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# ANALYSIS OF FLAVONOL GLYCOSIDES OF THIRTEEN *EPILOBIUM* SPECIES (ONAGRACEAE) BY LC-UV AND THERMOSPRAY LC-MS

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**Key Word Index**—*Epilobium*; Onagraceae; flavonoids; flavonol glycosides; LC-UV; LC-MS; chemotaxonomy.

**Abstract**—Analysis of the flavonol glycoside patterns of 13 *Epilobium* species was carried out by thermospray liquid chromatography-mass spectrometry (TSP/LC-MS) and high performance liquid chromatography coupled with a photodiode array detector (LC-UV). Mass-data and UV spectra recorded online, before and after post-column addition of shift reagents, provided useful structural information on the different compounds present in crude extracts. Nineteen flavonol glycosides were identified by this means. In order to confirm the on-line assignments, 13 of the compounds were isolated and their structures were elucidated. They were all 3-*O*-glycosides of kaempferol, quercetin and myricetin.

## INTRODUCTION

The genus *Epilobium* (Onagraceae) consists of over 200 species of willow-herbs found all over the world [1]. Sixteen *Epilobium* species are described in Switzerland [1] and 15 in southern Africa [2]. The genus is divided into two sections: *Epilobium* L. and *Chamaenerion* Tausch. [3, 4]. According to Haussknecht [4], the *Epilobium* L. section is divided into two parts, Schyzostigma and Synstigma.

Various members of the section *Epilobium* L., in particular *E. parviflorum* Schreb., *E. montanum* L., *E. roseum* L., and other European species have been used in folk medicine for the treatment of prostatic diseases, mainly benign prostatic hyperplasia [5, 6]. The dried aerial parts are used in infusions [6] and consumption of these herbal teas is very common due to the high prevalence of this disease in the population. In fact, over 80% of males 50–60 years of age have various degrees of bladder outlet obstruction resulting from benign prostatic hyperplasia [7].

The above-ground organs of willow-herbs are known to be rich in flavonoids and these can be successfully used as chemotaxonomic markers [8, 9]. Chemotaxonomy based on flavonoids allows identification of the different species, some of which are botanically very similar and can undergo hybridization [4]. Moreover, flavonoids have been shown to possess a variety of biological activities [10]. A flavonol glucuronide from *E. angustifolium*, for example, has very strong antiinflammatory properties [11]. Furthermore, it has been shown that some glucuronides have higher activities than the corresponding glucosides [12]. Besides antiinflammatory properties, natural and synthetic flavones inhibit aromatase and 5α-reductase, two enzymes implicated in the development of benign prostatic hyperplasia [13, 14].

By using liquid chromatography with UV photodiode-array detection (LC-UV) and post-column derivatization [15–17], it is possible to get rapid information about the aglycone moiety of a flavonol glycoside and the corresponding substitution pattern. Further structural information is provided by the combination of HPLC (LC) with mass spectrometry (MS). Using a thermospray (TSP) interface, it is possible to investigate crude plant extracts containing phenolic glycosides [18]. TSP is suitable for recording weak pseudomolecular $[M+H]^+$ ions and intense signals corresponding to the aglycone fragment $[A+H]^+$. In some cases it is also possible to observe the complementary ions of the sugar moiety.

## RESULTS AND DISCUSSION

A total of 13 species of *Epilobium* was studied. Nine species were collected in Switzerland and four in southern Africa. Plant parts were first extracted with $CH_2Cl_2$ and then with MeOH. The methanolic extracts were enriched in flavonoid constituents by a *n*-BuOH–$H_2O$ partition. The butanolic fractions were analysed by HPLC on RP-18 supports using a step gradient with an acetonitrile–water system containing TFA to avoid peak tailing. Photodiode-array detection allowed the on-line recording of UV spectra (200–500 nm) and a rapid attribution of

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peaks which corresponded to flavonoids in the chromatogram. The assignment of flavonol peaks was unambiguous, since these exhibit characteristic UV spectra [19]. An example is shown in Fig. 1 in which 11 peaks with UV spectra corresponding to flavonols are to be found. The peak X with a different UV spectrum was identified as ellagic acid by comparison with an authentic sample. Flavonoids which were isolated and fully characterized are denoted by numbers (**1–13**), while non isolated flavonoids are denoted by letters (**A–F**).

