PERGAMON

Phytochemistry 51 (1999) 991–994

PHYTOCHEMISTRY

Eudesmanolides and inositol derivatives from *Taraxacum linearisquameum*

C. Zidorn[a], E.P. Ellmerer-Müller[b], H. Stuppner[b,*]

[a]*Institut für Pharmakognosie der Universität Innsbruck, Josef-Möller-Haus, Innrain 52, A-6020, Innsbruck, Austria*
[b]*Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A-6020, Innsbruck, Austria*

Received 5 January 1999; received in revised form 23 February 1999; accepted 3 March 1999

Abstract

The methanol extract of subaerial parts of *Taraxacum linearisquameum* Soest afforded two eudesmane type sesquiterpene lactones, 2β-hydroxysantamarine-1β-D-glucopyranoside (**2**) and 3β-hydroxy-4αH-3-dihydrosantamarine-β-D-glucopyranoside (**3**) and two inositol derivatives, (1S,2S,4R,5S)-2,3,4,6-tetrahydroxy-5-[2-(4-hydroxyphenyl)acetyl]oxycyclohexyl-2-(4-hydroxyphenyl) acetate (**4**) and (2S,3R,5R,6S)-2,3,5,6-tetrahydroxy-4-[2-(4-hydroxyphenyl)acetyl]oxycyclohexyl-2-(4-hydroxy-phenyl)acetate (**5**). Additionally, the known compound taraxinic acid β-D-glucopyranosyl ester (**1**) has been isolated. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Taraxacum linearisquameum*; *Taraxacum* section Ruderalia; *Taraxacum officinale* agg; Asteraceae; Lactuceae; Sesquiterpene lactones; Eudesmanolides; *p*-Hydroxyphenylacetic acid inositol diesters

1. Introduction

Taraxacum linearisquameum Soest is a member of *Taraxacum* section Ruderalia Kirschner, (Ellgaard, Štěpánek, a group of far more than 100 mostly apomictic taxa, which have been formerly referred to as *Taraxacum officinale* agg. The members of this section occur naturally in meadows and on disturbed ground in Europe and Asia and are introduced worldwide to areas with temperate climate (Richards & Sell, 1976; Doll, 1994; Weihe, 1972). *T. linearisquameum* is a diploid and is one of the sexually reproducing taxa of the section. The drug Taraxaci Radix is extensively used in phyto- and folk-medicine as a diuretic and bile stimulant, an application seemingly based on the presence of sesquiterpene lactones, though few studies on the occurrence of these secondary metabolites have appeared (Willuhn, 1997; Hänsel, Kartarahardja, Huang & Bohlmann, 1980; Kisiel & Barszcz, 1998).

2. Results and discussion

The EtOAc fraction of the methanol extract of freeze-dried subaerial parts of *T. linearisquameum* was repeatedly chromatographed on silica gel to give three sesquiterpenoids (**1–3**) and two inositol derivatives (**4–5**).

Compound **1** was identified as taraxinic acid β-D-glucopyranosyl ester based on comparison of its molecular mass (ESIMS, negative mode, m/z: 423 $[M-H]^-$) and ^1H NMR and ^{13}C NMR data with those given in literature (Hänsel et al., 1980).

The high resolution FAB mass spectrum of **2** (positive mode, $m/z = 427.2083$ $[M+H]^+$) determined its molecular formula as $C_{21}H_{30}O_9$. The ^1H NMR spectrum (Table 1) shows characteristic signals of a β-D-glucoside moiety—one anomeric proton [δ_H 4.49 (*d*, $J_{1',2'} = 8.0$ Hz)], four signals in the range between δ_H

* Corresponding author. Tel.: +43-512-507-5307; fax: +43-512-507-2939.

