Xanthoxyloidin, a New Biscoumarin from *Fraxinus xanthoxyloides*

[1]Muhammad Iftikhar, [1]Naheed Riaz*, [1]Muhammad Saleem, [1]Sara Musaddiq, [1]Shahnawaz [1]Abdul Jabbar and [2]Rasool Bakhsh Tareen
[1]*Department of Chemistry, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan.*
[2]*Department of Botany, University of Baluchistan, Quetta, Pakistan.*
nrch322@yahoo.com; nrch322@hotmail.com*

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Summary: Xanthoxyloidin (**1**), a new biscoumarin together with esculetin (**2**), 5,7-dihydroxycoumarin (**3**) and 6,8-dihydroxy-7-methoxycoumarin (**4**) were isolated from the methanolic extract of the whole plant of *Fraxinus xanthoxyloides*. These compounds (**1-4**) were purified by using normal and reverse phase column and thin layer chromatographic techniques and their structures were determined using 1D (^{1}H and ^{13}C), 2D NMR (HSQC, HMBC, COSY, NOESY) spectroscopy and mass spectrometry (EIMS, HREIMS, FABMS, HRFABMS) and in comparison with reported data in literature.

Keywords: *Fraxinus xanthoxyloides*, Oleaceae; Coumarins, Secondary metabolites, Characterization.

Introduction

The genus *Fraxinus* of the plant family Oleaceae, is a group of about 63 flowering plants [1, 2], most of them are being used as diuretic, against constipation, dropsy, arthritis and rheumatic pain [3]. The roots of some species are reported to have anti-inflammatory, antimalarial and antipyretic properties, whereas, the bark is used as remedy against stomatitis, pyrexia and toothache [4]. The bark of *F. japonica* and *F. americana* is effective against rheumatism and gout [5, 6]. *F. bungeana* received the fame due to its use in diarrhea, arthritis and fever [7]. Previously, varieties of secondary metabolites like coumarins, secoiridoids, and phenylethanoids have been reported from the genus *Fraxinus* [8]. The diverse medicinal application of the genus *Fraxinus* promoted us to carry out the phytochemical studies on *Fraxinus xanthoxyloides*. In the present study we isolated and characterized xanthoxyloidin (**1**) a new biscoumarin (**1**) along with esculetin (**2**) [9], 5,7-dihydroxycoumarin (**3**) [10] and 6,8-dihydroxy-7-methoxycoumarin (**4**) [11] from the methanolic extract of the whole plant of *Fraxinus xanthoxyloides* (Fig. 1).

Experimental

General procedures

Column chromatography was carried out using silica gel F_{254} (230-400 mesh). Aluminium sheets pre-coated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E. Merck) were used for thin layer chromatography (TLC). TLC plates were visualized under UV lamp at 254 and 366 nm and by spraying with ceric sulphate solution followed by heating. UV spectra were scanned on a Hitachi UV-3200 spectrophotometer (λ_{max} in nm). IR spectra were measured on Shimadzu 460 spectrometer. ^{1}H and ^{13}C-NMRspectra were recorded on Bruker instrument operating at 400 and 100 MHz, respectively. Mass spectra were measured on JMS H×110 mass spectrometer with a data system and JMSA 500 mass spectrometers.

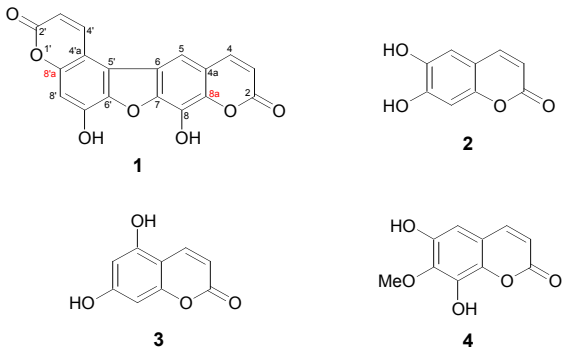

Fig. 1: Compounds (**1-4**) isolated from *Fraxinus xanthoxyloides.*

Plant Material

The whole plant material of *Fraxinus xanthoxyloides* Wall. was collected from Ziarat (Baluchistan) Pakistan in April 2011, which was identified by Prof. Dr. Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, Baluchistan University Quetta, Pakistan where a voucher specimen is deposited (FR-ZT-21/11).