*LC-MS Thermospray detection*

In order to get more information on the molecular weight of the flavonol glycosides and of their aglycones, TSP/LC-MS analysis of the extracts was carried out. The procedure is outlined with reference to *E. angustifolium* and involves HPLC coupled with UV and MS detection, using the same chromatographic conditions for both. Thus it was possible to compare directly the UV trace (350 nm) with the chromatogram (Fig. 2) showing the total ion current trace (TIC). Since TSP is a soft ionization technique, it yields a significant amount of structural information in the case of phenolic glycosides. Similar mass spectra to those measured under D/Cl-MS conditions with $NH_3$ as reactant gas are obtained with ammonium acetate as buffer. The TSP mass spectra of flavonol glycosides generally present a main peak corresponding to the aglycone $[A+H]^+$ ion and a weak peak corresponding to the glycoside $[M+H]^+$ pseudomolecular ion. In some cases, it is also possible to observe adducts with sodium $[M+Na]^+$. The mass difference between $[M+H]^+$ and $[A+H]^+$ ions gives useful information on the nature of the sugar moiety. From time to time, it is also possible to obtain the ions corresponding to the dehydrated ammonium adduct of the sugar moieties $[sugar-H_2O+NH_4^+]$.

As shown for **4** (Fig. 3), the signal at $m/z$ 449 corresponded to the $[M+H]^+$ pseudomolecular ion, while $[A+H]^+$ at $m/z$ 303, the most important peak, was attributed to the quercetin aglycone ($M_r$ 302) and $m/z$ 164 to the rhamnose complementary ion $[Rha-H_2O+NH_4^+]$. For **9**, ions were observed at $m/z$ 639, 617, 465, 303 (Fig. 4). The ions at $m/z$ 617 and 639 corresponded to $[M+H]^+$ and $[M+Na]^+$, respectively. The signal at $m/z$ 465 was due to the loss of a galloyl moiety ($-152$ amu), while the subsequent loss of a hexosyl unit ($-162$ amu) gave the $[A+H]^+$ ion at $m/z$ 303 corresponding to the aglycone (quercetin). The selectivity of the MS detection allowed resolution of multicomponent

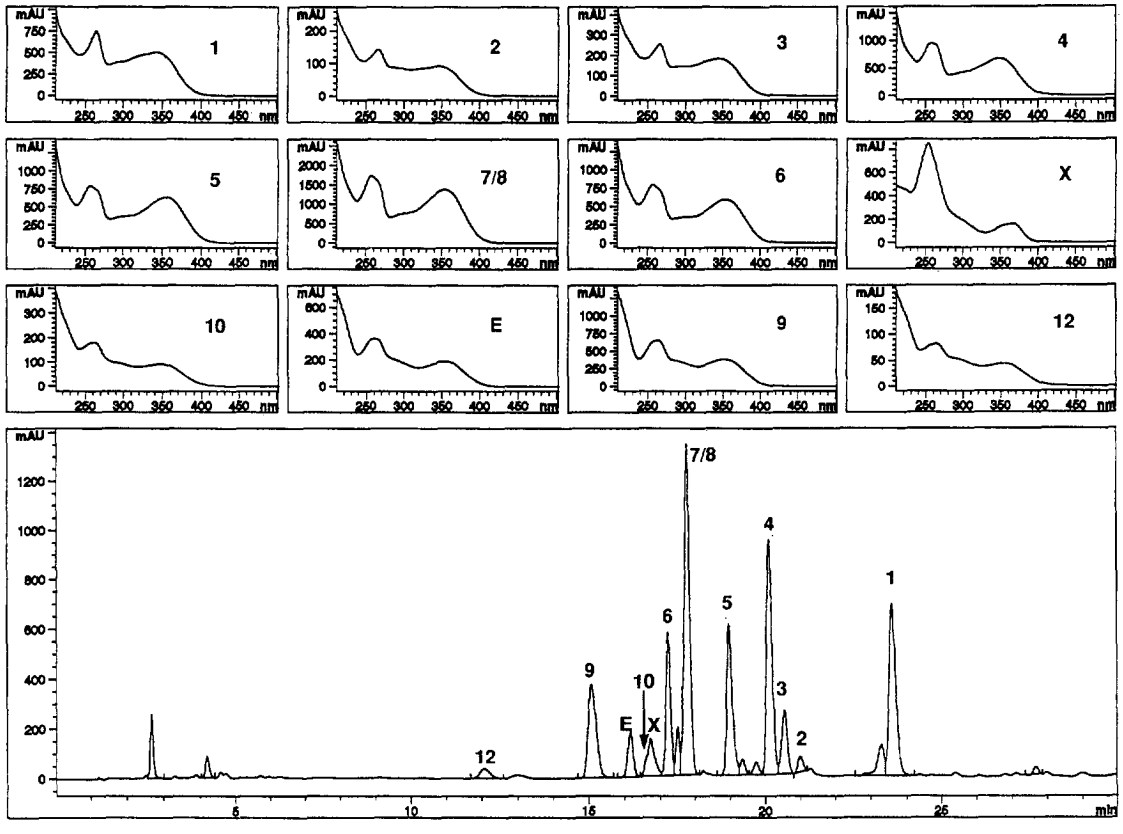



Fig. 1. HPLC-UV chromatogram of a methanolic extract of the aerial parts of *E. angustifolium* enriched by *n*-BuOH–$H_2O$ partition; UV trace recorded at 350 nm; column NovaPak RP-18, 4 μm (150 × 3.9 mm i.d.), equipped with a NovaPak Guard precolumn; step gradient of acetonitrile–water (containing 0.05% TFA): 0 min 10% $CH_3CN$, 4 min 12%, 12 min 12%, 16 min 18%, 30 min 25%; flow rate, 1 ml $min^{-1}$.

