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SESQUITERPENE LACTONES FROM *TANACETUM PRAETERITUM*

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Key Word Index—*Tanacetum praeteritum* subsp. *praeteritum*; Compositae; sesquiterpene lactones; sesquiterpene eudesmanolides; eudesmane.

Abstract—The aerial parts of *Tanacetum praeteritum* subsp. *praeteritum* afforded three new eudesmanolides and a new sesquiterpene ester, in addition to some known sesquiterpene lactones. The structures of the compounds were elucidated by spectral methods.

INTRODUCTION

Tanacetum praeteritum subsp. *praeteritum*, which grows on rocks and limestone slopes between 1200 and 2000 m and is endemic in Turkey, has been chemically investigated for the first time. Although other *Tanacetum* species which we have examined, gave mainly germacranolides, this plant afforded almost only eudesmanolides with the exception of *epi*-tatridin B. In addition to 10 known eudesmanolides and a known germacranolide, we have isolated four new eudesmanolides from *T. praeteritum* (Horwood) Heywood subsp. *praeteritum*.

RESULTS AND DISCUSSION

T. praeteritum subsp. *praeteritum* afforded the known compounds, douglanin (**5**) [1], santamarin (**6**) [2], reynosin (**7**) [3], *epi*-tatridin B (**8**) [4], arglanine (**9**) [5], ludovicin A (**10**) [5], ludovicin B (**11**) [5], armexine (**12**) [6], armefolin (**13**) [7], armexifolin (**14**) [7], 3α-hydroxyreynosin (**15**) [8] and the new compounds **1**–**4**.

Compound **1** showed IR bands of a hydroxyl group, a γ-lactone ring and unsaturation at 3420, 1760 and 1680 cm^{-1}, respectively. Its ^1H NMR spectrum (Table 1) contained the signals for an exocyclic methylene group conjugated with the γ-lactone ring system at δ6.18 (H-13, *d*, J = 3.5 Hz) and 5.50 (H-13, *d*, J = 3.0 Hz) and for three oxymethine protons at δ4.57, 4.31 and 3.81. Methyl signals at δ1.08 (H-14, s) and 2.04 (H-15, *d*, J = 1.5 Hz) and a lactone proton signal at δ4.57 (H-6, *dq*, $J_{5,6}$ = 12 and $J_{6,15}$ = 1.5 Hz) indicated a 6,12-lactonized eudesmanolide. According to the results of spin decoupling experiments, H-3 was at δ4.31 (*ddq*, J = 2, 4, 1.5 Hz) and H-1 at δ3.81 (*dd*, J = 4, 13 Hz.) The signal at δ8.19 indicated the presence of a hydroperoxy group in the molecule. Acetylation of **1** yielded the mono acetyl derivative having a keto group at C-3 which proved the presence of a hydroperoxy group at C-3 (**1a**) (Table 1). Acetylation of armexifolin (**14**) gave the same product. The HR-mass spectrum of **1a** gave the molecular formula $C_{17}H_{20}O_5$ ([M]$^+$ at m/z 304.1311) and confirmed the proposed structure.

