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A guaianolide alloside and other constituents from *Picris kamtschatica*

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Abstract

From *Picris kamtschatica* two new guaianolide glycosides, including a β-allopyranosyl analogue of ixerin F, were isolated, in addition to ten known sesquiterpene lactones and six phenolic compounds. This is the first time a sesquiterpene lactone alloside has been described from plants. The compounds were characterized based on mass, 1D and 2D NMR spectral data.

Keywords: *Picris kamtschatica*; Asteraceae; Sesquiterpenoids; Phenolics; Glucosides; Alloside

1. Introduction

In continuation of our chemical studies of plants belonging to the tribe Lactuceae (Asteraceae), we have undertaken an investigation of *Picris kamtschatica* Ledeb. Until now, this plant has not been a subject of phytochemical analysis, but some other *Picris* species have yielded germacrane-, eudesmane- and guaiane-type sesquiterpene lactone aglycones and glycosides as their characteristic secondary metabolites (Al-Easa et al., 1996; Bohlmann et al., 1981; Hafez et al., 1988; Kijjoa et al., 1992; Kisiel, 1992, Kisiel and Zielińska, 2000; Marco et al., 1992; Milovanović et al., 2000; Nishimura et al., 1986 and ref. cited herein). Roots and aerial parts of *P. kamtschatica* have yielded, in addition to known sesquiterpenoids and phenolics, two new guaianolide glycosides (**1** and **2**) containing glucose and allose as sugar moieties, respectively. The isolation and characterization of these compounds are described in this paper.

2. Results and discussion

The dried roots and aerial parts of *P. kamtschatica* were separately extracted with ethanol and the extracts were chromatographed on silica gel columns, and further separated and purified by preparative TLC and semipreparative RP-HPLC.

The root extract gave, in addition to **1** and **2**, the known guaianolides ixerin F (**3**), its aglycone **4**, 9α-hydroxy-3-deoxyzaluzanin C (**5**), its 11β,13-dihydroderivative **6**, scorzoside (**7**), 11β,13-dihydrozaluzanin C (**8**), its 3-*O*-β-glucopyranoside (**9**) and the known germacranolide picriside B (**10**). Moreover, eugenyl-4-*O*-β-glucopyranoside and dihydroconiferin were isolated. The extract from the aerial parts afforded 8-desoxylactucin (**11**) and jacquinelin (**12**), along with the flavonoids apigenin, luteolin and their 4′-*O*-β-glucopyranosides. The flavonoid glycosides were identified by comparison of their spectral data with those in the literature (Borai and Dayal, 1993; Iwashina et al., 1990). The remaining known compounds were characterized by direct comparison (HPLC, ^{1}H NMR, EIMS or ESIMS and $[\alpha]_D$ wherever possible) with compounds previously isolated in our earlier studies. With the exception of **8**, the sesquiterpene lactones were previously obtained from plants of the genus *Picris* (Nishimura et al., 1986; Kisiel, 1992; Kisiel and Zielińska, 2000), the guaianolides **11** and **12** being the most common constituents of their aerial parts (Bohlmann et al., 1981; Hafez et al., 1988; Kisiel, 1992; Marco et al., 1992; Milovanović et al., 2000). Luteolin and apigenin were isolated from *P. cyanocarpa* (Hafez et al., 1988). The other phenolic

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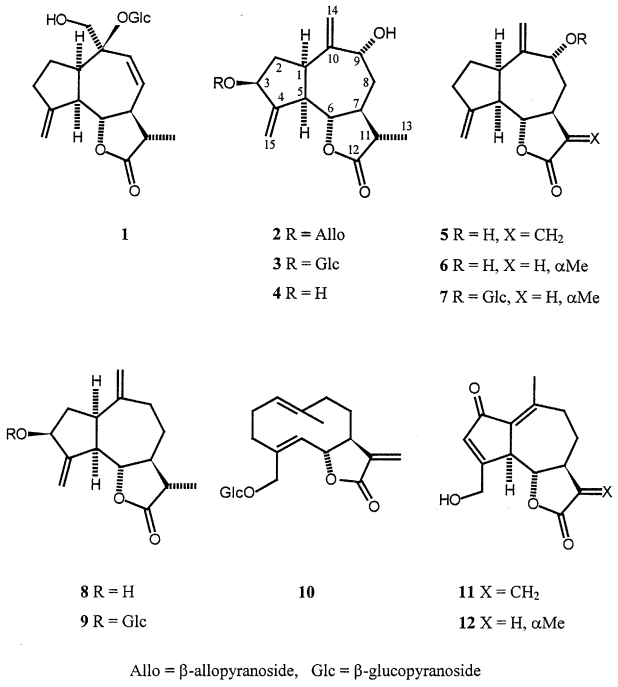
compounds are reported for the first time from *Picris* species.