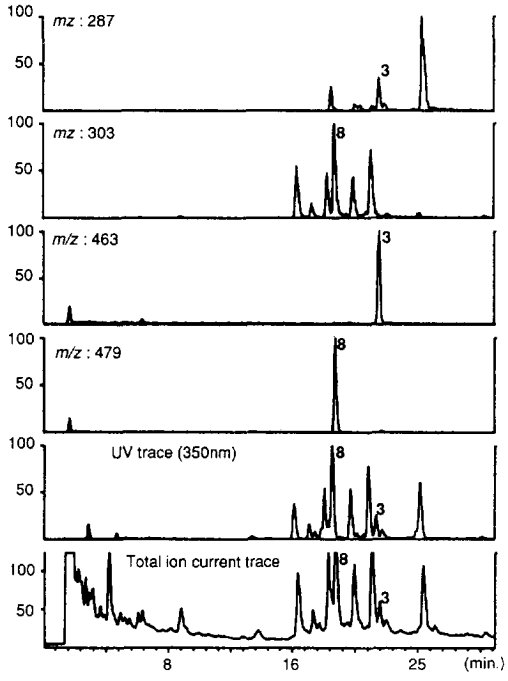



Fig. 2. TSP/LC-MS of the extract as in Fig. 1 using the same chromatographic conditions. Four masses were selected: ion trace at $m/z$ 479 of the pseudomolecular ion $[M+H]^+$ of **8**, the corresponding aglycone ion trace at $m/z$ 303, ion trace at $m/z$ 463 of the pseudomolecular ion $[M+H]^+$ of **3** and the corresponding aglycone ion trace at $m/z$ 287.

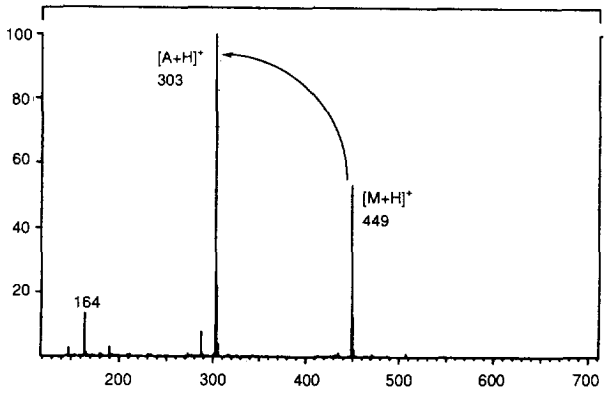



Fig. 3. TSP mass spectrum of **4** recorded on-line from the chromatogram of *E. angustifolium*.

peaks. Figure 5 illustrates the situation for the flavonol glycosides **7** and **8** which were observed as a single peak. Despite overlapping of these two compounds, their presence was demonstrated by the pairs of peaks at $m/z$ 465, 303 and 479, 303 in the mass spectrum.

All 13 *Epilobium* species were analysed in the same way and the data corresponding to each identified compound are reported in Table 1. In most species, **10** (myricitrin) appeared as the main peak, but in *E. angustifolium*, this was only observed as a small shoulder on the peak of

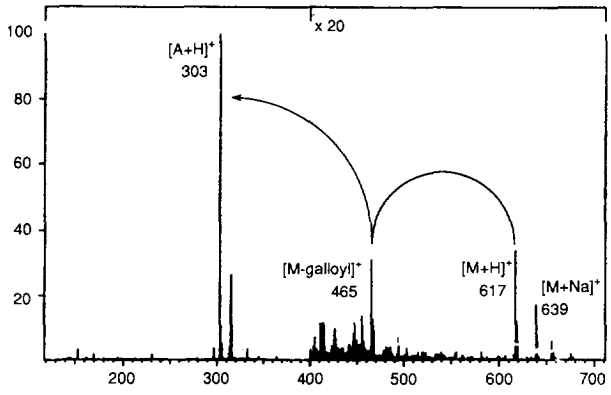



Fig. 4. TSP mass spectrum of **9** recorded on-line from the chromatogram of *E. angustifolium*.

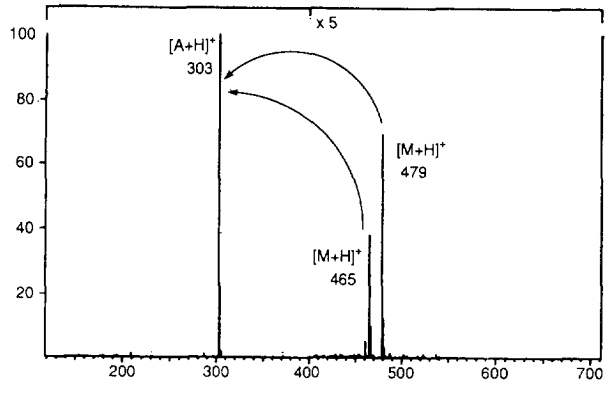



Fig. 5. TSP mass spectrum of co-eluting compounds **7** and **8** obtained on-line from the chromatogram of *E. angustifolium*.

ellagic acid. However, from the mass data ($m/z$ 465, 319), it was possible to confirm the presence of myricitrin in this taxon.