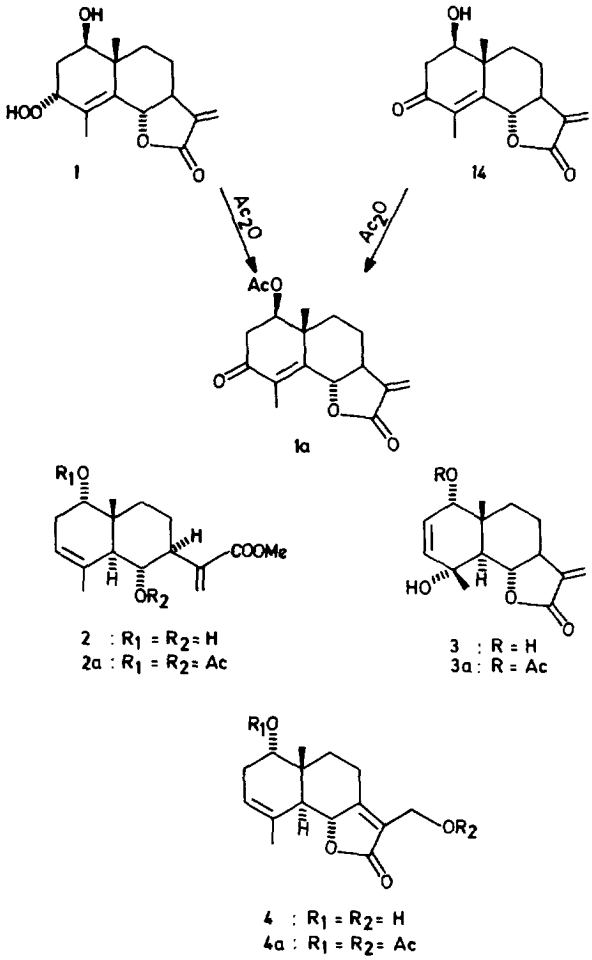

Table 1. ^{1}H NMR spectral data of 1–4 (200 MHz, $CDCl_3$)

H	1	1a	2	2a	3	3a	4	4a
1	3.81 *dd*	5.13 *dd*	3.35 *d*	4.57 *d*	3.42 *br s*	4.79 *d*	3.46 *br d*	4.69 *br d*
2	2.36 *ddd*	2.79 *m*	*	*	5.87 *dd*	5.88 *dd*	2.50 *m*	2.50 *m*
2′	1.97 *ddd*	2.60 *dd*	*	*	—	—	2.04 *ddd*	2.10 *m*
3	4.31 *ddq*	—	5.28 *m*	5.22 *m*	5.59 *d*	5.80 *d*	5.38 *m*	5.38 *m*
5	—	—	2.21 *br d*	*	2.37 *d*	2.54 *d*	2.26 *br d*	2.29 *br d*
6	4.57 *dq*	4.73 *dq*	3.85 *dd*	5.36 *dd*	3.98 *t*	4.04 *t*	4.83 *d*	4.84 *d*
7	2.65 *m*	2.79 *m*	2.48 *m*	2.50 *m*	2.55 *ddd*	2.64 *dddd*	—	—
8	*	*	*	*	2.10 *m*	2.10 *m*	2.95 *ddd*	3.03 *ddd*
8′	*	*	*	*	1.50 *dddd*	1.58 *m*	2.50 *m*	2.50 *m*
9	*	*	*	*	2.10 *m*	1.83 *m*	1.90 *m*	*
9′	*	*	*	*	*	*	1.50 *ddd*	1.56 *dd*
13	6.18 *d*	6.26 *d*	6.30 *s*	6.08 *s*	6.05 *d*	6.15 *d*	4.37 *s*	4.80 *br s*
13′	5.50 *d*	5.58 *d*	5.73 *d*	5.56 *s*	5.42 *d*	5.49 *d*		
14	1.08 *s*	1.33 *s*	0.85 *s*	0.93 *s*	0.88 *s*	1.05 *s*	0.94 *s*	1.03 *s*
15	2.04 *br d*	2.05 *d*	1.91 *br s*	1.87 *s*	1.33 *s*	1.41 *s*	1.92 *br s*	1.94 *br s*
O_2H	8.19 *br s*	—						
OMe			3.77 *s*	3.72 *s*				
OAc		2.10 *s*	—	2.01 *s*		2.06 *s*		2.07 *s*
OAc				2.02 *s*				2.04 *s*

J(Hz) = **1**, **1a**: 1,2 = 4; 1,2′ = 13; 2,2′ = 14; 2,3 = 2; 2′,3 = 4; 3,15 = 1.5; 5,6 = 12; 6,15 = 1.5; 7,13 = 3.5; 7,13′ = 3.0. **2**, **2a**: 1,2 = 5; 5,6 = 6,7 = 10. **3**, **3a**: 1,2 = 5.5; 2,3 = 10; 5,6 = 6,7 = 7,8 = 11; 7,8′ = 6; 7.13 = 3.5; 7,13′ = 3.0, **4**, **4a**: 1,2 = 4.5; 1,2′ = 2; 2,2′ = 14.5; 2,3 = 4.5; 5,6 = 11.5; 8,8′ = 14.5; 8,9 = 5; 8,9′ = 1.5; 9,9′ = 13.

