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Sesquiterpenoids from roots of *Taraxacum laevigatum* and *Taraxacum disseminatum*

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Abstract

Chromatographic separation of ethanolic root extracts of *Taraxacum laevigatum* and *Taraxacum disseminatum* afforded a total of eight germacrane- and eudesmane-type sesquiterpenoids, including new compounds, 1β,3β,6α-trihydroxy-4α(15)-dihydrocostic acid methyl ester and its 1-*O*-β-glucopyranoside. Their structures were established by spectroscopic analyses. In addition, the structure of 4α(15),11β(13)-tetrahydroridentin B-1-*O*-β-glucopyranoside was elucidated by extensive NMR studies.

Keywords: *Taraxacum laevigatum*; *Taraxacum disseminatum*; Asteraceae; Lactuceae; Sesquiterpenoids; Germacranolides; Eudesmanolides; Costic acid derivatives; Glycosides

1. Introduction

Plants of the genus *Taraxacum*, in particular *Taraxacum officinale*, have been used in herbal medicine for their choleretic, diuretic and anti-inflammatory properties (Blashek et al. 1998; Yang et al. 1996). For a long time, the only known sesquiterpene lactone constituents of the plants were two germacranolides, i.e. taraxinic acid β-glucopyranosyl ester (**1**) and its 11,13-dihydroderivative, and two eudesmanolides, i.e. 4α(15),11β(13)-tetrahydroridentin B (**5**) and taraxacolide-1-*O*-β-glucopyranoside, isolated from *T. officinale* (Hänsel et al. 1980). Recent reports from our and other laboratories showed the presence of further sesquiterpene lactones in dandelions. *T. platycarpum* afforded guaianolide desacetylmatricarin which showed anti-allergic activity (Ho et al., 1998). *T. linearisquameum* yielded **1** and two eudesmanolides glucosylated at the C-1 position, including **3** (Zidorn et al., 1999). Similar eudesmanolides were reported from *T. hallaisanensis* (Yang et al., 1996). In our study (Kisiel and Barszcz, 1998), *T. officinale* yielded three closely related germacranolide acids esterified with glucose, including the two above mentioned taraxinic acid derivatives, together with guaianolide ixerin D (**8**). Moreover, we could assign the stereochemistry at C-11 in 11,13-dihydrotaraxinic acid-β-glucopyranosyl ester, depicted in the formula **2**. No chemical studies appeared to have been reported for *T. laevigatum* (Willd.) DC. and *T. disseminatum* G.E. Haglund. The purpose of the present study was to characterize the compositions of sesquiterpenoids in both species, with the aim to provide more insight into the variation of the compounds in *Taraxacum* plants.

2. Results and discussion

The ethanol extracts from the roots of *T. laevigatum* and *T. disseminatum* were separately chromatographed on silica gel columns to give fractions which contained sesquiterpenoids and their glycosides. The fractions, after further separation and purification by prep. TLC

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and semiprep. HPLC, yielded a total of eight sesquiterpenoids, of which **1–3, 5** and **7** were found in both species, **6** and **8** originated from *T. laevigatum*, and **4** originated from *T. disseminatum*. The known germacranolides, **1** and **2**, and the guaianolide ixerin D (**8**) were identified by direct comparison (HPLC, ^{1}H-NMR) with the compounds previously isolated in our laboratory (Kisiel et al., 1998). Compound **5**, first reported from *T. officinale*, had spectral features in accord with those published (Hänsel et al., 1980). Its previously unreported ^{1}H-NMR spectral data in $CDCl_3$ are given in Table 2.

The ^{1}H-NMR spectra of compounds **3**, **4** and **6** (Table 1) showed characteristic signals of glucose moieties. The presence of β-glucosidic linkages followed from the large coupling constants of the anomeric proton doublets ($J > 7.6$ Hz). The ^{1}H-NMR and mass spectral data of compounds **3** and **4** pointed to the closely related structures depicted in the formulae. The structures **3** and **4** were originally assigned to eudesmanolides isolated from *T. hallaisanensis*. However, their published ^{1}H-NMR spectral data in pyridine-d_5 (Yang et al., 1996) were not comparable, even in part, with each other and were remarkably different from the corresponding data for **3** and **4** given in Table 1. Compound **3** was subsequently found in *T. linearisquameum*. Therefore, we recorded the ^{1}H-NMR spectrum of **3** in methanol-d_4 for further comparison and found the chemical shift values to be identical to those reported for 3β-hydroxy-4α(3)-dihydrosantamarine-1-*O*-β-glucopyranoside (4α(15)-dihydroridentin B-1-*O*-β-glucopyranoside) (Zidorn et al., 1999). Accordingly, the eudesmanolides from *T. hallaisanensis* (Yang et al., 1996) need reinvestigation.