As mentioned above, it was important to search for glucuronides in the different extracts, because of their potentially useful antiinflammatory properties. The selective detection of ions corresponding to quercetin and kaempferol glucuronides allowed their specific and sensitive detection in the crude extract of *E. angustifolium* (Fig. 2). On the other hand, no glucuronide was found in the other *Epilobium* species. Myricetin glucuronide, previously isolated from *E. angustifolium* [11], was not detected in the present analysed extract.

Concerning the mass difference between the $[M+H]^+$ and $[A+H]^+$ ions, hexose ($-162$ amu) was found for **7**, **12**, **B** and **F**, deoxyhexose ($-146$ amu, most probably rhamnose) for **1**, **4** and **10**, pentose ($-132$ amu) for **2**, **5**, **6**, **11**, **A**, **C**, and **D**, uronic acid for **3** and **8**. Compounds **9**, **13**, and **E** gave a difference of 304 amu. This was due to the loss of a hexosyl moiety substituted with a galloyl group.

### *LC-UV Photodiode-array detection*

LC-UV in combination with shift reagents added post-column allows the determination of the hydroxylation

Table 1. On-line UV and MS structural information of **1–13** and **A–F**

| Compound | TSP-MS | | UV spectra | | Shifted UV spectra | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | KOH | | $AlCl_3$ | | $AlCl_3$/TFA | | $H_3PO_4$ | | $H_3BO_3$/NaOAc | |
| | $[M+H]^+$ (amu) | $[A+H]^+$ (amu) | II | I | II | I | II | I | II | I | II | I | II | I |
| | | | | | | | (nm) | | | | | | | |
| **1** | 433 | 287 | 264 | 344 | 276 | 382 | 272 | 386 | 271 | 385 | 272 | 368 | 265 | 343 |
| **2** | 419 | 287 | 267 | 345 | 277 | 388 | 272 | 387 | 272 | 384 | 273 | 367 | 265 | 343 |
| **3** | 463 | 287 | 266 | 346 | 277 | 388 | 273 | 390 | 272 | 387 | 272 | 371 | 265 | 343 |
| **4** | 449 | 303 | 257, 263 sh | 348 | 275 | 394 | 271 | 404 | 270 | 390 | 271 | 374 | 262 | 363 |
| **5** | 435 | 303 | 257, 264 sh | 351 | 276 | 401 | 271 | 407 | 270 | 395 | 272 | 380 | 262 | 370 |
| **6** | 465 | 303 | 257, 263 sh | 351 | 277 | 404 | 271 | 407 | 269 | 394 | 272 | 380 | 262 | 368 |
| **7*** | 465 | 303 | 258, 264 sh | 352 | 276 | 403 | 271 | 407 | 270 | 393 | 271 | 379 | 262 | 370 |
| **8*** | 479 | 303 | 258, 264 sh | 352 | 276 | 403 | 271 | 407 | 270 | 393 | 271 | 379 | 262 | 370 |
| **9** | 617 | 303 | 262 | 352 | 278 | 406 | 271 | 410 | 269 | 396 | 270 | 395 | 264 | 367 |
| **10** | 465 | 319 | 262 | 350 | 277 | dec | 270 | 411 | 270 | 396 | 271 | 380 | 262 | 370 |
| **11** | 451 | 319 | 260 | 353 | 276 | dec | 268 | 423 | 268 | 381 | 275 | 380 | 262 | 372 |
| **12** | 481 | 319 | 264 | 354 | 278 | dec | 271 | 410 | 270 | 397 | 272 | 380 | 263 | 369 |
| **13** | 633 | 319 | 262 | 355 | 278 | dec | 270 | 423 | 270 | 395 | 270 | 395 | 263 | 370 |
| **A** | 419 | 287 | 263 | 342 | 275 | 387 | 273 | 387 | 272 | 386 | 273 | 360 | 265 | 345 |
| **B** | 449 | 287 | 261 | 347 | 275 | 390 | 272 | 388 | 272 | 387 | 272 | 365 | 277 | 361 |
| **C** | 435 | 303 | 260 | 350 | 273 | 390 | 271 | 403 | 271 | 387 | 274 | 362 | 263 | 360 |
| **D** | 435 | 303 | 264 | 348 | 273 | 390 | 271 | 403 | 270 | 391 | 274 | 362 | 276 | 363 |
| **E** | 617 | 303 | 263 | 354 | 278 | 407 | 271 | 407 | 269 | 393 | 271 | 405 | 263 | 368 |
| **F** | 481 | 319 | 262 | 355 | 270 | dec | 271 | 412 | 272 | 393 | 271 | 363 | 258 | 378 |