1

2 R = Allo
3 R = Glc
4 R = H

5 R = H, X = CH_2
6 R = H, X = H, αMe
7 R = Glc, X = H, αMe

8 R = H
9 R = Glc

10

11 X = CH_2
12 X = H, αMe

Allo = β-allopyranoside, Glc = β-glucopyranoside

Compound **1** appeared to be a new natural product. Its ^{1}H and ^{13}C NMR data (Table 1), all assignments based on 2D COSY, NOESY and HETCOR experiments, suggested a structure similar in part to the guaianolide glucopyranoside scorzoside (**7**), first reported from *Scorzonera hispanica* (Bryanskii et al., 1992). However, one pair of signals for exomethylene protons was absent in the ^{1}H NMR spectrum of **1**. Instead, the compound was concluded to have a primary hydroxyl group at C-4 or C-10 geminal with a glucosylated tertiary hydroxyl group. Furthermore, the COSY experiment established the spin system –CH=CH–CH(CH–CH_3)–CH(O)– in the seven-membered ring of **1**, suggesting a Δ^8-double bond. The primary hydroxyl group was assigned based on a pair of geminally coupled (J = 12.8 Hz) signals at δ 3.72 and δ 4.08, which proved to be further coupled (J = 8.3 and 4.5 Hz, respectively) with a hydroxyl proton signal at δ 4.96 by the COSY spectrum. The olefinic proton signals due to H-8 and H-9 appeared as two-fold doublets at δ 6.07 (J = 10.8 and 3.9 Hz) and δ 5.64 (J = 10.8 and 2.1), respectively. Their couplings with H-7 signal at δ 4.48, observed in the COSY spectrum, verified the location of the double bond. Additionally, the H-7 signal showed correlations with the signals at δ 3.91 (H-6) and δ 2.61 (H-11). The unusual low chemical shift for the C-7 proton signal can be attributed to the fact that the proton must be in the anisotropic range of the ring system. Initially, it was not clear whether the exomethylene group was located at the C-4 or the C-10 position. This was readily established by UV spectrum of **1**. The spectrum showed end absorption at 200 nm which excluded the presence of an 8, 10(14)-diene system in the molecule and indicated that the exomethylene group was attached to C-4. The structural skeleton of **1** was in agreement with the molecular formula $C_{21}H_{30}O_9$ confirmed by ESIMS which showed ion peaks at m/z 449 $[M+Na]^+$ and m/z 875 $[2M+Na]^+$, as well as with the ^{13}C NMR data. The β-linkage of the glucosyl moiety was deduced from the coupling constant (J = 7.8 Hz) of the anomeric proton signal at δ 5.06. The relative stereochemical assignment was inferred from the NOESY spectrum of **1**. In the spectrum H-6 correlated with H-11 and H-2 (δ 1.55), suggesting that these protons were on the β face of the molecule by analogy to those of **7**, isolated from the same source. The spectrum further confirmed the proximities of H-7 to H-5 and H-13; H-1 to H-5 and H-2α, as well as H-14a and H-14b to H-1 and H-2α, and H-9, respectively. These NOESY correlations led to the stereostructure **1** in agreement with the α-oriented primary hydroxyl group at the C-10 position. Thus, the new guaianolide **1** was characterized as a derivative of 3-deoxyzaluzanin C. Compound **1** represents the first example of a guaianolide containing an unconjugated Δ^8-double bond.

