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STRUCTURALLY RELATED GUAIANOLIDES FROM *INULA THAPSOIDES*

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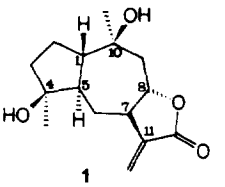
IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Inula thapsoides*; Compositae; sesquiterpene lactones; guaianolides.

Abstract—The whole plant extract of *Inula thapsoides* afforded seven new structurally related guaianolides. The structures and stereochemistry were determined by spectroscopic methods, particularly one- and two-dimensional NMR spectroscopy, and extensive NOE experiments.

INTRODUCTION

In previous studies of Turkish *Inula* species, we have obtained guaianolides and eudesmanolides as major components along with some sesquiterpene acids and flavonoids [1–3]. We have now investigated *Inula thapsoides*, subsp. *thapsoides* (Bieb. ex Willd. Sprengal) (Syn: *Conyza thapsoides* Bieb. ex Willd.), which is mainly found in central and south eastern Anatolia at elevations above 1600 m. From the whole plant extract, seven new guaianolides with an 8,12-*trans*-fused lactone ring were isolated. Their structures were determined as 4β,10β-dihydroxy-1β(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide (**1**), 4α,10β-dihydroxy-1β(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide (**2**), 4α-acetoxy-10β-hydroxy-1β(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide (**3**), 4β,10β-dihydroxy-1α(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide (**4**), 4α,10α-dihydroxy-5α(*H*)-guai-1,11(13)-dien-8α,12-olide (**5**), 3β-hydroxy-4β,10β-epoxy-1α(*H*),5α(*H*),11α-guaian-8α,12-olide (**6**) and 4β,10β-epoxy-1α(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide (**7**) by ^{1}H and ^{13}C NMR spectroscopic techniques.



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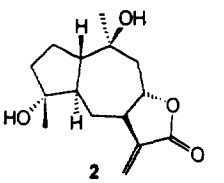

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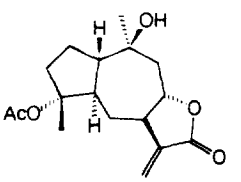

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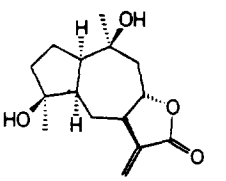

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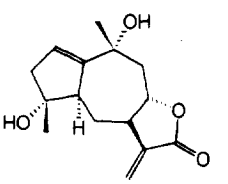

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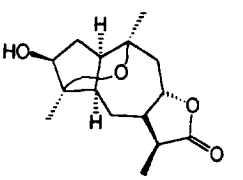

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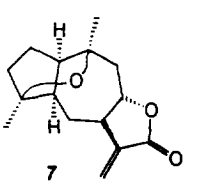

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RESULTS AND DISCUSSION

The IR spectrum of compound **1** exhibited frequencies corresponding to an α,β-unsaturated γ-lactone carbonyl at 1753 cm^{-1}, unsaturation at 1662 cm^{-1} and hydroxyl(s) at 3440 cm^{-1}. The CI mass spectrum of **1** showed a $[M + 1]^+$ ion peak at *m/z* 267 analysing for the molecular formula $C_{15}H_{22}O_4$. The ^{13}C NMR spectrum (Table 1) supported this formula, displaying two methyl quartets, five methylene triplets, four methine doublets and three quaternary singlets. The ^{1}H NMR spectrum (Table 2) showed the exocyclic methylene protons at δ6.09 (*d*, J = 3.8 Hz) and 5.41 (*d*, J = 3.0 Hz), the proton under the lactone oxygen at δ4.31 (*ddd*, J = 2, 9 and 11.5 Hz) and two methyl singlets at δ1.19 and 1.30. Irradiation of the signal at δ4.31 (H-8) and at δ1.95 (H-1) permitted identification of the sequences H_6–H_9 and H_1–H_5, respectively. Thus, irradiation of H-8 at δ4.31

Table 1. ^{13}C NMR spectral data of the sesquiterpene lactones **1**–**4** and **6** and **7***

