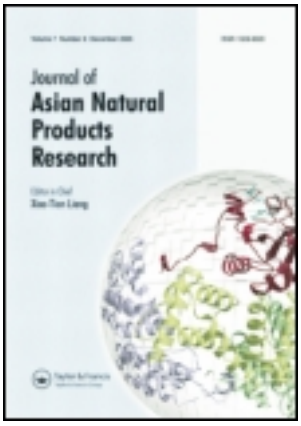

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Cichorin A: a new benzo-isochromene from *Cichorium intybus*

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One new benzo-isochromene, named cichorin A (**1**), together with three known compounds oleanolic acid, β-sitosterol, and β-sitosterol glucopyranoside, was isolated from *Cichorium intybus*. The structure of the new compound was elucidated by detailed spectroscopic analysis such as ^{1}H, ^{13}C NMR, COSY, HMQC, HMBC, and HR-EI-MS. Relative configuration of asymmetric centers of cichorin A (**1**) was determined by the analysis of the ^{1}H NMR coupling constants together with the NOESY experiment.

Keywords: benzo-isochromene; *Cichorium intybus*; Asteraceae; cichorin A

1. Introduction

Cichorium intybus L. is a medicinally important plant that belongs to the family Asteraceae (tribe Lactuceae). The root of *C. intybus* is used as anti-hepatotoxic, anti-ulcerogenic, and anti-inflammatory [1]. *Cichorium intybus* has a great value for its tonic effect upon the liver and the digestive tract, and it is also useful in the treatment of anorexia and dyspepsia [1]. Some of the compounds isolated from *C. intybus* play a role in chemical defense of chicory plant as antifeedants and possess cytotoxic activity toward cultured cancer cells. Pharmacological studies of the root extracts from *C. intybus* have shown their anti-inflammatory and hepatoprotective activities [2]. In the course of phytochemical studies of medicinal plants from Pakistan and Africa [3–11], we investigated *C. intybus* and obtained a new benzo-isochromene compound, cichorin A (**1**). Here, we describe the isolation and structural elucidation of cichorin A (**1**).

2. Results and discussion

Cichorium intybus was extracted with MeOH. The crude extract was fractionated on a silica gel column and yielded pure new compound cichorin A (**1**). The structure was elucidated by careful spectroscopic analysis (Figure 1).

Compound **1** was obtained as an amorphous powder. The IR spectrum showed absorption bands for hydroxyl group (3400 cm^{-1}) and aromatic ring (1600 cm^{-1}). A $[M]^{+}$ peak at *m/z* 284.1408 in the HR-EI-MS, along with

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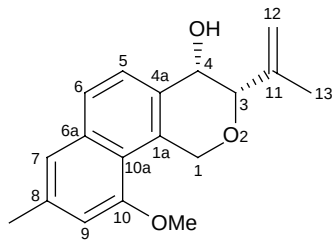

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Figure 1. Chemical structure of cichorin A (**1**).

the analysis of ^{1}H, ^{13}C NMR, and DEPT spectra, showed a molecular formula of $C_{18}H_{20}O_3$, indicating nine degrees of unsaturation.

The ^{1}H NMR spectrum of **1** (see Experimental section 3) showed an *ortho*-coupled aromatic proton signals at δ 7.50 (1H, d, $J = 9.0$ Hz, H-6), 6.85 (1H, d, $J = 9.0$ Hz, H-5), two *meta*-coupled aromatic proton signals at δ 6.83 (1H, d, $J = 2.0$ Hz, H-7), 6.35 (1H, d, $J = 2.0$ Hz, H-9), one methoxy singlet at δ 3.99 (3H, s, OMe), and one aromatic methyl singlet at δ 2.20 (3H, s, Me-8). The ^{1}H NMR spectrum in $CDCl_3$ also showed two oxymethine proton signals at δ 5.10 (1H, d, $J = 2.5$ Hz, H-4), 4.30 (1H, d, $J = 2.5$ Hz, H-3) and one oxymethylene signal at δ 5.01 (2H, br s, H-1). Furthermore, ^{1}H NMR spectrum (see Experimental Section) also showed one olefinic methyl at δ 1.67 (3H, s, H-13) and signals for exocyclic methylene at δ 4.85 (br s, H-12a) and 4.90 (br s, H-12b).

The ^{13}C NMR spectrum of compound **1** showed signals for six methines including four downfielded methine signals [δ 132.2 (C-6), 121.5 (C-5), 117.7 (C-9), and 117.5 (C-7)] for four aromatic protons, three methyls, two methylenes including one exocyclic methylene signal [δ 114.4 (C-12)], and seven quaternary carbons. In addition, the key HMBC correlations (Figure 2) of H-4 with C-3, C-5, and C-11, of H-3 with C-4, C-12, and C-13, of H-1 with C-3 and C-4a, of H-5 with C-4 and C-6, of H-9 with C-7, C-8, and C-10, and of H-7 with C-6, C-8, and C-9 verified the core benzo-isochromene. The HMBC correlations of aromatic methyl signal (δ 2.20) with C-7, C-8, and C-9 confirmed its attachment to C-8. Similarly, the position of methoxy group at C-10 was confirmed from its HMBC correlation to C-10. Attachment of aromatic system to C-1 and C-4 was confirmed from HMBC correlations of H-1 with C-1a and C-10a, and H-4 with C-4a and 5.

