Flavonoids from Ficaria verna Huds.

Michał Tomczyka,*, Jan Gudej a and Marek Sochackib

a Department of Pharmacognosy, Faculty of Pharmacy, Medical Academy of Białystok, Mickiewicza Str. 2a, 15–230 Białystok, Poland. Fax: +48-85-742 49 07. E-mail: tomczyk@amb.edu.pl

b Centre of Molecular and Macromolecular Studies, Department of Structural Studies, Mass Spectrometry Facility, Polish Academy of Sciences, 112 Sienkiewicza Str., 90-363 Łódź, Poland

* Author for correspondence and reprint requests

Z. Naturforsch. 57c, 440–444 (2002); received January 7/February 7, 2002

Ranunculaceae, Ficaria verna, Flavonoids

A phytochemical investigation of the flowers and leaves of Ficaria verna Huds. (Ranunculaceae) yielded four additional known flavonoid compounds including: kaempferol 3-O-β-D-(6'-α-L-rhamnopyranosyl)-glucopyranoside (nicotiflorin), apigenin 8-C-β-D-glucopyranoside (vitexin), luteolin 8-C-β-D-glucopyranoside (orientin) and apigenin 8-C-β-D-(2'-O-β-D-glucopyranosyl)glucopyranoside (flavosativaside). The characterisation of these compounds was achieved by various chromatographic and spectroscopic methods (UV, 1H NMR, 13C NMR and MS).

Introduction

The buttercup family, Ranunculaceae comprises 59 genera and about 1900 species (Evans, 1996). Members of the family are distributed throughout the world. Most of them are well represented as perennial plants widely occurring in all of Europe. The genus Ficaria (Ranunculaceae), often included in the genus Ranunculus, is represented in Poland only by two subspecies: Ficaria verna HUDS. (syn. Ranunculus ficaria L. subsp. bulbifer LAMBINON, Ficaria ranunculoides ROTH) and Ficaria nudicaulis A. KERN. (syn. Ranunculus ficaria L. subsp. calthifolius (RCHB.) ARCAD., Ficaria calthifolia (RCHB.) (Mirek et al., 1995; Tutin et al., 1964). F. verna known as pilewort is a common early-spring flowering perennial herb. It is used in folk medicine and homeopathy as an anti-inflammatory, astringent, antibiotic and anti-haemorrhagic treatment (Evans, 1996; Delacroix, 1969; Palliez et al., 1968; Docheva-Popova and Popov, 1955). The extracts of the plant are applied to haemorrhoids by topical application as ointment or suppository. Previous chemical studies of pilewort tubers proved the presence of triterpenoid saponins (Pourrat et al., 1979; Pourrat et al., 1982; Texier et al., 1984; Brisse-Le Menn et al., 1990). In the above-ground parts of the plant amino acids, mineral elements as well as vitamin C were detected (Perseca and Parvu, 1986; Rams, 1978; Franke and Kensbock, 1981; Istrătescu-Guti and Forstner, 1974). Pilewort is the first plant outside of the Gentianaceae family to be reported as containing the disaccharide – gentiobiose (Barthomeuf et al., 1987). In the fresh parts of the plant, ranunculin and products of its decomposition have been observed (Ruijgrok, 1966; Bonora et al., 1988). According to reviewed literature, little is known about the presence of undefined flavonoid compounds in F. verna (Cameroni and Bernabei, 1957; Lebreton, 1986). This has encouraged us to investigate the polyphenolic compounds of this plant growing in Poland. In the previous work we have initiated a phytochemical analysis of flavonoids and phenolic acids and reported the presence of derivatives of quercetin and kaempferol (Gudej and Tomczyk, 1999; Tomczyk and Gudej, 2000). The present paper describes isolation and structure elucidation of additional flavonoids from flowers and leaves of F. verna.

Materials and Methods

Plant material

F. verna flowers and leaves were collected in the Białystok area between March and April of 1997. A voucher specimen of plant has been identified by Dr. Jan Gudej and has been deposited in the herbarium of the Department of Pharmacognosy...
Medical Academy of Białystok, Poland (No. FV 97004).