C	1	2	3	4	6	7
1	52.13 *d*	51.88 *d*	50.31 *d*	52.15 *d*	61.74 *d*	64.34 *d*
2	41.44 *t*	40.90 *t*	37.79 *t*	38.08 *t*	31.78 *t*	29.10 *t*
3	23.60 *t*	23.35 *t*	24.11 *t*	24.39 *t*	78.10 *d*	23.46 *t*
4	73.85 *s*	80.49 *s*	89.87 *s*	82.18 *s*	63.30 *s*	71.88 *s*
5	50.13 *d*	49.76 *d*	48.84 *d*	49.90 *d*	61.74 *d*	63.47 *d*
6	29.94 *t*	28.89 *t*	29.10 *t*	29.57 *t*	25.89 *t*	29.69 *t*
7	50.06 *d*	49.10 *d*	47.52 *d*	42.10 *d*	45.23 *d*	45.90 *d*
8	79.14 *d*	80.16 *d*	80.24 *d*	82.62 *d*	81.82 *d*	82.14 *d*
9	49.65 *t*	48.21 *t*	47.98 *t*	43.22 *t*	45.12 *t*	46.20 *t*
10	72.15 *s*	71.72 *s*	71.82 *s*	73.15 *s*	57.68 *s*	58.11 *s*
11	138.32 *s*	140.12 *s*	140.01 *s*	140.12 *s*	40.43 *d*	134.75 *s*
12	not seen	169.97 *s*	172.24 *s*	170.65 *s*	178.08 *s*	174.14 *s*
13	119.36 *t*	119.16 *t*	119.43 *t*	119.60 *t*	10.72 *q*	122.59 *t*
14	25.55 *q*	30.74 *q*	31.19 *q*	31.86 *q*	19.08 *q*	17.96 *q*
15	23.09 *q*	23.94 *q*	22.21 *q*	24.27 *q*	11.81 *q*	17.97 *q*

*APT experiments were run at 50.34 MHz for **3** and **7**, and 125.4 MHz for **1**, **2**, **4** and **6**.

Table 2. ^{1}H NMR spectral data for compounds **1**–**7** (in $CDCl_3$, TMS as int. standard)

	1†	2	3	4	5	6	6a	6a‡	7
1	1.95 *m*	1.65 *m*	1.62 *m*	2.85 *ddd*	—	2.15 *dd*	2.14 *dd*	2.14 *dd*	2.16 *dd*
2	1.82 *m*	1.81 *m*	1.80 *m*	1.90 *m*	5.89 *d*	2.51 *ddd*	2.51 *brdd*	2.38 *ddd*	2.22 *m*
2′	1.68 *m*	1.7 *m*	1.72 *m*	1.45 *m*	—	1.53 *ddd*	1.53 *m*	1.21 *ddd*	1.38 *m*
3	1.65 *m*	1.6 *m*	1.58 *m*	1.88 *m*	2.26 *dd*	3.48 *dd*	4.57 *dd*	4.59 *dd*	1.58 *m*
3′	—	—	1.75 *m*	1.75 *m*	2.37 *dd*	—	—	—	2.20 *m*
5	1.58 *ddd*	1.97 *ddd*	1.93 *ddd*	2.22 *ddd*	2.16 *dd*	2.79 *dd*	2.68 *dd*	2.11 *br d*	2.44 *dd*
6	2.30 *ddd*	2.33 *ddd*	2.42 *ddd*	2.13 *ddd*	2.66 *dddd*	2.82 *dd*	2.77 *dd*	1.42 *brd*	1.56 *dd*
6	1.15 *ddd*	1.12 *ddd*	1.16 *ddd*	1.07 *ddd*	1.18 *ddd*	1.54 *dd*	1.53 *m*	0.83 *ddd*	2.82 *m*
7	2.47 *ddddd*	2.73 *ddddd*	2.80 *dddd*	3.35 *ddddd*	2.6 *dddd*	2.44 *brddd*	2.42 *brdd*	1.33 *m*	2.86 *m*
8	4.31 *ddd*	3.96 *ddd*	3.99 *ddd*	4.12 *ddd*	3.98 *ddd*	4.30 *ddd*	4.29 *ddd*	3.62 *ddd*	4.20 *ddd*
9	2.52 *dd*	2.56 *dd*	2.56 *dd*	2.47 *dd*	2.52 *dd*	2.72 *dd*	2.79 *qdd*	2.51 *dd*	2.80 *dd*
9′	1.95 *dd*	2.06 *dd*	2.02 *dd*	1.98 *dd*	2.27 *dd*	1.48 *dd*	1.44 *dd*	0.97 *dd*	1.52 *dd*
11	—	—	—	—	—	2.82 *dq*	2.84 *dd*	2.15 *dq*	—
13a	6.09 *d*	6.16 *d*	6.19 *d*	6.20 *d*	6.18 *d*	1.24 *d*§	1.25 *d*§	0.73 *d*§	6.38 *d*
13b	5.41 *d*	5.49 *d*	5.53 *d*	5.50 *d*	5.48 *d*	—	—	—	5.69 *d*
14	1.30 *s*	1.33 *s*	1.41 *s*	1.35 *s*	1.44 *s*	1.41 *s*	1.41 *s*	0.95 *s*	1.43 *s*
15	1.19 *s*	1.19 *s*	1.34 *s*	1.32 *s*	1.38 *s*	1.30 *s*	1.34 *s*	0.85 *s*	1.30 *s*
OAc	—	—	2.00 *s*	—	—	—	1.61 *s*	2.11 *s*	—