E-mail address: hermann.stuppner@uibk.ac.at (H. Stuppner)

0031-9422/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(99)00163-6

Table 1
NMR spectral data of **2** and **3** (^{13}C: 125 MHz, ^{1}H: 500 MHz)[a]

	2	**2**	**3**	**3**
Position	H	C	H	C
1	3.75 1H, *d* (5.5)	84.8	3.56 1H, *dd* (12.0, 4.5)	84.0
2	4.38 1H, br *s*	64.7	1.97 1H, *ddd* (12.0, 4.5, 4.5)	31.7
			1.77 1H, *ddd* (12.0, 12.0, 4.5)	
3	5.64 1H, br *s*	124.9	3.78 1H, *ddd* (12.0, 4.5, 4.5)	71.5
4		138.3	2.35 1H, *m*	35.5
5	2.41 1H, *d* (11.0)	52.5	1.64 1H, *dd* (11.5, 4.0)	49.3
6	4.06 1H, *dd* (11.0, 11.0)	83.0	4.17 1H, *dd* (11.5, 11.5)	81.6
7	2.60 1H, br *ddd* (11.0, 11.0, 3.0)	52.2	2.56 1H, br *ddd* (11.5, 11.5, 3.0)	52.1
8a	2.12 1H, *dd* (13.0, 3.0)	21.6	2.06 1H, *m*	22.5
8b	1.69 1H, *m*		1.55 1H, *ddd* (13.5, 13.5, 3.0)	
9a	2.13 1H, *dd* (13.5, 3.0)	35.9	2.06 1H, *m*	40.7
9b	1.51 1H, *ddd* (13.5, 13.5, 4.0)		1.34 1H, *ddd* (13.5, 13.5, 3.0)	
10		40.9		42.0
11		140.8		141.9
12		172.6		172.9
13a	6.03 1H, *d* (3.0)	117.2	6.01 1H *d* (3.0)	117.1
13b	5.51 1H, *d* (3.0)		5.49 1H, *d* (3.0)	
14	1.02 3H, *s*	14.2	1.07 3H, *s*	16.3
15	1.93 3H, *s*	23.6	1.02 3H, *d* (7.5)	9.3
Glucose				
1′	4.49 1H, *d* (8.0)	102.1	4.34 1H, *d* (8.0)	101.8
2′	3.27 1H, *dd* (8.0, 8.0)	75.3	3.14 1H, *dd* (8.0, 8.0)	75.0
3′	3.38 1H, *dd* (8.0, 8.0)	78.3	3.36 1H, *m**	78.2
4′	3.32 1H, *m**	71.7	3.33 1H, *m**	71.9
5′	3.31 1H, *m**	78.3	3.27 1H, *m**	77.9
6′a	3.91 1H, br *d* (12.0)	62.9	3.88 1H, br *d* (11.5)	63.0
6′b	3.69 1H, *dd* (12.0, 3.0)		3.66 1H, *dd* (11.5, 2.5)	

[a] In MeOH-d_4, ^{1}HNMR coupling constants are given in brackets, all assignments are confirmed by HSQC and HMBC experiments. *Signals are overlapping and may be exchangeable.

3.27–3.38 and signals for a oxygen-bearing methylene group [δ_H 3.91 (br $d(J_{6'a,6'b}=12.0$ Hz), δ_H 3.69 (*dd*, $J_{6'a,6'b}=12.0$ Hz, $J_{5',6'b}=3.0$ Hz)−, doublets for an exocyclic olefinic methylene group [δ_H 6.03 and δ_H 5.51 (*d*, $J_{13,7}=3.0$ Hz)], a vinylic proton [δ_H 5.64 (br *s*)], an olefinic methyl group [δ_H 1.93 (*s*)], three oxygen-bearing methine groups [δ_H 4.38 (br *s*), δ_H 4.06 (*dd*, $J_{6,5}=11.0$ Hz, $J_{6,7}=11.0$ Hz), δ_H 3.75 (*d*, $J_{1,2}=5.5$ Hz)] and an aliphatic methyl group [δ_H 1.02 (*s*)].

