

Full Paper

## The First Guaian-12-oic Acid Glucopyranosyl Ester and Other Constituents from *Picris rhagadioloides* (L.) Desf. (Asteraceae)

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**Abstract:** The first guaian-12-oic acid glucopyranosyl ester was isolated from the roots of *Picris rhagadioloides* (L.) Desf. (Asteraceae), in addition to five costuslactone-type guaianolides, four germacranolides and three phenolic compounds. This is the first time that the known phenolics syringaldehyde and syringaresinol, as well as the known sesquiterpene lactones glucozaluzanin C, cichorioside C and hypochoeroside A have been described from a *Picris* species. The compounds were characterized on the basis of physicochemical, 1D- and 2D-NMR spectroscopic, and mass spectrometric data.

**Keywords:** Sesquiterpenoids; phenolics; *Picris rhagadioloides*; Asteraceae; spectroscopic analysis.

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### Introduction

The genus *Picris* (Asteraceae, tribe Lactuceae) comprises about 40 species, which are distributed throughout Eurasia and extend to tropical Africa, Australia and New Zeland. Phytochemical investigations of several *Picris* taxa have revealed mainly sesquiterpene lactones, including a remarkable number of guaianolides, three germacranolides and two eudesmanolides, along with some other secondary metabolites [1-3]. Guaianolides represented by costuslactone- and lactucin-type

compounds are the most common constituents of this taxon. Following our recent chemotaxonomic work [2, 3] on *Picris* species, we report here the isolation and characterization of sesquiterpenoids and phenolics from roots of the hitherto not studied *Picris rhagadioloides* (L.) Desf. (syn. *Picris sprengeriana* (L.) Poir.), an annual herb of up to 50 cm height with yellow ligules, distributed throughout Europe, Asia Minor and North Africa.