*Obscured.

The IR spectrum of **2** showed a hydroxyl band at 3320 cm^{-1} and an ester carbonyl band at 1710 cm^{-1}. The HR-mass spectrum of the compound had a molecular peak $(C_{16}H_{24}O_4)[M]^+$ at *m/z* 280.1675. In the ^{1}H NMR spectrum of the compound, the signals of the exocyclic methylene protons at C-13 appeared at δ6.30 and 5.73 as singlets revealing an eudesmane acid. However, because of the methoxy singlet at δ3.77, it must be a methyl ester. In addition, the spectrum showed a quaternary methyl singlet at δ0.85 (H-14), an olefinic methyl singlet at δ1.91 (H-15), an olefinic proton signal at δ5.28 (H–3, *m*) and the other signals of the skeleton (Table 1). All the signals were assigned by spin decoupling experiments. The APT (Attached Proton Test) [9] spectrum of the compound supported the proposed formula giving a carbonyl signal, two olefinic quaternary carbons, an olefinic methine, an olefinic methylene, a methoxy, and two oxygen-bearing methine carbons and the other signals of the skeleton (Table 2). The carbon signals were assigned by means of the ^{1}H–^{13}C correlation (HETCOR) spectrum. Acetylation of **2** afforded the diacetyl derivative (**2a**) (Table 1).

Table 2. ^{13}C NMR spectral data of **2–4** (50.32 MHz, $CDCl_3$, * pyridine-d_5‡)

C	2†	APT	3*	APT	4	APT
1	71.2	(—)	70.9	(—)	72.4	(—)
2	33.4	(+)	126.9	(—)	32.2	(+)
3	120.8	(—)	135.8	(—)	119.7	(—)
4	136.6	(+)	70.2	(+)	133.2	(+)
5	46.3	(—)	50.0	(—)	47.0	(—)
6	73.1	(—)	81.3	(—)	81.4	(—)
7	52.3	(—)	49.6	(—)	165.8	(+)
8	27.6	(+)	21.4	(+)	22.4	(+)
9	34.5	(—)	35.0	(+)	34.0	(+)
10	40.5	(+)	40.3	(+)	39.0	(+)
11	144.8	(+)	139.4	(+)	123.4	(+)
12	166.0	(+)	170.4	(+)	174.0	(+)
13	125.1	(+)	117.7	(+)	54.4	(+)
14	17.23	(—)	19.9	(—)	16.4	(—)
15	25.8	(—)	24.9	(—)	23.6	(—)
16	51.4	(—)				

The IR spectrum of **3** contained a hydroxyl band at 3420 cm^{-1}, an α,β-unsaturated-γ-lactone band at 1760 cm^{-1} and an unsaturation band at 1680 cm^{-1}. Its EI–mass spectrum contained a molecular peak $(C_{15}H_{20}O_4)$ $[M]^+$ at *m/z* 264. The presence in the ^{1}H NMR spectrum of **3** of H_2-13 doublets at δ6.05 (*J* = 3.5 Hz) and 5.42 (*J* = 3.0 Hz), two quaternary methyl singlets at δ0.88 (H-14) and 1.33 (H-15) and the lactone proton at δ3.98 (H-6, *t*, *J* = 10 Hz) indicated a 6,12-lactonized eudesmanolide. The olefinic doublet of doublet at δ5.87 (H-2, *J* = 3, 10 Hz) and the olefinic doublet at δ5.59 (H-3, *J* = 10 Hz) which were coupled with each other showed that there was a double bound between C-2 and C-3 since the broadened singlet at δ3.42 (H-1, *J* = 3 Hz) was coupled with the doublet of doublet at δ5.87 (Table 1). The signals were assigned by spin decoupling experiments. The ^{13}C-decoupled and APT spectra confirmed the structure giving a carbonyl signal, two olefinic methine, an olefinic methylene, an olefinic quaternary carbon, a quaternary and two methine carbons bearing

oxygen atoms, two methyl and the other carbon signals (Table 2). The HETCOR spectrum of the compound allowed unambiguous assignments of the APT signals. Acetylation of the compound yielded the mono acetyl derivative **3a** (Table 1). The positioning of the hydroxyl group at C-4 was based on the ^1H and ^{13}C chemical shifts of the methyl at C-4 [10, 11].