J(Hz) for **1**, **2** and **3**: 1, 5 = 9.5; 5, 6 = 2; 5, 6 = 12; 6, 7 = 4; 6, 7 = 12; 6, 6 = 12; 7, 8 = 9.0; 7, 13a = 3.8 (or 3.5); 7, 13b = 3; 8, 9 = 11.5; 8, 9 = 2; 9, 9 = 14.5; for **4**: 1, 2 = 12; 1, 2 = 12; 1, 5 = 8; 5, 6 = 7; 5, 6 = 13; 6, 6 = 14; 6, 7 = 2; 6, 7 = 11.5; 7, 8 = 10.5; 8, 9 = 10; 8, 9 = 7; 9, 9 = 14; 7, 13a = 3.5; 7, 13b = 3; for **5**: 2, 3 = 1.8;5, 6 = 12; 5, 6 = 2; 6, 6 = 14; 6, 7 = 12; 6, 7 = 2; 7, 13a = 3.5; 7, 13b = 3; 7, 8 = 9.5; 8, 9 = 11.0; 8, 9 = 2.0; 9, 9 = 14; for **6** and **6a**: 1, 2 = 1; 1, 2 = 11; 2, 2 = 14; 2, 3 = 5.5; 2, 3 = 11.5; 1, 5 = 0.8; 2, 5 = 1; 5, 6 = 1.5; 5.6 = 11 (*q* in C_6D_6); 6, 6 = 15; 6. 7 = 0.8; 6, 7 = 11; 7. 8 = 10 (*q* in C_6D_6); 7, 11 = 8; 8, 9 = 9.5; 8, 9 = 2; 9, 9 = 13.5; 11, 13 = 8 for **7**: 1, 2 = 2; 1, 2 = 11; 2, 2 = 14.2; 2, 3 = 6; 2, 3 = 11.2; 1, 5 = 1.0; 2, 5 = 1.2; 5, 6 = 1.5; 5, 6 = 11; 6, 6 = 14.5; 6, 7 = 1.0; 6, 7 = 11; 7, 8 = 10.5; 7, 13a = 3.2; 7, 13b = 3; 8, 9 = 7.0; 8, 9 = 1.0; 9, 9 = 15.

*The spectra of **1**, **2**, **4** and **6a** were run at 500 MHz, the others at 200 MHz.

†In $CDCl_3$ + 2 drops pyridine.

‡In C_6D_6.

§Intensity three protons.

collapsed the double doublets of H-9a and H-9b at δ2.52 (*dd*, J = 2 and 1.45 Hz) and 1.95 (*dd*, J = 11.5 and 14.5 Hz) to doublets while also simplifying the H-7 multiplet at δ2.47. Conversely, irradiation at the frequency of H-7 collapsed the H-8 signal to a doublet of doublets (J = 2 and 11.5 Hz), and simplified the signals of H-6 protons at δ2.30 (*ddd*, J = 2, 4, 12 Hz) and 1.15 (*ddd*, J = 12, 12 and 12 Hz). Further irradiation of the better resolved signal of H-6 at δ2.30 converted the signal of H-5 at δ1.58 (*ddd*, J = 2, 9.5, 12 Hz) into a *dd* (J = 9.5

Table 3. 1D NOE difference spectra of **1**, **2**, **4** and **6a**'

