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DERIVATIVES OF MALONIC ACID IN *PARENTUCELLIA LATIFOLIA*

J. G. URONES, I. S. MARCOS, L. CUBILLO, V. A. MONJE, J. M. HERNÁNDEZ and P. BASABE

Departamento de Química Orgánica, Faculted de Químicas, 37008 Salamanca, Spain.

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Key Word Index—*Parentucellia latifolia*; Scrofulariaceae; diterpenes; labdanes; clerodanes; malonates.

Abstract-The aerial parts of *Parentucellia latifolia* contain four monoesters of malonic acid and diterpenoid alcohols, The esters were separated as methyl esters and were identified as: 7,13*E*-labdadien-15-yl-methyl malonic acid diester; ent-3,13*E*-clerodadien-15-yl-methyl malonic acid diester; 8-hydroxy-13*E*-labden-15-yl-methyl malonic acid diester and *ent*-2-oxo-3,13*E*-clerodadien-15-yl-methyl malonic acid diester.

INTRODUCTION

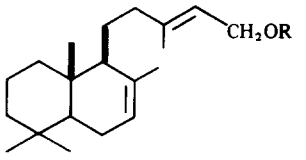
As part of a project designed to analyse the chemical components of toxic or endemic plants that affect grassland of the Iberian Peninsula, we have studied *Parentucellia latifolia* (L) Caruel, a semiparasitic plant occurring in the Euromediterranean region.

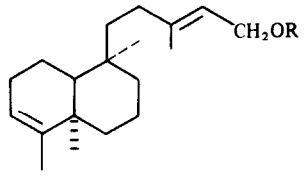
RESULTS AND DISCUSSION

The acid fraction of the hexane extract of *P. latifolia* was treated with an ethereal diazomethane solution. Column chromatography of the resultant mixture of methyl esters gave three fractions: I (60%), II (6%) and III (7.5%). CC (silica gel $AgNO_3$) of fraction I gave equal amounts of compounds **1** and **4**.

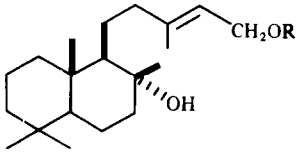
Compound **1** is an unsaturated diester (IR bands at 1770, 1675 and 840 cm^{-1}) whose ^{13}C NMR spectrum shows signals of 24 carbon atoms: six methyl groups, eight methylenes, four methynes (two sp^2) and six tetrasubstituted carbons (four sp^2, two carbonyl and two olefinic). The ^1H NMR spectrum of **1** shows signals of the following groups: CH=C–Me (δ5.38, 1H, *m*, 1.71, 3H, *s*); Me–C=CH–CH_2–OCOR (δ5.35, 1H, *t*, J = 6.8 Hz; 4.65, 2H, *d*, J = 6.84 Hz; 1.67, 3H, *s*); ROCO–CH_2–COOMe (δ3.38, 2H, *s*; 3.74, 3H, *s*)] as well as three methyl singlets (δ0.87, 0.85 and 0.75). Alkaline hydrolysis of **1** affords **2** a compound previously isolated in our laboratory [1]. As acetylation of **2** yields **3**, **1** is identified as 7,13*E*-labdadien-15-yl methyl malonic acid diester.

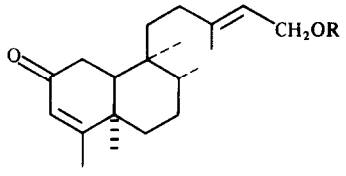
Compound **4** is also an unsaturated ester (IR bands at 1770, 1750, 1670, and 840 cm^{-1}) whose ^{13}C NMR spectrum shows signals of 24 carbon atoms: six methynes (two sp^2) and six tetrasubstituted carbons (four sp^2, two carbonyl and two olefinic). The ^1H NMR spectrum shows signals corresponding to the following groups: –CH=C–Me (δ5.1, 1H, *m*; 1.58, 3H, *s*); Me–C=CH–CH_2OCOR (δ5.33, 1H, *t*, J = 6.8 Hz; 4.65, 2H, *d* J = 6.8 Hz; 1.71, 3H, *s*); ROCO–CH_2–COOMe (δ3.39, 2H, *s*; 3.75, 3H, *s*) and those of three methyl groups (two C–Me and one CH–Me). Alkaline hydrolysis of **2** affords **5** [2] which after acetylation yields **6**. Thus the structure of **4** is established as *ent*-3,13*E*-clerodadien-15-yl-methyl malonic acid diester. Assignment of the ^{13}C NMR spectra of compounds **1**–**6** was carried out by DEPT experiments and ^1H–^{13}C, one bond and long range correlations.





