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SESQUITERPENE LACTONES AND GLUCOSIDES FROM *UROSPERMUM PICROIDES*

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Key Word Index—*Urospermum picroides*; Compositae; Lactuceae; sesquiterpene lactone; sesquiterpene glucoside; germacranolide; urospermal A; dihydrourospermal A.

Abstract—An extract of whole plants of *Urospermum picroides* afforded five new germacronolides and germacranolide glycosides. Their structures were elucidated by spectroscopic methods.

INTRODUCTION

Urospermum Scop. belongs to the Leontodontinae, one of the eight subtribes that comprise the tribe Lactuceae [1]. The genus contains only two species, *U. dalechampii* Schmidt and *U. picroides* (L.) Scop. ex. F.W. Schmidt, both of which have been investigated chemically [2–7].

Phytochemical reinvestigation of the whole *U. picroides* plants collected in the north of Egypt afforded, in addition to the known compounds urospermal A (**1**) [2], dihydrourospermal A (**2**) [2], a glucoside of urospermal A (**3**) [3], a *p*-hydroxyphenylacetate of the glucoside (**4**) [4], and five new germacronolides (**5–9**) which were identified by spectroscopic methods.

RESULTS AND DISCUSSION

Compounds were purified by chromatography (see Experimental); structures of the known compounds (**1–4**) were elucidated by comparison with the reported physical data. By HR-FAB-mass spectrometry, the elemental composition of **5** was determined as $C_{15}H_{20}O_5$ which was the same as that of **2**. The ^1H NMR data for **5** were similar to those of dihydrourospermal A (**2**), isolated from a related plant *U. dalechampii* [2], except for coupling constants between H-7 and H-11 (J = 11 Hz in **2** and 8 Hz in **5**) (Table 1). Together with the significant differences in methyl chemical shifts (δ_C 16.3 and 10.1 in **2** and **5**, respectively) in the ^{13}C NMR spectra (Table 2), compound **5** was elucidated to be an epimer of **2** at the 11-position, namely 11β,13-dihydrourospermal A as shown in Scheme 1 or its antipode.

Compound **6** was obtained as colourless crystals with empirical formula $C_{15}H_{18}O_5$. This demanded one more degree of unsaturation than **5**. The ^{13}C NMR spectrum was similar to that of **1**. However, an aldehyde group in **1** was replaced by a carboxyl group, which was then cyclized with the hydroxyl function at C-8 to set up another lactone ring. This was supported by a significant downfield shift of H-8 from δ_H 3.99 to 4.73 in the ^1H NMR spectrum and an upfield shift of C-1 from δ_C 160.2 to 135.6. The orientation of the methyl group at the C-11 position was expected to be the same as that in **2** from the H-H coupling constant between H-7 and H-11 (J = 13 Hz) [5]. Therefore, the structure of this compound was elucidated to be **6** or its antipode.

The ^1H NMR spectral data for compounds **7**, **8** and **9** indicated that these three compounds were sesquiterpene glucosides. Compound **7** was obtained as colourless crystals of elemental composition $C_{21}H_{26}O_{10}$. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) showed signals attributable to one β-glucopyranoside moiety, while an exo-methylene group appeared as two doublet signals at δ_H 5.80 and 6.18 (both H, both *d*, both J = 3 Hz), and δ_C 120.2 (*t*) and 139.6 (*s*). The ^{13}C NMR data showed the presence of two carbonyl signals at δ_C 173.0 and 171.0 for C-12 and C-14, respectively. Therefore, the structure of **7** was determined to be an 11,13-dehydroderivative of **6** as an aglycone with the β-glucopyranosyl unit on the hydroxyl group at the C-15 position.

