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A benzoquinone and flavonoids from *Cyperus alopecuroides*

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Abstract

A benzoquinone, named alopecuquinone, was isolated from the ethanol extract of the inflorescences of *Cyperus alopecuroides*. Its structure was primarily elucidated by spectroscopic analysis including ^{1}H, ^{13}C NMR, APT, HMQC, ^{1}H–^{1}H COSY and CIMS. The known flavonoids, vicenin 2, orientin, diosmetin, quercetin 3,3′-dimethyl ether and its 3,4′-dimethyl ether, were also isolated and characterized. The ethanol extract of the plant material showed moderate estrogenic activity using a strain of *Saccharomyces cerevisiae*.

Keywords: *Cyperus alopecuroides*; Cyperaceae; Benzoquinones; Alopecquinone; Flavonoides; Estrogenic activity

1. Introduction

Cyperus alopecuroids Rottb. (family Cyperaceae) is a huge sedge, widely distributed in tropical areas and occurs in Egypt in the Nile region, including the Delta and the Valley (Tackholm, 1974). It has been reported that *Cyperus* species have medicinal effects such as pectoral emollient, analgesic and anthelmintic (Boulos, 1983). A coumaran and quinones from *Cyperus* species have showed anti-feedant activity (Morimoto et al., 1999). The genus *Cyperus* is characterized by the presence of quinones (Allan et al., 1969, 1973, 1978; Alves et al., 1992), flavonoids (Seabra et al., 1995, 1997) and sesquiterpenes (Hikino and Aota, 1976; Nyasse et al., 1988). Continuing our previous studies on the genus *Cyperus* (Nassar et al., 1998, 2000), we report here on the isolation and identification of a new benzoquinone **1**, for which we propose the name alopecuquinone, together with five known flavonoids. The estrogenic activity of the ethanol extract of the inflorescences of *C. alopecuroides* and the major isolated compounds are described.

2. Results and discussion

The estrogenically active ethanol extract of *C. alopecuroides* was subjected to column chromatography of polyamide 6S. Several chromatographic runs on Sephadex LH-20 led to the isolation of a new benzoquinone, alopecuquinone (**1**), as well as five known flavonoids. The known flavonoids, vicenin 2, orientin, diosmetin, quercetin 3,3′-dimethyl ether and quercetin 3,4′-dimethyl ether were all identified by comparison of their UV, ^{1}H and ^{13}C NMR with literature data (Harborne, 1988; Agrawal, 1989). The ^{1}H NMR spectrum of compound 1 displayed six signals, each integrated for two equivalent protons. The CIMS spectrum, which showed a molecular ion peak at m/z 314.094, $C_{14}H_{18}O_8$, indicated that the compound has a symmetrical skeleton. The narrow doublet signal ($J=1.6$ Hz) at δ 6.6 can be attributed to the two olefinic protons H-2 and H-3 of the benzoquinone moiety. The three signals at δ 4.2 (2H, *br*. S, H-5, H-12), δ 3.8 (2 H, *dt*, $J=6.1$, 4.2 Hz, H-7, H-10) and δ 3.5 (2 H, *dd*, $J=5.8$, 4.3 Hz, H-6, H-11) are in accord with six protons, with each proton geminal to a hydroxyl group. The two methylene groups appeared as multiplets at δ 2.4 (H-8_a, H-9_a) and δ 2.0 (H-8_b, H-9_b). The coupling constants of protons at δ 3.5, δ 3.8 and δ 4.2 suggested a proton geminal to an exo (α) hydroxyl group at both C-7 and C-10 and a proton geminal to an

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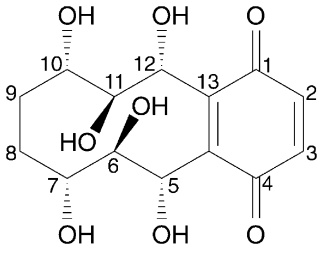

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endo (β) hydroxyl group at both C-6 and C-11 and a proton geminal to an exo (α) hydroxyl group at both C-5 and C-12. The location of the hydroxyl groups was confirmed by ^{1}H–^{1}H COSY. The protons at δ 3.8 (H-7, H-10) showed correlations with protons of the methylene groups at δ 2.0 and δ 2.4 as well as with the protons at δ 3.5 (H-6, H-11). Additionally, the olefinic protons at δ 6.6 showed correlations with the protons at δ 4.2 (H-5, H-12). The ^{13}C NMR spectrum of compound **1** exhibited seven carbon signals, with each signal corresponding to two equivalent carbon atoms. The protonated carbon signals were assigned by HMQC experiments whereas multiplicities were determined by APT experiments, which indicated four quaternary, eight methine and two methylene carbons. The relatively up-field shift of the two carbonyl carbons to δ 168.1 can be attributed to hydrogen bonding between the hydroxyl groups at C-5 and C-12 with the carbonyl groups C-4 and C-1, respectively, supporting the suggestion of the exo orientation of the hydroxyl groups at C-5 and C-12. Therefore, compound **1** is 5α,6β,7α, 10α,11β,12α-hexahydroxycyclodeca-1,4-benzoquinone, which is named here alopecuquinone.