^{13}C NMR and DEPT spectra (Table 1) displayed 21 signals, assignable to two methyl, four methylene and 11 methine groups and four quarternary carbons. The structure of an eudesmanolide glucoside and its substitution pattern has been established by ^{1}H–^{1}H COSY, HSQC, HSQC–TOCSY and HMBC experiments. Relative configurations of carbons 5, 6 and 7 have been deduced from coupling patterns of the corresponding protons in comparison with literature data (Glasl et al., 1995). Accordingly, H-5 and H-7 are α- and H-6 is β-oriented. This is confirmed by a NOESY experiment which revealed correlations between H-1 and H-6, H-5 and H-7 as well as H-6 and the C-14 methyl protons. Accordingly, in the three-dimensional model the protons H-1, H-5, H-6 and H-7 as well as the methyl group in position C-10 should show an axial position. In contrast H-2 has to be equatorial oriented since the small coupling constant of the doublet of H-1 at δ3.75 ($J=5.5$ Hz) is only consistent with an axial-equatorial arrangement of H-1 and H-2. Thus, **2** is 2β-hydroxysantamarine-1β-D-glucopyranoside, which represents a new natural compound.

High-resolution FABMS of **3** (positive mode, $m/z=429.2118$ $[M+H]^+$) results in a molecular formula of $C_{21}H_{32}O_9$. With the exception of the absence of the endocyclic double bond in position 3, ^{1}H and ^{13}C NMR spectral data (Table 1) are similar to those of **2**. However, one- and two-dimensional NMR data show that the second hydroxyl group has to be located in position C-3 instead of C-2 as established for compound **2**. Stereochemistry of carbons 5, 6 and 7 is in accordance with the one in compound **2**. β-Configurations of the substituents at C-1, C-3 and C-4 are deducible from multiplicities and coupling constants of the corresponding ^{1}H NMR signals. Thus, **3** is 3β-hydroxy-4α*H*-3-dihydrosantamarine-β-D-gluco-

Table 2
NMR spectral data of **4** and **5** (^{13}C: 125 MHz, ^{1}H: 500 MHz)[a]

	4	**4**		**5**	**5**
Position	H	C	Position	H	C
1	4.81 1H, *dd* (10.0, 3.7)	75.7	1/4	5.09 2H, *dt* (7.5, 2.0)	75.8
2	3.98. 1H, *t* (3.7)	68.0	2/5	4.02 2H, br *d* (2.0)	71.2
3	5.14 1H, *t* (3.7)	75.3	3/6	3.87 2H, *dd* (7.5, 2.5)	72.4
4	3.91 1H, *dd* (10.0, 3.7)	70.1			
5	3.56 1H, *t* (10.0)	74.9	α′/α″		172.6
6	3.82 1H, *t* (10.0)	71.9	β′/β″	3.65 4H, br *s*	41.6
α′		174.2	1′/1″		126.4
β′	3.61 2H, *d* (3.0)	40.9			
1′		126.4	2′/6′/		
				7.12 4H, br *d* (8.5)	131.4
			2″/6″		
2′/6′	7.10 2H, br *d* (8.5)	131.5			
3′/5′	6.73 2H, br *d* (8.5)	116.4	3′/5′/		
				6.74 4H, br *d* (8.5)	116.4
			3″/5″		
4′		157.5			
			4′/4″		157.1
α″		172.1			
β″	3.66 2H, *d* (3.0)	41.0			
1″		125.3			
2″/6″	7.13 2H, br *d* (8.5)	131.5			
3″/5″	6.71 2H, br *d* (8.5)	116.2			
4″	—	157.5			

[a] In MeOH-d_4, ^{1}HNMR coupling constants are given in brackets, all assignments are confirmed by HSQC and HMBC experiments.

pyranoside. This compound represents another new eudesmanolide which, like **1** and **2**, might be one of the active principles of the roots of dandelion.