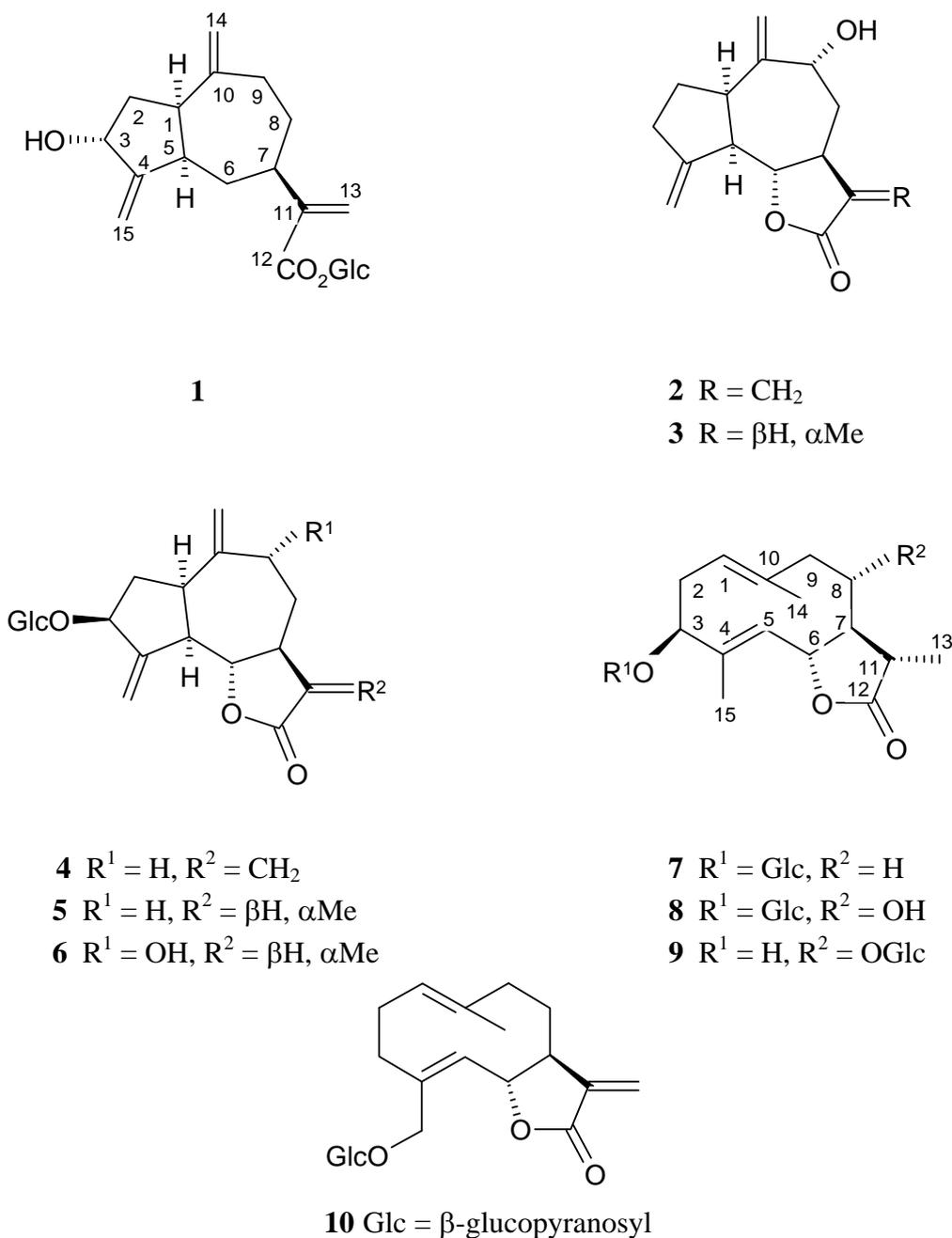
## Results and Discussion

The dried roots of the plant were extracted with ethanol and the extract was chromatographed on a silica gel column to give fractions which were submitted to preparative TLC and/or semipreparative RP-18 HPLC yielding the known phenolics eugenyl-4-O- $\beta$ -glucopyranoside, syringaldehyde and syringaresinol, as well as the new guaian-12-oic acid glucopyranosyl ester (**1**) and the known sesquiterpene lactones 9 $\alpha$ -hydroxy-3-deoxyzaluzanin C (**2**) in a mixture with its 11 $\beta$ ,13-dihydroderivative (**3**), glucozaluzanin C (**4**), its 11 $\beta$ ,13-dihydroderivative (**5**) in a mixture with picriside B (**10**), ixerin F (**6**), sonchuside A (**7**), cichorioside C (**8**), and hypochoeroside A (**9**) (Figure 1). Except for **9**, the known compounds were characterized by direct comparison of their spectra and retention time values with those of compounds previously isolated in our earlier studies [2-5]. The identity of compound **9**, first isolated from *Hypochoeris radicata* L., was established by comparison of its spectral data and the optical rotation value with those reported [6]. Here, the first detailed  $^1\text{H-NMR}$  evidence for the structure of this compound is provided (Table 1). In the original publication [6] only four  $^1\text{H-NMR}$  signals in pyridine- $d_5$  were given:  $\delta$  5.10 (*d*,  $J = 7.0$  Hz, H-1'), 1.83 (*d*,  $J = 7.0$  Hz, Me-13), 1.70 (*br. s*, Me-14) and 1.69 (*s*, Me-15), the latter was obviously misprinted. Similarly, no detailed  $^1\text{H-NMR}$  data are available for **8** [ $\delta$  4.87 (*d*,  $J = 7.0$  Hz, H-1'), 1.98 (*br. s*, Me-15), 1.77 (*d*,  $J = 7.0$  Hz, Me-13), 1.45 (*br. s*, Me-14)] [7]. They are also added to Table 1. The signal assignments were confirmed by COSY and NOESY correlations.