Compounds showing the same retention time.
dec = Decomposition.
I–II = UV absorption maxima bands.
sh = Shoulder.

pattern of flavonols and the position of the sugars on the aglycone. Weak bases deprotonate the more acidic phenolic hydroxyl groups. A strong base reacts with all the phenolic groups except those in the *peri* position to the keto function. $Al^{3+}$ forms a complex with *orthodihydroxyl* groups and keto groups having a hydroxyl in the *peri* position. The former are unstable in acidic media; *ortho*-dihydroxyl groups also form complexes with boric acid [19]. Analyses and, therefore, measurements of UV spectra in HPLC with post-column addition are normally performed in methanol [15, 16]. However, the conditions described here involved HPLC with an acidic acetonitrile–water system. All the shift reagents were tested on different reference flavonols under the same chromatographic conditions. The obtained values were comparable to those of the literature using methanol [19]. $Na_2HPO_4$ 0.1 M was used as weak base without neutralization since this concentration gives less precipitation problems than 0.5 M $Na_2HPO_4$ [17]. This last-mentioned concentration of base has been reported to give band I shifts of 7 to 14 nm [16] in comparison with the usual NaOAc spectrum [19]. With a weak base used at a lower concentration (0.1 M), the values obtained are more similar to those of the literature [19].

Figure 6 shows the UV spectra obtained on-line for **4** after the addition of the 5 different shift reagents. On band II, the shift of 11 nm with $Na_2HPO_4$ was characteristic for a non-substituted 7-hydroxyl group. A 15 nm shift with boric acid reagent was typical for *ortho*-dihydroxyl groups on the B ring. The shift of 42 nm on band I obtained with $AlCl_3$ without neutralization was specific for a 5-hydroxyl. Addition of $AlCl_3$ after neutralization gave a 56 nm shift of band I. This was due to a combination of an *ortho*-dihydroxyl group (C3′ and C4′) on the one hand and between the C-4 keto function and the 5-hydroxyl on the other hand complexing with $Al^{3+}$. As seen before, **4** showed a pseudomolecular ion corresponding to the aglycone $[A+H]^+$ at *m/z* 303. The identification of quercetin was confirmed since, according to the UV data, there was an *ortho*-dihydroxyl group on the B ring and free hydroxyl groups at C-7 and C-5.

The same measurements and interpretations have been made with all the other flavonol glycosides observed in the different *Epilobium* species studied. The results are listed in Table 1. Investigation of the glucosides in the extracts from the *Epilobium* species showed the presence of three different aglycones giving $[A+H]^+$ at *m/z* 287, 303 and 319. All the aglycones with $[A+H]^+$ at *m/z* 303 (**4**–**9**, **C**–**E**) showed the same UV values as **4**. The aglycones with a $[A+H]^+$ signal at *m/z* 287 (**1**–**3**, **A**, **B**) gave the same band II UV maxima as quercetin. However, band I was not shifted with boric acid reagent, proving the presence of kaempferol. For the compounds possessing an aglycone with $[A+H]^+$ at *m/z* 319 (**10**–**13**, **F**), the UV shifts were very similar to quercetin and kaempferol for band II. However, there was decomposition after addition of KOH, proving the presence of 3 adjacent OH groups [19]. According to these data, the third aglycone was myricetin. The three aglycones were all substituted at the C-3 position.

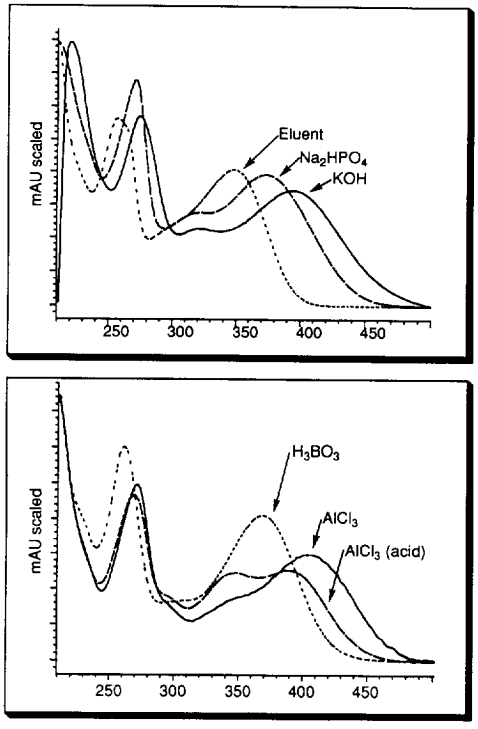



Fig. 6. UV spectra of **4** obtained after the post-column addition of different shift reagents.