The stereochemistry of asymmetric centers C-3 and C-4 of **1** was mainly determined by the coupling constant of H-3 and H-4 and NOESY experiment. The small coupling constant ($J = 2.5$ Hz) between them and the obvious NOESY correlation between H-3 and H-4 indicated their *cis* configuration.

Consequently, the structure was established to be 10-methoxy-8-methyl-3-(prop-1-en-2-yl)-3,4-dihydro-1*H*-benzo[-h]isochromen-4-ol (**1**, Figure 1), named cichorin A.

The known compounds β-sitosterol [12], β-sitosterol glucopyranoside [13], and oleanolic acid [14] were identified by comparing their physical and spectral properties with those reported in the literature.

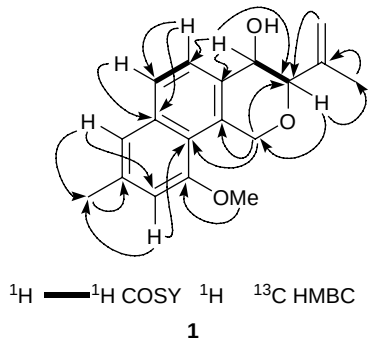

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Figure 2. Key COSY and HMBC correlations for cichorin A (**1**).

3. Experimental

3.1 General experimental procedures

IR spectra were recorded from Nicolet-510P spectrophotometer; ν_{max} in cm^{-1}. The ^1H NMR spectra were recorded on Bruker AMX-500 instruments using TMS as an internal reference. The chemical shifts are reported in parts per million (δ) while the coupling constants (J) are reported in hertz. The ^{13}C NMR spectra were recorded at 125 MHz on the same instrument. EI-MS and HR-EI-MS were carried out using MAT 8200 and Micromass LCT mass spectrometers in *m/z*.

Column chromatography (CC) was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Aluminum sheets precoated with silica gel 60 F 254 (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as the spray reagent.

3.2 Plant material

Whole plants of *C. intybus* were collected at Parachinar Kurram Agency, N.W.F.P Pakistan, in July 2005, and identified by Dr Jahandar Shah (plant taxonomist) of Peshawar University, Pakistan. A voucher specimen (No. ICP-29) has been deposited at the herbarium of the Botany Department, University of Peshawar.

3.3 Extraction and isolation

The air-dried whole plants (2 kg) of *C. intybus* were exhaustively extracted with MeOH at room temperature. The extract was evaporated to dryness yielding 60 g of residue. The residue was subjected to CC (silica gel, *n*-hexane, *n*-hexane–EtOAc and EtOAc, in an order of increasing polarity) yielding 13 fractions. Fraction F_5 (120 mg) was eluted with a mixture of *n*-hexane–EtOAc (2.5:7.5) yielding cichorin A (**1**) (6.0 mg), while fraction F_3 (200 mg) eluted with *n*-hexane–EtOAc (8.5:1.5) afforded oleanolic acid (80 mg). Similarly, β-sitosterol (11.1 mg) was isolated from the fraction F_2 (2 g), after elution with a mixture of *n*-hexane–EtOAc (8.5:1.5) and β-sitosterol glucopyranoside (40 mg) was isolated from fraction F_7 (160 mg) with *n*-hexane–EtOAc (2.5:7.5).

3.3.1 Cichorin A (*1*)

White solid. $[\alpha]_D^{29}$ +21 (*c* 0.20, CH_2Cl_2); IR (KBr) ν_{max}: 3400, 2963, 1600, 1420, 1000 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.67 (3H, s, 1H, H-13), 2.20 (3H, s, Me-8), 3.99 (3H, s, OMe), 4.30 (1H, d, $J = 2.5$ Hz, H-3), 4.85 (br s, H-12a), 4.90 (br s, H-12b), 5.01 (2H, br s, H-1), 5.10 (1H, d, $J = 2.5$ Hz, H-4), 6.35 (1H, d, $J = 2.0$ Hz, H-9), 6.83 (1H, d, $J = 2.0$ Hz, H-7), 6.85 (1H, d, $J = 9.0$ Hz, H-5), 7.50 (1H, d, $J = 9.0$ Hz, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.3 (C-10), 144.3 (C-11), 135.1 (C-8), 132.4 (C-6a), 132.3 (C-4a), 132.2 (C-6), 125.8 (C-1a), 121.5 (C-5), 119.4 (C-10a), 117.7 (C-9), 117.5 (C-7), 114.4 (C-12), 78.6 (C-3), 69.3 (C-4), 69.2 (C-1), 62.7 (OMe), 20.9 (Me-8), 18.5 (C-13). EIMS: *m/z* (%) 284.1 (17) $[M]^+$. HR-EI-MS: *m/z* 284.1412 $[M]^+$ (calcd for $C_{18}H_{20}O_3$, 284.1408).

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