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Flavonol glycosides from *Eschscholtzia californica*

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Abstract

The aqueous EtOH extract of aerial parts of *Eschscholtzia californica Cham.* yielded six flavonol 3-*O*-glycosides including two new compounds: quercetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] and isorhamnetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside]. Their structures were established on the basis of spectroscopic studies.

Keywords: Eschscholtzia californica; Papaveraceae; Flavonol 3-*O*-glycosides

1. Introduction

Eschscholtzia californica Cham. (Papaveraceae) is a traditional medicinal plant of Indians used by the rural population of California for its sedative and analgesic properties (Cheney, 1964). Pharmacological in-vivo studies with mice showed that only aqueous and EtOH extracts have sleep-inducing, sedative and anxiolytic effects (Rolland, 1991). Locomotion studies demonstrated that an aqueous extract of *E. californica Cham.* significantly reduced the spontaneous movement activity at doses >100 mg/kg mice. Moreover, the sleep-inducing and anxiolytic properties were shown in several behavior tests (e.g. novelty preference, two compartment tests and staircase test).

E. californica Cham. contains high amounts of isoquinoline alkaloids (Urzua & Mendoza, 1986). The flavonol glycosides rutin (Sando & Bartlett, 1920) and quercitrin (Jain, Tripathi, Pandey, & Rücker, 1996) and the isoflavones 2′-methoxyformononetin and 7-methoxy-2′,4′-dihydroxyisoflavone (Jain et al., 1996) have been earlier reported from this species. Here we report the isolation of five additional flavonol glycosides from an aqueous EtOH extract of *E. californica Cham.*

2. Results and discussion

HPLC fingerprint analysis of an aqueous EtOH extract of *E. californica Cham.* combined with photodiode array detection supplied the first indications of six different flavonoids. Their UV spectra show characteristic site bands for flavonol 3-*O*-glycosides in the range of 255 and 355 nm. To isolate these flavonoids the aqueous EtOH extract was initially fractioned on a Fractogel TSK HW 40 S column with MeOH–H_2O (8:2). The fractions showing a UV max at 350–370 nm, typical for flavonol 3-*O*-glycosides, were chromatographed using a preparative column with LiChrospher 60 RP select B as solid phase and with MeOH–H_2O mixtures as mobile phase. The isolated flavonoids (Fig. 1) were identified as three different quercetin (**1**–**3**) and three different isorhamnetin (**4–6**) derivatives by two-dimensional TLC in applying the corresponding reference substances after acid hydrolysis of the genuine compounds on the silica gel TLC plate (Heisig and Wichtl, 1988). It was observed that compounds **1** and **4** liberated glucose, compounds **2** and **5** glucose and rhamnose, whereas compounds **3** and **6** yielded rhamnose and glucose in ratios of 2:1. Interglycosidic linkage points were determined from the ^{13}C NMR spectrum. Besides the already known presence of rutin (**2**), we found for the first time quercetin 3-*O*-β-glucopyranoside (**1**), quercetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (**3**), isorhamnetin 3-*O*-β-glucopyrano-

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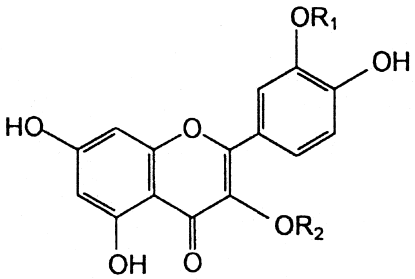

	R_1	R_2
1	H	β-D-Glc
2	H	α-L-Rha(1-6)-β-D-Glc
3	H	α-L-Rha(1-4)-α-L-Rha(1-6)-β-D-Glc
4	CH_3	β-D-Glc
5	CH_3	α-L-Rha(1-6)-β-D-Glc
6	CH_3	α-L-Rha(1-4)-α-L-Rha(1-6)-β-D-Glc

Fig. 1. Structures of flavonol glycosides.

