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PHENOLIC COMPOUNDS FROM *Clinopodium urticifolium*

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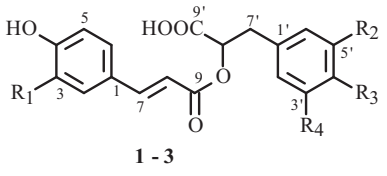
*A new phenolic compound, clinopodphenol A (**1**), together with six known ones, was isolated from the whole plant of* Clinopodium urticifolium*. The structure of **1** was elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. The anti-HIV-1 activity and cytotoxicity were evaluated for compound **1**. It showed significant potential cytotoxic ability and moderate anti-HIV-1 activity.*

Keywords: *Clinopodium urticifolium*, phenolic compounds, anti-HIV-1 activity, cytotoxicity.

Species of the genus *Clinopodium* are popular traditional Chinese medicinal herbs used to treat bruises and swelling, and are also purported to improve blood circulation [1, 2]. In recent years, several papers have described phytochemistry investigations of various species of *Clinopodium*, and it was found to be rich in saponins [3, 4], flavones [5, 6], polyphenols [7, 8], terpenes [9, 10], and the like. Saikosaponin-type saponins are characteristic of the genus *Bupleurum* (Umbelliferae) and are well known for their anti-hepatotoxic activity.

Clinopodium urticifolium belongs to the genus *Clinopodium*, which is widely distributed in Gansu, northwest China. In previous work, some bioactive compounds were isolated from this plant [9, 11, 12]. Motivated by a search for bioactive metabolites from this plant, a reinvestigation of the chemical constituents of the whole plant of *C. urticifolium* was carried out. As a result, a new phenolic compound, clinopodphenol A (**1**), together with six known phenols, was isolated from this plant. In addition, the anti-HIV-1 activity and cytotoxicity of compound **1** were evaluated. This article deals with the isolation, structural elucidation, and biological activities of the new compound.

The air-dried and powdered whole plant of *C. urticifolium* was extracted with 70% aqueous methanol and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (280 g). This crude extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and preparative HPLC to afford compounds **1–7**, including a new phenolic compound, clinopodphenol A (**1**), together with six known phenols, rosmarinic acid (**2**) [13], clinopodic acid B (**3**) [8], clinopodic acid F (**4**) [8], 4-*O*-methylcedrusin (**5**) [14], pinosylvin (**6**) [15], and catechin (**7**) [16].



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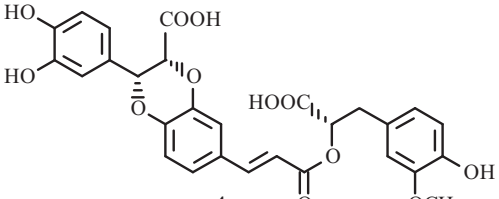
1: $R_1 = R_2 = R_3 = R_4 = OCH_3$
2: $R_1 = R_3 = R_4 = OH$, $R_2 = H$
3: $R_1 = R_3 = OH$, $R_2 = H$, $R_4 = OCH_3$



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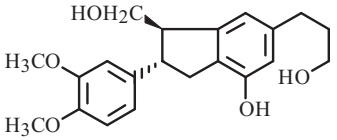

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TABLE 1. ^{1}H and ^{13}C NMR Data of Compound **1** (Py-d_5, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	129.8 s		4′	136.1 s	
2	113.2 d	6.52 s	5′	152.4 s	
3	146.6 s		6′	106.4 d	6.33 s
4	145.3 s		7′	38.5 t	2.86, m
5	111.3 d	6.74 (d, J = 8.0)			3.30 m
6	121.8 d	6.70 (d, J = 8.0)	8′	75.0 d	5.17 (t, J = 6.0)
7	143.9 d	7.69 (d, J = 15.3)	9′	173.4 s	
8	116.6 d	6.38 (d, J = 15.4)	3-OMe	55.8 q	3.83 s
9	168.1 s		3′-OMe	55.6 q	3.82 s
1′	134.8 s		4′-OMe	60.6 q	3.85 s
2′	106.4 d	6.33 s	5′-OMe	55.6 q	3.82 s
3′	152.4 s				

TABLE 2. Cytotoxicities of Clinopodphenol A (**1**)

Compound	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	3.67	6.47	7.52	8.24
Camptothecin	1.18	0.62	1.26	1.87

Data are IC_{50} values in μmol/L. For a compound to be deemed effective, an IC_{50} value < 100 μmol/L is required. Camptothecin was used as a positive control.

HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

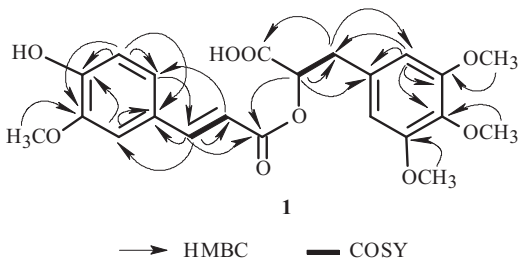

Fig. 1. Selected HMBC and ^{1}H-^{1}H COSY correlations of **1**.

