Cinerascenone, A New Flavonoid from *Vernonia cinerascens*

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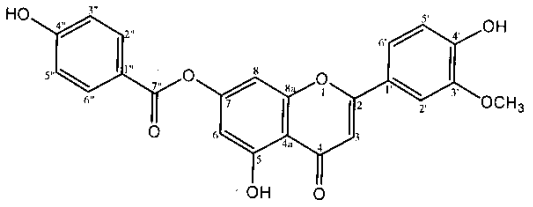
Summary: Cinerascenone **1** [7-{(4-hydroxybenzoyl)chrysoeriol}] a new flavonoid along with quercetin and 3′-methylquercetin have been isolated from the methanolic extract of the aerial parts of *Vernonia cinerascens*. The structures were assigned based on ^{1}H- and ^{13}C-NMR spectra (DEPT), 2D NMR analyses (COSY, HMQC and HMBC experiments) in combination with HRMS experiments and comparison with literature data of related compounds.

Introduction

The genus *Vernonia* belongs to family "Asteraceae" which consists of about 1300 genera and 25,000 species distributed all over the world and in almost all habitats [1]. The genus is enriched with pharmacological properties. *V. amygdalina* showed antimicrobial [2], immunomodulating [3], lipids lowering [4], antidiabetic [5], antioxidant and hepatoprotective [6] activities. *V. cinerea* displayed antimalarial [7], immunomodulating, anticancer [8], anti-arthritis [9], analgesic, anti-pyretic and anti-inflammatory [10] activities. Various species of this genus are used for the treatment of schistosomiasis, amoebic dysentery, gastrointestinal problems, malaria, venereal diseases, wounds, hepatitis, diabetes, colic, fever, stomachache, toothache, cough, nasal and bronchial pain along with anti-ulcer [11] and anti-leishmanial [12] activities. Literature survey revealed that a sesquiterpene is reported from *Vernonia cinerascens* [13]. The diverse medicinal applications of genus *Vernonia* has prompted us to carry out the phytochemical investigations on this species. Herein we report the isolation and characterization of a new flavonoid named as cinerascenone (**1**) along with quercetin [14] and 3′-methylquercetin [15].

Results and Discussion

Cinerascenone (**1**) was isolated as yellow amorphous powder. The IR spectrum showed the absorptions for hydroxyl and aromatic protons (3417, 2926 cm^{-1}) and substituted benzene rings (1655-1502, 831 cm^{-1}). The UV spectrum showed bands at 242, 250, 271 and 348 nm typical for substituted flavonoids and closely related to chrysoeriol [16]. The high resolution electron impact mass spectroscopy (HREIMS) deduced the molecular formula $C_{23}H_{16}O_8$ showing $[M]^+$ peak at *m/z* 420.0839 (calcd. for $C_{23}H_{16}O_8$, 420.0845) and indicated the presence of sixteen double bond equivalence. The ^{1}H-NMR spectrum (Table-1) of **1** displayed an ABX splitting pattern at δ 7.46 (1H, dd, J = 8.0, 1.5 Hz), 7.44 (1H, d, J = 1.5 Hz) and 6.91 (1H, d, J = 8.0 Hz) indicating the presence of a 1,3,4-trisubstituted benzene ring. The two doublets at δ 6.41 and 6.18 (1H each, J = 1.2 Hz) are typical for flavonoids having substitution at 5 and 7 position [17]. A singlet at δ 6.54 corresponds to H-3 in flavones. The ^{1}H-NMR spectrum also showed two 2H doublets at δ 7.81 (d, J = 8.5 Hz) and 6.90 (d, J = 8.5 Hz) indicating the presence of a *p*-hydroxybenzoyl moiety in the molecule [18]. The signal for a methoxy group appeared at δ 3.94. The ^{13}C-NMR spectrum (BB and DEPT) of **1** (Table-1) displayed 21 highly resolved carbon signals for one methyl, eight methine and twelve quaternary carbon atoms. The signal for the conjugated carbonyls was appeared at δ 184.0 along with ester carbonyl δ 166.3. The downfield signals at δ 166.7, 163.0,



Structure of compound **1**.

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Table-1: ^{1}H- and ^{13}C-NMR data and HMBC correlations of Cinerascenone **1** (CD_3OD).

