New Furanocoumarins and Other Chemical Constituents from *Ficus carica* Root Heartwood

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Two new furanocoumarins, 5-(1",1"-dimethylallyl)-8-methyl psoralen (**1**) and 2"-*O*-acetyl oxypeucedanin hydrate-3"-methyl ether (**2**), were isolated from the root heartwood of *Ficus carica* Linn. together with three known furanocoumarins, two triterpenoids, two long-chain compounds, and a steroid. Their structures and relative configurations were elucidated by spectroscopic methods (IR, HR-ESI-MS, and NMR) and by comparison of their NMR spectral data with those of related compounds.

Key words: Ficus carica, Furanocoumarins, Triterpenoids

Introduction

The characterization of plant metabolites by modern analytical methods is an important issue both in plant physiology and phytochemistry. A phytochemical screening usually marks the starting point for building a solid database for quality assessment and herbal drug authentication. Furthermore, the biological effects may thus be predicted and suitable marker compounds can be defined.

The genus *Ficus* (family: Moraceae) comprises mainly shrubs, often climbers with milky juice. About 800 species are known, of which 65 species are found in India, most of which are medicinally useful (Kirtikar and Basu, 1975; Chopra *et al*., 1956).

Ficus carica Linn., commonly known as fig tree, is one of the unique *Ficus* species with edible fruits of high commercial value. It is a moderate-sized deciduous tree, native to Carica in Asia Minor, and found in all tropical and sub-tropical countries. The fruit is antipyretic, tonic, purgative, aphrodisiac, and lithotriptic. In traditional medicine, it is useful in nose bleeding, blood leprosy, inflammation, weakness, paralysis, thirst, diseases of liver and spleen, pain in the chest, constipation, piles, and stimulates hair growth. The roots are used in the treatment of leucoderma, ring worm infections, bladder complaints, and visceral obstruction (Kirtikar and Basu, 1975; Chopra *et al*., 1956).

Previous investigations on this plant revealed the presence of triterpenoids, steroids, coumarins, and flavones in fruits (Rubnov *et al*., 2001), leaves (Peyron *et al*., 2000; Saeed and Sabir, 2002; Vaya and Mahmood, 2006), and stem (Weiping *et al*., 1997). The latex and fruits exhibit anticancer (Rubnov *et al*., 2001; Wang *et al*., 2008) and antioxidant (Oliveira *et al*., 2010) activities. The leaves demonstrate antidiabetic (Canal *et al*., 2000), anti-inflammatory, antioxidant (Ali *et al*., 2012), and antimicrobial (Jeong *et al*., 2009) activities, as well as antipyretic potential (Patil *et al*., 2010) and an anti-HSV effect (Wang *et al*., 2004).

The above survey indicates that no phytochemical work had been reported on the root heartwood of this plant. So, in the interest of identifying new natural products from *F. carica*, we investigated the chemical constituents of the root heartwood of this plant. This paper gives a description of the isolation of two new furanocoumarins, 5-(1",1"-dimethylallyl)-8-methyl psoralen and 2"-*O*-acetyl oxypeucedanin hydrate-3"-methyl ether along with eight known compounds, whose structures were unequivocally determined by ^{1}H, ^{13}C NMR, and IR spectroscopy as well as by mass spectrometry and by comparison with literature data. The phytochemical investigation of the root bark of this plant is underway in our laboratory and will be published in due course.

Experimental

General

IR spectra were recorded on a Shimadzu (Tokyo, Japan) FT IR-8400S spectrometer using KBr pellets. The UV spectra were taken in ethanol (95%) using a Shimadzu model Pharma Spec-1700 automatic recording spectrophotometer and a Beckmann (New York, USA) model DU spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as an internal standard on a Jeol (Tokyo, Japan) AL spectrometer at 300 MHz and 75 MHz, respectively. Mass spectra were recorded on a Waters (Millford, MA, USA) Xevo Q-TOF spectrometer. The GC-MS analysis was performed on a Shimadzu GC-MS-QP 2010 Plus instrument. C and H analyses of the compounds were done on Coleman (Cincinnati, OH, USA) and Carlo Erba (Milan, Italy) 1108 C and H analyzers. Melting points were recorded in soft glass capillaries in a Toshniwal (Mumbai, India) apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Merck (Darmstadt, Germany) silica gel G plates, and in the column chromatography (CC) fractionation Merck silica gel (60–120 mesh) was used.

Plant material

The roots of *Ficus carica* were collected from the University of Rajasthan Campus, Jaipur, Rajasthan, India in March, 2011, during daytime. The plant was authenticated at the herbarium of the Department of Botany, University of Rajasthan, Jaipur (Herbarium Sheet No. RUBL 19874).

