

Acetophenones from the Roots of *Cynanchum wilfordii* HEMSLEY

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Two acetophenones, cynandione A (**1**) and cynanchone A (**2**), were isolated from the roots of *Cynanchum wilfordii*. Their structures were identified by comparison of their physicochemical and spectral data with reported values.

Key words : *Cynanchum wilfordii*, Asclepiadaceae, Acetophenone, Cynandione A, Cynanchone A.

INTRODUCTION

Cynanchum wilfordii (Asclepiadaceae) is widely distributed in Korea, Japan, and China. The dried roots of this plant have been used as a tonic and to promote renal function (Perry, 1980). Study on the chemical constituents of this plant revealed several C/D-*cis*-polyoxypregnane derivatives and their glycosides of 2,6-dideoxy-3-*O*-methyl sugars (Hayashi *et al.*, 1975; Tsukamoto *et al.*, 1985a, b). In our study on the search for multidrug-resistance (MDR) modulator from natural products, methanol extract of *Cynanchum wilfordii* roots showed potent MDR reversing activity. We isolated two acetophenones (**1**, **2**) from the active fraction, but they did not show MDR modulating activity.

We describe the structure elucidation of two acetophenones isolated from the roots of *Cynanchum wilfordii* herein.

MATERIALS AND METHODS

Plant materials

Dried roots of *Cynanchum wilfordii* were obtained from a crude drug market in Taejeon and identified taxonomically with respect to morphology. A voucher specimen is deposited at Korea Research Institute of Bioscience and Biotechnology.

Instruments

Melting points were determined on Electrothermal IA 9100 Digital melting point apparatus without correction. UV spectra were obtained with Milton Roy

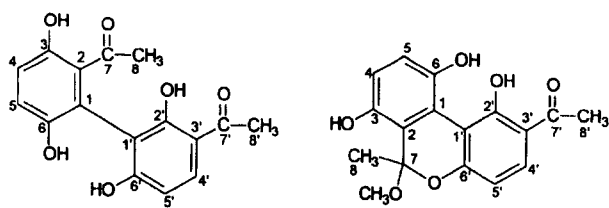
Spectronic 3000 Array. ¹H- and ¹³C-NMR, and Hetero-COSY spectra were run on Varian Unity 300 spectrometer in CDCl₃ or CD₃OD. EI-MS spectra were taken on a Hewlett-Packard MS Engine-5989 A. MPLC was run on Buchi 681 pump with UV/VIS filter photometer.

Extraction and isolation

Dried roots of *Cynanchum wilfordii* (3 kg) were extracted twice with MeOH at room temperature. Solvent was evaporated *in vacuo* to yield 480 g of MeOH extract. The MeOH extract was suspended in H₂O and extracted with dichloromethane (CH₂Cl₂). The CH₂Cl₂ extract (87 g) was subjected to a silica gel column chromatography with a step gradient solvent system of CH₂Cl₂-MeOH (CH₂Cl₂:MeOH=20:1, 10:1, 5:1, 2:1) as eluents to give six fractions. Fraction 2 was rechromatographed on a silica gel column using a hexane-acetone gradient system (6:4, 5:5, 4:6, 2:8, acetone) to give six fractions. The third fraction was further separated by reversed-phase MPLC with a H₂O-MeOH gradient system to give seven fractions. The second fraction was purified on a silica gel column with CH₂Cl₂:MeOH (30:1) afforded two acetophenones, compound **1** (14.3 mg) and compound **2** (9.1 mg).

Compound 1: Yellow needles (CH₂Cl₂), mp 198-200°C, UV λ_{max} (MeOH): 214, 282, 318, EI-MS *m/z* (rel. int.): 302 [M]⁺ (8), 284 [M-H₂O]⁺ (100), 266 (25), 237 (5), ¹H-NMR δ (CD₃OD, 300 MHz): 2.17 (3H, s, 8-CH₃), 2.56 (3H, s, 8'-CH₃), 6.49 (1H, d, *J*=8.9 Hz, 5'-H), 6.79 (1H, d, *J*=8.7 Hz, 4-H), 6.93 (1H, d, *J*=8.7 Hz, 5-H), 7.79 (1H, d, *J*=8.9 Hz, 4'-H), ¹³C-NMR δ (CD₃OD, 75 MHz): 26.3 (C-8'), 30.8 (C-8), 108.7 (C-5'), 113.1 (C-3'), 114.5 (C-1'), 118.2 (C-4), 120.3 (C-2), 121.7 (C-5), 127.8 (C-1), 133.9 (C-4'), 149.0 (C-6), 152.3 (C-3), 163.7 (C-2', 6'), 203.5 (C-7'), 207.3 (C-7).