	R
1	OCO—CH_2—COOMe
2	H
3	Ac
4	OCO—CH_2—COOMe
5	H
6	Ac





	R
7	OCO—CH_2—COOMe
8	Ac
9	OCO—CH_2—COOMe
10	Ac

Table 1. ^{1}H NMR data of compounds **1**–**10** (200 MHz, $CDCl_3$, TMS as internal standard)

H	1	2	3	4	5	6	7	8	9	10
3				5.19	5.15	5.18			5.71	5.71
				br s	*br s*	*br s*			*br s*	*br s*
7	5.38	5.37	5.38							
	br s	*br s*	*br s*							
14	5.35	5.41	5.35	5.33	5.35	5.32	5.35	5.34	5.31	5.30
	t(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)	*t*(6.8)
15	4.65	4.14	4.58	4.65	4.08	4.57	4.66	4.58	4.63	4.54
	d(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)	*d*(6.8)
16	1.71	1.68	1.72	1.71	1.63	1.70	1.72	1.71	1.68	1.67
	br s	*br s*	*br s*	*br s*	*br s*	*br s*	*br s*	*br s*	*br s*	*br s*
17	1.67	1.68	1.69	0.80	0.77	0.80	1.13	1.13	0.83	0.82
	br s	*br s*	*br s*	*d*(5.9)	*d*(5.9)	*d*(5.9)	*s*	*s*	*d*(5.9)	*d*(5.9)
18	0.85	0.84	0.85	1.58	1.54	1.58	0.80	0.79	1.88	1.87
	s	*s*	*s*	*br s*	*br s*	*br s*	*s*	*s*	*d*(1.5)	*d*(1.5)
19	0.87	0.87	0.88	1.00	0.96	0.99	0.87	0.86	1.10	1.10
	s	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
20	0.75	0.74	0.75	0.72	0.68	0.72	0.79	0.78	0.80	0.80
	s	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
2′	3.38			3.39			3.39		3.39	
OMe	3.74			3.75			3.75		3.73	
	s			*s*			*s*		*s*	
OAc			2.05			2.05		2.05		2.05
			s			*s*		*s*		*s*

From fraction II, an unsaturated hydroxyester **7** (IR bands at 3450, 1770, 1750, 1660 and 840 cm^{-1}) was isolated whose ^{13}C NMR spectrum shows signals of 24 carbon atoms, four of which correspond to the –OCO–CH_2–COOMe group (see Table 2). The remaining peaks correspond to five methyl groups, two methylenes, three methynes and four tetrasubstituted carbons (one of them sp^2). The ^{1}H NMR spectrum shows signals corresponding to the following groups: Me–C=CH–CH_2OCOR (δ5.35, 1H, *t*, J = 6.8 Hz; 4.66, 2H, *d*, J = 6.8 Hz; 1.72, 3H, *s*); OCO–CH_2–COOMe (δ3.39, 2H, *s*; 3.75, 3H, *s*); Me–C–OH (δ1.13, 3H, *s*) and three Me–C (δ0.87, 0.80 and 0.79). Hydrolysis of **7** followed by treatment with Ac_2O–pyridine affords **8** identified by comparison with an authentic sample [3]. This establishes that the structure of 8-hydroxy-13*E*-labden-15-yl-methyl malonic acid diester can be assigned for **7**.