Compound **8** was obtained as an amorphous powder and its elemental composition was found to be $C_{29}H_{32}O_{12}$. The ^1H and ^{13}C NMR data indicated that **8** was a *p*-hydroxyphenylacetate of **7**. The position of

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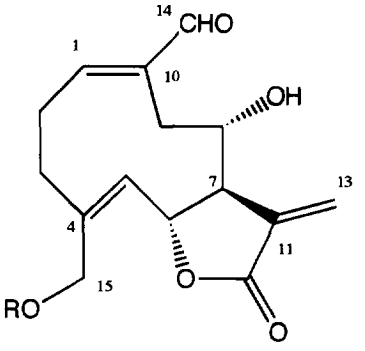
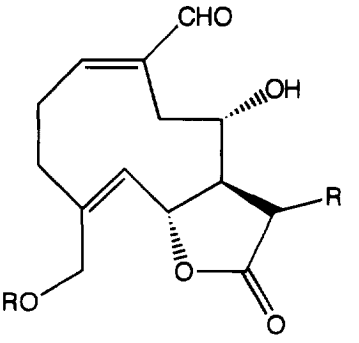
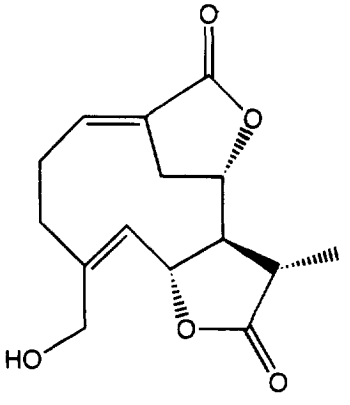


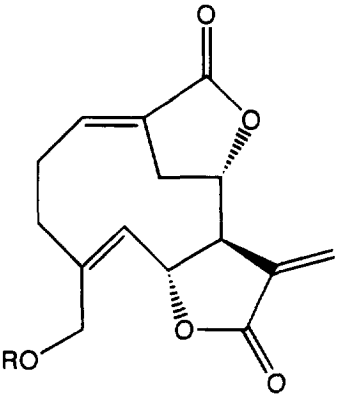
	R
1	H
3	Gluc
4	Gluc-6'-O-Pha
9	Gluc-3'-O-Pha



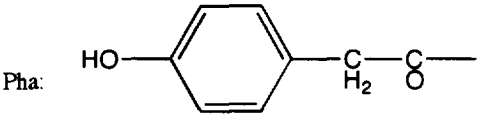
	R
2	α-Me
5	β-Me



6



	R
7	Gluc
8	Gluc-6'-O-Pha



esterification was determined to be the hydroxyl group at the 6′-position from the ^{13}C NMR chemical shift.

Compound **9** was isolated as an amorphous powder and its elemental composition was determined to be $C_{29}H_{34}O_{12}$. The NMR data showed the same characteristic signals of an aglycone as for **4** and a *p*-hydroxyphenylacetate moiety. The lack of respective upfield and downfield shifts at the C-1′ and C-6′ positions excluded the C-2′ and C-6′ positions as candidates for esterified sites. The signal at δ_C 69.8 must be assigned to C-3′ or C-4′. The most plausible assumption was that the esterification must occurred at the C-3′ position and, therefore, an upfield shift was observed from the general chemical shift for C-4, δ_C 71.5 to 69.8. Thus, the structure was elucidated to be **9**.

EXPERIMENTAL

General. Mps: uncorr.; ^1H and ^{13}C NMR (400 and 100 MHz, respectively) with TMS as int. standard. Prep. HPLC: ODS [Intertsil (GL Science, Tokyo): 20×250 mm with H_2O–MeOH at 6 ml min^{-1} (A) or 6×250 mm with H_2O–MeOH at 1.6 ml min^{-1} (B); detection, UV at 254 nm].

