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Novel Oxooxepane Derivatives and New Phorbic Acid Derivative from *Paederia scandens*

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Paederia scandens (Lour.) Merrill (Rubiaceae) is a climbing plant that is widely distributed in China, India, Japan, Korea, the Philippines, the USA, and Vietnam.[1,2] All parts of this plant have been used traditionally for the treatment of rheumatic arthritis, jaundice, dysentery, and dyspepsia and as an emetic and diuretic.[1,3,4] In previous studies, iridoid glycosides have been isolated and characterized as major secondary metabolites of *P. scandens*. Their extracts and individual constituents have been reported to exhibit various biological activities, including anti-*Helicobacter pylori*, antinociceptive, and antitumor promoting activities, as well as xanthine oxidase inhibition and uricosuric effect.[3-8] The present study describes the isolation of two new 7-oxooxepane derivatives having a naturally unprecedented skeleton and a new phorbic acid analog from the aerial parts of *P. scandens*.

Compound **1** was obtained as an amorphous solid, and its molecular formula of $C_{20}H_{22}O_{10}$ was deduced from the observation of the molecular ion $[M]^+$ at *m/z* 422.1212 (calcd for $C_{20}H_{22}O_{10}$, 422.1213) in the HR EI-MS. From the ^{1}H-NMR spectroscopic data, an oxymethine signal at δ_H 5.22 (1H, dd, *J* = 10.1, 4.8 Hz, H-1), three methylene signals at δ_H 2.92 (1H, dd, *J* = 12.8, 10.1 Hz, H-2), 2.57 (1H, dd, *J* = 12.8, 4.8 Hz, H-2), 2.37 (2H, m, H-4), and 2.64 (2H, m, H-5) were observed, which were ascribed to the proton signals on an oxooxepane skeleton with the aid of ^{1}H-^{1}H COSY and HMQC interpretation. HMBC correlations from H-1 to C-6 and C-7; from H-2 to C-4, C-7, and C-8; and from H-5 to C-3 and C-6 enabled the location of three ester groups (Fig. 1).

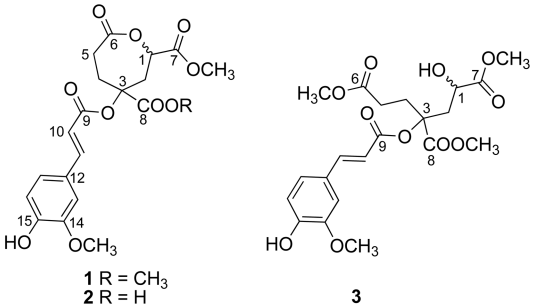

Figure 1. The chemical structures of **1**-**3**.

The positions of two methoxy groups were confirmed by the three-bond correlations between δ_H 3.82 and δ_C 172.6 (C-7) and between δ_H 3.69 and δ_C 176.2 (C-8) in the HMBC spectrum. In addition, the observation of the signals at δ_H 6.35 (1H, d, *J* = 15.9 Hz, H-10), 7.63 (1H, d, *J* = 15.9 Hz, H-11), 6.80 (1H, d, *J* = 8.1 Hz, H-16), 7.08 (1H, dd, *J* = 8.1, 1.6 Hz, H-17), 7.20 (1H, d, *J* = 1.6 Hz, H-13), and 3.88 (3H, s, OCH_3) indicated the presence of a ferulic acid moiety. This ferulic acid group was assumed to be affixed to a free hydroxy group of C-3 because this is the only available ether linkage that can position the ferulic moiety in the structure. Consequently, this compound was confirmed as (*E*)-dimethyl 4-(3-(4-hydroxy-3-methoxyphenyl)acryloyloxy)-7-oxooxepane-2,4-dicarboxylate and named paederoxepane A. This compound has an unprecedented natural structure.

