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 (caled 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 $\delta$H 5.22 (1H, dd, $J$ = 10.1, 4.8 Hz, H-1), three methylene signals at $\delta$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).

The positions of two methoxy groups were confirmed by the three-bond correlations between $\delta$H 3.82 and $\delta$C 172.6 (C-7) and between $\delta$H 3.69 and $\delta$C 176.2 (C-8) in the HMBC spectrum. In addition, the observation of the signals at $\delta$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)-di-methyl 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 $\delta$H 3.80 and 3.87 displayed three-bond correlations with $\delta$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)-7-oxooxepane-2,4-dicarboxylate and named paederoxepane B.

Compound 3 exhibited the molecular ion peak [M]$^+$ at m/z 454.1473 in the HR EI-MS, assignable to the molecular...
formula of C\textsubscript{21}H\textsubscript{26}O\textsubscript{11} (calcld 454.1475). The \textsuperscript{1}H-NMR chemical shifts of 3 displayed upfield-shifted signals at \(\delta\textsubscript{H} 4.34\) (1H, dd, \(J = 10.5, 2.6\)), \(2.53\) (1H, m, H-2a), and \(2.33\) (1H, m, H-2b) as compared to the signals of 1 [\(\delta\textsubscript{H} 5.22\) (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 \(\delta\textsubscript{C} 68.7\) in 3 instead of \(\delta\textsubscript{C} 75.2\) 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\textsubscript{50} > 20 \(\mu\)g/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. \textsuperscript{1}H-NMR and \textsuperscript{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 \(\mu\)m, 250 \(\times\) 10 mm i.d., Japan). TLC was conducted on pre-coated Kiesegel 60 F\textsubscript{254} plates (Art. 5715; Merck, Darmstadt, Germany). Spots on the TLC were detected under UV light.

**Plant Material.** The aerial parts of \(P\). \textit{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.

**Extraction and Isolation.** The aerial parts of \(P\). \textit{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, \(\text{CHCl}_3\), EtOAc, and \(n\)-BuOH, successively. The EtOAc-soluble fraction (5.5 g) was fractionated using reversed-phase column chromatography (\(\text{H}_2\text{O}-\text{MeOH} = 4:1 \rightarrow 1:1\)) into eleven fractions (PE\textsubscript{1}-11). The PE\textsubscript{5} fraction (380 mg) was chromatographed on Sephadex LH-20 (MeOH) and then through HPLC separation (MeCN-\(\text{H}_2\text{O} = 50:50\), 4 mL/min) to yield 2 (47 mg, \(t\)\textsubscript{R} 19.5 min) and 3 (4.8 mg, \(t\)\textsubscript{R} 22.0 min). The PE\textsubscript{6} (500 mg) was applied to the separation of HPLC (MeCN-\(\text{H}_2\text{O} = 50:50\), 4 mL/min) and furnished 1 (22.0 mg, \(t\)\textsubscript{R} 34.4 min).

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

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Table 1. The \(\textsuperscript{1}H\) and \(\textsuperscript{13}C\) NMR chemical shifts of 1-3 in CD\textsubscript{3}OD

\(\delta\textsuperscript{C}\) and \(\delta\textsuperscript{H}\) values were measured in \(\text{CDCl}_3\).
m/z 422.1212 (calcd for C_{20}H_{22}O_{10}: 422.1213); [\alpha]_D^{20} = -58.3^\circ (c 0.33, \text{MeOH}); UV \lambda_{\text{max}} (\text{MeOH}) nm (log e) 238 (0.71), 333 (1.48); IR \nu_{\text{max}} (\text{KBr, cm}^{-1}) 3441, 2955, 1790, 1739, 1629, 1595, 1272; \text{^1H NMR and ^13C NMR (CD}_3\text{OD)} 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^\circ (c 0.28, \text{MeOH}); UV \lambda_{\text{max}} (\text{MeOH}) nm (log e) 236 (0.83), 330 (1.62); IR \nu_{\text{max}} (\text{KBr, cm}^{-1}) 3430, 2956, 1789, 1708, 1629, 1514, 1272; \text{^1H NMR and ^13C NMR (CD}_3\text{OD)} see Table 1.

**\(E\)-Trimethyl-1-hydroxy-3-(3-(4-hydroxy-3-methoxy-phenyl) acrylloxy)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^\circ (c 0.16, \text{MeOH}); UV \lambda_{\text{max}} (\text{MeOH}) nm (log e) 234 (0.82), 330 (1.30); IR \nu_{\text{max}} (\text{KBr, cm}^{-1}) 3442, 2953, 1737, 1630, 1595, 1514, 1271, 1158; \text{^1H NMR and ^13C NMR (CD}_3\text{OD)} 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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**References**