*Distribution of the flavonol glycosides in the different* Epilobium *species*

All the flavonols of the different *Epilobium* species were identified by TSP/LC-MS and LC-UV with shift reagents. For example in *E. parviflorum*, **11**, **13**, **B** and **D** were observed in addition to glycosides **1**, **4**–**6**, **10** and **12** found in *E. angustifolium*. The same approach was applied to the other *Epilobium* species (Table 2). As the molar absorptivities ε were of the same order of magnitude in all the flavonols, it is possible to get semi-quantitative information from the UV chromatogram. Compound **F** was only present in *E. capense*; according to UV and mass data, it corresponded to a 3-*O*-hexoside of myricetin. As the 3-*O*-glucoside of myricetin has already been found in the same extract (it had a small difference in the retention time), **F** was probably the 3-*O*-galactoside of myricetin. Two other flavonoids, **A** and **C**, were also found in *E. capense*. **A** was a 3-*O*-pentoside of kaempferol and **B** a 3-*O*-pentoside of quercetin. Compound **B** had a retention time different from that of **D**, the isomer found in *E. parviflorum*.

In order to confirm the structures of the flavonoids characterized on-line, the main compounds (**1**–**13**) were isolated from *E. angustifolium* and *E. parviflorum*. The isolation of the flavonoids was performed by gel filtration on Sephadex LH-20 followed by separations on RP-18 phases, MPLC, Lobar and semi-preparative HPLC. The isolated compounds, except **4**, **7**, **10** and **12**, isolated by

Table 2. Distribution of flavonoids in the aerial parts of some *Epilobium* species

| *Epilobium* species | Compounds | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **A** | **B** | **C** | **D** | **E** | **F** |
| Section *Chamaenerion* Tausch. | | | | | | | | | | | | | | | | | | | |
| *E. angustifolium* L. | + + | + | + | + + | + + | + + | + + | + + | + + | + | – | + | – | – | – | – | – | + | – |
| *E. Dodonaei* Vill. | + + | – | – | + + | + | + | – | – | + | + + + | + | + + | + + | – | – | – | – | + | – |
| Section *Epilobium* L. | | | | | | | | | | | | | | | | | | | |
| Schyzostigma | | | | | | | | | | | | | | | | | | | |
| *E. capense* Buch.* | + | – | – | + | + + | + | + | – | – | + + | + + | + | + | + | + | + | – | – | + |
| *E. hirsurtum* L. | + | + | – | + + | – | + | – | – | – | + + + | + | + + | – | – | + | + | – | – | – |
| *E. hirsutum* L.* | + | + | – | + + | + + | + + | – | – | – | + + + | + + | + + | – | + | + | – | – | – | – |
| *E. montanum* L. | + | + | – | + + | + | + | – | – | – | + + + | + | + + | – | + | + | – | – | – | – |
| *E. parviflorum* Schreb. | + | – | – | + + | – | + | – | – | – | + + + | + | + + | + | – | + | – | + | – | – |
| Synstigma | | | | | | | | | | | | | | | | | | | |
| *E. alpinum* L. | + | – | – | + + | – | + | – | – | – | + + + | + | + + | + | – | + | + | – | – | – |
| *E. roseum* Schreb. | + | – | – | + + | – | + | – | – | – | + + + | + | + + | + | – | + | + | – | – | – |
| E. *salignum* Hausskn.* | + | + | – | + + | + + | + | + + | – | – | + + + | + + | + + + | + + | + | + | – | – | – | – |
| *E. stereophyllum* Fres.* | – | – | – | + | – | + | – | – | – | + | + | + + + | + + | – | + | + | – | – | – |
| *E. tetragonum* L. | + | – | – | + + | – | + | – | – | – | + + + | + | + + | + | – | + | + | – | – | – |
| Hybrid | | | | | | | | | | | | | | | | | | | |
| *E. roseum* × *E. parviflorum* | + | – | – | + + | – | + | – | – | – | + + + | + | + + | + | – | + | + | – | – | – |

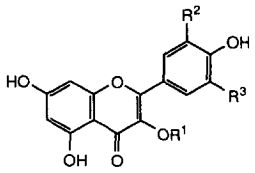
*Represents the species collected in Africa.

+ Low concentration, + + middle concentration, + + + high concentration, – compound absent.

Slacanin *et al.* [8], were characterized using different spectroscopic techniques (including $^1$H NMR, $^{13}$C NMR, D/CIMS) and identification of aglycones and sugars obtained after hydrolysis (Fig. 7). The obtained values were compared with literature references. [19, 20].