Equipment, reagents, solvents

All melting points (m.p.) were determined on Büchi 535 melting point apparatus and are uncorrected. All solvents of analytical grade were purchased from POCH (Gliwice, Poland). Polyamide (ROTH, Karlsruhe, Germany) and Sephadex LH-20 (FLUKA, Buchs, Switzerland) were used for column chromatography (CC). Chromatographic systems: PPC: 5% MeOH (system S1); TLCc: n-BuOH/HOAc/H2O 4:1.5 v/v/v upper phase (system S2), 15% HoAc (system S3), HOAc/HCl conc./H2O (30:3:10 v/v/v) (system S4), n-BuOH/pyridine/H2O (6:4:3 v/v/v) (system S5); TLCg: EtOAc/HCOOH/H2O 18:1:1 v/v/v (system S6). Visualisation of plates was performed using visible light, UV fluorescence and/or spraying with the following reagents: R1: 2% AlCl3, R2: Naturstoffreagenz A (ROTH, Karlsruhe, Germany), R3: aniline phthalate by heating at 110 °C for 5–10 min. CC: H2O/MeOH increasing gradient (system S7), C6H6/MeOH increasing gradient (system S8), EtOAc/MeOH increasing gradient (system S9). Acid hydrolysis: the pure compounds were treated with 5% HCl for compound I and with 10% HCl for compounds II, III, IV at 100 °C for 4 hrs. UV spectra were recorded on a SpecORD 40 UV-VIS Spectrophotometer (Jena Analytik AG, Germany) according to Mabry et al. (1970). 1H NMR and 13C NMR were taken on a Bruker instrument (200 and 50 MHz, respectively). MS were obtained with Finnigan MAT 95 mass spectrometer. All solvents from the extracts were evaporated to dryness using a Büchi Rotavapor R-200.

Extraction and isolation

Preparation of extracts from flowers has been described previously (Gudej and Tomczyk, 1999). Compound I was obtained from an EtOAc extract using a polyamide column (CC, system S7). The fractions eluted with 10% MeOH containing two compounds (mixture A) were repeatedly chro-matographed on a polyamide column (CC, system S8). Pure I (9 mg) was obtained from fractions eluted with C6H6/MeOH (8:2 v/v). Further fractions eluted with 15% MeOH from the same EtOAc extract on a polyamide column (CC, system S7) containing the mixture of compounds II and III (mixture B). Mixture B was further chromatographed on a polyamide column and eluted with system S9. This procedure led to the isolation of II and a mixture of II and III (mixture C). Compound I (35 mg) was obtained from the fraction eluting with EtOAc/MeOH (7.5:2.5 v/v). Using preparative PC, which was twice developed with system S1 from the mixture C, additional amounts of compounds II (8 mg) and III (4 mg) were separated. Both compounds were purified by column chromatography on Sephadex LH-20 using MeOH as eluent.

The air-dried and powdered leaves of F. verna (2 kg) were extracted with petrol and CHCl3 in a Soxhlet apparatus. Plant material purified in this way was successively extracted with MeOH. After solvent evaporation under reduced pressure, the MeOH extract was diluted with H2O and successively partitioned between Et2O, EtOAc and n-BuOH, affording 6.0, 20.0 and 119.0 g of each dried fraction, respectively. The EtOAc and n-BuOH extracts were chosen for further study. Initial isolation of compounds from the EtOAc extract was carried out by chromatography on polyamide eluting with solvents mixtures of increasing polarity (CC, system S7). Elution with 10% MeOH gave a mixture of two compounds (mixture D). Repeated chromatography of mixture D on a polyamide column (CC, system S8) yielded compound IV (72 mg). It was obtained from the fraction eluted with C6H6/MeOH (7:3 v/v) and was further purified by CC on Sephadex LH-20 using MeOH.