Irradiated proton	**1** obs. protons (%)	**2** obs. protons (%)	**4** obs. protons (%)	**6a*** obs. protons (%)
H-13a	H-13b (29.6)	H-13b (26.4)	H-13b (30.0)	H-8β (1.7), H-11 (3.8), H-6α (1.8)
H-13b	H-13a (35.2), H-6 (3.1)	H-13a (29.2), H-6 (3.0)	H-13a (38.3), H-6α (3.8)	—
H-8β	H-1β (7.3), H-6β (1.5)	H-9β (1.6), H-1β (1.8), H-6β (0.7)	H-7α (0.9), H-9β (3.1), H-6β (3.8)	H-9β (1.7), Me-14 (7.8), Me-13 (2.2)
H-7α	H-8 (1.5), H-9α (5.2), H-5α (2.1)	H-5α (2.4), H-6α (0.8), H-9α (1.9)	H-9α (1.9), H-5α (5.1), H-6α (1.7) H-13b (0.3)	H-1α (2.5), H-11 (2.4), H-5α (2.5), H-9α (0.1)
H-9β	H-9α (5.2), H-8β (1.5)	H-8β (2.4), H-9β (13.5)	H-8β (4,0), H-9α (14.0)	H-8β (3.1), H-9α (21.4), Me-14 (2.1)
H-9α	H-5α (2.1), H-9β (2.8), Me-14 (0.7)	H-7α (3.2), H-9β (8.7)	H-7α (1.3), H-1α (2.1), H-9β (8.0)	H-9β (6.3), H-1α (1.9), H-7α (1.3), H-2α (0.7)
H-6α	H-13b (1.8), H-5α (2.6), H-6β (8.1)	H-13b (1.6), H-7α (2.3), H-6β (5.4)	H-13b (2.3) H-7α (2.2), H-1α (2.3) Me-15 (0.3), H-6β (5.3)	H-1α + H-5α (0.4), H-6β (5.3)
H-6β	H-6α (2.6), H-8β (1.4), H-1β (1.2)	—	H-8β (5.2), H-6α (9.9)	H-8β (4.2), Me-14 (4.1), H-6α (3.0)
H-5α	H-7α (3.6), H-6α (4.2), H-9α (4.6)	H-7α (2.7) H-6α (1.6)	H-13b (1.4) H-7α (3.8) H-1α (3.2), H-6β (3.2)	H-3α (4.7), H-6α (1.7), H-7α (6.9), H-11 (1.9)
H-1	H-8β (4.3), H-9β (2.8), H-6β (5.0)	H-8β (0.6)	H-7α (0.8), H-5α (6.9) Me-14 (1.2), H-2α, H-9α + H-2α (5.3)	H-3α (4.7), H-2α (0.6), H-6α (1.4) H-7α (5.1), H-9α (1.9)
H-2β	H-1β (6.9), H-2α (13.0)			H-2α (3.0), Me-14 (0.7), Me-15 (0.1)
H-2α	H-2β (13.0)		H-1α (2.0), H-2β (0.6)	H-1α (3.9), H-2β (23.5), H-3α (5.8)
H-3α		—	H-1α (0.7), H-2α (0.8)	H-2α (3.9), H-1α + H-5α (13.9)
Me-14	H-9α (2.6), H-2α (2.9), H-5α (2.6)	H-8β (0.9), H-9β (1.8)	H-8β (0.6), H-1α (1.8) H-9β (1.4), H-9α (0.2)	H-8β (4.2), H-9β (1.3), H-2β (3.9)
Me-15	H-6α (8.3), H_2-3 + H-2α(1.9)	H-6α (1.5)	H-5α (0.9), H-6α (0.8) H-3α (0.5), H-6β (1.2)	
H-11α	—	—	—	H-6α (0.8), H-7α (4.2), Me-13 (2.1)

*Run at 500 MHz in $CDCl_3$ + C_6D_6.

and 12 Hz), while irradiation at the frequency of H-5 permitted identification of a multiplet at δ1.95 as originating from H-1.

The chemical shifts of the methyl singlets at δ1.19 and 1.30 indicated that these methyl groups should be adjacent to oxygen functions. The ^{13}C NMR spectrum (Table 1), which exhibited two singlets at δ73.85 and 72.15, also supported this finding. After acetylation under drastic conditions, **1** furnished a diacetate **1a** whose ^1H NMR spectrum (see Experimental) exhibited two acetate methyls at δ2.01 and 1.99, while the methyl singlets at δ1.19 and 1.30 were shifted to δ1.30 and 1.43, indicating the presence of two unsubstituted tertiary hydroxyl groups.

The sterochemistry at the centres C-8, C-5 and C-1 was deduced by extensive NOE experiments (Table 3). Since there was no NOE between H-7 and H-8, compound **1** should contain a *trans*-fused lactone ring as also followed from the coupling constants of H-13 and H-13'. Irradiation of H-8β enhanced the signals at δ1.95 (H-1) and 1.15 (H-6), indicating that these two protons were also β-oriented. Conversely, irradiation of H-6β at δ1.15 enhanced the signals of H-8 and H-1 as well as H-6α, and irradiation of H-6α at δ2.30 enhanced the signals at δ2.60 (H-5α), while no enhancement was observed for H-1. As a result, the fusion of five and seven membered rings was also *trans*. The orientations of the methyl singlets at C-4 and C-10 were deduced by NOE experiments and comparison of their chemical shifts to those reported in the literature [4,5] for similar guaianolides.

In view of these findings, **1** was 4β,10β-dihydroxy-1β(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide.