Compound **2** was assigned a molecular formula of $C_{19}H_{20}O_{10}$, corresponding to the molecular ion peak $[M]^+$ at *m/z* 408.1062 in the HR EI-MS. The ^{1}H- and ^{13}C-NMR spectroscopic data resembled those of compound **1**, except for the absence of a methoxy group. Two methoxy signals at δ_H 3.80 and 3.87 displayed three-bond correlations with δ_C 172.6 (C-7) and 150.2 (C-14), respectively, in the HMBC spectrum. Hence, the positions of the two methoxy groups were resolved, and it was found that the methoxy group at C-8 was missing. Accordingly, compound **2** was identified as (*E*)-4-(3-(4-hydroxy-3-methoxyphenyl)acryloyloxy)-2-(methoxycarbonyl)-7-oxooxepane-4-carboxylic acid (paederoxepane B).

Compound **3** exhibited the molecular ion peak $[M]^+$ at *m/z* 454.1473 in the HR EI-MS, assignable to the molecular

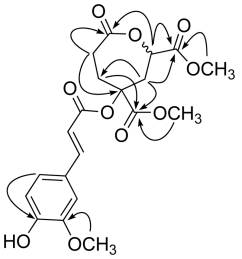

Figure 2. Selected HMBC correlations of **1**.

formula of $C_{21}H_{26}O_{11}$ (calcd 454.1475). The ^{1}H-NMR chemical shifts of **3** displayed upfield-shifted signals at δ_H 4.34 (1H, dd, J = 10.5, 2.6, H-1), 2.53 (1H, m, H-2a), and 2.33 (1H, m, H-2b) as compared to the signals of **1** [δ_H 5.22 (H-1), 2.92 (H-2a), 2.57 (H-2b)], implying the opening of the oxo-oxepane ring at C-1. In addition, this assumption was further supported by the facts that the carbon signal of C-1 appeared at δ_C 68.7 in **3** instead of δ_C 75.2 in **1** and that only the HMBC correlation from H-1 to carboxylic ester (C-7) was observed. Therefore, the structure of **3** was determined as (*E*)-trimethyl-1-hydroxy-3-(3-(4-hydroxy-3-methoxyphenyl) acryloyloxy)pentane-1,3,5-tricarboxylate, as shown in Figure 1.

All the isolates were tested for their cytotoxicity against the following three human cancer cell lines: Lu1 (lung cancer), LNCaP (prostate cancer), and MCF-7 (breast cancer), but the compounds were found to be inactive (ED_{50} > 20 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotation was measured with a JASCO DIP-1000 digital polarimeter (Tokyo, Japan). FAB-MS spectra were obtained on a JEOL JMS-AX505WA. UV and IR spectra were recorded on a Shimadzu UV-2101 and JASCO FT/IR-300E, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker spectrometer at 400 MHz and at 100 MHz, respectively. Column chromatography was performed using a Sephadex LH-20 (Pharmacia) and Kiesegel 60 (Art. 7734; Merck, Darmstadt, Germany). HPLC was performed on a column of YMC (J'sphere ODS-H80, S-4 μm, 250 × 10 mm i.d., Japan). TLC was conducted on pre-coated Kiesegel 60 F_{254} plates (Art. 5715; Merck, Darmstadt, Germany). Spots on the TLC were detected under UV light.

Plant Material. The aerial parts of *P. scandens* were collected from Chusan Experimental Station of Southern University Forest, College of Agricultural & Life Sciences, Seoul National University in 2002. A voucher specimen (SNUPC-012) was deposited at the College of Pharmacy at Seoul National University.

Extration and Isolation. The aerial parts of *P. scandens* (3.0 kg) were dried at room temperature and then extracted with MeOH. The MeOH extract (340 g) was concentrated *in vacuo* into a residue, which was suspended with water and then subsequently partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH, successively. The EtOAc-soluble fraction (5.5 g) was fractionated using reversed-phase column chromatography (H_2O-MeOH = 4:1→1:1) into eleven fractions (PE 1-11). The PE 5 fraction (380 mg) was chromatographed on Sephadex LH-20 (MeOH) and then through HPLC separation (MeCN-H_2O = 50:50, 4 mL/min) to yield **2** (47 mg, t_R 19.5 min) and **3** (4.8 mg, t_R 22.0 min). The PE 6 (500 mg) was applied to the separation of HPLC (MeCN-H_2O = 50:50, 4 mL/min) and furnished **1** (22.0 mg, t_R 34.4 min).

