Flavonoids from *Ficus pumila*

Luisa Pistelli*, Elisabetta E. Chiellini, Ivano Morelli

*Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy*

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1. Subject and source

The genus *Ficus* L. (Moraceae) consists of more than 850 species and is predominantly distributed in the tropical and sub-tropical areas. The latex is often anthelminthic, owing to the proteolic enzyme ficin. (Evans, 1989).

We have selected the aerial parts of *Ficus pumila* for phytochemical investigation, since flavonoid composition was not investigated previously. *Ficus pumila* L. (= *Ficus repens* Hort.) is a scadent shrub with evergreen coriaceous leaves, native to China, Japan and Formosa. Plant material (aerial parts) was collected in the autumn 1993 in Pisa (Italy) and a voucher specimen is deposited in the Herbarium of the Dipartimento di Scienze Botaniche [H.H.P. (PI) Nuove Acquisizioni (1961)/1], University of Pisa.

2. Previous work

Previous work on *Ficus pumila* had led to the isolation of the two furanocoumarins, bergapten and oxypeucedanin hydrate (Juan et al., 1997), and triterpenoids, sterols and the flavonoid glycosides: astragalin, isoquercitrin, apigenin 6-C-\(\alpha\)-L-rhamnopyranosyl-(1 → 2)-\(\beta\)-D-glucopyranoside, kaempferol 3-\(\alpha\)-L-rhamnopyranosyl-(1 → 6)-\(\beta\)-D-glucopyranoside and kaempferol 3-\(\alpha\)-L-rhamnopyranosyl-(1 → 6)-\(\beta\)-D-galactopyranoside (Katajama et al., 1998a,b).

*Corresponding author. Tel.: +39-050-44074; fax: +39-050-43321.
E-mail address: luipi@farm.unipi.it (L. Pistelli)*

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3. Present study

Dried stems and leaves (4.0 Kg) of *Ficus pumila* were powdered and extracted by Soxhlet successively with *n*-hexane (26.8 g), CHCl₃ (35.6 g), and CHCl₃–MeOH 9 : 1 (108.3 g); then the exhaustive material was macerated with MeOH (380 g). The *n*-hexane residue was partitioned between *n*-hexane and MeOH–H₂O 9 : 1. The methanolic soluble part (5.6 g), chromatographed on a Sephadex LH-20 column using CHCl₃–MeOH 1 : 9 as eluent, afforded bergapten (14 mg).

The *n*-hexane fraction was treated with warm alcoholic alkali solution, followed by Et₂O extraction to give a fat fraction. The compounds isolated from the nonsaponifiable fraction, after flash chromatography using *n*-hexane–EtOAc 7 : 3 as eluent, consist of ß-sitosterol (24.5 mg), ß-amyrin (12 mg), taraxasterol (9.2 mg) and an hitherto unreported pentacyclic triterpene, 11ß-hydroxy-ß-amyrin (11 mg), previously isolated from several other sources (Ikuta and Morikawa, 1992; Piozzi et al., 1975; Taylor, 1967), but never from *Ficus* species.

The CHCl₃ extract was subjected to gel permeation chromatography on Sephadex LH-20 (CHCl₃–MeOH 1 : 9) to yield a total of 10 fractions designated I–X. Fraction VI was further purified by flash chromatography (CHCl₃–MeOH 9 : 1) to give scopoletin (4 mg), while 7,4′-dimethoxy-5-hydroxyisoflavone (12 mg) was isolated from fraction IX (Jha et al., 1980). Flash chromatography of fraction X, eluting with CHCl₃–MeOH 9 : 1 mixture furnished 5,7,2′,5′-tetrahydroxyflavanone (3.9 mg) (Bar-nah et al., 1979) and an unresolved mixture of naringenin and genistein, identified by co-TLC with authentic samples.

Fractionation of the CHCl₃–MeOH (9 : 1) extract by column chromatography on Sephadex LH-20 (MeOH as eluent) and preparative TLC led to the isolation of apigenin (83 mg), taxifolin (14 mg), tricetin (5 mg) (Harborne, 1986) and luteolin (8.4 mg) and to the identification of hesperitin and chrysirn by co-TLC with authentic samples.

Rutin (42 mg) and isorhamnetin-3-O-glucoside (25 mg) (Agrawal, 1989) were isolated from the methanolic extract of the plant, after gel filtration on Sephadex LH-20 (MeOH) and repeated silica gel chromatography [CHCl₃–MeOH–H₂O (7 : 3 : 0.5)].

The structures of the isolates were established on the basis of their spectral evidences (mainly UV, ¹H and ¹³C NMR) in comparison with the previously reported data and by direct comparison with the authentic samples.

4. Chemotaxonomic significance

Investigation of the aerial parts of *Ficus pumila* (Moraceae) led to the isolation of two coumarins (scopoletin and bergapten), 10 flavonoids as aglycones (naringenin, genistein, hesperitin, chrysirn, apigenin, taxifolin, tricetin, luteolin, 7,4′-dimethoxy-5-hydroxyisoflavone, 5,7,2′,5′-tetrahydroxyflavanone) and two glycosides (rutin and isorhamnetin-3-glucoside).
None of the compounds is new. However the occurrence of some of the flavonoid aglycones (hesperitin, chrysin, taxifolin, tricetin, 7,4’-dimethoxy-5-hydroxyisoflavone, 5,7,2’,5’-tetrahydroxyflavanone, apigenin, luteolin) is quite significant, since these metabolites are reported in the *Ficus* genus for the first time. The isolates belong to several classes of flavonoids: flavones, isoflavones and flavanones, while flavonols resulted only as glycosides (as reported by Katajama, 1998). No morin was identified and isolated from this source according to Venkataraman chemotaxonomic study (Venkataraman, 1972).

Bergapten, scopoletin, β-sitosterol, taraxasterol, β-amyrin and 11α-hydroxy-β-amyrin were also obtained. These are expectable compounds in that they occur commonly in other species of the genus, except for 11α-hydroxy-β-amyrin.

The remarkable difference existing between the secondary metabolites of our plant material and those reported in the literature for specimens of *Ficus* from other geographical localities, may be related either to the real specific differences or more probably to a geographic or enviromental influence on biosynthesis.

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**References**