Pergamon

Phytochemistry, Vol. 46, No. 6, pp. 1103–1105, 1997

Printed in Great Britain
0031-9422/97 $17.00 + 0.00

PII: S0031-9422(97)00403-2

A BENZOQUINONE FROM *CYNANCHUM WILFORDII*

HOSUP YEO and JINWOONG KIM*

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

(*Received in revised form* 14 *March* 1997)

Key Word Index—*Cynanchum wilfordii*; Asclepiadaecea; acetophenones; 2-acetyl-3,6-diamino-1,4-benzoquinone.

Abstract—A new amino-substituted *p*-benzoquinone was isolated from the roots of *Cynanchum wilfordii*, together with the five known acetophenones: 2′,5′-dihydroxyacetophenone, 4′-hydroxyacetophenone, 2′,4′-dihydroxyacetophenone, 4′-hydroxy-3′-methoxyacetophenone and cynandione A. The structure of new compound was elucidated as 2-acetyl-3,6-diamino-1,4-benzoquinone. © 1997 Elsevier Science Ltd

INTRODUCTION

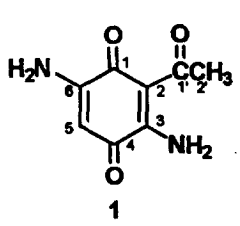
Cynanchum wilfordii Hemsley has been used as a tonic in Korea [1, 2]. The isolation of several C/D-*cis*-polyoxypregnane esters and their glycosides from this plant has already been reported [3–5]. In this paper, we report on the isolation and structural elucidation of a new benzoquinone, 2-acetyl-3,6-diamino-1,4-benzoquinone (**1**), along with five known acetophenones (**2–6**).

RESULTS AND DISCUSSION

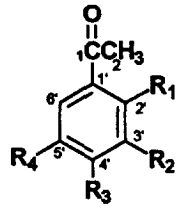
The 80% MeOH extract of dried root of *C. wilfordii* was extracted with *n*-hexane, chloroform and *n*-BuOH. Repeated chromatography of the first two extracts on silica gel afforded **1** and **2** from the chloroform extract and **3–6** from the *n*-hexane extract, respectively. Compounds **2–6** were identified as 2′,5′-dihydroxyacetophenone (**2**) [6], 4′-hydroxyacetophenone (**3**) [7], 2′,4′-dihydroxyacetophenone (**4**) [8], 4′-hydroxy-3′-methoxyacetophenone (**5**) [9] and cynandione A (**6**) [10] by comparison with previously reported spectroscopic data.

The molecular formula of **1** ($C_8H_8N_2O_3$) was assigned by HR EI mass spectroscopy and the ^{13}C NMR data. Compound **1** was isolated as a dark red amorphous powder and reacted positively to the ninhydrin test. Its mass spectrum showed fragment ion peaks at *m/z* 165, 137, 109 and 81 formed by the stepwise loss of an acetyl group and two molecules of carbon monoxide from the molecular ion (*m/z* 180). These findings indicated the presence of an acetyl-substituted benzoquinone moiety in **1** [11], and this was further supported by the quinonoid (δ 174.09, 177.22) and acetyl (δ 198.23, 32.18) signals in the ^{13}C NMR and DEPT spectra. In the UV-visible spectrum, the strong bathochromic shifts of the $\pi \rightarrow \pi^*$ transition at 304 and 325 nm, compared with those of a 1,4-benzoquinone (λ_{max} at 246 and 288 nm), suggested the presence of electron-donating substituents such as amino or polyalkoxy (polyhydroxy) groups [12]. Its IR spectrum exhibited the absorption bands for a primary amino group at 3339 and 3294 cm^{-1}. The three intensive bands at 1600, 1560 and 1543 cm^{-1}, shifted to lower wavenumbers when compared with the *p*-quinonoid carbonyl absorption of a parent 1,4-benzoquinone (1671 cm^{-1}), revealed the tautomeric interconversion of **1** to a coupled merocyanine structure as well as the presence of hydrogen bonding between the amino groups in the *ortho* position and the quinone carbonyl group [12–14]. ^1H-^1H and ^1H-^{13}C COSY experiments showed that **1** was a trisubstituted *p*-benzoquinone with an acetyl [δ_H 2.46 (3H, *s*)] and two primary amino [δ_H 10.85, 8.95, 8.26 and 7.61 (each 1H, *br s*)] groups, which were further supported by the two significant mass fragment peaks resulting from the fission of the diamino-benzoquinone ion into two halves with hydrogen transfers (*m/z* 68) and the formation of the diamino-cyclobutadiene(?) ion (*m/z* 81) [11, 12, 15]. As a result of restricted rotation, both NH_2 groups showed four broad signals which were lost on D_2O exchange [13, 14, 16]. In the ^{13}C NMR data, the chemical shifts for the quinone carbonyl carbons at δ 177.22 and 174.09 (C-1, C-4) and the amino substituted carbons at δ 157.39 and 154.34 (C-3, C-6) fell within the very narrow range of about 3 ppm. Thus, the two amino substituents must be located at C-3,6 or C-5,6 of the 2-acetyl-1,4-benzoquinone skeleton, which was supported by the small difference in frequencies for two