The IR spectrum of **2** was similar to that of **1** and its CI mass spectrum gave the same molecular formula ($C_{15}H_{22}O_4$) with a $[M + 1]^+$ ion at *m/z* 267 and the same prominent fragments at *m/z* 249 and 231, resulting from loss of two hydroxyl groups. The ^1H NMR spectrum (Table 2) was also similar, all assignments being made by spin decoupling in the manner described for **1**. Significant differences were seen in the chemical shifts of H-8 (δ3.96) and H-7 (δ2.73), although the *J* values remained unchanged. Like **1**, compound **2** also had two quaternary hydroxyl groups and a *trans*-fused lactone ring. The observed NOE (Table 3) between H-1 and H-8 showed that these two protons were also *cis*- and β-oriented, while no enhancement was observed for H-5, thus indicating that H-1 and H-5 were again *trans*. Further NOE experiments showed that the methyl signals at δ1.33 and 1.19 corresponded to H-14 and H-15, respectively. That H-5α of **2** is at lower field than H-5 of **1** was assumed to be due to α-orientation of the 4-OH function

in compound **2**, this supposition was reinforced by a significant change in the frequency of the C-4 signal, from δ73.85 in **1** to δ80.49 in **2**.

Hence, **2** was the C-4 epimer of **1**, i.e. 4α,10β-dihydroxy-1β(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide.

The IR spectrum of **3** showed absorbances at 1760 cm^{-1} (lactone) and at 3410 (hydroxyl), 1730 and 1250 cm^{-1} (acetyl). The ^{1}H NMR spectrum (Table 2) resembled that of **2** except for the presence of an extra methyl singlet at δ2.00 due to an acetate methyl. This was confirmed by the ^{13}C NMR spectrum (Table 1), which had extra signals at δ22.0 and 172.2, and the CI mass spectrum, which exhibited the $[M + 1]^+$ peak at *m/z* 309 for the molecular formula $C_{17}H_{22}O_5$ and a $[M + 1 - OAc]^+$ peak at *m/z* 249. Acetylation of **3** to **3a** under drastic conditions resulted in the appearance of an additional acetyl singlet at δ2.18; hence, **3** was a monoacetate of **2**. The location of the acetyl group could be deduced by the chemical shift of C-4 (δ89.15), which was at considerably lower field than in **1** and **2**, while the other chemical shifts were unchanged. Hence, **3** was the 4-acetate of **2**, i.e. 4α-acetoxy-10β-hydroxy-1β(*H*), 5α(*H*)-guai-11(13)-en-8α,12-olide.

Compound **4** accompanied **2**, but could be separated by successive preparative TLC. The CI mass spectrum (*m/z* 267) and ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed that it was a stereoisomer of **1** and **2**. In the ^{1}H NMR spectrum, the main differences compared with **1** and **2** were the coupling constants involving H-8 (δ4.12, *ddd*, J = 7, 10 and 10.5 Hz compared with 9, 11.5 and 2 Hz) and the chemical shifts of H-7 and H-1, which resonated further downfield at δ3.35 (*ddddd*, J = 2, 3, 3.5, 10.5 and 11.5 Hz) and 2.85 (*ddd*, J = 8, 12 and 12 Hz), respectively. The latter suggested that the junction of the five- and the seven- membered rings might be *cis*. Spin decoupling in the usual fashion beginning with H-7 again established the sequences H_6–H_9 and H_5–H_1. Acetylation furnished a diacetate whose ^{1}H NMR spectrum contained one acetate methyl singlet of double intensity while HREI mass spectra gave the molecular ion peak at *m/z* 350.1726 corresponding to $C_{19}H_{26}O_6$ thus confirming the presence of two tertiary hydroxyl groups.

Since **4** had the same molecular formula and the same substituents at the same locations as **1** and **2** and since the coupling constant involving H-7 showed that the lactone ring was *trans*-fused, the structural difference could only reside in the stereochemistry of the five–seven membered ring junction. Therefore, extensive 1D NOE difference spectroscopy experiments were performed (Table 3). H-1 showed a significant NOE with H-5, while there was no NOE between either H-8 and H-1 or H-8 and H-5, indicating that the junction of the five and the seven membered rings was *cis* (H-1α, H-5α) in an 8α,12-*trans*-fused guaianolide. Further, the NOEs observed between the methyl signal at δ1.32 and H-5α at δ2.22, H-6α at δ2.13 and H-3α at δ1.88 indicated that this methyl signal corresponded to the Me-4α group with the adjacent hydroxyl group β while NOEs between the methyl group at δ1.35 and H-1α at δ2.85, as well as H-9α at δ1.98 indicated that this methyl was the Me_{10} group and also α and that, therefore, the attached hydroxyl was β-oriented. Thus, **4** was a C-1 stereoisomer of **1**, i.e. 4β,10β-dihydroxy-1α(*H*),5α(*H*)-guai-11(13)-en-8α,12-olide.