The structure of our compound **4** was evident from direct comparison of its ^{1}H-NMR spectrum with that of **3**. The absence of proton signals associated with the exocyclic methylene group and the presence of a three-proton doublet at δ 1.15 and a doublet quartet at δ 2.36 indicated that **4** was a 11,13-dihydroderivative of **3**. The α configuration of the methyl group at C-11 was deduced from the observed large coupling $J_{7,11} = 11.3$ Hz. The proton signals assignments were supported by ^{1}H–^{1}H COSY spectrum. The NOESY spectrum verified the proximity of H-6 to Me-14 and Me-15, as well as the proximities of H-3 to H-5 and H-2α/4α (δ 2.60), and H-1 to H-8α/9α (δ 1.27). It also showed a cross peak between the anomeric sugar proton and H-1 of the aglycone indicating the attachment of the glucose moiety at the C-1 position. The ESIMS confirmed the structure with ion peaks at m/z 453 $[M + Na]^+$ and m/z 883 [2M +

Table 1
^{1}H-NMR spectral data of compounds **3**, **4** and **6**[a]

H	3	4	6
Aglycone moieties			
1	3.76 *dd* (11.6, 3.6)	3.75 *dd* (11.8, 3.8)	3.75 *dd* (11.6, 3.6)
2	2.59 *ddd* (12.0, 3.6, 3.6)	2.59 *ddd* (12.0, 3.8, 3.8)	2.61 *br d* (12.0)
2′	2.17 *ddd* (12.0, 12.0, 11.6)	2.17 *ddd* (12.0, 12.0, 11.8)	2.27 *m*
3	4.04 *m*	4.05 *m*	4.08 *m*
4	2.60 *m*	2.60 *m*	3.15 *m*
5	1.56 *dd* (11.3, 4.4)	1.43 *dd* (11.5, 4.3)	1.46 *dd* (10.4, 3.6)
6	4.13 *dd* (11.3, 10.8)	4.16 *dd* (11.5, 10.3)	4.33 *t*-like *m*
7	2.36 *m*	1.40 *m*	2.84 *ddd* (13.0, 9.4, 4.0)
8	1.63 *m*	1.27 *m*	1.59 *br dd* (13.5, 3.1)
8′	2.25 *m*[b]	1.44 *m*	1.79 *dddd* (13.5, 13.0, 13.0, 2.4)
9	1.27 *m*[b]	1.27 *m*	1.27 *m*
9′	2.17 *m*[b]	2.24 *m*	2.27 *m*
11	–	2.36 *dq* (11.3, 6.9)	–
13	5.27 *d* (2.9)	1.15 *d* (6.9)	5.82 *br s*
13′	6.08 *d* (3.1)		6.36 *br s*
14	1.06 *s*	1.06 *s*	1.19 *s*
15	1.23 *d* (7.3)	1.22 *d* (7.4)	1.34 *d* (7.3)
OMe	–	–	3.59 *s*
Glucosyl moieties			
1	4.92 *d* (7.7)	4.92 *d* (7.7)	4.94 *d* (7.8)
2	4.01 *m*	4.01 *dd* (8.6, 7.7)	4.01 *m*
3, 4	4.26 *m*	4.24 *m*	4.26 *m*
5	3.95 *m*	3.95 *m*	3.95 *m*
6	4.41 *dd* (11.6, 5.4)	4.40 *dd* (11.6, 5.3)	4.41 *dd* (11.4, 5.0)
6′	4.59 *dd* (11.6, 1.9)	4.59 br *d* (11.6)	4.55 dd (11.4, 2.0)

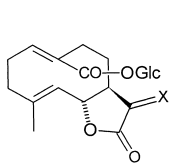
[a] Spectra were recorded in pyridine-d_5 (500.13 MHz), TMS as internal standard, δ values, coupling constants (parentheses) in Hz.
[b] Approximate values.

Na]$^+$. Thus, compound **4** was proved to be 4α(15),11β(13)-tetrahydroridentin B-1-*O*-β-glucopyranoside.

