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A novel iNOS and COX-2 inhibitor from the aerial parts of *Rodgersia podophylla*

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Abstract—A novel compound, rodgersinol (**1**), was isolated from the aerial parts of *Rodgersia podophylla* and its structure was elucidated with various spectroscopic methods. Rodgersinol (**1**) exhibited the significant inhibitory effects on iNOS and COX-2 expressions in LPS-activated macrophages.

Inducible nitric oxide synthase (iNOS) and cyclooxgenase-2 (COX-2) are important enzymes that mediate inflammatory processes and have been associated with pathogenesis of certain types of human cancers as well as inflammatory disorders.[1] Since proper regulation of iNOS and COX-2 expression could provide an effective and promising approach to various inflammation related to diseases, much attention has been given to the discovery of iNOS and COX-2 modulators, especially from plant sources in discovering modulators for iNOS and COX-2.[2,3] As a part of our ongoing search for biologically active materials of natural origin, a new compound with unprecedented structure in nature was isolated from the aerial parts of *Rodgersia podophylla* A. Gray (Saxifragaceae), which is distributed in China, Japan, and Korea. The rhizomes of this plant have traditionally been used to treat enteritis and bacillary dysentery, and are also reported to exhibit antipyretic and analgesic effects.[4] Recent investigation of *R. podophylla* revealed that flavonol glycosides of its aerial parts exhibited hepatoprotective activity in the primary cultures of rat hepatocyte injured by H_2O_2.[5] Previous phytochemical studies of this plant afforded fatty acids, flavonoids, lignans, monoterpenes, tannins, and triterpenes.[5–8] In this paper, we describe the structure elucidation of a novel compound and its inhibitory effects on protein expression of iNOS and COX-2 in vitro using Raw 246.7 cells.

The air-dried aerial parts of *R. podophylla* (2 kg), collected from Jinbu, Gangwon province, Korea, in September in 2001, were extracted with methanol. The *n*-hexane-soluble fraction of the methanol extract was subjected to fractionation using Sephadex LH-20, followed by silica gel column chromatography, and reversed-phase HPLC to yield compound **1** (4.8 mg).[9]

Compound **1** was obtained as a white powder and gave a molecular ion peak at *m/z* 284.1417 (calcd for $C_{18}H_{20}O_3$, 284.1412) in the HREIMS. The ^{1}H NMR spectrum of **1** in $CDCl_3$ as shown in Table 1, exhibited two kinds of aromatic rings, a 1,4-disubstituted benzene ring at δ 6.77 (2H, d, J = 8.9 Hz, H-3′, 5′) and 6.84 (2H, d, J = 8.9 Hz, H-2′, 6′), and 1,3,4-trisubstituted benzene ring at δ 6.72 (1H, d, J = 8.4 Hz, H-5), 7.12 (1H, dd, J = 8.4, 2.1 Hz, H-6) and 7.24 (1H, d, J = 2.1 Hz, H-2). The remaining protons revealed that there were two C_3 units in this compound. One C_3 unit was found to be a 1-propene comprised of a methyl proton at δ 1.87 (3H, dd, J = 6.6, 1.5 Hz, H-9), two olefinic protons at δ 6.15 (1H, dq, J = 15.9, 6.6 Hz, H-8) and 6.36 (1H, dq, J = 15.9, 1.5 Hz, H-7). Another C_3 unit was observed at δ 3.41 (1H, sextet, J = 6.9 Hz, H-10), 3.76 (2H, m, H-11), 1.29 (3H, d, J = 6.9 Hz, H-12), and assigned as a propyl alcohol. The links between C_3 units and benzene rings were verified using HMBC technique. The correlations from δ_H 3.41 (H-10) in a propyl alcohol moiety to δ_C 125.4 (C-2), 134.0 (C-3), and 154.6 (C-4) suggested that a propyl alcohol was connected to C-3 on the 1,3,4-trisubstituted benzene ring. The signal of H-7 at δ 6.36 exhibited long range correlations with C-1 at δ 133.3 and C-6 at δ 124.8, which implied that