Plant material. Whole plants of *Urospermum picroides* were collected in the West Desert, north of Egypt in 1992. A voucher specimen was deposited

Table 1. ^{1}H NMR spectral data for compounds **1**, **2**, **5**, **6**, **4** and **7–9***

H	1	2	5	6	4	7	8	9
1	6.84 (*ddd*, 1/2/11)	6.81 (*br t*, 9)	6.81 (*ddd*, 1/2/10)	6.60 (*br t*, 10)	6.89 (*ddd*, 1/8/11)	6.55 (*ddd*, 2/8/10)	6.52 (*ddd*, 2/8/10)	6.94 (*ddd*, 2/9/10)
2a	2.55 (*m*)	2.51 (*dt*, 2/10)	2.51 (*dt*, 2/10)	2.20 (*ddt*, 3/10/13)	†	2.37 (*ddt*, 2/10/12)	2.31 (*ddt*, 2/10/12)	2.59 (*m*)
2b	2.59 (*m*)	2.55 (*m*)	2.55 (*m*)	2.54 (*m*)	†	2.47 (*m*)	2.40 (*m*)	2.59 (*m*)
3a	2.00 (*dt*, 2/12)	1.99 (*dt*, 3/13)	2.01 (*dt*, 2/12)	2.03 (*dd*, 3/13)	2.00 (*dt*, 2/12)	2.05 (*dt*, 2/12)	2.06 (*dt*, 2/12)	2.06 (*dt*, 2/12)
3b	2.77 (*ddd*, 2/5/12)	2.74 (*ddd*, 3/6/13)	2.73 (*ddd*, 2/5/12)	2.64 (*ddd*, 3/6/13)	†	2.73 (*ddd*, 2/5/12)	2.60 (*ddd*, 2/5/12)	2.75 (*ddd*, 2/4/12)
5	5.09 (*br d*, 11)	5.00 (*d*, 10)	5.00 (*d*, 10)	4.99 (*d*, 10)	5.17 (*d*, 10)	5.20 (*d*, 10)	5.16 (*d*, 10)	5.22 (*d*, 10)
6	4.75 (*t*, 10)	4.76 (*t*, 10)	4.96 (*t*, 10)	4.96 (*t*, 10)	4.86 (*t*, 10)	4.98 (*t*, 7)	5.04 (*t*, 10)	4.96 (*t*, 10)
7	2.47 (*m*)	1.58 (*q*, 10)	1.99 (*dt*, 8/10)	1.83 (*ddd*, 6/10/13)	2.55 (*m*)	2.81 (*dd*, 3/7)	2.72 (*ddd*, 3/7/10)	2.61 (*dt*, 3/10)
8	3.99 (*m*)	3.83 (*dd*, 5/10)	3.85 (*ddt*, 2/6/10)	4.73 (*t*, 6)	4.06 (*dd*, 4/10)	5.05 (*dd*, 3/10)	†	4.03 (*ddd*, 2/5/10)
9a	2.51 (*m*)	2.69 (*dd*, 4/16)	2.46 (*m*)	2.74 (2H) (*m*)	2.87 (*dd*, 5/16)	†	2.80 (*ddd*, 3/10/16)	2.85 (*ddd*, 2/5/16)
9b	2.65 (*ddd*, 2/5/16)	2.41 (*br d*, 16)	2.64 (*ddd*, 1/6/15)		2.32 (*br d*, 16)	†	2.82 (*br d*, 16)	2.34 (*br d*, 16)
11		2.63 (*dd*, 7/12)	2.84 (*qui*, 8)	2.46 (*qd*, 7/13)				
13a	6.30 (*dd*, 1/3)	1.39 (*d*, 7)	1.36 (3H) (*d*, 8)	1.38 (3H) (*d*, 7)	6.17 (*dd*, 2/3)	5.80 (*d*, 3)	5.80 (*d*, 3)	6.17 (*dd*, 2/3)
13b	6.50 (*dd*, 1/3)				6.40 (*dd*, 2/3)	6.19 (*d*, 3)	6.18 (*d*, 3)	6.40 (*dd*, 2/3)
14	9.44 (*s*)	9.45 (*s*)	9.45 (*s*)		9.43 (*s*)			9.44 (*s*)
15a	4.35 (2H) (*br d*, 12)	4.30 (*d*, 13)	4.32 (*dd*, 5/12)	4.43 (2H) (*br s*)	4.30 (2H) (*br s*)	4.38 (*d*, 11)	4.34 (*dd*, 1/10)	4.28 (*d*, 11)
15b		4.37 (*d*, 13)	4.37 (*dd*, 5/12)			4.71 (*d*, 11)	4.37 (*dd*, 1/10)	4.63 (*d*, 11)
8-OH	5.74 (*d*, 11)		5.35 (*d*, 11)					
1′					4.32 (*d*, 8)	4.35 (*d*, 8)	4.32 (*d*, 8)	4.44 (*d*, 8)
2′					3.17 (*t*, 8)	3.23 (*dd*, 8/9)	3.17 (*dd*, 8/9)	3.40 (*t*, 8)
3′,4′					†	†	†	†
5′					3.45 (*ddd*, 2/6/8)	†	3.45 (*m*)	3.45 (*ddd*, 2/6/8)
6′a					4.24 (*dd*, 6/12)	3.67 (*dd*, 5/13)	4.25 (*dd*, 6/12)	3.68 (*dd*, 6/12)
6′b					4.46 (*dd*, 2/12)	3.85 (*dd*, 2/13)	4.48 (*dd*, 2/12)	3.83 (*dd*, 2/12)
2″,6″					7.10 (2H) (*d*, 9)		7.10 (2H) (*d*, 9)	7.1 (2H) (*d*, 9)
3″,5″					6.71 (2H) (*d*, 9)		6.71 (2H) (*d*, 9)	6.7 (2H) (*d*, 9)
7″					3.55 (2H) (*s*)		3.50 (2H) (*br s*)	3.60 (2H) (*br s*)