The molecular formula of compounds **4** was determined as $C_{22}H_{24}O_{10}$ based on high-resolution positive FABMS (m/z = 449.1448 $[M+H]^+$). The ^{1}H NMR spectrum indicated the presence of an inositol moiety esterified with two *p*-hydroxyphenylacetic acid units. This was evidenced by two pairs of downfield shifted broad doublets (4H, br *d*, J = 8.5 Hz) at δ6.71, 6.73, 7.10 and 7.13, two broad methylene group signals at δ3.61 and 3.66 (2H, *d*, J = 3.0 Hz) and signals of six oxygen-bearing methine groups in the range at δ3.56–5.14. The ^{13}C NMR spectrum showed two secondary, ten tertiary, two carboxylic and four non-carboxylic quarternary carbon signals. Signal assignments in Table 2 are confirmed by ^{1}H-^{1}H COSY and ^{1}H–^{13}C HETCOR experiments. Linkages of the *p*-hydroxyphenylacetic acid moieties with the inositol unit were obtained from HMBC results. H-1 and H-5 showed correlations with the carbonyl signals at δ_C 174.2 and 172.1, respectively. In addition, the chemical shifts of H-1 and H-5 are characteristic of an alcohol component of an ester. Stereochemistry of the cyclohexitol moiety has been deduced from multiplicities and coupling constants of the corresponding proton signals. The value α_D^{20} (in water) of −20°, is consistent with (−) chiro inositol as the basic cyclohexitol unit. Thus, **4** is 1S,2S,4R,5S)-2,3,4,6-tetrahydroxy-5-[2-(4-hydroxy phenyl)acetyl]oxycyclohexyl - 2 - (4 - hydroxyphenyl)acetate. The negative mode ESI mass spectrum of the inositol derivative **5** (m/z = 447 $[M-H]^-$) suggested the molecular formula of $C_{22}H_{24}O_{10}$ which is identical with that of compound **4**. However, ^{1}H and ^{13}C NMR spectra of **5** revealed only nine carbon and six hydrogen signals, including one methylene, three oxygen-bearing and two aromatic methine groups as well as one carboxylic carbon and two quarternary aromatic carbons. Therefore, compound **5** has to have a symmetric structure. Localization of the two ester groups has been established by a HBMC experiment which showed long-range correlations between H-1 and H-4 of the cyclohexitol unit and the carboxylic carbons of the *p*-hydroxyphenylacetic acid moieties. The cyclohexitol moiety has been identified as neo inositol as deduced from multiplicities and coupling constants of the corresponding ^{1}H NMR signals. Thus, **5** is (2S,3R,5R,6S)-2,3,5,6-tetrahydroxy-4-[2-[4-hydroxyphenyl)acetyl]oxycyclohexyl-2-(4-hydroxy-phenyl)-acetate, which as expected from the presence of a centre of symmetry, showed no optical activity. Both cyclohexitols and *p*-hydroxyphenylacetic acid are quite common in the plant kingdom and have already been reported from *Taraxacum* taxa, but, to our best knowledge, esters like **4** and **5** have been found neither in *Taraxacum* nor in any other genus. Evaluation of pharmacological activities of the isolated compounds **1–5** is in progress.

3. Experimental

Plant material. *T. linearisquameum* Soest was collected in April 1997 near Zirl/Tyrol/Austria. A voucher specimen is deposited at the Institute of Pharmacognosy.

Extraction and isolation of compounds **1–5**. Freeze-dried subaerial parts of *T. linearisquameum* (1190 g) were ground and extracted exhaustively at room temperature with MeOH yielding 150 g of residue after evaporation in vacuum. The residue was dissolved in methanol/water (1/2) and successively partitioned with petroleumether, CH_2Cl_2 and EtOAc. **1**(70 mg), **2**(280 mg), **3**(22 mg), **4**(10 mg) and **5**(7 mg) were isolated by repeated silica gel chromatography of the EtOAc phase with gradients of CH_2 Cl_2 and MeOH, CH_2Cl_2 and acetone and EtOAc and acetone, respectively.