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Table 1. ^{1}H and ^{13}C NMR chemical shifts of rodgersinol (**1**) in $CDCl_3$[a]

	δ_H	δ_C	HMBC
1	—	133.3	
2	7.24 (1H, d, J = 2.1)[b]	125.4	C-4, 6, 7, 10
3	—	134.0	
4	—	154.6	
5	6.72 (1H, d, J = 8.4)	118.4	C-1, 3, 4
6	7.12 (1H, dd, J = 8.4, 2.1)	124.8	C-2, 4, 7
7	6.36 (1H, dq, J = 15.9, 1.5)	130.3	C-1, 6, 8, 9
8	6.15 (1H, dq, J = 15.9, 6.6)	124.9	C-1, 7, 9
9	1.87 (3H, dd, J = 6.6, 1.5)	18.4	C-7, 8
10	3.41 (1H, sextet, J = 6.9)	35.6	C-2, 3, 4, 11, 12
11	3.76 (2H, m)	67.8	C-3, 10, 12
12	1.29 (3H, d, J = 6.9)	16.8	C-3, 10, 11
1′	—	151.3	
2′	6.84 (1H, d, J = 8.9)	116.3	C-1′, 3′, 4′
3′	6.77 (1H, d, J = 8.9)	119.7	C-1′, 2′, 4′
4′	—	151.1	
5′	6.77 (1H, d, J = 8.9)	119.7	C-1′, 2′, 4′
6′	6.84 (1H, d, J = 8.9)	116.3	C-1′, 3′, 4′

[a] ^{1}H and ^{13}C NMR data were measured at 400 MHz and 100 MHz, respectively.
[b] ^{1}H resonance integral, multiplicity, and coupling constants are give in parentheses.

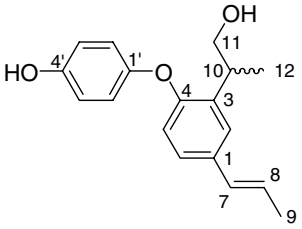

Figure 1. The structure of rodgersinol (**1**).

another C_3 unit, a propene, was also linked to C-1 of the 1,3,4-trisubstituted benzene ring. The remaining an 1,4-disubstituted benzene ring was assumed to be connected to either C-4 or C-11 via O-linkage. Since the hydroxy peaks of compound **1** did not appear in the original solvent, additional ^{1}H NMR experiments were conducted in DMSO-d_6. Two hydroxy peaks appeared at δ 9.25 (s) and 4.64 (t, J = 5.3 Hz), indicating the presence of an aromatic and a non-aromatic OH, respectively. A triplet peak at δ 4.64 was correlated with H-11 in the ^{1}H–^{1}H COSY spectrum, which proved that 1,4-disubstituted benzene ring was connected to C-4 on the trisubstituted benzene ring through an ether linkage. Therefore, the structure of compound **1** was elucidated as depicted in Figure 1 and named rodgersinol after the genus *Rodgersia*. Further synthetic experiments are necessary to determine the absolute configuration of the C-10 position in rodgersinol (**1**).

Since the traditional uses of *R. podophylla* were related to treating inflammatory diseases,[4] rodgersinol (**1**) was examined for the effects on iNOS and COX-2 enzymes.[10,11] The inhibitory effect of compound **1** on iNOS and COX-2 expressions in LPS-activated macrophages was demonstrated using the Western blot analyses. The levels of both iNOS and COX-2 proteins were