side (**4**), isorhamnetin 3-*O*-[α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (**5**) and isorhamnetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (**6**) in *E. californica Cham.* The identity of **1**, **2**, **4** and **5** were confirmed by comparison of the measured ^{1}H, ^{13}C NMR and ESI-MS data with spectroscopic data available from the literature and the data additionally obtained with authentic samples (Hörhammer, Wagner, & Probst, 1960; Hörhammer et al., 1966; Sosa & Sosa, 1966) (Roth, Karlsruhe). These compounds are widespread in flora whereas the two linear trisaccharides **3** and **6** are to our knowledge isolated for the first time and structurally elucidated by NMR and ESI-MS spectroscopy. Although the occurrence of these two compounds in Actinidia species was assumed (Webby & Markham, 1989), their structures were not confirmed by spectroscopic data (Harborne, 1986a). The ^{1}H NMR spectrum of compounds **3** and **6** indicated the presence of two rhamnosyl and one glucosyl moieties with characteristic signals at 4.53 and 5.18 ppm (H-1‴, H-1⁗, d, $J = 1.6$ Hz) and 1.07 and 0.91 ppm (H-6‴, H-6⁗, dd, $J = 6.19$ Hz) for rhamnose and the anomeric proton of β-glucose at 5.72 ppm (d, $J = 7.6$ Hz). The second rhamnose moiety must be attached to the rhamnosyl portion of rutinose, as the glucose ^{13}C signals were equivalent to those seen in rutin (see Table 1). Inspection of the ^{13}C shifts indicates that only one signal characteristic of a free rhamnose C-4‴ position is present at 73.9 ppm and indicates that the signal at 80.1 ppm must be ascribed to that of a substituted C-4 of an internal rhamnose moiety. Alternative substitution patterns involving either C-2 or C-3 of this rhamnose would require the presence of two signals in the region of 73–74 ppm and the absence of one of the signals of ca. 72 ppm. Consequently compound **3** is the linear quercetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside]. Similar arguments indicate compound **6** to be isorhamnetin 3-*O*-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside].

Due to the occurrence of the mono-, di- and triglycosides, we looked for the corresponding aglycones quercetin and isorhamnetin. Therefore authentic samples of quercetin and isorhamnetin (Roth, Karlsruhe) were chromatographed using the same HPLC conditions. Retention times and UV spectra did not coincide with any substance in the extract. It must be concluded that either the aglycones are not present in *E. californica Cham.*, or the amounts of quercetin and isorhamnetin are beyond the detection limit. However, it should be taken into account that it is well known that most flavonoids occur naturally in conjugated forms, usually bound to sugars (Harborne, 1986b).

3. Experimental

3.1. General

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were obtained in CD_3OD using a JEOL Eclipse + 500

Table 1
^{13}C NMR spectral data of **1**–**6** (δ ppm, CD_3OD, 125 MHz)

Position	1	2	3	4	5	6
2	158.5	158.5	158.4	158.5	158.5	158.4
3	135.6	135.6	134.4	135.3	135.5	134.2
4	179.5	179.4	179.3	179.4	179.3	179.1
5	163.1	162.9	163.1	163.1	163.0	163.0
6	99.9	99.9	99.8	100.0	100.1	100.2
7	166.0	166.0	165.8	166.2	166.2	166.7
8	94.8	94.9	94.8	94.8	95.0	94.9
9	158.9	159.3	158.9	158.6	158.9	158.5
10	105.9	105.6	105.9	105.7	105.7	105.6
1′	123.5†	123.1†	123.5†	123.1†	124.0†	123.4†
2′	117.4	116.0	117.4	114.3	114.6	114.5
3′	145.9	145.8	145.9	150.8	150.9	150.6
4′	149.5	149.8	149.5	148.4	148.3	148.3
5′	117.4	117.7	117.4	116.0	116.1	116.1
6′	123.5†	123.5†	123.5†	123.8†	123.0†	123.7†
OCH_3-3′				56.8	56.8	56.9
Glc-1″	102.6	104.7	102.6	103.6	104.5	102.7
2″	75.7	75.7	74.1	75.9	75.9	73.9
3″	78.2	78.2	78.9	78.5	78.2	78.8
4″	71.9	71.3‡	71.9‡	71.5	71.6‡	71.9‡
5″	77.1	77.1	77.1	78.1	77.4	77.2
6″	62.6	68.5	68.3	62.5	68.6	68.1
Rha-1‴		102.4	102.2		102.5	102.4
2‴		72.0‡	72.3‡		72.1‡	72.3‡
3‴		72.2‡	72.3‡		72.3‡	72.3‡
4‴		73.9	80.1		73.9	80.1
5‴		69.7	69.9		69.8	69.8
6‴		17.9	17.8		17.9	17.8
Rha-1⁗			100.5			100.5
2⁗			72.1‡			72.1‡
3⁗			72.4‡			72.4‡
4⁗			73.9			73.8
5⁗			69.7			69.9
6⁗			17.5			17.5

†‡Signals interchangeable within the column.

spectrometer. δ are expressed as ppm downfield from TMS. ESI-MS was recorded on a Micromass AutoSpec spectrometer, sample was introduced in MeOH (10 μl min^{-1}). CC was performed on a Fractogel TSK HW 40S column (Superformance® 500-100 Merck). HPLC was carried out on a preparative LiChrospher 60 RP select B column (10 × 100 mm, 3 μm, Merck). 2D-TLC (silica gel; EtOAc–EtOH–H_2O (8:2:1); then $C_6H_5CH_3$–$HCO_2C_2H_5$–HCO_2H (5:4:1)) after acid hydrolysis was performed as described in (Heisig and Wichtl, 1988).