Extraction

Air-dried and finely powdered root heartwood (3 kg) was exhaustively extracted with ethanol (95%) on a steam bath for three times, 8 h each. The extract was concentrated by rotary evaporation, and a dark brown semisolid (110 g) was obtained, which was fractionated with petroleum ether followed by benzene and dichloromethane. A yellow petroleum ether fraction (1.52 g), yellowish-brown benzene fraction (3.80 g), and brown dichloromethane fraction (8.68 g) were obtained upon evaporation of the solvents.

Isolation of compounds

TLC of the petroleum ether, benzene, and dichloromethane fractions in benzene/ethyl acetate (1:1) exhibited a similar profile, hence, these were mixed and chromatographed over a silica gel column. Elution with solvents of increasing polarity, *viz.* petroleum ether, benzene, ethyl acetate, and methanol, afforded eleven fractions (fractions A – K). Fractions B, D, E, F, G, I, J, and K were purified by a combination of CC, TLC, and recrystallization using suitable solvent systems to yield compounds **5**, **6**, **7**, **8**, **9**, **10**, **1**, and **2**, respectively. Fraction C yielded a white residue on evaporating the solvent which on crystallization from petroleum ether/benzene afforded white crystals. These were further subjected to GC-MS in which five compounds were indicated with retention times of 25.84, 26.45, 26.63, 27.18, and 28.14 min. Amongst these, the last two compounds, *viz*. **3** (RT 27.18 min) and **4** (RT 28.14 min), had reasonable difference in their retention time and were identified on the basis of their mass spectral data. Fractions A and H did not yield any crystallizable compound.

β-Sitosteryl palmitate (**5**) ($C_{45}H_{80}O_2$): White needles. – M.p. 83–85 °C (King and Jurd, 1953). – IR (KBr): ν_{max} = 1720, 1640 (C=O), 1220, 1180 (C–O), 730, 720 cm^{-1} (long chain). – FAB-MS: *m/z* = 675 $[M + Na]^+$, 397 $[M^+ – C_{16}H_{31}O_2]$, 273, 255.

Nonadecan-10-one (**6**) ($C_{19}H_{38}O$): White crystals. – M.p. 57–59 °C (Cadogan and Buckingham, 1996). – IR (KBr): ν_{max} = 1720 cm^{-1} (C=O). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (6H, t), 1.48 (28H, m), 2.36 (4H, t). – MS: *m/z* = 282 $[M^+]$, 267 $[M^+ – CH_3]$, 170 $[M^+ – C_8H_{16}]$, 155 $[M^+ – C_9H_{19}]$, 127 [155 – CO].

Psoralen (**7**) ($C_{11}H_6O_3$): White needles. – M.p. 160–162 °C (Lin *et al*., 2007). – IR (KBr): ν_{max} = 1720 (C=O), 1580 and 1570 cm^{-1} (aromatic). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (1H, d, *J* = 10 Hz), 7.73 (2H, m), 7.53 (1H, s), 6.84 (1H, d), 6.40 (1H, d, *J* = 10 Hz). – MS: *m/z* = 186 $[M^+]$, 157 $[M^+ – H – CO]$, 130 $[M^+ – 2CO]$.

β-Sitosterol (**8**) ($C_{29}H_{50}O$): White needles. – M.p. 135–137 °C (King and Jurd, 1953). – IR (KBr): ν_{max} = 3400 (O–H), 1090 cm^{-1} (C–O). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.27 (1H, t), 3.48 (1H, m) 1.16–0.70 (6×CH_3, s,d). – MS: *m/z* = 414 $[M^+]$, 88], 399 $[M^+ – CH_3$, 18], 396 $[M^+ – H_2O$, 43], 381 $[M^+ – CH_3 – H_2O$, 37], 273 $[M^+ – C_{10}H_{21}$, 28], 255 [273 – H_2O, 33], 231 [273 – C_3H_6, 26].

8-Methyl psoralen (**9**) ($C_{12}H_8O_3$): Light yellow needles. – M.p. 150–152 °C. – IR (KBr): ν_{max} = 1720 (C=O), 1580 and 1570 cm^{-1} (aromatic). – ^1H NMR (300 MHz, DMSO-d_6): δ = 7.98 (1H, d, J = 10 Hz), 7.83 (1H, d, J = 3 Hz), 7.49 (1H, s), 6.92 (1H, d, J = 3 Hz), 6.35 (1H, d, J = 10 Hz), 2.51 (1H, s). – MS: m/z = 200 [M^+], 185, 171, 129, 111, 98, 83, 69 etc. A literature survey indicates that even though this compound had been synthesized earlier and shown to be phototoxic (Rodighiero *et al*., 1969), this appears to be its first isolation from a natural source.