Position	$^{1}H^{a}$ multiplicity (*J*, Hz)	$^{13}C^{b}$ (DEPT)	HMBC^{c} (H→C)
2	–	166.2	–
3	6.54 s	104.1	C-2, C-4, C-10, C-1′
4	–	184.0	–
4a	–	105.2	–
5	–	163.0	–
6	6.18 d (1.2)	100.2	C-5, C-7, C-8, C-10
7	–	166.7	–
8	6.41 d (1.2)	95.1	C-6, C-7, C-9, C-10
8a	–	159.0	–
1′	–	123.0	–
2′	7.44 d (1.5)	110.6	C-2, C-1′, C-3′, C-4′, C-6′
3′	–	149.2	–
4′	–	152.5	–
5′	6.91 d (8.0)	116.7	C-1′, C-3′, C-4′, C-6′
6′	7.46 dd (8.0, 1.5)	121.7	C-2, C-1′, C-2′, C-4′, C-5′
1″	–	117.5	–
2″, 6″	7.81 d (8.5)	129.4	C-1″, C-3″, C-4″, C-5″, C-7″
3″, 5″	6.90 d (8.5)	117.0	C-1″, C-2″, C-4″, C-6″
4″	–	163.0	–
7″	–	166.3	–
OCH_3	3.94, s	56.5	C-3′

[a] ^{1}H NMR spectrum was recorded at 500MHz
[b] ^{13}C NMR spectrum was recorded at 125MHz
[c] HMBC spectrum was recorded at 500MHz

159.0, 152.5, 149.2 were due to the presence of oxygenated aromatic quaternary carbons. The presence of *p*-hydroxybenzoyl group was also supported by ^{13}C-NMR due to the signals at δ 166.3, 163.0, 129.4, 117.5 and 117.0 [18]. The methoxy carbon signal appeared at δ 56.5. The above data conclude that the compound **1** was closely related to chrysoeriol [17, 19] with the addition of a *p*-hydroxybenzoyl group. The position of the methoxy and *p*-hydroxybenzoyl group was finally confirmed by Heteronuclear Multiple Bond Correlations (HMBC) in which CH_3O- (δ 3.94) showed ^{3}J correlation with C-3′ (δ 149.2). The position of the *p*-hydroxybenzoyl group was deduced by the RDA fragment in HREIMS at *m/z* 272.0325 ($C_{14}H_8O_6$) and 148.0529 ($C_9H_8O_2$) indicating the presence of the methoxy group in ring B and *p*-hydroxybenzoyl group in ring A [20]. The downfield shift of C-7 (δ 166.7) indicated the presence of the *p*-hydroxybenzoyl group at this position which was confirmed by the HMBC correlations (Table-1) of H-6 (δ 6.18) and H-8 (δ 6.41) with C-7 (δ 166.7). On the basis of above data compound **1** was [5,4′-dihydroxy-7-(4-hydroxybenzoyl)-3′-methoxyflavone] and named as cinerascenone.

Experimental

General

Column chromatography was carried out using silica gel of 70-230, 230-400 mesh and sephadex LH-20. Aluminium sheets pre-coated with silica gel 60 F_{254} (20×20 cm, 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 spraying reagent. The UV spectrum was recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectrum was recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HREIMS spectra were recorded on Jeol JMS-HX 110 spectrometer. The ^{1}H-NMR spectrum was recorded on Bruker AMX-500 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (*J*) are in Hz. The ^{13}C-NMR spectra were recorded at 125 MHz on the same instrument.

Plant Material

The aerial parts of *Vernonia cinerascens* Sch. Bip. (2 kg, dried) was collected from Peruwal (District Khaniwal) in May 2006 and identified by Prof. Dr. Altaf Ahmad Dasti, Plant Taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan, where a voucher specimen (VC09/CIDS/06) is deposited.

Isolation

The shade dried, ground aerial parts of *V. cinerascens* (2 kg) were extracted with methanol (3×6 L). The combined MeOH extracts were concentrated, and the residue (20 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol along with water in increasing order of polarity. The fraction obtained by ethyl acetate-methanol-water (9.2:0.7:0.1) gave two spots on TLC subjected to Sephadex LH-20 eluted with methanol and finally purified by PTLC using ethyl acetate-methanol-water (8.9:1.0:0.1) provided 3'-methylquercetin (17 mg) and quercetin (13 mg), respectively. The fraction obtained from ethyl acetate-methanol-water (7.7:1.3:1) was chromatographed over Sephadex LH-20 using methanol as eluent and finally purified by preparative TLC with solvent system ethyl acetate-methanol-water (7:2:1) to afford cinerascenone (**1**) (6 mg).

Cinerascenone (**1**)

Yellow amorphous powder (6 mg), R_f value 0.43. UV (CH_3OH) λ_{max} nm (log ε): 242 (3.7), 250

(4.01), 271 (3.82), 348 (3.91). IR (KBr) ν_{max} cm^{-1}: 3417, 2926, 1655-1502, 831. EIMS *m/z* (rel.int., %) 420 (5), 300 (100), 272 (16), 228 (28), 148 (13), 121 (15). ^{1}H- and ^{13}C-NMR data and HMBC correlations, see Table-1. HREIMS $[M]^{+}$ *m/z* 420.0839 (calcd. for $C_{23}H1_6O_8$, 420.0845).

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