Compound **1** was isolated as an amorphous solid. Its molecular formula was deduced to be  $\text{C}_{21}\text{H}_{30}\text{O}_8$  from the positive ESI MS which showed ion peaks at  $m/z$  433  $[\text{M} + \text{Na}]^+$  and 843  $[2\text{M} + \text{Na}]^+$  and by high-resolution mass spectral measurements.  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectra of **1** (Table 2) exhibited signals for a glucopyranosyl unit and a sesquiterpene moiety. The signals could be assigned unambiguously from a combination of COSY, HETCOR and HMBC spectra, and previously reported data of related metabolites. The chemical shifts of C-1 and C-2 signals of the sugar unit at  $\delta$  96.52 and 74.30, respectively, and the anomeric proton doublet ( $J = 7.7$  Hz) at  $\delta$  6.49 indicated the presence of an ester  $\beta$ -glucosidic linkage. This conclusion was supported by the HMBC correlation between the glucose anomeric proton (H-1) signal and the aglycone carbonyl (C-12) signal. After subtraction of the six carbon resonances of the glucopyranosyl unit, the remaining 15 signals, including six signals assigned to olefinic carbons and one to an ester carbonyl, were attributable to a sesquiterpene framework ( $\text{C}_{15}\text{H}_{20}\text{O}_3$ ), indicating the presence of six degrees of unsaturation, *i.e.* three double bonds, one carbonyl functionality and two ring systems. The  $^1\text{H-NMR}$  spectrum displayed signals assignable to three exocyclic methylene protons, an oxygenated methine proton, three further methine protons and four pairs of endocyclic methylene protons suggesting the presence of a hydroxylated 4(15), 10(14), 11(13)-guaiatrien-12-oic acid residue. The COSY spectrum established the spin system H-5  $\rightarrow$  H-1  $\rightarrow$  H-2  $\rightarrow$  H-3 and allylic couplings of H-3 and H-5 with H-15a/H-15b, thus allowing the assignment of

the hydroxyl group at C-3. The resonances of C-1 to C-5 and C-10 are similar to those of dehydrocostuslactone (1 $\alpha$ , 5 $\alpha$ , 7 $\alpha$ (H)-guaia-4(15), 10(14), 11(13)-trien-6,12-olide) derivatives having a hydroxyl group at C-3 [8, 9], while the resonances of C-11 to C-13 are in good agreement with those published for the acrylic acid ester moieties at C-7 of eudesmane-type sesquiterpenoids [10, 11].

**Figure 1.** Chemical structures of compounds **1** – **10**.



The relative stereochemistry of **1** was resolved by a combination of NOESY data and coupling constant values, based on the assumption that H-7 is  $\alpha$ , as established for closely related guaianolides of known absolute stereostructure. In the NOESY spectrum H-5 correlated with H-7 and H-1, suggesting that these protons are on the  $\alpha$  face of the molecule. No effect was observed between H-1

and H-3. The spectrum further confirmed proximities of H-6 $\beta$  to H-8 $\beta$ ; H-9 $\beta$  to H-14a ( $\delta$  4.86), and H-2 $\beta$  to H-3 $\beta$ /H-14b ( $\delta$  4.83). The configurations of H-5 $\alpha$  and H-7 $\alpha$  were also confirmed on the basis of the values of  $J_{5\alpha,6\beta}$  (11.0 Hz),  $J_{6\beta,7\alpha}$  (11.0 Hz) and  $J_{7\alpha,8\beta}$  (11.0 Hz) were indicative of *anti*-diaxial relationships of the respective protons. Concerning the configuration of the hydroxyl group at C-3 in epimeric 3 $\alpha$ -hydroxy- and 3 $\beta$ -hydroxydehydrocostuslactone derivatives, recently isolated from leaves of artichoke [12], a notable difference in the chemical shifts of H-1 was observed in pyridine- $d_5$  ( $\delta$  3.43 vis 2.74, respectively). The H-5 signal was less deshielded. We also noted that the presence of the hydroxyl group at C-3 induced a downfield shift of the resonance of H-1 ( $\delta$  3.43), thus supporting the 3 $\alpha$ -OH stereochemistry. On the basis of these findings the structure of **1** was established as 3 $\alpha$ -hydroxy-1 $\alpha$ ,5 $\alpha$ ,7 $\alpha$ (H)-guaia-4(15),10(14),11(13)-trien-12-oic acid  $\beta$ -glucopyranosyl ester, a new natural product.