*1, 2, 5 and 6 in $CDCl_3$, and 4, 7, 8 and 9 in CD_3OD. Letters and figure are multiplicities and coupling constants in Hz.
†Undetectable due to overlapping with solvent or HDO signals, or overlapping of signals with each other.

in the Herbarium of the Faculty of Science, Minia University, El-Minia, Egypt.

Extraction and isolation. The air-dried whole *U. picroides* plants were extracted with $CHCl_3$–MeOH (1 : 1, 3l), 3× for 48 hr. The combined extract was concentrated *in vacuo* and the residue was dissolved in 95% MeOH (1 l). The soln was washed with *n*-hexane (1 l) and the aq. MeOH layer was concd *in*

Table 2. ^{13}C NMR data for compounds **1**, **2**, **5**, **6**, **4** and **7**–**9***

C	1	2	5	6	4	7	8	9
1	160.3	159.3	159.2	135.6	162.4	137.1	137.1	162.2
2	27.9	27.8	27.7	27.9	29.0	28.8	28.9	28.7
3	32.8	32.9‡	32.4‡	31.6	33.9	32.6	32.5	33.9
4	141.3	140.2	139.7	142.2	140.6	142.6	142.4	140.5
5	127.0	128.0	128.4	127.6	129.5	130.2	130.3	129.5
6	75.8	75.8	75.2	78.1	77.8‡	79.7	79.7	77.7‡
7	51.6	56.1	52.2	56.5	52.7	54.5	54.4	52.6
8	70.1	71.5	66.7	73.5	71.3	75.9	75.9	71.3
9	33.2	33.2‡	32.8‡	34.4	34.6	35.5	35.7	34.2
10	144.3	144.7	144.7	129.9	145.5	132.0	131.3	145.5
11	136.9	41.3	39.0	42.0	139.8	139.6	139.6	139.7
12	†	179.2	†	176.6	172.7	173.0	173.7	†
13	125.3	16.4	10.1	13.4	124.2	120.2	120.2	124.1
14	199.6	199.3	199.4	170.5	202.2	171.0	171.1	202.1
15	61.2	61.1	61.2	62.5	69.3	69.3	69.4	69.1
1′					104.2	104.5	104.2	104.3
2′					75.0	75.2	75.0	73.4
3′					78.2‡	78.2‡	78.0	79.6
4′					71.5	71.7	71.5	69.8
5′					75.6	78.3‡	75.7	78.1‡
6′					64.8	62.8	64.8	62.5
1″					126.3		126.3	126.5
2″,6″					131.4		131.4	131.6
4″					157.6		157.6	†
3″,5″					116.4		116.4	116.2
7″					41.2		41.2	41.1
8″					173.8		173.1	174.0