All 19 flavonols characterized were monoglycosides, substituted at the C-3 position, the aglycones being kaempferol, quercetin and myricetin. Complex glycosylation and *O*-methylation were absent in the studied species. According to the information obtained here, the genus *Epilobium* can be considered as a relatively advanced genus of the Onagraceae family, other genera showing a greater diversity of flavonoids [21]. The flavonoids found in the *Epilobium* species investigated here are reported in Table 2. All species showed the presence of **4**, **6**, **10** and **12**. Flavonol glycosides **9** and **E** are specific for the section *Chamaenerion* Tausch. Compound **B** was found in all studied species of the section *Epilobium* L. but was not found in the section *Chamaenerion* Tausch. Glucuronides (**3** and **8**) were found only in *E. angustifolium*. In all the *Epilobium* species studied, except *E. angustifolium* and *E. stereophyllum*, myricitrin was the main constituent. Compound **1** was absent from *E. stereophyllum* but present in all the other species. The same flavonoids as for *E. roseum* were found in the hybrid obtained between *E. roseum* and *E. parviflorum*. For *E. hirsutum*, **5** and **A** absent in the *E. hirsutum* collected in Switzerland, were present in the species collected in Kenya. Compound **C** present in *E. hirsutum* from Switzerland was absent from Kenyan species.

According to these data, there is no clear distinction between Schyzostigma and Synstigma and no special rule could be formulated. Some differences were found between the section *Chamaenerion* Tausch. and the section *Epilobium* L. Noteworthy is that *E. angustifolium* is very different from the other *Epilobium* species of the section *Epilobium* L. One species, *E. Dodonaei*, possesses characteristics of both sections in that it contains **9** and **E** as in *E. angustifolium* but glucuronides are absent. Glycosides **4**, **10**, **12** and **13** are the main flavonol constituents of *E. Dodonaei*, like most other species of the section *Epilobium* L.




| | R1 | R2 | R3 | trivial name |
|---|---|---|---|---|
| 1 | Rha | H | H | |
| 2 | Ara | H | H | |
| 3 | GlcA | H | H | |
| 4 | Rha | OH | H | quercitrin |
| 5 | Ara | OH | H | guajaverin |
| 6 | Gal | OH | H | hyperosid |
| 7 | Glc | OH | H | isoquercitrin |
| 8 | GlcA | OH | H | |
| 9 | Gal6-galloyl | OH | H | |
| 10 | Rha | OH | OH | myricitrin |
| 11 | Ara | OH | OH | |
| 12 | Glc | OH | OH | isomyricitrin |
| 13 | Gal6-galloyl | OH | OH | |

Fig. 7. Compounds isolated from *E. angustifolium* and *E. parviflorum*.

Furthermore, the flavonols identified in this chemotaxonomic study can be used for the standardization of *Epilobium* extracts used in cases of benign prostatic hyperplasia. This is important because characterization of the active principles responsible for antiinflammatory activity and hormone metabolism inhibition of the *Epilobium* species is still underway. These results will be the subject of a future publication.

## EXPERIMENTAL

*Plant material and samples. E. angustifolium* L. was collected in Mayens de Riddes (Valais, Switzerland) in August 1992, *E. Dodonaei* Vill. in Grimisuat, (Valais, Switzerland) in 1989, *E. roseum* Schreb. in Sion (Valais, Switzerland) in 1989, *E. alpinum* L. in Gruyères (Fribourg, Switzerland) in 1989, *E. hirsutum* L., *E. tetragonum* L. and the hybrid, obtained from *E. parviflorum* Schreb. and *E. roseum* Schreb., were cultivated in Bruson (Valais, Switzerland) and collected in July 1989. *E. parviflorum* Schreb. was cultivated in Centre les Fougères (Conthey, Valais, Switzerland) in 1991, *E. montanum* was collected at Pont-de-Nant (Vaud, Switzerland) in 1985. Voucher specimens of all the *Epilobium* species collected in Switzerland have been deposited at the Institute of Pharmacognosy and Phytochemistry, Lausanne University. *Epilobium capense* Buch. and *E. salignum* Hausskn. were collected on the Nyika plateau, in Malawi, in 1989 and voucher specimens have been deposited at the National Herbarium, Zomba, Malawi. *Epilobium stereophyllum* Fres. and *E. hirsutum* L. were collected in Kenya in 1992 and voucher specimens have been deposited at the National Herbarium, Nairobi, Kenya. Aerial plant parts were extracted at room temp. with solvents of increasing polarity: $CH_2Cl_2$ and MeOH. The methanolic extracts were enriched in flavonoid constituents by *n*-BuOH–$H_2O$ partition. Solns to be analysed were prepd by dissolving 30 mg of the butanolic fr. in 1 ml of a MeOH–$H_2O$ mixt. (1:3) The samples were filtered (0.45 $\mu$m).

*LC conditions.* Analytical sepns were performed on a Waters NovaPak RP-18 column, 4 $\mu$m (150 × 3.9 mm i.d.), equipped with a NovaPak pre-column. A step gradient of acetonitrile–$H_2O$ was used: 0 min, 10% $CH_3CN$, 4 min 12%, 12 min 12%, 16 min 18%, 30 min 25%. The flow rate was 1 ml min$^{-1}$. To avoid the tailing of phenolic compounds, 0.05% of TFA was added to the solvents to give a pH of 3. The vol. of sample injected was 20 $\mu$l.