The ^{1}H-NMR spectral data of compound **6** were in part close to that of **3** but the two exocyclic methylene doublets were replaced by broadened singlets shifted much more downfield and a three-proton singlet at δ 3.59 appeared, suggesting the presence of a methyl ester function and, consequently, a derivative of costic acid methyl ester. Again, the proton signal assignments were supported by ^{1}H–^{1}H COSY spectrum and the structural elucidation was confirmed by NOESY experiment and by ESIMS which showed peaks at *m*/*z* 483 [M + Na]$^+$ and *m*/*z* 943 [2M + Na]$^+$. Based on these data, compound **6** was deduced to be 1β,3β,6α-trihydroxy-4α(15)-dihydrocostic acid methyl ester-1-*O*-β-glucopyranoside, a new natural product.

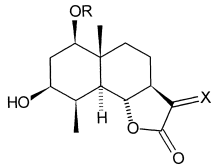
Mixtures of less polar eudesmane sesquiterpenoids, containing aglycones of **3**, **4** and **6** (by ^{1}H-NMR) could not be completely separated by HPLC. Therefore, spectral data of still impure compound **7** were examined. The ^{1}H-NMR spectrum of **7** also showed two broadened singlets at δ 5.72 and δ 6.30, ascribable to the exocyclic methylene protons at C-13 and a three-proton singlet at δ 3.77, characteristic of the methyl ester group. Other spectral features of **7**, confirmed by ^{1}H–^{1}H COSY experiment, were compatible with the structure of the aglycone of **6**. This was strongly supported by ESIMS which showed significant peaks of ions at *m*/*z* 321 [M + Na]$^+$ and *m*/*z* 299 [M + H]$^+$, and ions formed by successive loss of three molecules of water from the [M + H]$^+$ ion. Thus, compound **7** was found to be another new sesquiterpenoid obtained from dandelions, i.e. 1β,3β,6α-trihydroxy-4α(15)-dihydrocostic acid methyl ester. Closely related compounds, 1β,3β,6α-trihydroxycostic acid methyl ester and its derivative at C-6 were reported from *Artemisia rutifolia* (Jakupovic et al., 1991; Tan and Jia, 1992).

Similar to some earlier reports on sesquiterpenoids in *Taraxacum* species, we found *T. laevigatum* and *T. disseminatum* to contain predominantly taraxinic acids esterified with glucose and eudesmane type sesquiterpenoids glucosylated at the C-1 position.



1 X = CH_2

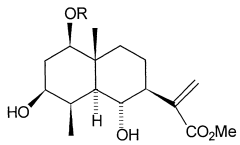
2 X = H, αMe



3 R = Glc, X = CH_2

4 R = Glc, X = H, αMe

5 R = H, X = H, αMe



6 R = Glc

7 R = H

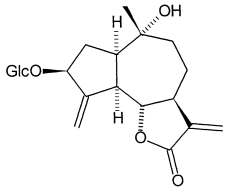


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Table 2
^{1}H-NMR data of compounds **5** and **7**[a]

H	**5**	**7**
1	3.37 *dd* (11.7, 4.0)	3.32 *dd* (11.7, 4.2)
2	1.87 *m*	1.87 *ddd* (11.9, 4.2, 4.2)
2′	1.75 *ddd* (11.9, 11.9, 11.7)	1.74 *ddd* (11.9, 11.9, 11.7)
3	3.84 *m*	3.80 *m*
4	2.36 *m*	2.49 *m*
5	1.40 *dd* (11.4, 4.4)	1.14 *dd* (10.7, 4.3)
6	4.08 *dd* (11.4, 10.1)	3.90 *dd* (10.7, 4.3)
7	1.59 *m*	2.49 *m*
8	1.87 *m*	1.74 *m*
8′	1.45 *m*	1.65 *m*
9	1.14 *m*	1.14 *m*
9′	1.95 *ddd* (13.3, 3.0, 3.0)	1.86 *ddd* (12.9, 3.3, 3.3)
11	2.36 *dq* (12.4, 6.9)	–
13	1.22 *d* (6.9)	6.30 *br s*
13′		5.72 *br s*
14	1.02 *s*	0.98 *s*
15	0.98 *d* (7.4)	0.93 *d* (7.4)
OMe	–	3.77 *s*

[a] Spectra were recorded in $CDCl_3$ (500.13 MHz), TMS as internal standard, δ values, coupling constants (parentheses) in Hz.

3. Experimental

3.1. Plant material

Roots of *T. laevigatum* and *T. disseminatum* were collected in June 1998 from plants growing in the Garden of Medicinal Plants, Institute of Pharmacology, Polish Academy of Sciences, Kraków, where voucher specimens have been deposited.