**Table 1.**  $^1\text{H-NMR}$  (500.13 MHz) data of **8** and **9**. In pyridine- $d_5$ ;  $\delta_{\text{H}}$  in ppm,  $J$  in Hz.

Position	<b>8</b>	<b>9</b>
1	4.85-4.90 ( <i>m</i> ) <sup>a</sup>	4.95-5.05 ( <i>m</i> ) <sup>a</sup>
2 $\alpha$	2.52 ( <i>m</i> ) <sup>a</sup>	2.61 ( <i>m</i> )
2 $\beta$	2.46 ( <i>ddd</i> , $J = 12.0, 11.0, 10.7$ )	2.57 ( <i>ddd</i> , $J = 12.0, 11.0, 10.1$ )
3	4.80 ( <i>dd</i> , $J = 10.7, 5.9$ )	4.54 ( <i>m</i> ) <sup>a</sup>
5	4.85-4.90 ( <i>m</i> ) <sup>a</sup>	4.92 (br. <i>d</i> , $J = 10.4$ )
6	4.85-4.90 ( <i>m</i> ) <sup>a</sup>	4.95-5.05 ( <i>m</i> ) <sup>a</sup>
7	2.17 ( <i>ddd</i> , $J = 11.0, 11.0, 10.9$ )	2.36 ( <i>ddd</i> , $J = 11.5, 8.5, 8.0$ )
8	4.11 ( <i>m</i> ) <sup>a</sup>	4.21 ( <i>m</i> ) <sup>a</sup>
9 $\alpha$	2.52 ( <i>m</i> ) <sup>a</sup>	2.47 ( <i>dd</i> , $J = 12.3, 11.3$ )
9 $\beta$	2.81 (br. <i>d</i> , $J = 12.0$ )	3.41 (br. <i>d</i> , $J = 12.3$ )
11	3.16 ( <i>dq</i> , $J = 10.9, 6.9$ )	3.64 ( <i>dq</i> , $J = 11.5, 6.8$ )
Me-13	1.77 ( <i>d</i> , $J = 6.9$ )	1.83 ( <i>d</i> , $J = 6.8$ )
Me-14	1.45 (br. <i>s</i> )	1.70 (br. <i>s</i> )
Me-15	1.98 ( <i>s</i> )	1.95 ( <i>d</i> , $J = 0.9$ )
1'	4.87 ( <i>d</i> , $J = 7.5$ )	5.07 ( <i>d</i> , $J = 7.8$ )
2'	4.11 ( <i>m</i> ) <sup>a</sup>	4.05 ( <i>dd</i> , $J = 9.0, 7.8$ )
3'	4.24 ( <i>m</i> ) <sup>a</sup>	4.24 ( <i>m</i> ) <sup>a</sup>
4'	4.24 ( <i>m</i> ) <sup>a</sup>	4.24 ( <i>m</i> ) <sup>a</sup>
5'	3.95 ( <i>m</i> )	3.98 ( <i>m</i> )
6'a	4.41 ( <i>dd</i> , $J = 12.3, 5.2$ )	4.38 ( <i>dd</i> , $J = 12.1, 5.3$ )
6'b	4.62 (br. <i>d</i> , $J = 12.3$ )	4.54 ( <i>m</i> ) <sup>a</sup>

<sup>a</sup>) Overlapped signals.

