Phytochemicals from *Ipomoea carnea* collected from Murree Hills

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Summary: *Ipomoea carnea* has been identified as a medicinal shrub and produces a variety of secondary metabolites. Present study deals with the isolation and identification of a naturally occurring new compound, dodecyl-*p*-coumarate (**1**), together with seven known compounds: methyl-*p*-coumarate (**2**), octyl-*p*-coumarate (**3**), umblelliferone (**4**), escopoletin (**5**), 3-oleanone (**6**), *β*-sitosterol (**7**) and stigmasterol (**8**). The structures of all these compounds could be established by the combination of 1D and 2D NMR spectroscopy and mass spectrometry, or by comparison with the literature values. Compounds **2-8** have been reported for the first time from this source.

Introduction

Ipomoea carnea is a dicot shrub with long and heart shaped green leaves and funnel shaped flowers, relative to the family Convolvulaceae, a large family comprising of about 2000 species. The plant is toxic and dangerous to cattle [1]. *I. carnea* has the Central Nervous System depressant activity which appears to fall under the sedative-hypnotic category of central depressants with muscle relaxant property [1]. It contains the sedatative and anti-convulsant component identical to marsilin. A saponin has been isolated from *I. carnea* with anti-carcinogenic and oxytoxic properties [2]. Roots of this plant are used as laxative and to irritate menstruation and produces the milky juices which are used for the treatment of skin diseases and leucoderma [3]. The plant is known to produce two toxic metabolites acting as mild purgative [3]. *I. carnea* is a source of swainsonine, which is an indolizine alkaloid and a potent inhibitor of Golgi-α-mannosidase II, an immunomodulator, and a potential chemotherapy drug [4]. Anticonvulsant and anticarcenogenic agents have also been reported from this plant [2].

Present work deals with the isolation of eight secondary metabolites from the *n*-hexane soluble part of the ethanolic extract of *I. carnea*, which were identified as dodecyl-*p*-coumarate (**1**), methyl-*p*-coumarate (**2**), octyl-*p*-coumarate (**3**) [5,6], umbelliferone (**4**) [7], escopoletin (**5**) [8], 3-oleanone (**6**) [9], *β*-sitosterol (**7**) [10] and stigmasterol (**8**) [11].

Results and Discussion

The FD-MS of **1** displayed the molecular ion at *m/z* 332, whereas the EI-MS showed, other than molecular ion, fragment ions at *m/z* 147 and 119 characteristic of coumarates [12]. The molecular formula ($C_{21}H_{32}O_3$) was determined based on the HR-EI-MS (*m/z* 332.23487) and exhibited six degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 3385, 3145, 2934, 1705, 1670, 1632, 1515 and 1470 cm^{-1}.

The aromatic region of the ^{1}H-NMR spectrum of **1** displayed two signals at δ 7.42 (d, J = 8.4 Hz) and 6.83 (d, J = 8.4 Hz) splitted into an AA′BB′ system, indicating a *p*-substituted benzene ring. Signals for protons typical for a conjugated *trans*-double bond were observed at δ 7.63 (d, J = 15.9 Hz) and 6.31 (d, J = 15.9 Hz). A signal for two hydrogen atoms appeared in the same spectrum at δ 4.16 as triplet which showed COSY correlation with a multiplet of two hydrogens at δ 1.71. Further, the high field region of the spectrum displayed a broad singlet of 18 hydrogen atoms at δ 1.23-1.36 and a triplet methyl at δ 0.85, indicating a long chain hydrocarbon part in the molecule. The ^{13}C-NMR spectrum of **1** was in agreement with the mass and ^{1}H-NMR data. These data in conjunction with the HMBC information to established the structure of **1** as dodecyl-*p*-coumarate.

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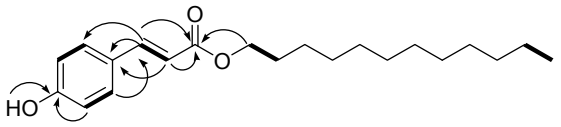

COSY correlations

HMBC correlations

Fig. 1: COSY 45 and HMBC correlations observed in the spectra of 1.