Compound **4** contained bands at 3420 (OH), 1740 (γ-lactone) and 1680 cm^{-1} (C=C) in its IR spectrum. The EI-mass spectrum contained a molecular peak ($C_{15}H_{20}O_4$) $[M]^+$ at *m/z* 264. The ^1H NMR spectrum was similar to that of douglanin, apart from the presence of a broadened singlet at δ4.37 instead of the two exocyclic methylene protons (H-13) indicating a CH_2OH group at C-13. The lack of the signal of H-7 and the appearance of H-6 signal as a doublet ($J_{5,6} = 11$) at δ4.83 indicated a double bond between C-7 and C-11 (Table 1). All the signals were assigned by spin decoupling experiments. The APT spectrum of **4** confirmed the proposed structure showing a carbonyl signal, for sp^2 carbons, three of which were quaternary, three sp^3 carbons bearing oxygen atoms and the other signals of the molecule (Table 2). Acetylation of the compound afforded the diacetyl derivative (**4a**) (Table 1).

EXPERIMENTAL

General. CC: Kieselgel 60 (0.063–0.200 mm, Merck) and Sephadex LH-20 (Pharmacia); TLC precoated silica gel 60 F_{254}, 0.2 mm plates (Merck), spots were detected under UV and by spraying acidified ceric sulphate followed by heating. ^1H and ^{13}C NMR 200 and 50.32 MHz, respectively, $CDCl_3$ and pyridine-d_5 as solvents and TMS as int. standard.

Plant material. Tanacetum praeteritum (Horwood) Heywood subsp. *praeteritum* was collected from the southwest part of Turkey (Fethiye). A voucher specimen (ISTE 64370) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul, Turkey.

Extraction and isolation. Dried and powdered aerial parts (4.9 kg) were extracted successively with petrol (40–60°), $CHCl_3$ and EtOH. The extracts were combined and treated with MeOH. The residue was applied to a silica gel column and eluted with petrol, a gradient of Et_2O being added up to 100% followed by MeOH. The frs from CC were further sepd by prep. TLC and/or Sephadex LH-20: 7 mg **1**, 255 mg **2**, 200 mg **3**, 20 mg **4**, 27 g **5**, 300 mg **6**, 50 mg **27**, 13 mg **8**, 15 mg **9**, 665 mg **10**, 15 mg **11**, 45 mg **12**, 85 mg **13**, 85 mg **14**, 130 mg **15** were obtained.

3α-*Peroxyarmefolin* (**1**). Compound. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 1760 (γ-lactone), 1680 (C=C); ^1H NMR: Table 1.

Acetylation of compound **1**. Compound **1** (7 mg) was treated with 1 ml pyridine and 1 ml Ac_2O overnight. After vacuum evapn it was sepd by prep. TLC and 4 mg acetyl derivative (**1a**) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775 (γ-lactone), 1735, 1260 (OAc), 1710 (C=O), 1670 (C=C); ^1H NMR: Table 1; HR-MS *m/z* (rel. int.): 304.1311 ($C_{17}H_{20}O_5$) $[M]^+$ (64), 245 $[M - MeCO_2]^+$ (19), 244 $[M - MeCO_2H]^+$ (85), 229 $[244-Me]^+$ (24), 216 (23), 190 (26), 189 (35), 167 (26), 161 (44), 149 (100), 135 (36), 111 (38), 109 (28), 95 (34), 91 (38), 85 (32), 83 (37), 81 (30), 71 (52), 69 (41), 57 (81), 55 (54).