***To whom all correspondence should be addressed.**

Extraction and Isolation

The shade dried, ground whole plant of the *F. xanthoxyloides* (04 kg) was extracted with MeOH (3 × 15 L). The combined methanolic extract (85 g) was subjected to column chromatography (CC) over silica gel eluting with *n*-hexane, *n*-hexane:dichloromethane (DCM), DCM, DCM:methanol and methanol in increasing order of polarity to get 6 fractions (1-6). The fraction 2 obtained at *n*-hexane:DCM (6:4) was subjected for further purification on CC to get esculetin (**2**). The fraction 3 eluted with *n*-hexane:DCM (5.5:4.5) from main column, was chromatographed over silica gel using an isocratic of *n*-hexane-DCM (7.8:2.2) that yielded 5,7-dihydroxycoumarin (**3**). The fraction 4 from first column, collected with an isocratic of *n*-hexane:DCM (4.5:5.5), on further purification afforded 6,8-dihydroxy-7-methoxycoumarin (**4**). The fraction 5 obtained from main column with *n*-hexane-DCM (3:7) was also processed further using the same conditions as for compound **4** to get compound **1**.

Xanthoxyloidin (**1**)

White amorphous powder (55 mg); UV (MeOH) λ_{max} nm: 223, 255, 287, 300, 325. IR (KBr) ν_{max} cm^{-1}: 3365, 1712, 1610-1500, 1645. The ^{1}H and ^{13}C-NMRdata, see Table-1; HRFABMS *m/z*: 337.2571 $[M+H]^+$ (calcd. for $C_{18}H_9O_7$, 337.2599).

Table-1: ^{1}H and ^{13}C-NMRdata HMBC and COSY correlations of **1** (CD_3OD, 400 and 100 MHz).

Position	δ_H (*J* in Hz)	δ_C	HMBC (H→C)	COSY (H→H)
2	–	160.7	–	–
3	6.16 (d, 9.6)	111.4	2, 4, 4a	H-3/H-4
4	7.95 (d, 9.6)	144.7	1a, 2, 5	H-4/H-3, 5 (allylic)
4a	–	110.5	–	–
5	7.09 (s)	111.5	1a, 4, 5′,7	H-5/H-4 (allylic)
6	–	109.1	–	–
7	–	148.7	–	–
8	–	142.3	–	–
8a	–	146.8	–	–
2′	–	160.5	–	–
3′	6.07 (d, 9.6)	111.3	1a′, 2′, 4a′	H-3′/H-4′
4′	7.14 (d, 9.6)	142.7	1a′, 2′, 5′	H-4′/H-3′
4′a	–	110.2	–	–
5′	–	116.3	–	–
6′	–	141.2	–	–
7′	–	148.5	–	–
8′	6.85 (s)	101.8	1a′, 4a′, 6′, 7′	–
8′a	–	150.1	–	–

Results and Discussion

Compound **1** was isolated as white amorphous powder, which showed diagnostic absorption bands in its IR spectrum at 3365 and 1712 cm^{-1} for hydroxyl functional group and a lactone moiety, respectively, and at 1600, 1545, 1425 cm^{-1} due to the aromatic system. The UV absorption bands at 223, 255, 287, 300 and 321 nm was indicative of a biscoumarin [12]. The HRFABMS (+*ve* mode) of **1** showed molecular ion peak at *m/z* 337.2597 $[M+H]^+$ depicting the molecular formula $C_{18}H_9O_7$ with 15 double bond equivalence (DBE) where as its ^{1}H-NMRspectrum (Table 1), showed six signals in the aromatic region including two pairs of doublets at δ [7.95 (d, J = 9.6 Hz), 6.16 (d, J = 9.6 Hz)] and [7.14 (d, J = 9.6 Hz), 6.07 (d, J = 9.6 Hz)] and two sharp singlets at δ 7.09 and 6.85. The amount of coupling constants (J = 9.6 Hz) of first two pairs led to the determination of at least two coumarin nuclei in **1** [13].

The ^{13}C-NMRspectrum (BB and DEPT) of **1** substantiated the ^{1}H-NMRinformation as it disclosed 18 carbon signals for six methine and twelve quaternary carbons. Therefore, 14 DBE was attributed to two coumarin moieties, whereas, the remaining 01 DBE could be justified for a condensed furan ring between two coumarin nuclei. The presence of furan ring was further substantiated due to HMBC correlation of H-5 (δ 7.09) with the carbons at δ 109.1 (C-6), 148.7 (C-7) and 116.3 (C-5′). The HMBC interaction of H-4′ (δ 7.14) with C-5′ (δ 116.3) also provided evidence for the above deduction. Above discussed HMBC interaction and that of H-8′ (δ 6.85) with C-6′ (δ 141.2) also confirmed the sites for attachment of coumarin moieties with furan ring [12, 14, 15]. The confirmation of both the hydroxyl moieties at C-7′ and C-8 was also done by the diagnostic HMBC correlation of H-8′ (δ 6.85) with C-7′ (δ 148.5) and missing of the said connectivity with C-8 (δ 142.3) either of H-5 (δ 7.09) or of H-8′ (δ 6.85). Other important HMBC correlations are shown in Table 1.

The above discussed data accomplished structure as **1**, which is a new natural product and is named as xanthoxyloidin.

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