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Original Contribution

Nonallergenic urushiol derivatives inhibit the oxidation of unilamellar vesicles and of rat plasma induced by various radical generators

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ABSTRACT

Urushiols consist of an *o*-dihydroxybenzene (catechol) structure and an alkyl chain of 15 or 17 carbons in the 3-position of a benzene ring and are allergens found in the family Anacardiaceae. We synthesized various veratrole (1,2-dimethoxybenzene)-type and catechol-type urushiol derivatives that contained alkyl chains of various carbon atom lengths, including –H, $-C_1H_3$, $-C_5H_{11}$, $-C_{10}H_{21}$, $-C_{15}H_{31}$, and $-C_{20}H_{41}$, and investigated their contact hypersensitivities and antioxidative activities. 3-Decylcatechol and 3-pentadecylcatechol displayed contact hypersensitivity, but the other compounds did not induce an allergic reaction, when the ears of rats were sensitized by treatment with the compounds every day for 20 days. Catechol-type urushiol derivatives (CTUDs) exerted very high radical-scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl radical and inhibited lipid peroxidation in a methyl linoleate solution induced by 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). However, veratrole-type urushiol derivatives did not scavenge or inhibit lipid peroxidation. CTUDs also acted as effective inhibitors of lipid peroxidation of the egg yolk phosphatidylcholine large unilamellar vesicle (PC LUV) liposome system induced by various radical generators such as AMVN, 2,2′-azobis(2-amidino-propane) dihydrochloride, and copper ions, although their efficiencies differed slightly. In addition, CTUDs suppressed formation of cholesteryl ester hydroperoxides in rat blood plasma induced with copper ions. CTUDs containing more than five carbon atoms in the alkyl chain showed excellent lipophilicity in a *n*-octanol/water partition experiment. These compounds also exhibited high affinities to the liposome membrane using the ultrafiltration method of the PC LUV liposome system. Therefore, CTUDs seem to act as efficient antioxidative compounds against membranous lipid peroxidation owing to their localization in the phospholipid bilayer. These results suggest that nonallergenic CTUDs act as antioxidants to protect against oxidative damage of cellular and subcellular membranes.

Urushiols are major components contained in the sap of the lacquer tree (*Rhus verniciflua* Stokes, Anacardiaceae) [1]. Urushiols consist of *o*-dihydroxybenzene (catechol) coupled with a saturated or unsaturated alkyl side chain of 15 or 17 carbons and are amphipathic compounds [1–3]. These compounds exert various biological effects such as antioxidant [4,5], antimicrobial [6,7], and anticancer [8] activities. However, urushiols cause skin redness, swelling, inflammation, and irritation on contact, which is known as urushiol-induced contact dermatitis [9–11]. Therefore, nonallergenic urushiol derivatives would be useful as a bioactive compound in the human body.

Excessive reactive oxygen species generated in the body cause oxidative damage. In particular, lipid peroxidation has been implicated in various human diseases, including atherosclerosis, cancer, and aging [11–16]. α-Tocopherol and flavonoids (quercetin, luteolin, catechins, etc.) are distributed widely in food, including cereals, vegetables, and fruits, and are excellent antioxidants in humans [17–20]. In addition, the catechol structure of various phenolic compounds including flavonoids is important as an active site for their antioxidative activity [21,22]. The lipophilicity and localization of antioxidants in biological systems are generally considered important to understanding the biological activities of these compounds on cellular and intracellular membranes. Natural urushiols possessing a catechol structure and an alkyl side chain of 15 or 17 carbons are amphipathic compounds; therefore, attention has been focused on the inhibitory effects of these compounds on biomembrane peroxidation. However, the urushiols have a fatal

Abbreviations: AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); AsA, ascorbic acid; *n*-BuLi, *n*-butyllithium; CE-OOH, cholesteryl ester hydroperoxide; CTUD, catechol-type urushiol derivative; DPPH, 1,1-diphenyl-2-picrylhydrazyl; PC LUV, phosphatidylcholine large unilamellar vesicle; MeL-OOH, methyl linoleate hydroperoxide; NMR, nuclear magnetic resonance; THF, tetrahydrofuran; α-Toc, α-tocopherol; VTUD, veratrol-type urushiol derivative

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defect, as they induce strongly allergic reactions on contact, although natural urushiols act as excellent antioxidants. Therefore, the functions of urushiol are desirable but unattainable.