3.2. Extraction and isolation

Roots of *T. laevigatum* (254 g) and *T. disseminatum* (182 g) were ground and exhaustively extracted with EtOH at room temperature with shaking. Concentration of the extracts under reduced pressure provided 23 g and 12 g of residues, respectively. In each case, the residue was chromatographed on a silica gel (Merck, Art. 7754) column using hexane–EtOAc (up to 100% EtOAc), followed by EtOAc–MeOH (up to 15% MeOH) gradient solvent systems. Less polar frac-

tions eluted with EtOAc and more polar fractions eluted with EtOAc–MeOH (19:1 and 9:1) mixtures containing sesquiterpenoid aglycones and glycosides, respectively, were further separated and purified by prep. TLC (Merck, Art. 5553, $CHCl_3$–MeOH mixtures) and by semiprep. HPLC (Delta-Pak C-18 cartridge column, particle size 15 μm, 25 mm × 100 mm, H_2O–MeOH mixtures, flow rate of 3 ml min^{-1}, UV photodiode-array detector), as described below.

T. laevigatum. The less polar fractions, after separation by prep. TLC ($CHCl_3$–MeOH, 9:1), afforded a mixture (13.1 mg) of eudesmane sesquiterpenoids, containing aglycones of **3**, **4** and **6** (by ^{1}H-NMR). The mixture was very difficult to separate by HPLC (H_2O–MeOH, 3:2) and compounds **7** (6.0 mg) and **5** (3.4 mg), still contaminated mainly with each other, were obtained. The more polar fractions were subjected to prep. TLC ($CHCl_3$–MeOH, 17:3 or 4:1) to give a mixture (6.4 mg) of **1** and **2** in a ratio ca. 5:3, respectively, **8** (3.4 mg) and a mixture (22.5 mg) of **3** and **6**. The latter mixture was processed by semiprep. HPLC (H_2O–MeOH, 13:7) to yield only small amounts of pure **3** (2.4 mg) and **6** (4.3 mg).

T. disseminatum. Similar separation procedures were used. The less polar fractions afforded aglycone mixture (4.7 mg) described above. The more polar fractions, after separation by prep. TLC, yielded a mixture (28.1 mg) of **1** and **2** in a ratio ca. 1:1, **4** (5.0 mg) and **3** (4.2 mg).

Compound **4**. Solid. ESIMS *m/z*: 453 $[M + Na]^+$, 883 $[2M + Na]^+$. ^{1}H-NMR: Table 1.

Compound **6**. Solid. ESIMS *m/z*: 483 $[M + Na]^+$, 943 $[2M + Na]^+$. ^{1}H-NMR: Table 1.

Compound **7**. Solid. ESIMS *m/z*: 321 $[M + Na]^+$, 299 $[M + H]^+$, 281 $[M - H_2O + H]^+$, 263 $[M - 2H_2O + H]^+$, 245 $[M - 3H_2O + H]^+$. ^{1}H-NMR: Table 2.

References

Blashek, W., Hänsel, R., Keller, K., Reichling, J., Rimpler, H., Smeider G., 1998. Hagers Handbuch der Pharmazeutischen Praxis, Bd 3, Drogen L-Z., Springer–Verlag, Berlin–Heidelberg–New York p. 897.

Hänsel, R., Kartarahardja, M., Huang, J.-T., Bohlmann, F., 1980. Sesquiterpen-lacton-β-D-glucopyranoside sowie ein neues Eudesmanolid aus *Taraxacum officinale*. Phytochemistry 19, 857–861.

Ho, Ch., Choi, E.J., Yoo, G.S., Kim, K.-M., Ryu, S.Y., 1998. Desacetylmatricarin, an anti-allergic component from *Taraxacum platycarpum*. Planta Medica 64, 577–578.

Jakupovic, J., Tan, R.X., Bohlmann, F., Jia, Z.J., Huneck, S., 1991. Sesquiterpene lactones from *Artemisia rutifolia*. Phytochemistry 30, 1714–1716.

Kisiel, W., Barszcz, B., 1998. In: Poster G 49 at the 46th Annual Congress of the Society for Medicinal Plant Research in Vienna.

Tan, R.X., Jia, Z.J., 1992. Sesquiterpenes from *Artemisia rutifolia*. Phytochemistry 31, 2534–2536.

Yang, D.S., Whang, W.K., Kim, I.H., 1996. The constituents of *Taraxacum hallaisanensis* roots. Arch. Pharmacal Research 19, 507–513.

Zidorn, C., Ellmerer-Müller, E.P., Stuppner, H., 1999. Eudesmanolides and inositol derivatives from *Taraxacum linearisquameum*. Phytochemistry 51, 991–994.