3.2. Plant material

The aerial parts of *E. californica Cham.* were collected from plants cultivated in the Botanical Garden of Marburg, Germany. A voucher specimen is deposited at the Department of Pharmaceutical Biology, Marburg.

3.3. Extraction and isolation

The dried plants (100 g) were cut up and extracted with EtOH (30%) in a percolation system for 16 h at 50–53°C. The extract was prefractioned on a Fractogel TSK HW 40S column (Superformance® 500-100 Merck) with MeOH–H_2O (8:2) as eluent. The fractions showing a UV max at 350–370 nm were purified by HPLC on a preparative LiChrospher 60 RP select B column with MeOH–H_2O systems to afford **1** (116 mg), **2** (204 mg), **3** (56 mg), **4** (55 mg), **5** (15 mg) and **6** (69 mg).

3.4. Quercetin 3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (*3*)

UV (λ_{max}, nm, MeOH) 256, 266 (sh), 356; (NaOMe) 269, 327, 404; (NaOAc) 274, 323, 385; (NaOAc–H_3BO_3) 261, 300 (sh), 301 (sh), 378; ($AlCl_3$) 274, 295

(sh), 331, 430. ESI-MS m/z: 757 $[M + H]^+$. ^1H NMR: δ 7.93 (1H, d, $J_{2',6'} = 2.2$ Hz, H-2′), 7.62 (1H, dd, $J_{5',6'} = 8.5$ Hz, H-6′), 6.89 (1H, d, H-5′), 6.40 (1H, d, $J_{6,8} = 2.1$ Hz, H-8), 6.20 (1H, d, H-6), 5.72 (1H, d, $J_{1',2'} = 7.6$ Hz, H-1′), 5.18 (1H, d, $J_{1'''',2''''} = 1.4$ Hz, H-1′′′′), 4.53 (1H, d, $J_{1''',2'''} = 1.4$ Hz, H-1′′′), 3.00–4.00 (H-2′′- H-5′′, H-2′′′-H-5′′′, H-2′′′′-H-5′′′′), 1.07 (3H, d, $J_{5''',6'''} = 6.2$ Hz, CH_3-6′′′), 1.00 (3H, d, $J_{5'''',6''''} = 6.2$ Hz, CH_3-6′′′′). Multiplicities of most sugar resonances were not determined, because of signal overlap.

3.5. Isorhamnetin 3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-glucopyranoside] (**6**)

UV (λ_{max}, nm, MeOH) 257, 266 (sh), 306 (sh), 357; (NaOMe) 272, 328, 415; (NaOAc) 272, 320, 397; (NaOAc–H_3BO_3) 257, 268 (sh), 306 (sh), 360; ($AlCl_3$) 269, 278 (sh), 301 (sh), 369 (sh), 403. ESI-MS m/z: 761 $[M + H]^+$. ^1H NMR: δ 7.93 (1H, d, $J_{2',6'} = 2.1$ Hz, H-2′), 7.56 (1H, dd, $J_{5',6'} = 8.5$ Hz, H-6′), 6.90 (1H, d, H-5′), 6.35 (1H, d, $J_{6,8} = 2.1$ Hz, H-8), 6.16 (1H, d, H-6), 5.72 (1H, d, $J_{1'',2''} = 7.6$ Hz, H-1′′), 5.18 (1H, d, $J_{1'''',2''''} = 1.4$ Hz, H-1′′′′), 4.53 (1H, d, $J_{1''',2'''} = 1.6$ Hz, H-1′′′), 3.96 (3H, s, OCH_3-3′), 3.00–4.00 (H-2′′-H-5′′, H-2′′′-H-5′′′, H-2′′′′-H-5′′′′), 1.07 (3H, d, $J_{5''',6'''} = 6.2$ Hz, CH_3-6′′′), 0.91 (3H, d, $J_{5'''',6''''} = 6.2$ Hz, CH_3-6′′′′). Multiplicities of most sugar resonances were not determined, because of signal overlap.

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