# Flavonoids in flowers of *Calendula officinalis* L.

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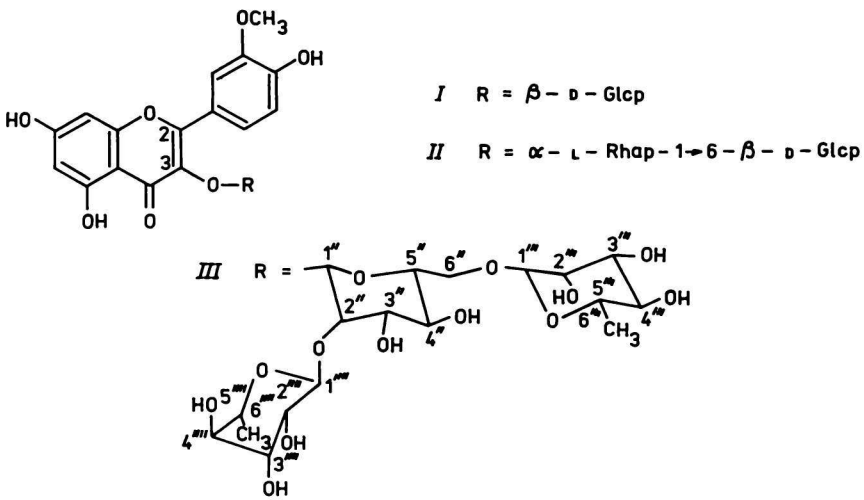
The triglycoside isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (*III*) from flowers of *Calendula officinalis* L. was isolated together with the already known glycosides isorhamnetin-3-*O*-β-D-glucopyranoside (*I*) and isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside (narcissine, *II*). The total content of flavonoids in ligulate ray-florets and tubular disc-florets inclusively involucra was found to be 0.88 and 0.25 %, respectively.

So far, essential oil [1], sesquiterpenes [2], carotenoids [3], pentacyclic triterpenes [4, 5], organic acids [6], and flavonoids have been reported to be constituents of marigold (*Calendula officinalis* L., *Asteraceae*). Flavonoids were investigated by *Friedrich* [7], *Biryuk* and coworkers [8] and recently by *Komissarenko* and coworkers [9], who identified several flavonoids from the ethanolic extract of flowers. During our studies on constitution of isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (*III*) the paper by *Vidal-Ollivier et al.* [10] appeared presenting its structure; it left us, therefore, only to supplement this paper by more detailed spectral data of this triglycoside obtained in an alternative way.

The crude ethanolic extract of flavonoids separated on a Sephadex LH-20 column afforded three glycoflavonoids belonging to the isorhamnetin group.

Compounds *I* (yellow crystals, m.p. = 248—250 °C) and *II* (yellow crystals, m.p. = 176 °C) were identified by $^1$H NMR spectral data consistent with those reported [7—9] for isorhamnetin-3-*O*-β-D-glucopyranoside (*I*) and isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside (narcissine, *II*).

Compound *III* (pale-yellow crystals from methanol—water), m.p. = 187 °C, $[\alpha](578\,\text{nm},\ 23\,°\text{C},\ \varrho = 2.5\,\text{g dm}^{-3},\ \text{MeOH}) = -76°$ Acid hydrolysis of *III* yielded isorhamnetin, glucose, and rhamnose; the electron impact mass spectrum of the former showed a peak at $m/z = 317$ (protonated aglycone) and at $m/z = 772$ (molecular radical ion). The IR spectrum was indicative of the presence of hydroxyl, conjugated carbonyl, aryl alkyl ethereal, and glycoside (broad) groupings. The UV spectrum measured in methanol and that recorded after addition of diagnostic reagents [11] were almost identical with those of the above-mentioned glycoflavonoids. A bathochromic shift evoked by NaOMe, NaOAc, and $AlCl_3$ evidenced the presence of hydroxyl groups at the flavonoid backbone in positions C-4′, C-5, and C-7, and also of a methoxyl group at C-3′ Binding site and structure of the saccharide moiety were estimated on the basis of $^{13}C$ NMR spectral data, which were in accordance with those published in [10]. The structure of this compound can be illustrated by formula *III*, confirming the assumption of Friedrich [6] who presumed the presence of a trisaccharide




in the molecule of one of the isorhamnetin derivatives. Similarly *Parker* and *Bohm* presumed such a compound in *Limnanthes douglasii*; due to a small amount of the isolate they were unable to solve the structure [12].