Paederoxepane A (1): An amorphous solid; HR EI-MS

Table 1. The ^{1}H and ^{13}C NMR chemical shifts of **1-3** in CD_3OD

	1		**2**		**3**	
	δ_C[a]	δ_H[b]	δ_C	δ_H	δ_C	δ_H
1	75.2	5.22, dd (10.1, 4.8)	75.2	5.22, dd (10.4, 4.6)	68.7	4.34, dd (10.5, 2.6)
2	36.9	2.92, dd (12.8, 10.1) 2.57, dd (12.8, 4.8)	36.8	2.94, dd (12.3, 10.4) 2.45, m	40.6	2.53, m 2.33, m
3	79.4	-	79.5	-	82.8	-
4	34.4	2.37, m	34.5	2.22, m	31.9	2.49, m
5	29.6	2.64, m	29.7	2.57, m	29.9	2.50, m 2.41, m
6	175.5	-	176.4	-	175.7	-
7	172.6	-	172.6	-	176.6	-
8	176.2	-	176.4	-	173.8	-
9	168.6	-	168.6	-	168.4	-
10	114.4	6.35, d (15.9)	114.4	6.36, d (15.9)	115.9	6.35, d (15.9)
11	149.7	7.63, d (15.9)	149.7	7.63, d (15.9)	148.5	7.59, d (15.9)
12	128.1	-	128.1	-	128.4	-
13	112.7	7.20, d (1.6)	112.7	7.19, d (1.5)	112.6	7.20, d (1.7)
14	150.2	-	150.2	-	150.3	-
15	152.0	-	151.9	-	151.7	-
16	125.5	6.80, d (8.1)	125.5	6.80, d (8.2)	125.1	6.81, d (8.2)
17	117.3	7.08, dd (8.1, 1.6)	117.3	7.09, dd (8.2, 1.5)	117.3	7.08, dd (8.2, 1.7)
6-OCH_3	-	-	-	-	53.1	3.64, s
7-OCH_3	54.2	3.82, s	54.2	3.80, s	53.6	3.82, s
8-OCH_3	53.2	3.69, s	-	-	53.7	3.73, s
14-OCH_3	57.3	3.88, s	57.3	3.87, s	57.3	3.89, s

[a]Spectroscopic data were measured in 100 MHz. [b]Spectroscopic data were measured in 400 MHz.

m/z 422.1212 (calcd for $C_{20}H_{22}O_{10}$: 422.1213); $[\alpha]_D^{20}$ −58.3° (*c* 0.33, MeOH); UV λ_{max} (MeOH) nm (log ε) 238 (0.71), 333 (1.48); IR ν_{max} (KBr, cm^{-1}) 3441, 2955, 1790, 1739, 1629, 1595, 1272; ^1H NMR and ^{13}C NMR (CD_3OD) see Table 1.

Paederoxepane B (2): An amorphous solid; HR EI-MS *m/z* 408.1062 (calcd for $C_{19}H_{20}O_{10}$: 408.1056); $[\alpha]_D^{20}$ −48.9° (*c* 0.28, MeOH); UV λ_{max} (MeOH) nm (log ε) 236 (0.83), 330 (1.62); IR ν_{max} (KBr, cm^{-1}) 3430, 2956, 1789, 1708, 1629, 1514, 1272; ^1H NMR and ^{13}C NMR (CD_3OD) see Table 1.

(*E*)-Trimethyl-1-hydroxy-3-(3-(4-hydroxy-3-methoxyphenyl) acryloyloxy)pentane-1,3,5-tricarboxylate (3): An amorphous solid; HR EI-MS *m/z* 454.1473 (calcd for $C_{21}H_{26}O_{11}$: 454.1475); $[\alpha]_D^{20}$ −15.3° (*c* 0.16, MeOH); UV λ_{max} (MeOH) nm (log ε) 234 (0.82), 330 (1.30); IR ν_{max} (KBr, cm^{-1}) 3442, 2953, 1737, 1630, 1595, 1514, 1271, 1158; ^1H NMR and ^{13}C NMR (CD_3OD) see Table 1.

***In vitro* Cytotoxicity Assay.** All the isolates were tested in Lu1, LNCaP, and MCF-7 cells according to the established method.[9]

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