Compound **1** was obtained as a pale yellow gum. Its molecular formula was determined as $C_{22}H_{24}O_9$ by HR-ESI-MS *m/z* 433.1495 $[M + H]^+$ (calcd 433.1499). Its ^{1}H and ^{13}C NMR spectra (Table 1) showed signals to 24 hydrogens and 22 carbons, respectively, corresponding to two aromatic rings with five aromatic protons (δ_C 106.4–152.4, δ_H 6.33–6.74), a pair of (*Z*) double bond with a couple of *cis*-olefinic protons (δ_C 143.9, 116.6; δ_H 7.69, d, J = 15.3, 6.38, d, J = 15.4), a methylene (δ_C 38.5, δ_H 2.86 and 3.30), an oxidated methine (δ_C 75.0, δ_H 5.17), an ester carbonyl group (δ_C 168.1), a carboxyl group (δ_C 173.4), and four methoxyl groups (δ_C 55.8, 55.6, 60.6, 55.6; δ_H 3.83, 3.82, 3.85, 3.82). Strong absorption bands accounting for the carboxyl group (3600–3050 cm^{-1}) and aromatic groups (1608, 1521, 1452 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed maximum absorption at 230, 279, and 305 nm, which confirmed the existence of the aromatic functions. The HMBC correlations of H-7 (δ_H 7.69, d, J = 15.3) with C-1 (δ_C 129.8), C-2 (δ_C 113.2), and C-6 (δ_C 121.8), and H-8 (δ_H 6.38, d, J = 15.4) with C-1 (δ_C 129.8), indicated that the double bond was linked to C-1. The HMBC correlations of H-7 (δ_H 7.69, d, J = 15.3) and H-8 (δ_H 6.38, d, J = 15.4) with C-9 (δ_C 168.1) indicated that the ester carbonyl was linked to the double bond. In addition, by the analysis of HMBC and ^{1}H–^{1}H COSY correlations (Fig. 1), the methylene can be assigned to C-7′, the oxidated methine can be assigned to C-8′, and the carboxyl can be assigned to C-9′. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of rosmarinic acid [17] (Table 1). The obvious differences are the

chemical shift and the substituent groups on aromatic rings. The ^{1}H and ^{13}C NMR data indicated that a 1,3,4-trisubstituted phenyl group bearing two oxygen atoms (δ_H 6.52 s, 6.74 d, J = 8.0, 6.70 d J = 8.0; δ_C 113.2, 111.3, 121.8), and a 1,3,4,5-tetrasubstituted phenyl group bearing three oxygen atoms (δ_H 6.33 s (2H), δ_C 106.4 d) existed in compound **1**. The HMBC correlations of four methoxyl groups (δ_H 3.83 s, 3.82 s, 3.85 s, 3.82 s) with C-3 (δ_C 55.8), C-3′ (δ_C 55.6), C-4′ (δ_C 60.6), and C-5′ (δ_C 55.6) indicated that the four methoxyl groups should be located at C-3, C-3′, C-4′, and C-5′, respectively. Thus, the structure of compound **1** was determined as shown, and this compound was given the name clinopodphenol A.

EXPERIMENTAL

General. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HR-ESI-MS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) and Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany) and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating the Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The whole plant of *C. urticifolium* was collected in Qudang Prefecture of Gansu Province, People′s Republic of China, in September 2009. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-09-09-15) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered whole plant of *C. urticifolium* (2.5 kg) was extracted three times with 70% aqueous MeOH (3 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (280 g). The crude extract was subjected to silica gel (200–300 mesh) column chromatography eluting with a $CHCl_3$–MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A–F. The separation of fraction C (22.6 g) by Si gel column chromatography eluted with $CHCl_3$–acetone (20:1–1:2) yielded mixtures B1–B6. Fraction B3 (6.87 g) was subjected to Si gel column chromatography using petroleum ether–acetone and preparative HPLC (50% MeOH-H_2O, flow rate 12 mL/min) to give compounds **1** (16.2 mg), **5** (28.2 mg), and **6** (46.4 mg). Fraction B4 (2.82 g) was subjected to Si gel column chromatography eluting with petroleum ether–acetone and then run on preparative HPLC (30% MeOH–H_2O, flow rate 12 mL/min) to yield compounds **2** (8.36 mg), **3** (9.27 mg), **4** (16.4 mg), and **7** (36.8 mg).

Clinopodphenol A (1): obtained as a pale yellow gum; $[\alpha]_D^{15.8}$ + 11.5° (*c* 0.2.2, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 305 (3.22), 279 (4.08), 230 (4.25). IR (KBr, ν_{max}, cm^{-1}): 3345, 3082, 292, 2952, 2886, 1608, 1521, 1452, 1378, 1326, 1255, 1228, 1105, 1042, 854, 812. ^{1}H NMR and ^{13}C NMR data, see Table 1; positive ESI-MS *m/z* 433 $[M + H]^+$; HR-ESI-MS *m/z* 433.1495 $[M + H]^+$.

Anti-HIV-1 Assay. Cytotoxicity against C8166 cells (CC50) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) [18]. Clinopodphenol A shows anti-HIV-1 activity with EC_{50} 1.87 μg mL^{-1}, CC_{50} 186.2 μg·mL^{-1}, and TI (therapeutic Index) 99.6.

Cytotoxicity Assays. The cytotoxicity tests for the isolates were performed using a previously reported procedure [19]. All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic ability against HL-60, Hep-G2, KB, and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) is shown in Table 2.

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