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The glucosinolate (GSL) profiles (inflorescence, stem, root, and fruit) of the wild-growing plant *Lepidium graminifolium* L. (Brassicaceae) from Croatia was established by LC-MS analysis. During this investigation, we confirmed the presence of benzyl- (**1**), 3-methoxybenzyl- (**2**), 4-hydroxybenzyl- (**4**), 4-methoxyindol-3-ylmethyl- (**7**) GSLs and reported for the first time in the plant the presence of (2*R*)-hydroxybut-3-enyl- (**11**), (2*S*)-hydroxybut-3-enyl- (**12**), but-3-enyl- (**13**), and 2-phenylethyl- (**14**) GSLs. Finally, 3-hydroxybenzyl GSL (**3**) was isolated for the first time from *L. graminifolium* inflorescence and characterized by spectroscopic data interpretation.

Keywords: *Lepidium graminifolium*; Brassicaceae; glucosinolate; glucolepigramin; LC-MS; NMR.

1. Introduction

Lepidium graminifolium L. (grassleaf pepperweed) is a wild and annual plant (Brassicaceae) of the Mediterranean region and Eastern Europe, used as a medicinal, food, or ornamental plant (Agelet et al. 2000; Savo et al. 2011; Guarrera & Savo 2016).

The fatty acid content of the seed oil of *L. graminifolium* and flavonoids in the aerial parts of the plant have been reported previously (Fursa et al. 1981; Miller et al. 1965; Nilsson et al. 1998). Using paper chromatography (PC), benzyl isothiocyanate (ITC) was detected in root and aerial part extracts of a wild *L. graminifolium* (origin France), suggesting that benzyl glucosinolate (GSL) (**1**) (Figure 1) was present in those plant organs (Delaveau, 1958). 3-Methoxybenzyl- (**2**) and 3-hydroxybenzyl- (**3**) GSLs were identified by PC, IR spectroscopy, and mass spectrometry of the hydrolysis products from seeds of plants cultivated in Denmark (Friis & Kjær 1963). PC of a seed extract detected two more GSLs – one of which was supposed to be 4-hydroxybenzyl GSL (**4**) (Danielak & Borkowski 1969). 3-Methylsulfanylpropyl ITC, benzyl ITC, and phenylacetonitrile were identified by GC-MS in 8-week-old seedlings grown in a glasshouse, indicating the presence of 3-methylsulfanylpropyl GSL (**5**) and **1** (Cole 1976). Indol-3-ylmethyl- (**6**), 4-methoxyindol-3-ylmethyl- (**7**), and 1-methoxyindol-3-

ylmethyl- (**8**) GSLs were detected by HPLC analysis of 10-15-day-old seedlings (Bäuerle et al. 1986). Finally, a GC analysis of the hydrolysis products of the compounds extracted from the seeds (origin Spain) showed the presence of 3-hydroxybenzyl-, methoxybenzyl-, 3,4-dimethoxybenzyl-, 3,4-5-trimethoxybenzyl ITCs and thiocyanate ion, suggesting that the seeds contained **2**-**4**, 3,4-dimethoxybenzyl- (**9**), and 3,4,5-trimethoxybenzyl- (**10**) GSLs (Figure 1) (Daxenbichler et al. 1991).

As part of our continued interest in the chemistry of the *Lepidium* sp. (Montaut et al. 2017), the aim of this study was to identify the GSLs present in inflorescence, stem, root, and fruit of *L. graminifolium* wild-growing in Croatia by HPLC-ESI-MS coupled with a photodiode-array detector. Finally, the major GSL in the fruit, 3-hydroxybenzyl GSL (**3**), was isolated and characterized using spectroscopic techniques.

2. Results and discussion

The inflorescence, stem, root, and fruit of *L. graminifolium* were harvested on Rab Island (Croatia). The plant parts were extracted and analyzed by HPLC-ESI-MS for intact GSLs (Tutin et al. 1968-1980; Zrybko et al. 1997). The t_R, mass, and UV data of the products were compared with those of standards from our HPLC-ESI-MS library including the authenticated isolated GSLs **2** and **7** (Baird et al. 1988; Montaut et al. 2010) and the commercial standards **1**, **4**, (2*R*)-hydroxybut-3-enyl- (**11**), (2*S*)-hydroxybut-3-enyl- (**12**), but-3-enyl- (**13**), and 2-phenylethyl- (**14**) GSLs (see supplementary material section). In the inflorescence (Figure S1a), the minor compounds at t_R 7.4 min, t_R 9.1 min, and t_R 23.4 min were determined to be **11** (Figures 1 and S1, Tables S1 and S2), **12**, and **1**, respectively. The other minor compound at t_R 25.6 min was found to be **2**. GSLs **2**, **11**, and **12** were also present in the stem (Figure S1b), root (Figure S2a), and fruit (Figure S2b) while **1** was also identified in the root and fruit. In addition, **7** (t_R 29.1 min) was identified in the stem. GSLs **13** (t_R 18.2 min) and **14** (t_R 26.9 min) were found in the root. Finally, a minor compound at t_R 17.9 min observed in the fruit and stem was found to be **4**. The compound at t_R 20.3 min found in the inflorescence, stem, root, and fruit extracts (Figures S1 and S2) did not match any GSLs in our library. Therefore, it was isolated (see supplementary material section) and its structure was elucidated using UV, NMR (^{1}H, ^{13}C, HMBC, COSY, and HSQC), and HRMS data. The data reported in the supplementary material section suggest that the peak at t_R 20.3 min corresponds to 3-hydroxybenzyl GSL (**3**) (Figure 1).

The presence of **3** in *L. graminifolium* seeds was previously proposed thanks to the structural elucidation of its hydrolysis product (Friis & Kjær 1963). It was shown in our study that **3** is not only produced in the seed but in all parts of the plant. Like other *Lepidium* species, *L. graminifolium* appears to be a source of arylaliphatic GSLs (Montaut et al. 2017); in fact, **1** and **2** are the major GSLs in the root while **2** and **3** are the major GSLs in the fruit. In previous investigations, **2** was only reported in the seed of *L. graminifolium* (Friis & Kjær 1963; Daxenbichler et al. 1991); our study additionally showed that **2** is found in all plant parts. Furthermore, **4** was only detected in the seeds in a previous study (Danielak & Borkowski, 1969), whereas it was also found by us to be a minor GSL in both stem and fruit. While **7** was previously detected in 10-15-day-old seedlings of *L. graminifolium* (Bäuerle et al. 1986), we could only detect it in the stem. Contrary to previous reports, we neither detected any **5**, **6**, **9** nor **10** in *L. graminifolium*. The differences in GSL profiles may be due to genetic, geographical and ecological impacts, analytical methods, or the fact that we have analyzed a wild *L. graminifolium*, since some previous investigations were carried out on cultivated plants or samples.

3. Conclusion

We report for the first time in *L. graminifolium* harvested in Croatia the presence of four known GSLs (**11-14**). Furthermore, **3** was isolated and characterized by spectroscopic data interpretation for the first time from the inflorescence. Finally, we have confirmed by LC-MS analyses the presence of four previously reported GSLs (**1**, **2**, **4**, and **7**).

Supplementary material

Experimental details related to this paper are available online, alongside Tables S1-S2 and Figures S1-S2.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure caption

Figure 1. Structures of glucosinolates **1-14** from *Lepidium graminifolium*