Identification of isolated flavonoid compounds

Compound I: Pale-yellow needles; m.p. 187–189 °C; Rf, TLCc: S2-0.51; S3-0.56; TLCg: S6-0.35; UV λmax MeOH 267, 297sh, 301, 350; +NaOMe 277, 326, 405; +AlCl3 274, 306, 352, 398; +AlCl3/HCl 276, 303sh, 348, 397; +NaOAc 275, 326, 362; +NaOAc/H3BO3 267, 304sh, 351 nm. 1H NMR (DMSO-d6): 12.56 (1H, brs, H-5), 7.98 (2H, d, J = 8.8 Hz, H-2’ and H-6’), 6.87 (2H, d, J = 8.8
Hz, H-3’ and H-5’), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6), 5.30 (1H, d, J = 7.1 Hz, H-1’), 5.11–4.44 (6H, m, H-12–H-17-sugars), 4.37 (1H, s, H-1”), 4.12–3.49 (10H, m, H-sugars), 0.79 (3H, d, J = 6.0 Hz, H-6”), CH2-rhamnose ppm; 13C NMR (DMSO-d6): 177.34 (C-4), 164.29 (C-7), 161.17 (C-5), 159.88 (C-4”), 156.80 (C-9), 156.49 (C-2), 133.18 (C-3), 130.86 (C-2’ and C-6’), 120.87 (C-1’), 115.08 (C-3’ and C-5’), 103.90 (C-10), 101.32 (C-1”), 100.76 (C-1”), 98.76 (C-6), 93.76 (C-8), 76.31 (C-3”), 75.70 (C-5’), 74.13 (C-2’), 71.77 (C-4”), 70.55 (C-3”), 70.30 (C-2”), 69.88 (C-4”), 68.21 (C-5”), 67.38 (C-6”), 17.72 (C-6”) ppm. Rf value of the aglycone (coTLCc, S4, R1) after acid hydrolysis of I was identical with kaempferol. Rf values of sugars (coTLCc, S5, R3) after hydrolysis were identical with the standards, glucose and rhamnose.

Compound II: Yellow needles; m.p. 275–276°C; Rf TLCC: S2-0.41; S3-0.23; TLCg: S6-0.38; UV λmax MeOH 269, 302sh, 332; +NaOMe 277, 329, 393; +AlCl3 276, 304, 346, 383; +AlCl3/HCl 278, 303, 343, 383; +NaOAc 280, 301, 356; +NaOAc/H3BO3 271, 301sh, 332 nm. 1H NMR (DMSO-d6): 13.17 (1H, s, H-1), 8.03 (2H, d, J = 8.6 Hz, H-2’ and H-6’), 6.89 (2H, d, J = 8.6 Hz, H-3’ and H-5’), 6.79 (1H, s, H-3), 6.27 (1H, s, H-6), 4.68 (1H, d, J = 10 Hz, H-1’) ppm; 13C NMR (DMSO-d6): 182.10 (C-4), 163.94 (C-2), 162.55 (C-7), 161.12 (C-4’), 160.38 (C-9), 155.98 (C-5), 128.96 (C-2’ and C-6’), 121.60 (C-1’), 115.80 (C-3’ and C-5’), 104.59 and 104.03 (C-8 and C-10), 102.45 (C-3), 98.12 (C-6), 81.84 (C-5’), 78.64 (C-1’), 73.37 (C-2’), 70.82 (C-3’), 70.51 (C-4’), 61.27 (C-6”) ppm. LSIMS calcd for C21H20O10 432.379; negative-ion 431 [M-H]–; positive-ion 433 [M+H]+, 313 [M+H+120]–, 271 [M+H-162]–. Total acid hydrolysis of II yielded vitexin accompanied by its Wessely-Moser isomer isovitexin (Rf, coTLCc, S2, S3, R1, R2) and sugar–glucose (Rf, coTLCc, S5, R3).

Results and Discussion

The EtOAc extracts of F. verna flowers and leaves were fractionated on polyamide columns. Further separation and purification was achieved by combining PPC, polyamide columns and Sephadex LH-20 and gave pure flavonoid compounds I–IV. The identification of those compounds was carried out on the basis of Rf values, products of acid hydrolysis and spectroscopic methods (UV, 1H NMR, 13C NMR, MS). The flavonoids isolated from F. verna are listed in Table I. Acid hydrolysis of I released glucose and rhamnose in sugar’s residue and kaempferol as an aglycone. The UV spectral data of I with diagnostic shift reagents indicated a flavonol substituted at position C-3, free C-7 and absence of an ortho-dihydroxyl pattern at B ring. The 1H NMR spectrum suggested that I is a disaccharide of kaempferol on the basis of two signals in the sugar region at 5.30 (d, J = 7.1 Hz)
Table I. Flavonoids from Ficaria verna Huds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R'–</th>
<th>O–rut</th>
<th>R&quot;–</th>
<th>H</th>
<th>R&quot;–</th>
<th>OH</th>
<th>R&quot;–</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound I</td>
<td>R'–</td>
<td>H</td>
<td>R&quot;–</td>
<td>H</td>
<td>R&quot;–</td>
<td>OH</td>
<td>R&quot;–</td>
<td>H</td>
</tr>
<tr>
<td>Compound II</td>
<td>R'–</td>
<td>H</td>
<td>R&quot;–</td>
<td>H</td>
<td>R&quot;–</td>
<td>OH</td>
<td>R&quot;–</td>
<td>glc</td>
</tr>
<tr>
<td>Compound III</td>
<td>R'–</td>
<td>H</td>
<td>R&quot;–</td>
<td>OH</td>
<td>R&quot;–</td>
<td>OH</td>
<td>R&quot;–</td>
<td>glc</td>
</tr>
<tr>
<td>Compound IV</td>
<td>R'–</td>
<td>H</td>
<td>R&quot;–</td>
<td>H</td>
<td>R&quot;–</td>
<td>OH</td>
<td>R&quot;–</td>
<td>glc (1&quot;→ 2&quot;) glc</td>
</tr>
</tbody>
</table>

rut – rutinoside.
glc – glucose.