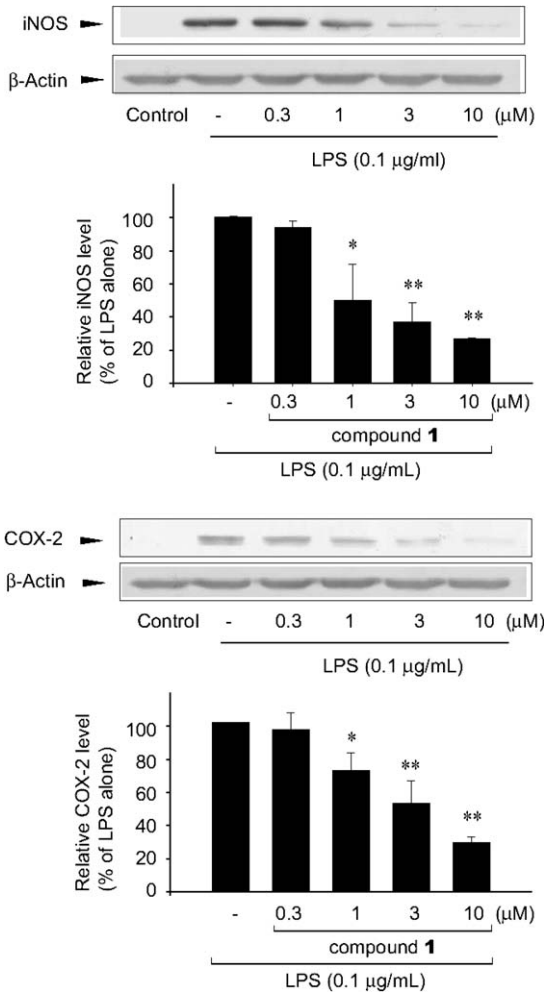

Figure 2. The effects of compound **1** on the iNOS and COX-2 protein expression. The levels of iNOS and COX-2 were monitored 12 h after treatment of Raw 264.7 cells with LPS (0.1 μg/mL) with or without compound **1** (i.e., 1 h before LPS). The relative protein levels were measured by scanning densitometry of the band intensities in immunoblots. Data represent means ± SD from four separate experiments (significant as compared to LPS alone, $*P < 0.05$, $**P < 0.01$).

gradually decreased in a dose-dependent manner, with increasing concentrations of rodgersinol (**1**) (Fig. 2). At a concentration of ca. 2 and 3 μM, compound **1** inhibited 50% of the protein expression of iNOS and COX-2, respectively.

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9. The aerial parts of *R. podophylla* were collected from Jinbu, Gangwon province, on September in 2001. A voucher specimen (SNUPC-011) was deposited at the Seoul National University. Dried materials (2 kg) were extracted with MeOH giving a crude extract (173 g). The MeOH extract was partitioned with *n*-hexane, CH_2Cl_2, and *n*-BuOH, successively. The *n*-hexane extract (46 g) was subjected to Sephadex LH-20 (Pharmacia, 400 g) chromatography with the solvent system (*n*-hexane/CH_2Cl_2/MeOH = 10:10:1) which gave four fractions (F01–F04). The F04 fraction (1.1 g) was applied to silica gel (230–400 mesh, Merck, 40 g) column chromatography using a *n*-hexane/EtOAc (10:1, 7:1, 5:1, 3:1, 1:1, 0:1, 500 mL each) gradient that afforded four fractions (F0401–F0404). Separation of the F0401 fraction (70 mg) by HPLC (YMC-Pack Ph, MeCN/H_2O = 45:55 (v/v), 2 mL/min) gave compound **1** (4.8 mg, t_R: 22.4 min). Rodgersinol (**1**): a white powder; mp 178–179 °C (MeOH); $[\alpha]_D^{20}$ − 14.6° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 261 (ε 8987); IR (film) υ_{max} 3362, 2963, 1605, 1498, 1212, 1021, 616 cm^{-1}; ^1H NMR, ^{13}C NMR and HMBC data see Table 1; HREIMS *m*/*z* 284.1417 $[M]^+$ (calcd for $C_{18}H_{20}O_3$ 284.1412).
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11. Raw 264.7 cells, murine macrophage cells (American Type Culture Collection, Menassa, VA, USA), were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were plated at a density of $2–3 \times 10^6$/mL and preincubated for 24 h at 37 °C. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. For all experiments, cells were grown to 80–90% confluency and were subjected to no more than 20 passages. Raw 264.7 cells were incubated with 0.1 μg/mL LPS to stimulate the COX-2 and iNOS gene expression. Compound **1** was dissolved in DMSO and added to the incubation medium 1 h prior to the addition of LPS. For measuring the protein levels of iNOS and COX-2, Western blotting technique was used according to Ref. 10.