A very different quantitative representation of flavonoids in ligulate ray-florets as opposed with tubular disc ones inclusively involucra was estimated by the method of *Christ* and *Müller* [13]. This finding showing the ligulate ray-florets to be more valuable is important when collecting the drug.

## Experimental

The melting points were measured on a Kofler micro hot-stage, the mass spectra were taken with a ZAB-EQ (VG Analytical Ltd. Manchester), the UV and IR spectra were recorded with the respective Specord UV VIS (Zeiss, Jena), and Perkin—Elmer, model 477 spectrophotometers. The $^1H$ and $^{13}C$ NMR spectra of dimethyl sulfoxide-$d_6$ solution containing tetramethylsilane as an internal reference were run with a Bruker AM-300 apparatus at 300 and 75.47 MHz, respectively. *Calendula officinalis* was grown in Eastern Slovakia, flowers were dried in the shade.

### *Isolation*

Dried ligulate ray-florets of *Calendula officinalis* L. (318 g) were worked up according to [14]. The obtained sum of flavonoids (4.96 g) was separated by gel permeation chromatography on Sephadex LH-20 (eluent methanol). The separation was repeated with 80 % methanol (vol. %) and the single portions were crystallized from dilute methanol to give three compounds of characteristic $R_f$ values on thin-layer chromatograms on silica using ethyl acetate—formic acid—water ($\varphi_r = 10:2:3$) as eluent. Compound *I*: 16 mg, $R_f = 0.45$; *II*: 95 mg, $R_f = 0.20$; *III*: 260 mg, $R_f = 0.08$.

Spectral data of compound *III*: mass spectrum, $m/z$ ($I_r$/%): 772 ($M^+ + 2$) (19), 625 (12), 317 (100), 257 (27), 224 (78). IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3400, 2900, 1660, 1600, 1500, 1445, 1430, 1360, 1210, 1130, 1060 (broad), 925, 810. UV spectrum (methanol), $\lambda_{max}$/nm: 255, 268 sh, 306, 356; (NaOMe): 272, 329, 410; ($AlCl_3$): 272, 306, 366 sh, 412; ($AlCl_3$/HCl): 273, 301, 364, 409; (NaOAc): 275, 289, 317, 378; ($H_3BO_3$/NaOAc): 254, 268, 317, 359. $^1H$ NMR spectrum, $\delta$ (40 °C): 12.6 (br. s, 1H, C-5—OH), 10.3 (br. s, $OH_{aglyc.}$), 9.7 (br. s, 1H, $OH_{aglyc.}$), 7.83 (d, 1H, $J_{2',6'} = 2.1$ Hz, C-2′—H), 7.49 (dd, 1H, $J_{5',6''} = 8.4$ Hz, C-6′—H), 6.90 (d, 1H, C-5′—H), 6.41 (d, 1H, $J_{6,8} = 2.1$ Hz, C-8—H), 6.19 (d, 1H, C-6—H), 5.62 (d, 1H, $J_{1'',2''} = 7.5$ Hz, C-1″—H), 5.03 (d, 1H, $J_{1''',2'''} = 1.5$ Hz, C-1‴—H), 4.39 (d, 1H, $J_{1'''',2''''} = 1.6$ Hz, C-1⁗—H), 5.27, 5.06, 4.50, 4.42, 4.35, 4.27, 4.27 (7 × OH, $OH_{sacch.}$), 3.86 (s, 3H, C-3′—$OCH_3$), 0.97 (d, 3H, $J_{6'',5''} = 6.2$ Hz, C-6‴—H), 0.73 (d, 3H, $J_{6'''',5''''} = 6.2$ Hz, C-6⁗—H). $^{13}C$ NMR spectrum, $\delta$(40 °C): 177.1 (C-4), 163.9 (C-7), 161.1 (C-5), 156.3 (C-9), 156.3 (C-2), 149.2 (C-3′), 146.8 (C-4′), 132.4 (C-3), 122.1 (C-6′), 121.0 (C-1′), 115.1 (C-5′), 113.3 (C-2′), 104.0 (C-1), 100.8 (C-1‴), 100.7 (C-1⁗), 98.6 (C-6), 98.5 (C-1″), 93.7 (C-8), 77.6 (C-2″), 76.9 (C-3‴), 75.7 (C-5″), 71.7 (C-4‴), 71.7 (C-4⁗), 70.6 (C-3‴), 70.6 (C-3⁗), 70.5 (C-2⁗), 70.4 (C-2‴), 70.2 (C-4″), 68.2 (C-5‴), 68.2 (C-5⁗), 66.6 (C-6″), 55.7 ($OCH_3$), 17.5 (C-6‴), 17.0 (C-6⁗).

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