and 4.37 (s), corresponding to the anomeric protons of β-glucose and to the anomeric proton of the α-linked rhamnose, respectively. That fact indicated that α-rhamnose was attached to the C-6 at β-glucose moiety which was confirmed by its 13C NMR spectrum and this also defined the disaccharide as a 3-O-rutinoside. The structure of compound I is therefore established as a kaempferol 3-O-β-D-(6’-O-α-L-rhamnopyranosyl)-glucopyranoside (nicotiflorin). Compounds II and IV showed the same UV spectra and diagnostic shifts as apigenin with free 5, 7 and 4’-hydroxyl groups. With the two former compounds acid hydrolysis yielded vitexin (identified by co-TLC with authentic standards) accompanied by small amounts of its Wessely-Moser isomer. The 1H NMR spectrum confirmed that II is a derivative of apigenin substituted by glucose at C-8. The presence of a C-glucosyl bond at C-8 of apigenin was shown by the 13C NMR spectrum. In addition, the structure of II was confirmed by LSIMS spectrum. The spectrum of II gave a molecular ion peak [M+H]+ at m/z 433 indicating a molecular mass of 432. A second prominent ion was obtained at m/z 271 [M+H-162]+ resulting from the loss of a sugar chain consisting of one hexose. The presence of a peak at m/z 313 [M+H-120]+ suggests that hexose is linked to the aglycone by a C-8 linkage, similarly as in IV. Compound II was identified as apigenin 8-C-β-D-glucopyranoside (vitexin). The UV spectral data of III with diagnostic shift reagent indicated a luteolin. The bathochromic shift observed in band II in the presence of NaOAc indicated a free 7-hydroxyl group. The presence of an ortho-dihydroxyl group in the B-ring of III was detected by comparing the spectrum in the presence of AlCl3 with that obtained in AlCl3/HCl and additionally confirmed by a bathochromic shift observed in band I in the presence of NaOAc/H3BO3. Structure of III was additionally recognized as luteolin 8-C-β-D-glucopyranoside (orientin) by analysing the mixture isomers after acid hydrolysis (Wessely-Moser rearrangement) and analysis of the 1H NMR spectrum. The chromatographic behaviour of IV suggested a diglycosidic structure. Acid hydrolysis of IV gave compound II (co-TLC with authentic standards) and the sugar was identified as glucose. The 1H NMR spectrum of IV exhibited two sugar anomeric protons at 4.79 (d, J = 10 Hz) and 4.09 (d, J = 8.5 Hz) with coupling constants appropriate for β-glucopyranose. The presence of the C-glycosidic bond in C-8 position in IV was confirmed by 13C NMR. In this case the signal at 105.15 ppm indicates substitution at C-8 position of the aglycone. In addition, in the LSIMS spectra of IV peaks at m/z 595 (positive mode) and m/z 593 (negative mode) were observed. In order to obtain information about the structure of the sugar moiety, collisionally induced dissociation of [M+H]+ ion was performed and linked scan spectra at constant B/E were recorded. The presence of peaks at m/z 433 [M+H-162]+ and m/z 271 [M+H-161-162]+ confirmed the presence of two hexose units. The formation of relative intense ions at m/z 475 [M+H+120]+ and m/z 313 [M+H+162-120]+ suggests that sugar chain is linked by a C-8 linkage (Gluchhoff-Fiasson et al., 1989; Qimin et al., 1991). Compound IV was, based on products of acid hydrolysis and of its spectrosopic data, identified as apigenin 8-C-β-D-(2”-O-β-D-glucopyranosyl)-glucopyranoside (flavosativaside). The spectral properties of compounds I–IV, in-

Mabry T. J. 1970; Markham et al., 1978; Agrawal, 1989; Kartnig et al. 1991; Harborne, 1996). This is the first report of these compounds from F. verna.