Compound **5** exhibited IR absorbances at 3410 (hydroxyl), 1750 (lactone) and 1660 cm^{-1} (unsaturation). The HREI mass spectrum showed the molecular ion peak at *m/z* 264.1358 corresponding to $C_{15}H_{20}O_4$, i.e. two mass units less than those of **1**, **2** and **4**, while in the CI mass spectrum the $[M + 1]^+$ peak at *m/z* 265 and high intensity peaks corresponding to the sequential loss of two hydroxyl groups were observed at *m/z* 247 $[M + 1 - H_2O]^+$ and 229 $[M + 1 - 2H_2O]^+$. The ^{1}H NMR spectrum (Table 2) showed the signal of an olefinic proton at δ5.89 (H-2) (*dd*, J = 1.8 and 4 Hz) coupled with the two protons of a two proton multiplet at δ2.34 along with the signals typical of H-13 and H-13′ at δ6.18 (*d*, J = 3.5 Hz) and 5.48 (*d*, J = 3.0 Hz), the signal of lactone proton at δ3.98 (*ddd*, J = 2, 9.5 and 11 Hz, H-8), and two methyl singlets at δ1.44 and 1.38. That the double bond was located between C-1 and C-2 was clear from the absence of an H-1 signal and the fact that the adjacent two proton signal (H-3a,b) was not coupled further. Because of the small quantity of H-5 neither ^{13}C NMR nor NOE spectrometry could be performed, however, the stereochemistry of the two hydroxyl groups could be determined indirectly by comparison with literature data for a similar guaianolide with a *cis*-fused lactone ring from *Jasonia candicans* [6]. Thus, the structure of **5** was 4α,10α-dihydroxy-5α(*H*)-guai-1,11(13)-dien-8α,12-olide.

Compound **6** exhibited lactone and hydroxyl absorbances at 1762 and 3420 cm^{-1}, but it did not show unsaturation. This was supported by the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum (Table 2) showed the lactone proton signal at δ4.30 (*ddd*, J = 2, 9.5 and 10 Hz, H-8), a carbinol methine at δ3.48 (*dd*, J = 5.5 and 11.5 Hz, H-3), two methyl singlets at δ1.41 and 1.30 and a methyl doublet (J = 7 Hz) at δ1.24. The absence of the usual signals of an exocyclic methylene group indicated that the methyl group was attached to C-11. Irradiation of the signal at δ4.30 (H-8) collapsed the signals at δ2.72 (*dd*, J = 2 and 13.5 Hz) and at δ1.48 (*dd*, J = 9.5 and 13.5 Hz) to doublets (each J = 13.5 Hz), thus locating H-9a and H-9b, which were not coupled further. Irradiation of the signal at δ3.48 (H-3) collapsed the signals at δ2.51 (*ddd*,J = 1.0, 5.5 and 14 Hz) and at δ1.53 (*ddd*, J = 11, 11.5 and 14 Hz) to doublets, thus identifying H-2a and H-2b, each of which was also coupled with H-1.

The ^{13}C NMR spectrum (Table 1) displayed three methyl quartets, three methylene triplets, four methine doublets and three singlets. Of the quaternary carbons, one at δ177.9 was clearly due to the lactone carbonyl, while the other two at δ57.65 and 63.98 could be assigned to two carbons carrying an oxygen bridge for the following reason. Acetylation of **6** furnished a monoacetate **6a** in whose ^{1}H NMR spectrum the signal formerly at δ3.48 was shifted to 4.56, indicating the presence of only one secondary hydroxyl group in **6**, while the chemical shifts of the two methyl singlets were unaffected. Thus, the

oxygen bridge linked C-4 and C-10. The stereochemistry assigned to **6** followed from the coupling constants and extensive NOE spectrometry of **6a** in $CDCl_3 + C_6D_6$ (Table 3). Based on the coupling constants (Table 2) and NOEs the lactone ring had to be *trans*-fused ($J_{7,8} = 10$ Hz) and the C-11 methyl group had to be β-oriented ($J_{7,11} = 8$ Hz and NOEs). In such a lactone with an oxygen bridge linking C-4 and C-10, H-1 and H-5 and therefore C-14 and C-15 must of necessity be *cis* and α although superposition of the signals of H-1 and H-11 in $CDCl_3$ and H-1, H-5 and H-11 in C_6D_6 interfered with the unambiguous demonstration of this. An attempt to construct a Drieding model with H-1 and H-5 both β and the oxygen bridge α proved to be difficult. Hence, **6** was 3β-hydroxy,4β,10β-epoxy-1α(*H*),5α(*H*),11α(*H*)-guaian-8α,12-olide.