Compound **9** was isolated from fraction III. It is an unsaturated diester with an α,β-unsaturated carbonyl group (IR bands at 1770, 1750, 1690, 1660 and 840 cm^{-1}; UV 242 nm). The ^{13}C NMR spectrum also shows signals of 24 carbon atoms, four corresponding to –OCO–CH_2–COOMe (see Table 2) and the rest distributed as five methyls, six methylenes, four methynes and five completely substituted carbons. The ^{1}H NMR spectrum shows signals corresponding to the groupings:–CO–CH =C–Me (δ5.71, 1H, *d*, J = 1.5 Hz; 1.88, 3H, *d*, J = 1.5 Hz); Me–C=CH–CH_2–OCOR (δ5.31, 1H, *t*, J = 6.8 Hz; 4.63, 2H, *d*, J = 6.8 Hz; 1.68, 3H, *s*); –OCO–CH_2–COOMe (δ3.39, 2H, *s*; 3.75, 3H, *s*) and those of two Me–C (δ1.10, 0.80) and a Me–CH (δ0.83, *d*, J = 5.9 Hz). Alkaline hydrolysis of **9** followed by treatment with Ac_2O–pyridine affords **10**, which is also obtained by oxidation of **6** with sodium chromate. Thus **9** must have a structure of *ent*-2-oxo-3, 13*E*-clerodadien –15–yl–methyl malonic acid diester.

EXPERIMENTAL

^{1}H NMR: 200 MHz, $CDCl_3$, TMS as int. standard; ^{13}C NMR: 50.3 MHz.

Extraction and isolation. 8.3 kg of *Parentucellia latifolia*, collected at Arabayona (Salamanca, Spain), were dried and extracted with *n*-hexane in a Soxhlet apparatus for 12 hr. The extract (103 g) was dewaxed with MeOH (49%) and then extracted with 12% Na_2CO_3 (25.8%) and 4% NaOH (5.2%). The neutral fraction represented 20.0% of the original extract.

3.5 g of the Na_2CO_3– soluble fraction was esterified with CH_2N_2 and then fractionated by CC to give three fractions: I (60.0%, *n*-hexane–EtOAc 9:1), II (6.1%, *n*-hexane–EtOAc 7:3) and III (7.5%, *n*-hexane–EtOAc 7:3). The remaining acid fraction was a mixture of straight chain compounds. 500 mg of fraction I were chromatographed over silica gel impregnated with 10% $AgNO_3$. Elution with *n*-hexane–Et_2O (9:1) gave compounds **1** (200 mg) and **4** (198 mg). Compounds **7** (259 mg) and **9** (140 mg) were isolated by CC from fractions II and III respectively.

7,13E-*Labdadien*-15-*yl-methyl malonic acid diester* (**1**). Colourless oil, $[\alpha]_D$ + 3° ($CHCl_3$; *c* 1.2). IR ν_{max}^{film} cm^{-1}: 1750, 1720, 1670 and 840; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Hydrolysis of **1**. **2**. 200 mg of **1** were treated with 5 ml 5% NaOH–MeOH for 3 hr at room temp. Usual work-up and extraction with Et_2O yielded 172 mg of **2**, $[\alpha]_D$ + 5° ($CHCl_3$; *c* 1.0) IR ν_{max}^{film} cm^{-1}: 3360, 1670, 1010, 840; ^{1}H NMR: Table 1; ^{13}C NMR Table 2.

Acetylation of **2**, **3**. To 172 mg **2** dissolved in 1 ml of pyridine 2 ml of Ac_2O were added and the mixture was kept at room

Table 2. ^{13}C NMR date of compounds **1–10** ($CDCl_3$)*

C	1	2	3	4	5	6	7	8	9	10
1	39.28	39.32	39.29	18.41	18.39	18.40	39.34	39.34	35.90	35.89
2	18.87	18.18	18.88	26.94	26.93	26.92	18.53	18.53	200.08	199.97
3	42.42	42.45	42.45	120.48	120.48	120.46	42.90	42.91	125.61	125.62
4	33.02	33.01	33.02	144.50	144.44	144.50	33.31	33.30	172.13	172.18
5	50.29	50.33	50.33	38.25	38.27	38.30	56.28	56.28	39.97	39.95
6	23.90	23.91	23.92	36.94	36.99	36.99	20.67	20.67	35.04	35.04
7	122.50	122.40	122.45	27.65	27.63	27.63	44.84	44.85	27.02	27.02
8	135.14	135.24	135.19	36.35	36.41	36.41	74.05	74.04	36.15	36.15
9	54.58	54.68	54.58	38.71	38.71	38.72	61.44	61.42	38.81	38.79
10	36.90	36.93	36.92	46.52	46.60	46.60	39.95	39.92	45.89	45.86
11	25.50	25.67	25.50	36.65	36.94	36.72	23.97	23.94	35.77	35.76
12	42.04	42.12	42.03	32.96	32.95	32.97	42.11	42.11	32.50	32.45
13	143.58	140.21	142.70	144.50	140.43	143.22	144.16	143.41	143.07	142.18
14	117.89	123.61	118.59	117.31	123.25	118.03	117.67	118.23	117.89	118.51
15	62.44	59.41	61.37	62.51	59.31	61.44	62.53	61.47	62.34	61.32
16	16.59	16.37	16.54	16.74	16.46	16.70	16.62	16.62	16.78	16.77
17	22.15	22.13	22.12	16.00	15.96	15.98	23.56	23.53	15.72	15.74
18	33.18	33.18	33.18	17.97	17.91	17.92	33.43	33.45	17.85	17.87
19	21.88	21.86	21.86	20.00	20.00	19.99	21.54	21.55	18.82	18.84
20	13.64	13.63	13.64	18.36	18.34	18.34	15.48	15.50	18.44	18.45
1′	166.96			166.95			166.96		166.94	
2′	41.43			41.43			41.43		41.39	
3′	166.45			166.45			166.45		166.44	
OMe	52.39			52.46			52.37		52.35	
Ac			20.97			20.94		21.01		20.98
Ac			170.94			170.94		174.09		172.18