The preliminary testing of the ethanolic extract of the *C. alopecuroides* inflorescences showed some estrogenic activity with a strain of *Saccharomyces cereviseae* as measured by the activation of the transcription of β-galactosidase. The transcription system in *S. cereviseae* is a powerful screening tool for phytoestrogens (Maier et al., 1995). The estrogenic activity, measured in Miller Units [Δabs.(1000) min^{-1} protein mg^{-1}], for 2.73×10^{-3} g of plant extract was equivalent to 2×10^{-9} g of 17-β-estradiol. Additionally, the activity of the three major isolated compounds were measured. Relative to estradiol, both quercetin 3,4′-dimethyl ether and diosmetin showed weak activity and orientin none.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Bruker AMX and/or a Varian Unity Inova with TMS as an internal reference and DMSO-d_6 as solvent. Mass spectra were obtained using a Finigan MAT TSQ 700 spectrometer, while UV in MeOH were determined using a Shimadzu UV 240 spectrophotometer and IR spectra were recorded in Mattson 5000 FTIR spectrometer.

3.2. Plant material

The inflorescences of *C. alopecuroides* were collected in March 1999 from Mansoura, Egypt and identified by Dr. I.A. Mashaly, Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt. A voucher specimen is deposited in the Herbarium of the National Research Centre, Dokki, Cairo, Egypt.

3.3. Extraction and isolation

The dried powdered inflorescences of *C. alopecuroides* (1300 g) were extracted with 70% ethanol. The ETOH extract was evaporated under reduced pressure affording 110 g of crude extract. The latter was extracted with CH_2Cl_2 and the residue (50 g), which showed estrogenic activity, was subjected to a polyamide 6S column chromatography. The column was eluted with water/ethanol step gradient. The eluted fractions were examined by PC using *n*-butanol–acetic acid–water (BAW) and 15% acetic acid for elution. Final purification was effected by a Sephadex LH-20 column using MeOH–H_2O (1:1) as eluent to give 10 mg alopecuquinone, 15 mg vicenin 2, 30 mg orientin, 25 mg diosmetin, 10 mg quercetin 3,3′-dimethyl ether and 40 mg quercetin 3,4′-dimethyl ether.

3.4. 5α,6β,7α,10α,11β,12α-Hexahydroxycyclodeca-1,4-benzoquinone, (alopecuquinone, **1**)

Orange amorphous material. UV/λ_{max} (MeOH) 260, 320, 350, 400 nm; IRν_{max} (film) 3458 (OH), 2920, 1648 (C=O), 1700, 1620, 1403, 945 cm^{-1}; ^{1}H NMR (DMSO-d_6); δ 6.6 (2H, *d*, $J=1.6$ Hz, H-2,3), 4.2 (2H, *brS*, H-5, 12), 3.8 (2H, *dt*, $J=6.1$, 4.2 Hz, H-7, 10), 3.5 (2H, *dd*, $J=5.8$, 4.3, H-6, 11), δ 2.4 (2H, *m*, H-8_a, 9_a), 2.0 (2H, *m*, H-8_b, 9_b); ^{13}C NMR (125 MHz, DMSO-d_6): 168.1 (C-1,4), 138.7 (C-2,3), 128.5 (C-13, 14), 70.4 (C-6,11), 66.9 (C-7, 10), 65.5 (C-5, 12), 29.9 (C-8, 9). CIMS: (*m*/*z*) 314.094 ($C_{14}H_{18}O_8$, ([M^+]).

3.5. Estrogenic activity test with a β-galactosidase assay

The estrogenic activity of the ethanol extract under investigation and of the three major isolated compounds were determined by using a strain of *S. cereviseae* BJ3505 and measuring the level of β-galactosidase expressed in the yeast cells. The *S. cereviseae* strain, which was transformed with the human estrogen receptor expression plasmid and estrogen responsive reporter

plasmid, was employed as a screening technique for phytoestrogen activity (Santiso-Mere, et al., 1991). Ampicillin-resistant yeast cultures were selected for and grown in liquid media overnight at 30 °C and 200 rpm in the presence of $CuSO_4$ to induce formation of the estrogen receptors (Maier et al., 1995). In addition either estradiol or known phytoestrogen (positive control), ethanol (negative control) or plant extract were added to the assay tubes. When an approximate density of $A_{600} = 1.0$ was reached, the solution was centrifuged and supernatant discarded. The yeast cells were resuspended in 1 ml transcriptional-assay buffer (6×10^{-2} M $Na_2HPO_4{\cdot}H_2O$, 4×10^{-2}M $NaH_2PO_4{\cdot}H_2O$, 10^{-2}M $MgSO_4{\cdot}7H_2O$ and 0.27% (v/v) mercaptoethanol, pH 7.0) and lysed by glass bead homogenization in a microcentrifuge at 4 °C and 10,000 rpm for 10 min. The supernatant was then analyzed for crude protein content using the Bio-Rad/Bradford Protein Concentration Assay using bovine serum albumin as a standard (Bradford, 1976). β Galactosidase levels are dependant on the binding of the compound to the estrogen receptor and can be measured according to Miller (1972). Equivalent amounts of yeast cell protein (1–100 μg dependant on expected activity) were incubated with 1 ml transcriptional-assay buffer for 5–10 min at room temperature; 200 μg of ONPG (4 mg/ml) were added and allowed to react at room temperature for 30 min. The reaction was stopped by the addition of 500 μl of 1 M Na_2CO_3. β-Galactosidase activity was measured at 420 nm and converted into Miller units [Δabs. (1000) min^{-1} protein mg^{-1}].

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