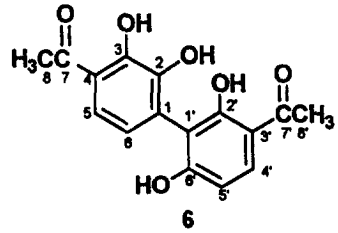
* Author to whom correspondence should be addressed.



1



	R_1	R_2	R_3	R_4
2	OH	H	H	OH
3	H	H	OH	H
4	OH	H	OH	H
5	H	OMe	OH	H



6

quinonoid carbonyl bands in the IR spectrum [12]. The positions of the acetyl and amino substituents in **1** were confirmed from the proton-detected heteronuclear multiple bond correlation (HMBC) spectrum, which showed distinct cross-peaks of correlation through three bonds from the quinonoid proton at δ 5.61 (H-5) to the amino-substituted carbon at δ 157.39 (C-3) and the quinone carbonyl carbon at δ 177.22 (C-1) signals, whereas the carbonyl carbon of the acetyl group at δ 198.23 (C-1′) was not connected with the quinonoid proton (H-5). Moreover, the cross-peaks were not observed from the quinonoid proton (H-5) to the carbon signals at δ 154.34 (C-6) and δ 174.09 (C-4) since the two-bond C—H coupling constant is about zero in 1,4-benzoquinone [17]. From all the above data, the structure of **1** was elucidated as 2-acetyl-3,6-diamino-1,4-benzoquinone. Compound **1** has not been reported previously in nature although **1** was known as a synthetic compound [14].

EXPERIMENTAL

General. Mps: uncorr.; EIMS and HR-EIMS: VG-Trio 2 and Hewlett-Packard 5890-JMS AX505WA mass spectrometer at 70 eV, respectively; NMR: 400 MHz (^{1}H) and 100 MHz (^{13}C), with reference to the residual solvent signals; TLC: precoated silica gel 60 F_{254} (Merck) and detection by spraying with anisaldehyde–10% H_2SO_4 following by heating; CC: silica gel (230–400 mesh, Merck).

Plant material. The roots of *Cynanchum wilfordii* Hemsley were purchased from a commercial supplier in Seoul, Korea, and identified by Dr Dae Suk Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the herbarium of our institute.

Extraction and isolation. The air-dried roots of *C. wilfordii* (5 kg) were cut into pieces and extracted with 80% MeOH. The MeOH extract was evapd *in vacuo* to give a crude extract (950 g), which was successively extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH. The $CHCl_3$ layer (126 g) was fractionated by CC over silica gel using a $CHCl_3$–MeOH gradient to give 15 frs. Fr. 6 (14.7 g) was subjected to repeated CC over silica gel with *n*-hexane-EtOAc (20:1) and *n*-hexane-Me_2CO (4:1) to give 2-acetyl-3,6-diamino-1,4-benzoquinone (**1**) (3.4 mg) and 2′,5′-dihydroxyacetophenone (**2**) (12 mg). The *n*-hexane layer (10.5 g) was chromatographed on silica gel, eluting with a *n*-hexane-EtOAc gradient to afford 4′-hydroxyacetophenone (**3**) (44 mg), 2′,4′-dihydroxyacetophenone (**4**) (143 mg), 4′-hydroxy-3′-methoxyacetophenone (**5**) (13 mg) and cynandione A (**6**) (27 mg).