Compound **2**. Yellowish crystals; mp 184–186° (dec.); FTIR (microspectrometry) ν_{max}^{ZnSe} cm^{-1}: 3350, 1765, 1709, 1267, 1244, 1076; ESIMS (negative ion), (rel. int.): *m/z*851.3 $[2\ M-H]^-$ (4), 689.3 $[2\ M-glucose-H]^-$ (6), 461.1 $[M+2H_2O-H]^-$ (44), 425.1 $[M-H]^-$ (24), 327.2 $[M-glucose+2MeOH-H]^-$ (100), 263.1 $[M-glucose-H]^-$ (24).

Compound **3**. Colorless crystals; mp 142–146° (dec.); FTIR (microspectrometry) ν_{max}^{ZnSe} cm^{-1}: 3350, 1763, 1261, 1234, 1077, 1032; ESIMS (negative ion), (rel. int.): *m/z*855.3 $[2\ M-H]^-$ (100), 487.1 $[M+HOAc-H]^-$ (72), 427.2 $[M-H]^-$ (38).

Compound **4**. Colorless crystals; mp 190–193° (dec.); FTIR (microspectrometry) ν_{max}^{ZnSe} cm^{-1}: 3350, 1726, 1615, 1599, 1517, 1447, 1357, 1229, 1161, 1103; $[\alpha]_D^{20}-20°$ (H_2O; *c*0.128). ESIMS (negative ion), (rel. int.): *m/z*1343.1 $[3\ M-H]^-$ (8), 895.2 $[2\ M-H]^-$ (72), 447.1 $[M-H]^-$ (100).

Compound **5**. Colorless crystals; mp 108–110° (dec.); FTIR (microspectrometry) ν_{max}^{ZnSe} cm^{-1}: 3350, 1726, 1617, 1598, 1517, 1449, 1355, 1226, 1150, 1103; $[\alpha]_D^{20}-20°$ (H_2O; *c*0.120). ESIMS (negative ion), (rel. int.): *m/z*1343.6 $[3\ M-H]^-$ (12), 895.2 $[2\ M-H]^-$ (42), 447.1 $[M-H]^-$ (100).

Acknowledgements

The authors wish to thank Dr J. Kirschner and Dr J. Štěpánek (both Institute of Botany, Pruhonice, Czech Republic) for the identification of plant material, S. Schwaiger (Inst. f. Pharmakognosie, Innsbruck) for valuable technical assistance, Dr S. Sturm (Inst. f. Pharmakognosie) and Professors Dr K.-H. Ongania (Inst f. Organische Chemie) for MS measurements and Mr. C. Van den Boom for IR measurements.

References

Doll, R. (1994). Taraxacum Wiggers. In W. Rothmaler, *Exkursionsflora Band 4. 8. Aufl* (pp. 570–580). G. Fischer: Jena.

Glasl, S., Kastner, U., Baumann, A., Robien, W., Jurenitsch, J., & Kubelka, W. (1995). *Phytochemistry*, *38*, 159.

Hänsel, R., Kartarahardja, M., Huang, J-T., & Bohlmann, F. (1980). *Phytochemistry*, *19*, 857.

Kisiel W., Barszcz B. 1998 Poster G49 at the 46th Annual Congress of the Society for Medicinal Plant Research in Vienna.

Richards, A. J., & Sell, P. D. (1976). Taraxacum Weber. In T. G. Tutin et al., *Flora Europaea*, *vol. 4* (pp. 332–343). Cambridge: Cambridge University Press.

Weihe, Kv (1972). In *Garcke Illustrierte Flora* (23rd ed.) (p. 1516). Parey: Berlin und Hamburg.

Willuhn, G. (1997). Taraxaci radix cum herba. In M. Wichtl, *Teedrogen und Phytopharmaka* (3rd ed.) (pp. 571–574). Stuttgart: Wissenschaftliche Verlagsgesellschaft.