′), 98.7 (C-2), 81.1 (C-5), 74.0 (C-6), 61.6 (C-7), 56.0 (C-6′), 53.9 (OCH_3).
14. Anti-complement activity was determined by modified method of Mayer. For the classical pathway assay, a diluted solution of normal human serum (80 μL) collected from a healthy volunteer (Man) was mixed with gelatin veronal buffer (80 μL) with or without sample. Each sample was dissolved in DMSO and used as a negative control. The mixture was pre-incubated at 37 °C for 30 min, and 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 405 nm. Rosmarinic acid was employed as positive controls.