*Compounds **1**, **2**, **5** and **6** for $CDCl_3$ and **4**, **7**, **8** and **9** for CD_3OD.

†Not observed.

‡The same symbols in each column maybe interchanged.

vacuo. The residue was suspended in H_2O (1 l), and the suspension was extracted with EtOAc (1 l) and *n*-BuOH (1 l) successively. Both layers were concd to give residues of 13 and 10 g, respectively.

The EtOAc fraction was chromatographed over silica gel (200 g) with a mixture of $CHCl_3$ and MeOH, with increasing MeOH contents [$CHCl_3$ (1.5 l), $CHCl_3$–MeOH (199 : 1, 1.5 l), $CHCl_3$–MeOH (99 : 1, 1.5 l), $CHCl_3$–MeOH (49 : 1, 1 l) $CHCl_3$–MeOH (24 : 1, 1 l), $CHCl_3$–MeOH (47 : 3, 1 l), $CHCl_3$–MeOH (9 : 1, 1 l), $CHCl_3$–MeOH (7 : 1, 1 l), $CHCl_3$–MeOH (17 : 3, 1 l), $CHCl_3$–MeOH (7 : 3, 1 l) and $CHCl_3$–MeOH–H_2O (700 : 300 : 1, 1 l)] successively, 250 ml fractions being collected.

Fractions 20–24 were combined and evaporated *in vacuo* to give a residue (1.5 g), which was rechromatographed over silica gel (50 g) with a solvent system [$CHCl_3$ (1 l) → $CHCl_3$–MeOH (99 : 1, 1 l) and $CHCl_3$–MeOH (99 : 1, 1 l → 94 : 6, 1 l), fractions of 8 g being collected. The residue (394 mg) of fractions 149–160 was further separated on silica gel (50 g) with a gradient solvent system, $CHCl_3$–MeOH (99 : 1, 1 l → 96 : 4, 1 l)], fractions of 15 g being collected. The residue (25 mg) of fractions 45–50 was collected and evaporated *in vacuo* to give urospermal A (**1**, 20 mg), while the residue (100 mg) of fractions 51–62 was separated by prep. HPLC (B) (40% MeOH) to give compounds **5** and **6** as a mixture (5.9 min), and then this mixture was further separated by HPLC (B) with a solvent (30% MeOH) to give compounds **5** (1.0 mg, 9.3 min) and **6** (2.5 mg, 8.0 min). Fractions 161–171 of the same EtOAc fraction were combined to give a residue (396 mg), which was chromatographed similarly to the previous fraction to give dihydrourospermal A (**2**), fractions 54–60, as a crystalline material (10 mg).

Fractions 31–35 of the EtOAc fraction were also combined and evaporated *in vacuo* to give a residue of 1.8 g, which was chromatographed on silica gel (50 g) with solvent systems [$CHCl_3$, 500 ml, $CHCl_3$–MeOH (24 : 1, 1 l → 9 : 1, 1 l) and $CHCl_3$–MeOH (7 : 1–7 : 3, 500 ml)] fractions of 15 g being collected. The residue (120 mg) of fractions 43–49 was separated by HPLC (A) using 40% MeOH to give compound **9** (2.0 mg, 42 min).

The residue (120 mg) of fractions 50–56 of the same EtOAc fraction was evaporated *in vacuo* to give the 6′-*p*-hydroxyphenylacetate of urospermal A 15-*O*-β-D-glucopyranoside (**4**, 100 mg) in a pure state. The residue (100 mg) of fractions 57–68 was separated by HPLC (B) using 40% MeOH as a solvent to give compound **8** (2.0 mg, 26 min).