Lactone **7** was the 7,11-dehydro analogue of lactone **6**. Its IR spectrum showed absorbances at 3430 (hydroxyl), 1758 (lactone) and 1660 cm^{-1} (unsaturation), while the ^1H NMR spectrum (Table 2) exhibited typical α,β-unsaturated-γ-lactone methylene proton signals at δ6.38 (*d*, $J = 3.2$ Hz) and 5.69 (*d*, $J = 3$ Hz), a lactone proton signal at δ4.20 (*ddd*, $J = 1$, 7 and 10.5 Hz), two methyl singlets at δ1.30 and 1.43 and a methyl doublet. Spin decoupling permitted confirmation of the sequence H_5–H_9 and, in part the sequence H_1–H_4. In accordance with this the ^{13}C NMR spectrum (Table 1) displayed four methine doublets, six methylene triplets, two methyl quartets and three quaternary carbons one of which, at δ169.79, could be assigned to the lactone carbonyl, while the other two, at δ58.11 and 62.06, could be attributed to C-4 and C-10 bearing an oxygen bridge since acetylation with acetic anhydride and pyridine under drastic conditions failed. On the basis of the molecular ion peak at *m/z* 248 in the mass spectrum combined with other spectroscopic data, **7** was 4β,10β-epoxy-1α,5α(*H*)-guai-11(13)-en-8β,12-olide, which is obviously derived from **4**.

EXPERIMNTAL

Plant material. Inula thapsoides subsp. *thapsoides* was collected in July 1992 in central Turkey (Yıldızeli, Sivas) and identified by Prof. Dr. Semra Kurucu. A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Ankara (AEF: 17512).

Extraction and isolation. Whole plant (1.7 kg) of *I. thapsoides* was extracted with petrol–Et_2O–EtOH (1:1:1) at room temp. and the solvent evapd. *in vacuo*, giving 32 g of extract. The extract was chromatographed on a silica gel (350 g, 100–200 mesh) column and eluted with petrol and a gradient of petrol–EtOAc (0–100% EtOAc) followed by MeOH. Fr. 16 (5.5 g) from petrol–EtOAc (1:1) was subjected to CC and eluted with CH_2Cl_2 and a gradient of EtOAc. Subfr 8–9 were combined and a further fractionation was carried out on a Sephadex column (petrol–$CHCl_3$–MeOH, 7:4:1). All guaianolides were isolated from the latter column and further purified by repeated prep. TLC. The yields were as follows: **1** (27 mg), **2** (20 mg), **3** (9 mg), **4** (35 mg), **5** (7 mg), **6** (24 mg), **7** (11 mg).

4β,10β-*Dihydroxy*-1β(*H*),5α(*H*)-*guai*-11(13)-*en*-8α-12-*olide* (**1**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1753, 1662, 1130, 995, 760; CIMS *m/z* (rel. int.): 267 $[M + 1]^+$ (33.4), 249 $[M + 1 - H_2O]^+$ (92.7), 231 $[M + 1 - 2H_2O]^+$ (100), 207 (34.1), 189 (16.5), 163 (24.2). 79 (62.8), 59 (58.6); ^1H and ^{13}C NMR: Tables 2 and 1.

Diacetate of **1** (**1a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1730, 1660, 1250, 1125, 990, 770; ^1H NMR δ6.18 (1H, *d*, $J = 3.1$ Hz, H-13), 5.50 (1H, *d*, $J = 3$ Hz, H-13′), 4.33 (1H, *ddd*, $J = 2$, 10.5, 11.5 Hz, H-8), 2.54 (1H, *m*, H-7), 2.51 (1H, *dd*, $J = 2$, 15 Hz, H-9β), 2.36 (1H, *ddd*, $J = 2$, 4.5, 12 Hz, H-6α), 2.01 (3H, *s*, OAc), 1.99 (3H, *s*, OAc), 1.43 (3H, *s*, Me-14), 1.30 (3H, *s*, Me-15).

4α,10β-*Dihydroxy*-1β(*H*)-5α(*H*)-*guai*-11(13)-*en*-8α,12-*olide* (**2**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3415, 1760, 1662, 1030, 990, 760; CIMS *m/z* (rel. int.): 267 $[M + 1]^+$ (11.2), 249 $[M + 1 - H_2O]^+$ (44.1), 231 $[M + 1 - 2H_2O]^+$ (42.3), 207 (18.2), 189 (8.4), 79 (9.9), 57 (100); ^1H and ^{13}C NMR: Tables 2 and 1.