The structure **2** of the second new natural product was established by mass, ^{1}H and ^{13}C NMR spectral

Table 1
^{1}H (500.13 MHz) and ^{13}C (125.76 MHz) NMR data of **1** in pyridine-d_5

Position	δ_H, J (Hz)	δ_C
Aglycone moiety		
1	2.89 *ddd* (12.0, 7.5, 6.4)	46.53
2α	1.78 *br ddd* (12.0, 7.5, 7.5)	24.69
2β	1.55 *dddd* (12.0, 12.0, 9.0, 8.0)	
3α	2.20 *ddd* (17.5, 9.0, 7.5)	28.87
3β	2.46 *br dd* (17.5, 8.0)	
4	–	151.30
5	3.31 *dd* (10.6, 6.4)	52.09
6	3.91 *dd* (10.6, 10.6)	77.29
7	4.48 *dddd* (13.0, 10.6, 3.9, 2.1)	45.71
8	6.07 *dd* (10.8, 3.9)	131.29
9	5.64 *dd* (10.8, 2.1)	135.58
10	–	83.29
11	2.61 *dq* (13.0, 6.9)	41.82
12	–	178.25
13	1.40 *d* (6.9)	12.81
14a	3.72 *dd* (12.8, 8.3)	66.77
14b	4.08 *dd* (12.8, 4.5)	
15a	4.87 *br s*	108.48
15b	5.11 *br s*	
14-OH	4.96 *m*	–
Glucosyl moiety		
1	5.06 *d* (7.8)	98.37
2	4.03 *m*	74.91
3	4.14 *m*	78.68
4	4.14 *m*	71.46
5	3.96 *m*	77.89
6a	4.23 *m*	62.35
6b	4.55 *br d* (11)	

analyses (Table 2), including 2D COSY, NOESY and HETCOR experiments. The ^1H and ^{13}C NMR spectral data of **2** were essentially identical to those of ixerin F (**3**), the guaianolide glucopyranoside first reported from *Ixeris tamagawaensis* (Asada et al., 1984), except for the signals arising from sugar moieties, thus suggesting the presence of the same aglycone structure with different sugars attached to the C-3 position of the aglycone. However, the carbon resonances at δ 36.5, δ 41.8 and δ 45.3 assigned to C-7, C-1, and C-11 of **3** in the earlier report needed to be corrected to C-1, C-11 and C-7, respectively. The ESI mass spectra of both compounds showed ion peaks at m/z 449 $[M+Na]^+$ and m/z 875 $[2M+Na]^+$, in accord with the molecular formula $C_{21}H_{30}O_9$ and ^{13}C NMR data. Examining the cross peaks in the COSY and HETCOR spectra of **2**, the sequences of all sugar proton and carbon signals were obtained. A close comparison of the signals with those of **3** indicated that a stereochemical difference at C-3 occurred in their sugar rings. The sugar hydroxyl group at the C-3 position of **2** proved to be axially oriented due to small coupling constants of the C-3 proton signal at δ 4.79. This configuration indicated the presence of an allopyranoside (an epimer of glucopyranoside at C-3). The C-2 proton signal of the allosyl unit gave a broad two-fold doublet at δ 4.05 ($J=8.0$ and 3.0 Hz) with the large coupling to the anomeric proton doublet at δ 5.51, indicating the β-glycosidic linkage with the aglycone. The coupling pattern of the remaining proton signals was comparable to that of reported allopyranosides (Dai et al., 2001; Jensen, 1996; Lee et al., 1991). The stereostructure depicted in the formula **2** was proposed by analogy to that of **3** and other zaluzanin C derivatives, also isolated from the plant roots. Compound **2** was optically inactive, the optical rotation value of **3** was very small ($[\alpha]_D=1.0$). Therefore, **2** was characterized as an allopyranoside analogue of ixerin F (**3**). To date, there have been no sesquiterpene lactone allopyranosides identified from plants. Allose, once thought to be a rare plant sugar, is now more frequently found linked to various compounds (Dai et al., 2001; Jensen, 1996; Lee et al., 1991).

The composition of the sesquiterpene lactones found in the roots of *P. kamtschatica* is similar to those isolated previously from *P. evae* (Kisiel and Zielińska, 2000), a species originating from Australia.

Table 2
^1H (500.13 MHz) and ^{13}C (125.76 MHz) NMR data of **2** in pyridine-d_5

