A New Galloylglucoside from *Cleyera ochnacea* DC.

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Received June 29, 2001; accepted September 1, 2001

A new galloylglucoside, 3-hydroxy-5-methylphenol 1-*O*-β-D-(6′-galloyl)glucopyranoside (1) was isolated from *Cleyera ochnacea* DC. (Theaceae). Its structure was elucidated on the basis of chemical and spectral analysis. Compound 1 showed inhibitory activity against rat cerebellar nitric oxide synthase (NOS).

Key words galloylglucoside; nitric oxide synthase (NOS); *Cleyera ochnacea* DC.

Cleyera ochnacea DC. (Theaceae) (Japanese name: Sakaki) is a small evergreen tree. This tree is common in the forests of southwestern Japan up to 1400 m elevation,[1] and it has been traditionally reserved for Shinto-shrine worship. The chemical constituents of the plant have been investigated, and acids and glucosides have been isolated.[2,3] As part of our continuous search for novel biologically active compounds from plants, crude extract of *C. ochnacea* DC. was found to show inhibitory activity against rat cerebellar nitric oxide synthase (NOS). Bioactivity-guided chromatographic fractionation of the active extract led to the isolation and characterization of a new compound, 3-hydroxy-5-methylphenol 1-*O*-β-D-(6′-galloyl)glucopyranoside. Herein, we report the isolation and structural elucidation of this new compound and its inhibitory activity against rat cerebellar NOS.

The MeOH extract of *C. ochnacea* DC. was suspended in water and then extracted with AcOEt and *n*-BuOH, successively. The *n*-BuOH extract was treated as described in Experimental to isolate compound **1**.

Compound **1**, obtained as an off-white amorphous powder, $[\alpha]_D$ −20.3°C (MeOH), gave a positive response to the ferric chloride reagent, and its molecular formula was established as $C_{20}H_{22}O_{11}$ by high resolution (HR)-FAB-MS. The liquid secondary ion (LSI)-MS of **1** exhibited an $[M+H]^+$ ion peak at *m*/*z* 439, together with a minor peak at *m*/*z* 153, suggestive of the presence of a galloyl group. This was supported by a two-proton singlet at δ 7.10 in the ^{1}H-NMR spectrum and five characteristic carbon signals (δ 166.7, 146.1, 138.8, 121.7, 110.0) in the ^{13}C-NMR spectrum. The remaining 13 signals indicated one methyl (δ 21.5) and six aromatic (δ 159.9, 158.9, 140.8, 111.0, 109.3, 102.2) carbons, as well as six carbons corresponding to a glucose moiety (δ 101.9, 77.8, 75.1, 74.7, 71.2, 64.4). The configuration of the anomeric center in the glucose moiety was assigned as β from coupling constant (J=7.5 Hz) of the anomeric proton in the ^{1}H-NMR spectrum. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of 3-hydroxy-5-methoxylphenol 1-*O*-β-D-(6′-galloyl)glucopyranoside except for some signals assignable to the 5-methoxyresorcinol moiety.[4] The ^{1}H-NMR spectrum of **1** showed an aromatic methyl group (δ 2.08, 3H, s) and three aromatic protons [(δ 6.26, 1H, br s), (δ 6.35, 2H, br s)], indicative of a 5-methylresorcinol. In addition, the LSI-MS data of **1** showed a fragment ion peak at *m*/*z* 315, which was derived by elimination of the 3-hydroxy-5-methylphenoxy group.

This was confirmed by enzymatic hydrolysis. Treatment of **1** with β-glucosidase gave the aromatic compound (**2**). Compound **2** was identified as 5-methylresorcinol by comparison with the ^{1}H- and ^{13}C-NMR spectrum of an authentic sample.

The connections between the above units were assigned on the basis of ^{1}H–^{13}C long-range correlations in the heteronuclear multiple bond connectivity (HMBC) spectrum. (Fig. 1) HMBC showed correlations of the anomeric proton (δ 4.89) to C-1 of the 5-methylresorcinol moiety (δ 159.9), and 6′-H_2 of the β-glucose moiety (δ 4.32, 4.56) to the ester carbonyl carbon of the gallic acid moiety (δ 166.7), indicating the location of the β-glucose and gallic acid moieties to be at C-1 and C-6′, respectively. Thus, compound **1** was determined to be 3-hydroxy-5-methylphenol 1-*O*-β-D-(6′-galloyl)glucopyranoside.

Inhibitory activity of **1** against inducible NOS (iNOS) and neuronal NOS (nNOS) was evaluated according to the methods in the indicated references.[5,6] The IC_{50} values of reference compound, N^G-monomethyl-L-arginine,[7] were 10.0 μM for iNOS and 1.1 μM for nNOS. Compound **1** showed weak inhibitory activity; its IC_{50} values were 152 μM for iNOS and 515 μM for nNOS.