A number of coumarates have already been identified as constituents of the members of the genus *Ipomoea* [13, 14], such as compound **1**, which is a synthetically known compound [15] but now was isolated for the first time from natural sources. Such kind of phenolic acids and their various esters are getting great attention due to their potential biological activity, such as antioxidant, anti-inflammatory, antiallergic, antimicrobial [16-18], antiviral, anticarcinogenic and UV blocking properties [19-22]. The lipophilic character of these kinds of compounds plays a very important role in their biological potential. Some of the synthetic *p*-coumarates have also been reported to inhbit α- and β-DNA polymerases [15].

Compounds **2**-**5** have already been reported from natural sources but isolated for the first time from *Ipomoea carnea*. The structures of compounds **2** and **3** were established by comparison with literature values for their mass and NMR spectra. Compounds **4** and **5** are well-known phytochemicals; they were isolated as a mixture and identified on the basis of their ^{1}H-NMR and mass spectra and by comparison with the TLC profile of mixed standard compounds.

Experimental

General Experimental Procedures

IR spectra were recorded as neat film on Shimadzu IR-460 spectrophotometer. UV spectrum was measured in methanol of analytical grade on U-3200 Shimadzu UV-240 spectrophotometer. EI-MS and HR-EI-MS experiments were performed on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were measured on Bruker AMX-300 MHz instrument 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 75 MHz on the same instrument. 2D NMR data was determined on Bruker AMX-300 MHz instrument. Column chromatography was carried out using flash silica gel of 70-100 mesh, purchased from the local distributor of E-Merck. Aluminium sheets pre-coated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck; purchased from a local authorized dealer of 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.