Position	δ_H, J (Hz)	δ_C
Aglycone moiety		
1	3.65 *ddd* (9.4, 8.5, 6.8)	36.23
2α	2.44 *ddd* (13.7, 8.5, 8.5)	37.51
2β	2.20 *ddd* (13.7, 6.8, 6.8)	
3	4.81 *br dd* (8.5, 6.8)	80.83
4	–	151.63
5	2.91 *br dd* (9.7, 9.4)	49.29
6	4.14 *dd* (9.7, 9.7)	84.20
7	2.55 *dddd* (12.0, 12.0, 9.7, 3.2)	45.30
8α	2.32 *ddd* (13.4, 3.2, 3.2)	40.84
8β	1.49 *ddd* (13.4, 12.0, 3.2)	
9	4.75 *dd* (3.2, 3.2)	73.15[a]
10	–	153.74
11	2.28 *dq* (12.0, 7.0)	41.90
12	–	178.72
13	1.19 *d* (7.0)	13.34
14a	5.12 *s*	110.55
14b	5.15 *s*	
15a	5.49 *d* (1.4)	111.71
15b	5.92 *d* (1.5)	
Allosyl moiety		
1	5.51 *d* (8.0)	102.44
2	4.05 *br dd* (8.0, 3.0)	72.54
3	4.79 *br s*	73.15[a]
4	4.26 *br d* (9.5)	69.30
5	4.47 *ddd* (9.5, 5.0, 2.5)	75.88
6a	4.39 *br dd* (11.2, 5.0)	63.23
6b	4.52 *br d* (11.2)	

[a] Signals overlapped.

3. Experimental

3.1. Plant material

The aerial parts and roots of *P. kamtschatica* were collected in June 1999 from plants growing in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, where a voucher specimen was deposited (No 99/66). Seeds of wild origin were obtained from the Botanical Garden, Russian Academy of Sciences, Moscow.

3.2. Extraction and isolation

The dried and finely powdered aerial parts (412 g) and roots (179 g) were exhaustively extracted with ethanol at room temperature with shaking and the solvent was evaporated under reduced pressure to give 40 g and 22 g of residues, respectively. The latter was chromatographed on silica gel (Merck, Art. 7754) column using hexane–EtOAc (up to 100% EtOAc), followed by EtOAc–MeOH (up to 10% MeOH) gradient solvent systems.The relevant fractions were combined, as shown by TLC, and further separated and purified by preparative TLC (Merck, Art. 5553) and semipreparative RP-HPLC on a Delta-Pak C-18 column (particle size 15 μm, 25×100 mm) coupled to a photodiode array detector using a H_2O–MeOH (11:9) mixture at a flow rate of 3.0 ml min^{-1}. Elution of the column with hexane–EtOAc (4:1) afforded **8** (10.1 mg) and a mixture (19.9 mg) of **5**, **6** and **8** in the ratio ca. 1:1:1.5, after purification by preparative TLC (hexane–EtOAc, 3:2). Further elution with hexane–EtOAc (1:1) followed by TLC purification ($CHCl_3$–MeOH, 9:1) yielded **4** (9.7 mg). More

polar fractions, eluted with EtOAc and EtOAc–MeOH (19:1) were separated by TLC ($CHCl_3$–MeOH, 9:1 or 17:3) and HPLC to give **1** (3.2 mg), **2** (4.8 mg), **3** (17.0 mg), **7** (1.3 mg), a ca. 3:1 mixture (2.2 mg) of **9** and **10**, eugenyl-4-*O*-β-glucopyranoside (1.0 mg) and dihydroconiferin (3.2 mg). The residue from the aerial part extract was chromatographed on a silica gel column as described above. Fractions eluted with hexane-EtOAc (3:2) were further separated by TLC (hexane–EtOAc, 1:1) to yield a mixture (24.7 mg) of **11** and **12** in the ratio ca. 1:1.8, apigenin (17.2 mg) and luteolin (26.6 mg). Purification of EtOAc fractions by TLC using $CHCl_3$-MeOH (17:3) furnished a mixture (ca. 1:3, 4.3 mg) of apigenin- and luteolin-4′-*O*-β-glucopyranosides and 13.8 mg of the latter glucoside.

*3.3. 10β,14-Dihydroxy-10(14), 11β (13)-tetrahydro-8,9-didehydro-3-deoxyzaluzanin C-10-O-β-glucopyranoside (**1**)*

Solid; $[\alpha]_D^{25.8} = -41.8$ ($c = 0.63$, MeOH); UV on-line: 200 nm; ^1H and ^{13}C NMR, see Table 1; ESIMS: *m*/*z* 449 $[M + Na]^+$, 875 $[2M + Na]^+$.

*3.4. 9α-Hydroxy-11β (13)-dihydrozaluzanin C-3-O-β-allopyranoside (**2**)*

Solid; UV on-line: 200 nm; ^1H and ^{13}C NMR, see Table 2; ESIMS: *m*/*z* 449 $[M + Na]^+$, 875 $[2M + Na]^+$.

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