In this study, we chemically synthesized veratrole- and catechol-type urushiol derivatives (compounds **1–12**) that contain alkyl side chains of different lengths. The urushiol derivatives (**1–12**) were subjected to a contact hypersensitivity evaluation using rats. We also measured radical-scavenging activity of the urushiol derivatives (**1–12**) using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)[2] radical and methyl linoleate system. In addition, the inhibitory effects of the urushiol derivatives (**1–12**) against lipid peroxidation on phosphatidylcholine large unilamellar vesicles (PC LUV) and copper ion-induced rat blood plasma were investigated.

Materials and methods

General experimental procedures

Nuclear magnetic resonance (NMR) spectral data were measured with Varian ^{Unit}INOVA 300 and 500 (Varian, Walnut Creek, CA, USA) spectrometers using tetramethylsilane in $CDCl_3$ as the internal standard. Mass spectral data were obtained by electrospray ionization mass spectrometry (API 3200Q trap, Applied Biosystems, Foster City, CA, USA) under the following conditions: ion source temperature, 0 °C; electron voltage of positive and negative mode, 5000 and −4500 V, respectively. Column chromatography was performed with silica gel (Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) resin. The high-performance liquid chromatography (HPLC) analysis was performed on a Shimadzu LC-6AD with a SPD-M20A detector and silica gel (4.6 × 250 mm, TSK-gel, Silica-60, Tosoh Co., Tokyo, Japan), LiChroprep Lobar (RP-8, 40–63 μm, 25 × 310 mm, Merck), and Octyl-80Ts (4.6 × 150 mm, TSK-gel, Tosoh Co.) columns. Thin-layer chromatography was performed on silica gel 60 F_{254} (0.25 mm thickness, Merck).

Chemicals

Veratrole (1,2-dimethoxybenzene, **1**), 3-methylveratrole (**2**), 3-methylcatechol (**8**), 1-bromopentane, 1-bromodecane, 1-bromopentadecane, 1-bromoeicosane, butyllithium (1.6 M solution in *n*-hexane), boron tribromide (BBr_3; 1.0 M solution in *n*-hexane), 2,6-di-*tert*-butyl-4-methylphenol (BHT), 70% perchloric acid ($HClO_4$), diethylenetriaminepentaacetic acid (DTPA), and methyl linoleate were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Pyrocatechol (catechol, **7**) was purchased from Kanto Chemical Co. (Tokyo, Japan). *n*-Octanol was obtained from Samchun Pure Chemical Co. (Pyeongtaek, Korea). DPPH, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), ascorbic acid (AsA), and egg yolk 3-*sn*-phosphatidylcholine were obtained from Wako (Osaka, Japan). (±)-α-Tocopherol (α-Toc) was purchased from Fluka (Buchs SG, Lucerne, Switzerland). All other chemicals and solvents were of analytical grade.