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Compound 1

Compound 2

Fig. 1. Chemical structures of the isolated compounds

Compound 2: Yellow needle crystals, UV λ_{\max} (MeOH): 222, 263, 307, EI-MS m/z (rel. int.): 316 $[M]^+$ (4), 285 $[M-Ome]^+$ (20), 284 (100), 266 (45), 237 (10), 1H -NMR δ ($CDCl_3$, 300 MHz): 1.60 (3H, s, 8- CH_3), 2.67 (3H, s, 8'- CH_3), 3.52 (3H, s, $-OCH_3$), 6.74 (1H, d, $J=8.7$ Hz, 5'-H), 6.91 (1H, d, $J=8.9$ Hz, 5-H), 7.03 (1H, d, $J=8.9$ Hz, 4-H), 7.46 (1H, s, 6-OH), 7.77 (1H, d, $J=8.7$ Hz, 4'-H), 8.18 (1H, s, 3-OH), 15.57 (1H, s, 2'-OH), ^{13}C -NMR δ ($CDCl_3$, 75 MHz): 24.0 (C-8), 26.2 (C-8'), 51.5 ($-OCH_3$), 104.8 (C-7), 110.9 (C-1', 5'), 119.0 (C-1, 5), 115.1 (C-3'), 115.3 (C-2), 122.3 (C-4), 131.8 (C-4'), 146.2 (C-6), 147.2 (C-3), 158.6 (C-2'), 159.1 (C-6'), 204.5 (C-7').

RESULTS AND DISCUSSION

Compound 1, $C_{16}H_{14}O_6$, gave a yellow needle crystals. The UV spectrum of 1 have λ_{\max} at 214, 282 and 318 nm. The EI-MS spectrum of 1 exhibited a signal for molecular ion peak at m/z 302 and a base peak at m/z 284 $[M-H_2O]^+$. The 1H -NMR spectrum of 1 showed two acetyl signals at δ 2.17 and 2.56, and two pairs of ortho coupled aromatic protons; one coupling between δ 6.49 (1H, d, $J=8.9$ Hz) and 7.79 (1H, d, $J=8.9$ Hz), the other between δ 6.93 (1H, d, $J=8.7$ Hz) and 6.79 (1H, d, $J=8.7$ Hz). ^{13}C -NMR spectrum of 1 showed 16 carbon signals including 4 phenolic carbons and 8 aromatic carbons, suggesting dimeric structure of dihydroxyacetophenone. All the chemical shifts of 1 was well agreed to those of cyandione A which was isolated from *Cynanchum taiwanianum* (Lin *et al.*, 1997; Lin *et al.*, 1997a; Huang *et al.*, 1995). Based on the physico-chemical and spectral data, 1 was identified to be as cyandione A (2,3'-diacetyl-3,6,2',6'-tetrahydroxybiphenyl). Recently cyandione A was isolated from *Cynanchum wilfordii* along with five benzoquinone derivatives but the position of one acetyl group was determined differently from 1 (Yeo *et al.*, 1997).

Compound 2, $C_{17}H_{16}O_6$, gave a yellow needle crystals and UV spectrum showed similar pattern of λ_{\max} to those of 1. Its EI-MS spectrum exhibited a signal for molecular ion peak at m/z 316 and fragment ion peaks at 284 (base peak), 266 and 237, which were similar to those of 1. 1H -NMR spectrum of 2 also

showed two pairs of ortho coupled aromatic protons; one set between δ 6.74 (d, $J=8.7$ Hz) and 7.77 (d, $J=8.7$ Hz) and the other between δ 6.91 (d, $J=8.9$ Hz) and 7.03 (1H, d, $J=8.9$ Hz), which were similar to those of 1. But, three methyl signals were appeared at δ 1.60 (3H, s) for a methyl signal, at δ 2.67 (3H, s) for an acetyl group and at δ 3.52 (3H, s) for a methoxyl signal. This compound revealed one intramolecular hydrogen-bonded proton signal at δ 15.57 arising from the carbonyl group at C-7' and C-2' hydroxy group. ^{13}C -NMR spectrum of 2 showed 17 carbon signals which were assigned by comparison with those reported from *Cynanchum taiwanianum* (Lin *et al.*, 1997; Lin *et al.*, 1997b; Huang *et al.*, 1996). The structure of 2 was identified as cynanone A, which was isolated for the first time from *Cynanchum wilfordii*.

ACKNOWLEDGEMENTS

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