*HPLC-UV analyses and shift reagents.* Eluent delivery was provided by a Shimadzu LC-9A pump equipped with a low pressure mixing valve FCV-9AL and a Rheodyne 7125 injection valve with 20 $\mu$l loop. Post-column addition of the bases (for neutralization of the mobile phase) and of the shift reagents, was achieved by two Waters M-6000 pumps, respectively. Neutralization

of the mobile phase was done in an Upchurch mixing tee while reaction with shift reagents was performed in a 10 μl visco mixer (Lee, Westbrook, CO), followed by a reaction coil. UV spectra were recorded on a HP 1040 photodiode array detector.

KOH, $AlCl_3$ and $H_3BO_3$ were prepd as described by Hostettmann *et al.* [15] and the conditions of the post-column addition were the same as those described by Wolfender and Hostettmann [17], except for the weak base where 0.1 M $Na_2HPO_4$ was added at a flow rate of 0.8 ml $min^{-1}$ without neutralization.

*HPLC-MS analyses.* A Finnigan MAT TSQ-700 triple quadrupole instrument equipped with a TSP 2 interface was used for the data acquisition and processing. The temps of the source block and of the vaporizer were 300° and 95°, respectively. The electron multiplier voltage was 1.8 kV, dynode 15 kV and the filament and discharge were off. Full scan spectra from *m/z* 120 to 700 in PI mode were obtained (scan time 1.2 sec). Concerning the LC part, the eluent delivery was provided by an HPLC pump equipped with a gradient controller. The UV spectra were recorded on-line with a programmable multiwavelength detector. Post-column addition of buffer (ammonium acetate 0.5 M) was achieved by a programmable HPLC pump (0.2 ml/min).

*Isolation of flavonol glycosides.* The dried aerial parts of *E. angustifolium* (1.1 kg) were extracted at room temp. successively with $CH_2Cl_2$ and MeOH. The methanolic extract (80.5 g) was enriched in flavonoid constituents by *n*-BuOH–$H_2O$ partition. The *n*-BuOH fr. (23.0 g) was then fractionated by gel filtration with Sephadex LH-20 (MeOH). Nine frs were collected (1–9). Fr. 4 (711 mg) was subjected to MPLC on RP-18 (LiChroprep 15–25 μm, Merck) with MeOH–$H_2O$ (35:65) and yielded **1** (26 mg), **2** (12 mg), **4** (31 mg) and after purification by semi-prep. HPLC on a LiChrospher 100 RP-18 column (5 μm, i.d. 1.6 × 25 cm) with MeCN–$H_2O$ (3:7) (containing 0.1% TFA), **3** (13 mg). Fr. 5 (596 mg) subjected to MPLC with MeOH–$H_2O$ (25:75) yielded **5** (25 mg), **6** (15 mg), **7** (16 mg) and **8** (29 mg). Fr. 7 (150 mg) was sepd into 2 frs (7a, b) on Lobar RP-18 (LiChroprep 40–63 μm, Merck). Compound **9** (25 mg) was purified from fr. 7a by semi-prep. HPLC on a Lichrosorb. RP-18 column (7 μm, i.d. 1.6 × 25 cm) with MeOH–$H_2O$ (3:7) (containing 0.1% TFA).

The dried aerial parts of *E. parviflorum* (520 g) were extracted at room temp. successively with $CH_2Cl_2$ and MeOH. Fractionation of the methanolic extract (31.7 g) by filtration on Sephadex LH-20 (MeOH) gave 15 frs (1–15). Fr. 8 gave **10** (680 mg). Frs 5 (220 mg), 12 (180 mg) and 15 (90 mg) were purified on Lobar RP-18 (LiChroprep 40–63 μm, Merck) with MeOH–$H_2O$ (4:6) giving **12** (23 mg), **11** (14 mg) and **13** (13 mg), respectively.

All the isolated flavonol glycosides were analysed by $^1$H NMR, $^{13}$C NMR and D/CIMS. For all these compounds, $^1$H NMR and $^{13}$C NMR values were compared with literature references [19, 20]. Sugars and aglycones obtained after hydrolysis of the isolated flavonol glycosides were compared with authentic samples. Hydrolysis to obtain aglycones and sugars was carried out in 2 N HCl:MeOH (1:1). Sugars and aglycones were sepd by EtOAc–$H_2O$ partition. Sugars were analysed by TLC on precoated silica gel 60 F 254 aluminium sheets with EtOAc–$H_2O$–MeOH–HOAc (13:3:4:3) and detected with *p*-anisidine phthalate. Aglycones sepd on silica gel TLC plates with $CHCl_3$–MeOH–HOAc (90:10:3) were detected by NP/PEG reagent (diphenyl–boryloxyethyl–amine/polyethyleneglycol-4000) under UV 350 nm.

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