Synthesis of veratrole-type urushiol derivatives

Synthesis of veratrole-type urushiol derivatives (VTUDs) was carried out according to the method of Niimura et al. [23] and Satoh et al. [24]. Exactly 3.82 ml veratrole (0.03 mol) was added to a solution (130 ml) of dry tetrahydrofuran (THF) and the mixture was stirred for 30 min at 0 °C. A solution of 28.2 ml *n*-BuLi (1.6 M solution in *n*-hexane, 0.045 mol) in 5 ml THF was slowly added to the mixture and then stirred successively for 1 h at 0 °C and for 1 h at room temperature. A solution (5.56 ml) of 1-bromoalkanes (0.045 mol), with different carbon chain lengths, was slowly poured into the solution and then refluxed for 5 h at 210 °C. The resulting mixture was added to a saturated NH_4Cl solution (150 ml, twice) and partitioned with ethyl acetate (EtOAc; 200 ml, twice). The organic layer was washed with brine (200 ml, twice) and added to anhydrous K_2CO_3. After filtration, the organic layer was evaporated in vacuo at 35 °C. The concentrates was purified by silica gel (100 g, 2.3 × 67 cm) column chromatography eluting with toluene to give the VTUDs. Four VTUDs (**3, 4, 5,** and **6**) were synthesized using 1-brompentane, 1-bromodecane, 1-bromopentadecane, and 1-bromoeicosane by the same procedure described above.

Synthesis of catechol-type urushiol derivatives

VTUDs (**3**–**6**, 2.85 g, 13.68 mmol), with different side chain lengths, were dissolved respectively with 29.4 ml dry methylene chloride (CH_2Cl_2) at 0 °C. The sample solution was added to a solution (19.38 ml) of BBr_3 (1.0 M solution in hexane, 19.38 mmol) in dry CH_2Cl_2, and the mixture was stirred successively for 2 h at 0 °C and then for 12 h at room temperature. Each solution was partitioned with H_2O to give the CH_2Cl_2 layer. The aqueous layer was partitioned with CH_2Cl_2 (60 ml, twice). The combined organic layer was washed with brine (200 ml, twice) and added to anhydrous K_2CO_3. After filtration, the organic layer was evaporated in vacuo at 35 °C. The crude products were purified by silica gel (Kieselgel 60, Merck, 2.3 × 59 cm, benzene/EtOAc 6/1, v/v) column chromatography to give catechol-type urushiol derivatives (CTUDs). 3-Pentylcatechol (**9**), 3-decylcatechol (**10**), 3-pentadecylcatechol (**11**), and 3-eicosylcatechol (**12**) were synthesized using 3-pentylveratrole (**3**), 3-decylveratrole (**4**), 3-pentadecylveratrole (**5**), and 3-eicosylveratrole (**6**) by the same procedure described above.

Induction of contact hypersensitivity by the urushiol derivatives on rat ears

Sprague–Dawley rats (male, 6 weeks of age, 180–200 g) were obtained from Samtako Bio Korea (Osan, Korea). The rats were housed under controlled humidity (55 ± 5%), room temperature (25 ± 1 °C), and light cycle (12 h light/12 h dark). Food and water were available ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chonnam National University (CNU IACUC-YB-R-2012-26). All rats were acclimated for 1 week with a standard rodent diet (Harlan Rodent diet, 2018S) before experiments. An ethanol (EtOH) solution (3 μmol/50 μl) of one of the VTUDs or CTUDs (**1–12**) was applied to the rear (1 cm^2) of the left ear of the rats ($n = 6$) daily for 20 days. The erythema visualized on the rat ears treated with the VTUDs and CTUDs was reflective of contact hypersensitivity.

Determination of hematological biomarkers in the blood of rats treated with the urushiol derivatives

After the VTUD and CTUD treatments for 20 days, rats ($n = 6$) were anesthetized with diethyl ether, the abdominal wall was opened, and blood was collected from the abdominal aorta into glass tubes. The numbers of white blood cell, neutrophil, and eosinophil in the whole blood were determined by Veterinary Multi-species Hematology System (Hemavet 850, CDC Technologies Inc., Oxford, MI, USA). Blood serum was obtained by centrifugation (1500 *g*) at 4 °C for 20 min and stored at −70 °C until analysis. Serum IgE and histamine levels were measured using Rat IgE ELISA kit (Komabiotec, Seoul, Korea) and histamine level was measured using a histamine enzyme-linked immunosorbent assay kit (Oxford Biomedical Research, Rochester Hills, MI, USA) according to the manufacturer's instructions.