To our knowledge, this represents the first time a guaian-12-oic acid esterified with glucose has been encountered in plants. The co-occurrence of guaiane-type sesquiterpenoids, which have different configurations of hydroxyl groups at C-3, in the same taxon, is rather unusual, albeit preceded in naturally occurring guaianolides from *Cynara scolymus* L. [12] and *Centaurea canariensis* Brouss. (var. *subexpinnata* Burch.) [13]. Only a few further examples of costuslactone derivatives with 3 $\alpha$ -hydroxyl groups have been reported in the literature, mainly from *Saussurea* species [14 – 17].

**Table 2.**  $^1\text{H}$ - (500.13 MHz) and  $^{13}\text{C}$ -NMR (125.76 MHz) data of **1** (pyridine- $d_5$ ;  $\delta$  in ppm,  $J$  in Hz).

Position	<b>1</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	3.43 ( <i>ddd</i> , $J = 10.0, 8.6, 6.6$ )	47.00
2 $\alpha$	2.17 ( <i>ddd</i> , $J = 12.9, 6.6, 2.6$ )	38.73
2 $\beta$	1.90 ( <i>m</i> ) <sup>a)</sup>	-
3	4.83 ( <i>br. s</i> ) <sup>a)</sup>	74.22
4	-	153.02
5	3.10 ( <i>br. dd</i> , $J = 11.0, 8.6$ )	45.60
6 $\alpha$	1.81 ( <i>br. d</i> , $J = 13.9$ )	39.61
6 $\beta$	1.56 ( <i>ddd</i> , $J = 13.9, 11.0, 11.0$ )	-
7	2.92 ( <i>br. dd</i> , $J = 11.0, 11.0$ )	42.71
8 $\alpha$	1.96 ( <i>m</i> ) <sup>a)</sup>	38.00
8 $\beta$	1.29 ( <i>ddd</i> , $J = 13.0, 13.0, 11.0, 3.0$ )	-
9 $\alpha$	1.90 ( <i>m</i> ) <sup>a)</sup>	36.64
9 $\beta$	2.39 ( <i>m</i> )	-
10	-	150.60
11	-	147.16
12	-	166.31
13a	5.57 ( <i>br. s</i> )	124.00
13b	6.39 ( <i>br. s</i> )	-
14a	4.86 ( <i>br. s</i> )	110.31
14b	4.83 ( <i>br. s</i> ) <sup>a)</sup>	-
15a	5.15 ( <i>br. s</i> )	109.34
15b	5.40 ( <i>dd</i> , $J = 1.0, 1.0$ )	-
1'	6.49 ( <i>d</i> , $J = 7.7$ )	96.52
2'	4.29 ( <i>m</i> ) <sup>a)</sup>	74.30
3'	4.35 ( <i>m</i> ) <sup>a)</sup>	78.66
4'	4.38 ( <i>m</i> ) <sup>a)</sup>	71.04
5'	4.11 ( <i>m</i> )	79.64
6'a	4.39 ( <i>dd</i> , $J = 12.0, 4.6$ )	62.18
6'b	4.50 ( <i>dd</i> , $J = 12.0, 2.2$ )	-

<sup>a)</sup> Overlapped signals.

## Conclusions

Our chemical study of the roots of *Picris rhagadioloides* has led to the isolation of the new guaiane derivative **1**, nine known sesquiterpene lactones (**2** – **10**) and three known phenolics. The production of compound **1** and its distribution within the hitherto not studied *Picris* species might be of taxonomic importance. Moreover, *P. rhagadioloides* seems to be characterized by the occurrence of the costuslactone-type of guaianolides (**2** – **6**) and the germacranolides **7** - **10**, accumulated mainly as glycosides. This is the first time that the sesquiterpene lactones **4**, **8** and **9**, and the phenolics syringaldehyde and syringaresinol have been isolated from a taxon of the genus *Picris*. The

germacranolides **8** and **9**, known as constituents of some other representatives of the tribe Lactuceae, are rare natural products. Compound **8** has been reported from *Cichorium intybus* L. [7], *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz. [18] and *Hypochoeris radicata* L. [6], while compound **9** has been previously found in the latter species.