2-Acetyl-3,6-diamino-1,4-benzoquinone (**1**). Dark red amorphous powder, mp > 300°; HR-EIMS, Found, *m/z* 180.0536; Calcd. for M^+, $C_8H_8N_2O_3$: 180.0535; EIMS *m/z* (rel. int.): 180 $[M]^+$ (47), 165 (11), 152 (6), 137 (45), 124 (8), 109 (18), 82 (9), 81 (7), 68 (100), 55 (9), 54 (22); UV λ_{max}^{DMSO} nm (log ε): 477 (2.87), 325 (4.34), 304 (4.21); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3339 and 3294 (NH_2), 1600, 1560, 1543, 1459; ^{1}H NMR (DMSO-d_6): δ 2.46 (3H, *s*, 2′-Me), 5.61 (1H, *s*, H-5), 7.61, 8.26, 8.95, 10.85 (each 1H, *br s*, 3-NH_2 and 6-NH_2); ^{13}C NMR (DMSO-d_6): δ 32.18 (C-2′), 96.65 (C-5), 104.77 (C-2), 154.34 (C-6), 157.39 (C-3), 174.09 (C-4), 177.22 (C-1), 198.23 (C-1′).

Acknowledgements—This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the Research Centre for New Drug Development at Seoul National University.

REFERENCES

1. Namba, T., *The Encyclopedia of Wakan-Yaku with Color Pictures*, Vol. 1. Hoikusha, Osaka, 1993, p. 77.
2. Han, D. S., *Korean Journal of Pharmacognosy*, 1973, **4**, 83.
3. Tsukamoto, S., Hayashi, K. and Mitsuhashi, H., *Chemical and Pharmaceutical Bulletin*, 1985, **33**, 2294.
4. Hayashi, K. and Mitsuhashi, H,., *Chemical and Pharmaceutical Bulletin*, 1975, **23**, 139.
5. Tsukamoto, S., Hayashi, K. and Mitsuhashi, H., *Tetrahedron*, 1985, **41**, 927.
6. Patra, A., Ghosh, G., Sengupta, P. K. and Nath, S., *Magnetic Resonance Chemistry*, 1987, **25**, 734.
7. Li, J., Kadota, S., Kawata, Y., Hattori, M., Xu, G.-J. and Namba, T. *Chemical and Pharmaceutical Bulletin*, 1992, **40**, 3133.

8. Pelter, A., Ward, R. S. and Bass, R. J., *Journal of the Chemical Society, Perkin Transactions I*, 1978, 666.
9. Junior, P., *Planta Medica*, 1986, **52**, 218.
10. Huang, P.-L., Lu, C.-M., Yen, M.-H., Wu, R.-R. and Lin, C.-N., *Phytochemistry*, 1995, **40**, 537.
11. Williams, D. H., Budzikiewicz, H. and Djerassi, C., *Mass Spectrometry of Organic Compounds*. Holden-Day, San Francisco, 1967, p. 527.
12. Berger, S., Hertl, P. and Rieker, A., in *The Chemistry of Quinonoid Compounds*, Vol. 2, ed. S. Patai and Z. Rappoport. Wiley, Chichester, 1988, p. 29.
13. Moore, H. W., Shelden, H. R. and Shellhamer, D. F., *Journal of Organic Chemistry*, 1969, **34**, 1999.
14. Schäfer, W. and Schlude, H., *Tetrahedron Letters*, 1967, 4307.
15. Thomson, R. H., *Naturally Occurring Quinones*, 2nd edn. Academic Press, London, 1971, p. 39.
16. Tresselt, D., Eckardt, K., Ihn, W., Radics, L. and Reinhardt, G., *Tetrahedron*, 1981, **37**, 1961.
17. Kalinowski, H.-O., Berger, S., Braun, S., *Carbon-13 NMR Spectroscopy*. Wiley, Chichester, 1988, p. 523.