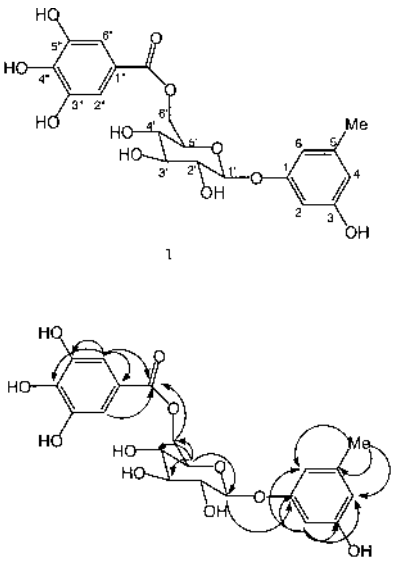

Fig. 1. HMBC Correlations for Compound **1**

Experimental

General Procedures Optical rotation was recorded on a Perkin-Elmer 241 polarimeter. MS were obtained by use of a Hitachi M-90 (LSI-MS) or a JEOL JMS-SX/SX102A (HR-FAB-MS) mass spectrometer. One- and two-dimensional (1D, 2D) NMR spectra were measured with a JEOL JNM A-400 spectrometer. Chemical shifts were expressed in ppm downfield from internal tetramethylsilane. Chromatorex (Fuji Silysia Chemical Ltd.) was used for column chromatography. Precoated silica gel Kieselgel 60 F_{254} plates (0.25 mm thickness) were used for TLC, and the spots were detected

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by spraying with 1% $Ce(SO_4)_2$/10% H_2SO_4, followed by heating. Preparative HPLC was carried out on a Develosil ODS 10/20 column (i.d. 20 mm×250 mm) and guard column (i.d. 20 mm×50 mm) (Nomura Chemical) with detection by UV absorption at 220 nm (Shinadzu SPD 6A).

Plant Material The plant material *Cleyera ochnacea* DC. was collected from the botanical garden of Aburahi Laboratories, Shionogi & Co., Ltd. in January 1996.

Extraction and Isolation Fresh leaves and branches (1.2 kg) were chopped into small pieces and extracted twice with MeOH at room temperature for 7 d. Then the extract was evaporated *in vacuo* to yield the MeOH extract (93.7 g). The MeOH extract (20 g) was then suspended in distilled water and successively extracted with AcOEt, *n*-BuOH. The *n*-BuOH soluble fraction was evaporated under reduced pressure to give brown oil (8.1 g). The *n*-butanol-soluble fraction (1.0 g) was subjected to Chromatorex (ODS) column chromatograph with H_2O–MeOH (80 : 20→0 : 100) to afford four fractions. Fraction 2 (228 mg) was subjected to HPLC (ODS) (CH_3CN : H_2O=20 : 80) to give **1** (45 mg).

3-Hydroxy-5-methylphenol 1-*O*-*β*-D-(6′-Galloyl)glucopyranoside (1) An off-white amorphous powder, $[\alpha]_D^{26}$ −20.3° (*c*=0.22, MeOH). LSI-MS *m*/*z* (rel. int. %): 439 (M+H, 48), 341 (65), 315 (70), 219 (100), 153 (70). HR-FAB-MS *m*/*z*: 439.1243 $[M+H]^+$ (Calcd for $C_{20}H_{23}O_{11}$, 439.1240). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 2.08 (3H, s, Me-5), 3.40—3.54 (3H, m, H-2′, 3′, 4′), 3.77 (1H, m, H-5′), 4.32 (1H, dd, *J*=6.1, 11.7 Hz, H-6′a), 4.56 (1H, dd, *J*=2.0, 11.7 Hz, H-6′b), 4.89 (1H, d, *J*=7.5 Hz, H-1′), 6.26 (1H, br s, H-4), 6.35 (2H, br s, H-2, 6), 7.10 (2H, s, H-2″, 6″). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 21.5 (q, Me), 64.4 (t, C-6′), 71.2 (d, C-4′), 74.7 (d, C-2′), 75.1 (d, C-5′), 77.8 (d, C-3′), 101.9 (d, C-1′), 102.2 (d, C-2), 109.3 (d, C-6), 110.0 (d, 2C, C-2″, 6″), 111.0 (d, C-4) 121.7 (s, C-1″), 138.8 (s, C-4″), 140.8 (s, C-5), 146.1 (s, 2C, C-3″, 5″), 158.9 (s, C-3), 159.9 (s, C-1), 166.7 (s, CO).

Enzymatic Hydrolysis of 1 Compound **1** (60 mg) and *β*-glucosidase (SIGMA G-0395, 120 mg) were suspended in 0.1 M acetate buffer (pH 5.0, 24 ml) and incubated at 37°C for 24 h. The reaction mixture was extracted with Et_2O (20 ml×2) and the organic layer was dried and further evaporated to dryness. The Et_2O extract (12 mg) was subjected to chromatography on silica gel (*n*-hexane : AcOEt=2 : 3) to obtain 5-methylresorcinol (6 mg).

Acknowledgments We are grateful to Dr. Masafumi Fuzimoto, Director of Aburahi Laboratories of Shionogi & Co., for his encouragement and permission to publish this work. We also wish to express our thanks to Professor Isao Kitagawa (Kinki University, Osaka) for discussions and helpful support.

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