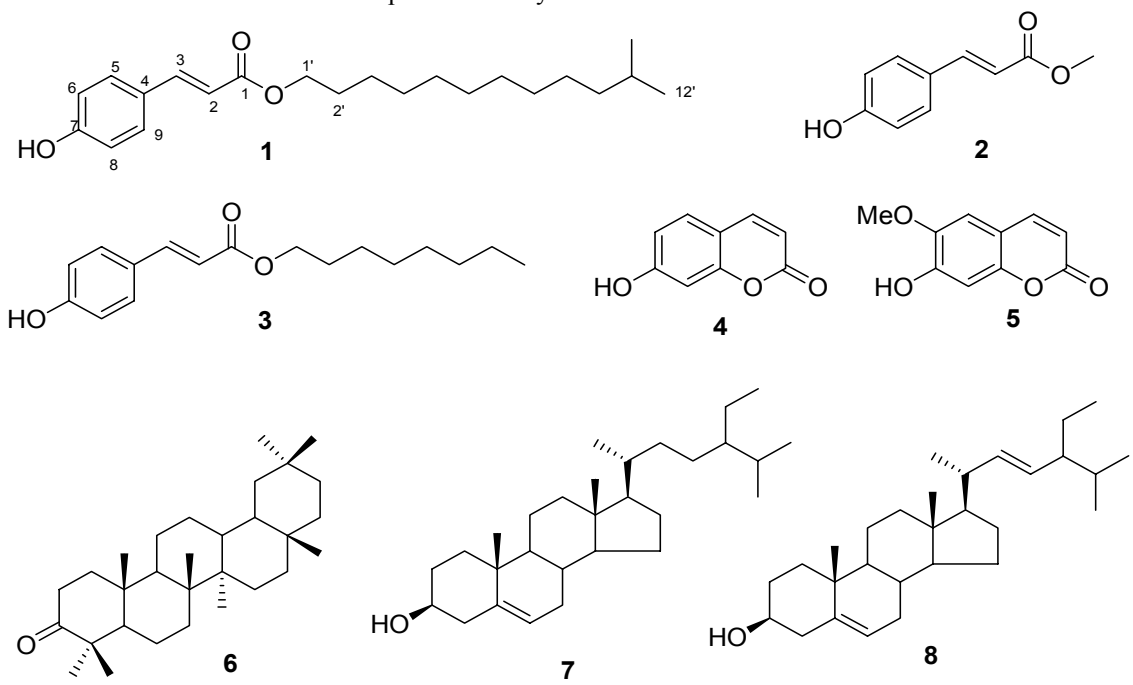

Fig 2: Metabolites (1-8) isolated from *Ipomoea carnea.*

Collection and Extraction of Plant Material

The aerial parts of *I. Carnea* Jacq. were collected from the Murree Hills in July 2008 and were identified by Mr. Muhammad Farrukh Nisar, Plant Taxonomist, Department of Botany, University of Gujrat, Gujrat, Pakistan, where the voucher specimen (IC-GUG-21-08) has been deposited. The shade dried plant material (5 kg) was extracted in ethanol (15 L) thrice at room temperature. The concentrated extracts were combined to get a gummy mass (103 g).

Isolation and Purification

The 100 g from the crude extract was suspended in water and was extracted successively with *n*-hexane, dichloromethane (DCM) and ethyl acetate (EtOAc). The *n*-hexane soluble fraction (25 g) was subjected to column chromatography over flash silica gel using gradient eluting system of *n*-hexane: DCM (100:0 to 0:100) to get eight fractions (1-8). Fraction No 6 on further silica gel column chromatography with 35% DCM in *n*-hexane yielded **1** (15 mg), **2** (25 mg) and **3** (11 mg). The TLC profile of fraction 4 showed only two UV-active components, which were separated by preparative TLC developed with a mobile phase of *n*-hexane-DCM (1:1) to get compounds **4** (13 mg) and **5** (30 mg). Fraction 2 from the main column when further purified by repeated silica gel column chromatography eluting with 22% DCM in *n*-hexane gave **6** (20 mg), whereas, fraction 5 eluting with gradient of *n*-hexane: DCM (9:1 to 1:1) yielded compounds **7** and **8** (65 mg). Compounds **7** and **8** were obtained as mixture, showing a single spot on TLC and were identified by NMR and mass spectral data of the mixture.

*Characterization of Dodecyl-p-coumarate (**1**)*

IR (neat) ν_{max}: 3385 (O-H), 3145 (C-H), 2934 (C-H), 1705 (C=O), 1670 (*trans* C=C), 1605, 1515, (aromatic system), 1475 and 1370 cm^{-1}; UV (MeOH) λ_{max} (log ε): 205 (3.89), 228 (3.82), 311 (3.99); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.63 (1H, d, *J* = 15.9 Hz, *trans*-H-3), 7.42 (2H, d, *J* = 8.4 Hz, H-2,6) 6.83 (2H, d, *J* = 8.4 Hz, H-3,5), 6.31 (1H, d, *J* = 15.9 Hz, *trans*-H-2), 5.33 (1H, s, 7-OH), 4.16 (2H, t, *J* = 6.6 Hz, H-1′), 1.71 (2H, m, H-2′), 1.36-1.23 (18H, br s, H-3′–H-11′), 0.85 (3H, t, *J* = 6.3 H-8′); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 167.5 (C-1), 157.4 (C-7), 144.2 (C-3), 129.9 (C-5,9), 127.3 (C-4), 115.9 (C-6,8), 115.8 (C-2), 64.6 (C-1′), 31.9 (C-11′), 29.6 (C-4′–C-8′), 29.3 (C-9′), 28.7 (C-3′), 25.9 (C-2′), 22.6 (C-10′) and 14.0 (C-12′); EI-MS *m/z* (%): 332 (7), 247 (6), 303 (8), 289 (15), 147 (60), 119 (100); HR-EI-MS *m/z*: 332.23491 (calcd. 332.23487 for $C_{21}H_{32}O_3$).

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