Bergapten (**10**) ($C_{45}H_{80}O_2$): White needles. – M.p. 180–182 °C (Harkar *et al*., 1984). – IR (KBr): ν_{max} = 1720 (C=O), 1590, 1550 (aromatic), 1130 cm^{-1} (C–O). – ^1H NMR (300 MHz, $CDCl_3$): δ = 8.24 (1H, d, J = 10 Hz), 7.69 (1H, d, J = 2 Hz), 7.24 (1H, s), 7.16 (1H, d, J = 2 Hz), 6.36 (1H, d, J = 10 Hz), 4.28 (3H, s). – MS: m/z = 216 [M^+], 215 [M^+ – H], 200 [M^+ – H – CH_3], 185 [M^+ – OCH_3].

Results and Discussion

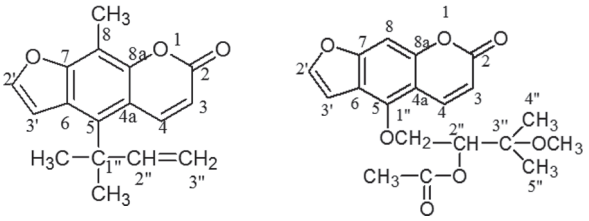
Purification and crystallization of fractions J and K yielded two new furanocoumarins, 5-(1",1"-dimethylallyl)-8-methyl psoralen (**1**) and 2"-*O*-acetyl oxypeucedanin hydrate-3"-methyl ether (**2**), respectively, structurally identified by ^1H and ^{13}C NMR, IR, and mass spectral data.

Compound **1** was obtained as light yellow crystals with a melting point of 215–217 °C. The elemental analysis and molecular weight determination by HR-ESI-MS established its molecular formula to be $C_{17}H_{16}O_3$. Absorption bands of the coumarin lactone group (1720, 1130 cm^{-1}) and aromatic ring (1620, 1580, and 1570 cm^{-1}) were observed in its IR spectrum. The UV absorption bands at 306, 266, 249, and 220 nm indicated it to be a furanocoumarin derivative (Harkar *et al*., 1984). In the ^1H NMR spectrum (Table I), the doublets at δ_H 7.15 and 7.54 ppm both with J = 2.4 Hz corresponded to the furan ring protons H-3' and H-2', respectively, and the doublets at δ_H 6.16 and 8.26 ppm (J = 9.7 Hz) could be attributed to the protons on C-3 and C-4, respectively. The singlet at δ_H 2.59 ppm could be attributed to the methyl group present at C-8 (Kalidhar, 1990). The dimethylallyl group appeared in the form of a multiplet in the region δ_H 6.32–6.42 ppm for one olefinic proton (C**H=**CH_2), δ_H 4.92–4.96 ppm for the other two olefinic protons (CH=C$\mathbf{H_2}$), and a singlet due to two methyl groups at δ_H 1.74 ppm. The confirmation of the position of the methyl group on C-8 clearly indicated that the dimethylallyl group is positioned on C-5. In its ^{13}C NMR spectrum, 11 carbon atom signals in the lower field region, consisting of signals of four aromatic methane groups, six quarternary carbon atoms, and one carbonyl group, suggested the presence of a furanocoumarin framework. In addition, a quarternary carbon atom, a vinyl group, and three methyl groups were also observed (Table I). In the mass spectrum, the molecular ion peak was observed at m/z 268. Prominent peaks were observed at m/z 253 [M^+ – CH_3], 239 [M^+ – H – CO], 225 [253 – CO], 212 [M^+ – 2CO], and 199 [M^+ – C_5H_9]. The above spectral data led to the identification of compound **1** as 5-(1",1"-dimethylallyl)-8-methyl psoralen (Fig. 1).

Compound **2**, which has a melting point of 76–78 °C, was assigned the molecular formula $C_{19}H_{20}O_7$ based on HR-ESI-MS data. Its UV absorption spectrum (310, 265, 253, 221 nm) revealed its furanocoumarin nature (Harkar *et al*., 1984). Its IR absorption spectrum showed a cou-

Table I. NMR spectroscopic data of **1** and **2**.

Position	**1**		**2**	
	^{13}C	^1H	^{13}C	^1H
2	161.9	-	162.4	-
3	117.0	6.16, d	114.2	6.33, d
4	138.5	8.26, d	140.8	8.19, d
5	136.6	-	151.9	-
6	112.7	-	116.2	-
7	155.4	-	156.5	-
8	110.9	-	96.3	7.27, s
4a	119.1	-	108.7	-
8a	147.4	-	151.4	-
2'	144.8	7.54, d	145.1	7.62, d
3'	106.4	7.15, d	105.9	7.01, d
1"	30.9	-	72.8	4.52, m
1"-Me	29.7	1.74, s	-	-
1"-Me	29.7	1.74, s	-	-
2"	141.7	6.32–6.42, m	88.6	3.95, m
3"	111.4	4.92–4.96, m	76.5	-
8-Me	22.9	2.59, s	-	-
$H_3C-\mathbf{C}-$ (=O)	-	-	169.1	-
$\mathbf{H_3C}-C-$ (=O)	-	-	17.8	2.11, s
4"	-	-	21.5	1.36, s
5"	-	-	21.5	1.31, s
-OMe	-	-	51.7	2.77, s