*Assignments based on DEPT experiments and particularly in the case of **3** and **6** on C/H (one bond and long range) two dimensional correlations.

temp. overnight. Usual work-up yielded 170 mg of **3**, colourless oil, $[\alpha]_D+1°$ ($CHCl_3$; *c* 1.1). IR ν_{max}^{film} cm^{-1} 1750, 1670, 1250, 840; ^{1}H NMR Table 1; ^{13}C NMR: Table 2.

ent-3,13E-*Clerodadien*-15-*yl-methyl malonic acid diester* (**4**). Colourless oil, $[\alpha]_D-28°$ ($CHCl_3$; *c* 0.98). IR ν_{max}^{film} cm^{-1} 1770, 1750, 1670, 840; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Hydrolysis of **4**. Alkaline hydrolysis of **4** (198 mg) by the standard procedure yielded 153 mg of **5**, colourless oil, $[\alpha]_D-32°$ ($CHCl_3$; *c* 1.5). IR ν_{max}^{film} cm^{-1} 3360, 1670, 1010, 840; ^{1}H NMR: Table 1: ^{13}C NMR: Table 2. Acetylation of **5** (153 mg) yielded **6** (170 mg), colourless oil $[\alpha]_D-30°$ ($CHCl_3$, *c* 1.7). IR ν_{max}^{film} cm^{-1} 1750, 1670, 1250, 840; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

8-*Hydroxy*-13*E*-*labden*-15-*yl-methyl malonic acid diester* (**7**). Colourless oil, $[\alpha]_D-20°$ (Cl_3CH; *c* 1.4). IR ν_{max}^{film} cm^{-1} 3450, 1770, 1750, 1670, 840; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2. By hydrolysis of **7** (43 mg) and then acetylation with Ac_2O–pyridine **8** (31 mg) was obtained as a colourless oil, $[\alpha]_D+22°$ ($CHCl_3$; *c* 1.2). IR ν_{max}^{film} cm^{-1} 3450, 1750, 1670, 1250, 840; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

2-*Oxo*-3,13E-*clerodadien*-15-*yl-methyl malonic acid diester* (**9**). Colourless oil, $[\alpha]_D-9°$ ($CHCl_3$; 0.9). IR ν_{max}^{film} cm^{-1} 1770, 1750 1670, 840; ^{1}H NMR Table 1; ^{13}C NMR Table 2; UV λ_{max} 242 nm. Hydrolysis of **9** followed by acetylation gave **10** as a colourless oil, $[\alpha]_D-11°$ ($CHCl_3$; *c* 1.3). IR ν_{max}^{film} cm^{-1} 1750, 1670, 1250, 840; ^{1}H NMR Table 1; ^{13}C NMR: Table 2.

Oxidation of **6** *with sodium chromate.* To **6** (130 mg) dissolved in benzene (3 ml), Ac_2O (0.5 ml), HOAc (0.5 ml), NaOAc (70 mg) and Na_2CrO_4 (112 mg) were added. The mixture was stirred for 24 hr at 45° C, then ice and water were added and the mixture was extracted with Et_2O to afford 9, (90 mg).

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