## Experimental

### General

Column chromatography: Merck silica gel 60 (0.063 – 0.2 mm). Prep. TLC: Merck silica gel 60 plates (0.25 mm). Semiprep. HPLC: Delta-Pak C-18 column (particle size 15  $\mu\text{m}$ , 25 x 100 mm) coupled to a dual wavelength UV/VIS detector operating at 210 and 260 nm, isocratic, H<sub>2</sub>O-MeOH mixtures, flow rate 3.0 mL min<sup>-1</sup>. Optical rotations: PolAAR31 polarimeter. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra: Bruker AMX spectrometer (at 500.13 MHz and 125.76 MHz) in pyridine-*d*<sub>5</sub>,  $\delta$  in ppm rel. to TMS, *J* in Hz. MS: ESI MS, pos. mode: Finnigan MAT-95S, HR-ESI MS, pos. mode: Mariner Biospectrometry Work Station.

### Plant Material

The roots of *P. rhagadioloides* were collected in June 2006 from plants cultivated in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences, Krakow, where a voucher specimen (06/106) was deposited. Seeds of the plant were obtained from the Botanical Garden of the University of Copenhagen, Denmark.

### Extraction and Isolation

The dried plant material (144 g) was powdered and exhaustively extracted with ethanol at room temperature to give a crude extract (10 g), after evaporation of the solvent under reduced pressure. This extract was chromatographed on a silica gel column eluted with hexane-EtOAc, followed by EtOAc-MeOH (up to 10 % MeOH) gradient solvent systems to give fractions which were submitted to prep. TLC and/or semiprep. HPLC. Elution of the silica gel column with hexane-EtOAc (4:1) afforded a mixture of **2** and **3** (*ca.* 2:1, 2.2 mg), and syringaldehyde (1.2 mg), after separation by prep. TLC (hexane-EtOAc, 7:3). Fractions eluted with hexane-EtOAc (3:2), followed by semiprep. HPLC purification (H<sub>2</sub>O-MeOH, 1:1), gave syringaresinol (6.7 mg). Fractions from EtOAc elution were further purified by prep. TLC (CHCl<sub>3</sub>-MeOH, 17:3). Initial fractions were separated by semiprep. HPLC (H<sub>2</sub>O-MeOH, 11:9) to afford **1** (11.3 mg), eugenyl-4-O- $\beta$ -glucopyranoside (1.4 mg), **7** (4.5 mg), a mixture of **5** and **10** (2.6 mg, *ca.* 2:1) and **4** (3.4 mg), in that order. Further fractions were purified by semiprep. HPLC (H<sub>2</sub>O-MeOH, 7:3) to give **8** (6.1 mg), a mixture of **6** and **8** (9.1 mg, *ca.* 1:6), and **9** (4.8 mg). The mixtures were not separated further, as the <sup>1</sup>H-NMR signals could be readily assigned to the respective compounds.

3 $\alpha$ -Hydroxy-1 $\alpha$ , 5 $\alpha$ , 7 $\alpha$  (H)-guaia-4(15), 10(14), 11(13)-trien-12-oic acid  $\beta$ -glucopyranosyl ester (**1**). White, amorphous solid;  $[\alpha]_D^{24} = -7.1$  ( $c = 1.57$ , MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: Table 2; ESI MS (pos. mode)  $m/z$ : 433  $[\text{M}+\text{Na}]^+$ , 843  $[2\text{M}+\text{Na}]^+$ ; HR-ESI MS (pos. mode)  $m/z$ : 433.18502 ( $[\text{C}_{21}\text{H}_{30}\text{O}_8 + \text{Na}]^+$ ; calc. 433.18329).

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*Sample availability:* Available from the authors.

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