1 **2**

Fig. 1. Chemical structures of 5-(1",1"-dimethylallyl)-8-methyl psoralen (**1**) and 2"-*O*-acetyl oxypeucedanin hydrate-3"-methyl ether (**2**).

marin lactone (1720, 1130 cm^{-1}) and aromatic ring (1620, 1580, and 1570 cm^{-1}) absorption bands. In the ^1H NMR spectrum, two typical coumarin pyrone ring protons (H-3, H-4) were observed as doublets (J = 10 Hz) at δ_H 6.33 and 8.19 ppm, respectively. The furan ring proton H-2' was observed as a doublet at δ_H 7.62 ppm (J = 2 Hz), and a doublet for the H-3' proton appeared at δ_H 7.01 ppm (J = 2 Hz). In the side chain, singlets for the two methyl groups on C-3" appeared at δ_H 1.36 and 1.31 ppm, for the methoxy group at δ_H 2.77 ppm, and for the acetate group at δ_H 2.11 ppm. The proton on C-2" appeared as a multiplet at δ_H 3.95 ppm. A multiplet at δ_H 4.52 ppm corresponded to the C-1" protons. The chemical shift of H-4 distinguishes the C-8- and C-5-substituted furanocoumarins. A literature survey indicates that, if the compound was substituted at C-5, then the H-4 proton signal would appear downfield, between δ_H 8.03 and 8.32 ppm (Kalidhar, 1990). In our case, the H-4 proton signal appeared at δ_H 8.19 ppm indicating the substitution at C-5, and hence the aromatic singlet at δ_H 7.27 ppm corresponded to the C-8 proton. The ^{13}C NMR spectrum showed 11 carbon atoms in the lower field region indicating the presence of a furanocoumarin skeleton. In addition, signals of two methyl groups, one acetyl group, two carbon atoms attached to an oxygen atom, one methoxy group, and a quarternary carbon atom were also observed (Table I). Compound **2** was assumed to be a linear furanocoumarin derivative, and its ^{13}C NMR spectrum was similar to that of oxepeucedanin hydrate (Harkar *et al.*, 1984) except for a methoxy and an acetyl group. In the mass spectrum, the molecular ion peak was observed at *m/z* 360. Other important peaks were observed at *m/z* 329 [$M^+ - OCH_3$], 287 [$M^+ - C_4H_9O$], 201 [$M^+ - C_8H_{15}O_3$], and 159 [$M^+ - C_{11}H_5O_4$]. On the basis of the above mentioned spectral data, compound **2** was characterized as 2"-*O*-acetyl oxypeucedanin hydrate-3"-methyl ether (Fig. 1). Although the non-methylated product has been reported earlier from *Angelica officinalis* (Harkar *et al.*, 1984), this oxypeucedanin derivative has been isolated for the first time from nature by us.

In the gas chromatogram of fraction C, compound **3**, having a retention time of 27.18 min, had the abundance 60,00000. In the mass spectrum, the molecular ion peak appeared at *m/z* 468 (12). The mass fragmentation pattern closely resembled to that of neolup-12-ene triterpenoids (Shiojima *et al.*, 1992). The base peak appeared at *m/z* 218 (a) by cleavage in ring C which gives rise to fragments at *m/z* 203 (85) [a – 15], 189 (98) [a – 29], and 175 (29) [a – 43]. Other important peaks appeared at *m/z* 453 (8) [$M^+ - CH_3$] and 393 (11) [453 – CH_3COOH]. Based on this mass fragmentation pattern, compound **3** was characterized as neolup-12-en-3β-yl acetate.

Further, compound **4**, having a retention time of 28.14 min in the gas chromatogram, had the abundance 16,00000. Interestingly, the molecular ion peak of this compound also appeared at *m/z* 468 (19), but the mass fragmentation pattern closely resembled to that of lup-20(29)-ene series (Shiojima *et al.*, 1992). Peaks at *m/z* 189 (100) and 203 (43) were formed by the loss of C_2H_5 and CH_3 moieties, respectively, from the fragment appearing at *m/z* 218 (30), the latter being obtained by cleavage in ring C. The other important peaks were observed at *m/z* 408 (24) [$M^+ - CH_3COOH$] and 393 (22) [$M^+ - CH_3 - CH_3COOH$]. On the basis of this mass fragmentation pattern, compound **4** was characterized as lup-20(29)-en-3β-yl acetate.

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