1α,6α-*Dihydroxyisocostic acid methyl ester* (**2**). Compound. IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1710 (C=O), 1620 (C=C); ^1H NMR: Table 1; ^{13}C NMR (APT): Table 2; HR-MS: *m/z* (rel. int.): 280.1675 $[C_{16}H_{24}O_4]$ $[M]^+$ (4), 262 $[M - H_2O]^+$ (15), 244 $[262 - H_2O]^+$ (100), 230 $[245 - Me]^+$ (34), 229 $[244 - Me]^+$ (23), 215 $[230 - Me]^+$ (20), 197 [212 – Me] (24), 185 (32), 184 (28), 169 (32), 143 (31), 135 (34), 121 (44), 119 (45), 109 (28), 107 (72), 105 (30), 97 (34), 91 (38), 81 (30), 71 (29), 69 (34), 67 (29), 54 (70), 55 (70), 55 (33).

Acetylation of compound **2**. Compound **2** (25 mg) was treated with 1 ml pyridine and 1 ml Ac_2O overnight. After vacuum evapn it was sepd by prep. TLC and 14 mg acetyl derivative (**2a**) was obtained. IR ν_{max}^{KBr} cm^{-1}: 1740, 1250 (OAc), 1720 (ester), 1630 (C=C); ^1H NMR: Table 1, HR-MS *m/z* (rel. int.): 244.1463 ($C_{16}H_{20}O_2$) $[M - 2 \times MeCO_2H]^+$ (18), 229 $[244 - Me]^+$ (8), 185 $[229 - CO_2]^+$ (10), 158 $[185 - C_2H_3]^+$ (13), 149 (100), 132 (10), 119 (25), 107 (9), 105 (17), 97 (15), 95 (13), 91 (18), 83 (16), 77 (10), 71 (24), 69 (23), 67 (12), 57 (80), 55 (44), 53 (10).

1α-*Hydroxy-1-desoxo-arglanine* (**3**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 1760 (γ-lactone), 1680 (C=C); ^1H NMR: Table 1; ^{13}C NMR (APT): Table 2; EI-MS *m/z* (rel. int.): 264 ($C_{15}H_{20}O_4$) $[M]^+$ (13), 249 $[M - Me]^+$ (100), 231 $[249 - H_2O]^+$ (52); 213 $[231 - H_2O]^+$ (60), 185 $[213 - CO]^+$ (79), 175 (21), 164 (71), 147 (41), 131 (34), 119 (47), 107 (37), 100 (95), 91 (55), 77 (43), 69 (68).

Acetylation of compound **3**. Compound **3** (25 mg) was treated with 1 ml pyridine and 1 ml Ac_2O overnight. After vacuum evapn it was sepd by prep. TLC and 15 mg acetyl derivative (**3a**) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1770 (γ-lactone), 1730, 1250 (OAc), 1670sh (C=C); ^1H NMR: Table 1.

Praeteritenolide (**4**). Compound. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 1740 (γ-lactone), 1680 (C=C), 750; ^1H NMR: Table 1; ^{13}C NMR (APT): Table 2; EI-MS *m/z* (rel. int.): 264 ($C_{15}H_{20}O_4$) $[M]^+$ (21), 246 $[M - H_2O]^+$ (46), 228 $[246 - H_2O]^+$ (42), 213 $[228 - Me]^+$ (32), 202 (19), 185 (18), 175 (11), 167 (18), 57 (14), 149 (20), 131 (15), 119 (42), 109 (43), 105 (35), 91 (31), 83 (100), 77 (22), 67 (16).

Acetylation of compound **4**. Compound **4** (17 mg) was treated with 1 ml pyridine and 1 ml Ac_2O overnight. After vacuum evapn it was sepd by prep. TLC and 8 mg acetyl derivative (**4a**) was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), 1735, 1240 (ester) 1680 (C=C), 1440, 1375, 1020; ^1H NMR: Table 1; EI-MS *m/z* (rel. int.): 348 ($C_{19}H_{24}O_6$) $[M]^+$ (0.2), 288 $[M - MeCO_2H]^+$ (60), 273 $[288 - Me]^+$ (14), 245 $[243 - CO]^+$(38), 228 $[288 - MeCO_2H]^+$ (90), 213 $[228 - Me]^+$ (89), 199 (69), 185 (44), 171 (23), 67 (34), 157 (36), 143 (31), 133 (2), 119 (100), 109 (45), 105 (59), 97 (10), 91 (41), 84 (78), 67 (19), 63 (27), 58 (82).

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