4α-*Acetoxy*-10β-*hydroxy*-1β(*H*)-*guai*11(13)-*en*-8α,12-*olide* (**3**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1765, 1730, 1670, 1490, 1385, 1245; CIMS *m/z* (rel. int.): 309 $[M + 1]^+$ (12.3), 265 $[M - Ac - 1]^+$ (13.2), 249 $(M - OAc]^+$ (21.9), 231 $(249 - H_2O]^+$ (19.4), 58 (74.8), 57 (100); ^1H and ^{13}C NMR: Tables 2 and 1.

Acetate of **3** (**3a**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1735, 1700, 1485, 1390, 1250, 1100; HREIMS *m/z* (rel. int.): 350.1726 $[M]^+$ (1.2), 308.8986 $[M - Ac]^+$ (0.2), 307.0036 $[M - Ac - 1]^+$ (1.3), 248 $[M - OAc]^+$ (14.6), 230 $[248 - H_2O]^+$ (72.1), 91 (55.3).

4β,10β-*Dihydroxy*-1α(*H*)-5α(*H*)-*guai*-11(13)-*en*-8α,12-*olide* (**4**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1760, 1690, 1650, 1470, 1380, 1260, 1180, 1120, 1000, 930, 910, 770; CIMS *m/z* (rel. int.): 267 $[M + 1]^+$ (65.1), 249 $[M + 1 - H_2O]^+$ (63.4), 232 (100), 213 (58.3), 203 (45.0), 189 (57.4), 95 (39.6), 71 (20.9), 58 (95.4); ^1H and ^{13}C NMR: Tables 2 and 1.

Diacetate of **4** (**4a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1725, 1245, 1680, 1645, 1485, 1460, 1385, 1155, 990, 910, 770; HREIMS *m/z* (rel. int.) 350.1726 $[M]^+$ (0.5), 307.0036 $[M - 43]^+$ (4.1), 248 (14.3), 230 (70.4), 91 (83.3), 79 (77.9).

4α,10α-*Dihydroxy*-5α(*H*)-*guai*-1,11(13)-*dien*-8α,12-*olide* (**5**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1755, 1710, 1660, 1460, 1380, 1260, 1145, 1005, 900; CIMS *m/z* (rel. int.): 265 $[M + 1]^+$ (9.8), 247 $[M + 1 - H_2O]^+$ (17.2), 229 $[M + 1 - 2H_2O]^+$ (7.1), 57 (100); ^1H NMR: Table 2.

3β-*Hydroxy*-4β,10β-*epoxy*-1α(*H*),5α(*H*),11α(*H*)-*guaian*-8α,12-*olide* (**6**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1765, 1465, 1385, 1210, 1055, 990, 950; HREIMS *m/z* (rel. int.): 266.1541 $[M]^+$, EIMS *m/z* 266 $[M]^+$ (6.4), 265 $[M - 1]^+$ (29.7), 250 $[M - 16]^+$ (7.3), 181 (24.2), 95 (27.5), 83 (100), 69 (34.2); ^1H and ^{13}C NMR: Tables 2 and 1.

4β,10β-*Epoxy*-1α(*H*),5α(*H*)-*guai*-11(13)-*en*-8α,12-*olide* (**7**). Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1758, 1660, 1480, 1385, 1270, 1150, 1120, 1080, 1000, 970, 920, 895, 835, 815, 760, 725; EIMS *m/z* (rel. int.): 264 $[M]^+$ (4.1), 239 $[M - Me]^+$ (13.7), 229 (8.5), 211 (9.8), 175 (16.8), 135

(36.7), 11 (64.3), 97 (73.4), 83 (78.9), 69 (100), 57 (74.6); ^{1}H and ^{13}C NMR: Tables 2 and 1.

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REFERENCES

1. Topcu, G. and Öksüz, S. (1990) *Phytochemistry* **29**, 3666.
2. Öksüz, S. and Topcu, G. (1992) *Phytochemistry* **31**, 195.
3. Topçu, G., Öksüz, S., Shieh, H.-L., Cordell, G. A. Pezzuto, J. M. and Bozok-Johansson, C. (1993) *Phytochemistry* **33**, 407.
4. Zdero, C., Bolmann, F. and Müller, M. (1987) *Phytochemistry* **26**, 2763.
5. Zdero, C. and Bohlmann, F. (1989) *Phytochemistry* **28**, 1653.
6. Ahmed, A. A., Mahmoud, A. A., Williams, H. J. Scott, A. I., Relbenspies, J. H. and Mabry, T. J. (1993) *J. Nat. Prod.* **56**, 1276.