The *n*-BuOH fraction (10 g) was treated as the EtOAc fraction. Fractions 34–38 were combined and

evaporated *in vacuo*. The residue (1.15g) was chromatographed over ODS [Cosmosil 75C_{18}-OPN, (Nakarai Tesque, Kyoto), 4×25 cm] with a mixture of H_2O–MeOH, with increasing MeOH contents (H_2O–MeOH, 9:1, 1 l → 1:1, 1 l), H_2O–MeOH (1:1, 1 l → 7:3, 1 l), and H_2O–MeOH (7:3, 1 l → 100% MeOH, 1 l), fractions of 10 g being collected.

The residue (90 mg) of fractions 72–90 was crystallized with hot MeOH and the crystals obtained were collected by filtration to give compound **7** (60 mg), while the residue (386 mg) of fractions 91–111 was evaporated *in vacuo* to give the 15-*O*-β-D-glucopyranoside of urospermal A (**3**, 186 mg).

Known compounds isolated. Urospermal A (**1**), ^{1}H and ^{13}C NMR: Tables 1 and 2 [2], dihydrourospermal A (**2**), $[\alpha]_D^{22}$ ~0° (MeOH, *c* 0.29) [2], urospermal A 15-*O*-β-D-glucopyranoside (**3**), $[\alpha]_D^{22}$ + 83.8° (MeOH, *c* 0.44) [3], urospermal A 15-*O*-β-D-glucopyranoside 6′-*p*-hydroxyphenylacetate (**4**), $[\alpha]_D^{22}$ +71.4° (MeOH, *c* 0.35) [4].

8,15-*Dihydroxygermacra*-1(10), 4-*dien*-(12,6)-*olide*-14-*al* (**5**). Colourless oil, UV λ_{max}^{MeOH} nm: 207, 227; ^{1}H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 279.1263 $[M-H]^-$ ($C_{15}H_{19}O_5$ requires 279.1232).

15-*Hydroxy*-1(10),4-*germacra*-1(10),4-*dien*-(12,6); (14,8)-*diolide* (**6**). Colourless crystals, mp 220–222°, UV λ_{max}^{MeOH} nm: 207, 220sh; ^{1}H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 277.1085 $[M-H]^-$ ($C_{15}H_{18}O_5$ requires 277.1076).

15-*Hydroxygermacra*-1(10),4,11(13)-*trien*-(12,6); (14,8)-*diolide* 15-*O*-β-D-*glucopyranoside* (**7**). Colourless crystals, mp 189–191°, $[\alpha]_D^{25}$ + 14.9° (MeOH, *c* 0.60), IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1750, 1660, 1290, 1225, 1155, 1080–950; UV λ_{max}^{MeOH} nm (logε): 209 (4.37); ^{1}H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 437.1445 $[M-H]^-$ ($C_{21}H_{25}O_9$ requires 437.1448).

15-*Hydroxygermacra*-1(10),4,11(13)-*trien*-(12,6); (14,8)-*diolide* 15-O-β-D-*glucopyranoside* 6′-p-*hydroxyphenylacetate* (**8**). Colourless powder, UV λ_{max}^{MeOH} nm: 211, 220sh, 277; ^{1}H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 571.1804 $[M-H]^-$ ($C_{29}H_{31}O_{12}$ requires 571.1815).

8,15-*Diydroxygermacra*-1(10),4,11(13)-*trien*-(12,6)-*olide*-14-*al* 15-O-β-D-*glucopyranoside* 2′-p-*hydroxyphenylacetate* (**9**). Colourless powder UV λ_{max}^{MeOH} nm: 211, 271; ^{1}H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 573.1977 $[M-H]^-$ ($C_{29}H_{33}O_{12}$ requires 573.1972).

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