Note
1,2-Di-O-α-linolenoyl-3-O-β-galactosyl-sn-glycerol as a Superoxide Generation Inhibitor from Perilla frutescens var. crispa

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Using a superoxide (O2•−) generation assay system with differentiated HL-60 cells, 1,2-di-O-α-linolenoyl-3-O-β-galactosyl-sn-glycerol (DLGG) was identified as an O2•− generation inhibitor from Perilla frutescens var. crispa (a local variety, kida-chirimen shiso). DLGG suppressed the O2•− level in a dose-dependent manner with an IC50 value of 21 μM, comparable to those of rosmarinic acid (RoA, IC50 = 29 μM) and caffeic acid (CA, IC50 = 30 μM). While RoA and CA also dose-dependently inhibited O2•− generation in a xanthine-xanthine oxidase system, DLGG had no effect in the same system. Thus DLGG appeared to decrease the O2•− level in the HL-60 assay system by mechanisms different from those of RoA and CA, which appeared to act as O2•− scavengers.

Key words: Perilla frutescens; 1,2-di-O-α-linolenoyl-3-O-β-galactosyl-sn-glycerol (DLGG); superoxide; xanthine oxidase; HL-60 cells

Phagocytes produce superoxide anions (O2•−) and nitric oxide (NO) in response to infectious stimuli. While reactive oxygen and nitrogen species are important toxic molecules acting against pathogens in the immunological defense system, excessive production in chronic inflammation is known to cause various inflammation-related diseases, including cancer, diabetes, and atherosclerosis.1–4 Because O2•− is the initial reactive oxygen species generated by NADPH oxidase and other enzymes, the release of O2•− is a key event in the development of various diseases, including cardiovascular diseases, diabetes, cancer, and inflammatory diseases.5–8

The active principle was traced based on inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced O2•− generation in an assay system with human promyelocytic HL-60 cells, which had been differentiated into neutrophil-like cells by dimethylsulfoxide (DMSO).3) The differentiated cells release a significant amount of O2•− in response to TPA and are useful for testing inhibitory compounds that suppress the release of O2•−.6) Repeated column chromatography of the ethyl acetate-soluble part of the methanol extract of fresh leaves of P. frutescens var. crispa (1.5 kg) followed by reversed-phase preparative HPLC afforded active compound I (85 mg) as a glassy resin; FAB-MS: 797 [(M + Na)+] corresponding to the molecular weight of C36H32O10; IR: 3,700–3,000, 1,730, and 1H-NMR (400 MHz, CDCl3, δppm from TMS): 0.97 (6H, t, J = 6.7 Hz), 1.2–1.4 (br.), 1.5–1.7 (br.), 2.0–2.1 (8H, m.), 2.33 (4H, q, J = 6.7 Hz), 2.8 (8H, br. m.), 3.5–3.7 (3H, br.), 3.72 (1H, dd., J = 11.0, 6.6 Hz), 3.8–3.9 (1H, br.), 3.91 (1H, dd., J = 11.0, 5.3 Hz), 3.95 (1H, br.), 4.02 (1H, br. s), 4.22 (1H, dd., J = 12.0, 6.6 Hz), 4.27 (1H, d., J = 7.5 Hz), 4.39 (1H, dd., J = 12.0, 3.3 Hz), 5.25–5.45 (2H (olefins) + 1H (sn-2), br.).

The 1H-NMR signals in the region of 3.5–4.5 ppm suggested the presence of sugar and partly acylated glycerol moieties. In addition, the signals at 0.97, 1.2–1.4, 2.0–2.1, 2.33, 2.80, and 5.3–5.5 ppm indicated the presence of two residues of an unsaturated fatty acid, strongly suggesting that I is a glycero-glycerolipid. In the base-catalyzed methanalysis of I, the liberation of only methyl α-linolenate was confirmed by GLC analysis on an Inertcap-225 capillary column. The presence of a galactosyl moiety was also determined by GLC analysis (Inertcap-225 capillary column), in which TMS-galactose, derived from the acid hydrolysate of the products (alcohol fraction) of base-catalyzed methanalysis, was detected. One of the two unsaturated fatty acids was determined to be esterified to the secondary hydroxyl group (2-OH) of glycerol based on the chemical shift of a multiplet signal at 5.7 ppm on 1H-NMR (400 MHz, δ5-pyridine). This signal was assignable to the proton attached to the acylated carbon (C-2 of glycerol moiety). In addition, the β-anomer form of the galactose moiety was deduced from the coupling constant (J = 7.5 Hz) of the proton at the anomeric position of the galactosyl group at 4.27 ppm. Also, it was suggested that the galactoside linkage was biosynthetically formed with the

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Abbreviations: O2•−, superoxide; NO, nitric oxide; SOD, superoxide dismutase; TPA, 12-O-tetradecanoylphorbol-13-acetate; α-LA, α-linolenic acid; XA, xanthine; XOD, xanthine oxidase; RoA, rosmarinic acid; CA, caffeic acid; DMSO, dimethylsulfoxide
hydroxyl group at the sn-3 carbon of glycerol (S-configuration at C-2 of glycerol). This configuration was further confirmed by the specific rotation [α]D2° = −4.5° (c 0.22, CHCl3), which was identical to previous data. Hence the structure of 1 was elucidated to be 1,2-di-O-α-linolenoyl-3-O-β-galactosyl-α-glycerol (DLGG), as shown in Fig. 1.

As shown in Fig. 2, DLGG dose-dependently inhibited TPA-induced O₂⁻ generation in the assay system with DMSO-differentiated HL-60 cells, and the IC₅₀ value (21 µM) was comparable to those of rosmarinic acid (RoA, 29 µM) and caffeic acid (CA, 30 µM), representative dietary O₂⁻ scavengers. RoA has been reported in P. frutescens and CA is a related phenolic compound (Fig. 1). Moreover, α-linolenic acid (α-LA, IC₅₀ = 9.2 µM), the constituent fatty acid of DLGG, showed approximately 2 times stronger activity than DLGG.

When O₂⁻ generation was investigated using WST-1 in a noncellular xanthine-xanthine oxidase (XA-XOD) system to test O₂⁻ scavenging activity, neither DLGG nor α-LA had any effect (Fig. 3), but RoA and CA markedly and dose-dependently inhibited WST-1 formation, which was abrogated by superoxide dismutase (SOD). Therefore, DLGG is widely distributed in plants, and has been reported to have several bioactivities, including suppression of NO radical production in macrophage cells via suppression of inducible NO synthase production. We also confirmed that DLGG inhibited NO production in RAW264 macrophage cells stimulated by lipopolysaccharide and interferon-γ at an IC₅₀ value of 20 µM, identical to the results of a previous report. Thus, it is interesting that DLGG can inhibit both O₂⁻ and NO generation from activated phagocyes.

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References