Phytochemistry, Vol 28, No 10, pp 2717–2721, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00

SESQUITERPENOIDS AND PHENOLICS OF *PULICARIA PALUDOSA*

ARTURO SAN FELICIANO,* MANUEL MEDARDE, MARINA GORDALIZA, ESTHER DEL OLMO and JOSÉ M MIGUEL DEL CORRAL

Departamento de Química Orgánica, Facultad de Farmacia, 37007 Salamanca, Spain

(*Received in revised form* 3 *February* 1989)

Key Word Index—*Pulicaria paludosa*; Compositae, Inuleae, sesquiterpenoids; flavonoids

Abstract—Thirteen sesquiterpenoids of the skeletal types caryophyllane, cadinane, oplopane, eudesmane, *allo*-aromadendrane and 4-*epi*-guaiane, were isolated from *Pulicaria paludosa*. Their structures were established mainly by NMR techniques and chemical transformations. Four of them are new natural products Three flavonoids and some simple phenolic derivatives were also isolated.

INTRODUCTION

Pulicaria paludosa (Compositae, Inuleae) is a herbaceous plant native to the West of the Iberian Peninsula. In previous papers on the composition of its hexane extract we have described the structural determination of the major component, which was named paludolone and displayed a new sesquiterpenoid skeleton [1] and also the structural assignment of pulicaral, pulicaric acid and two triquinanic glycosides [2]. In the present report we describe the identification of minor components, that are mainly sesquiterpenoids and phenolic derivatives.

RESULTS AND DISCUSSION

The hexane extract of aerial parts was fractionated into acid and neutral parts, as described previously [1, 2]. Flavonoids **1–3** were isolated from the acid part by CC and were identified by comparison of their physicochemical and spectral data with those previously reported for these compounds [3, 4].

By repeated chromatographic separations of the dewaxed neutral part, in addition to the substances previously described [1, 2], sesquiterpenoids **4–15** were isolated. Compounds **4** and **5** were identified respectively as T-cadinol and oplopanone by direct comparison with authentic samples isolated from *Juniperus* specia [5, 6]. Caryophyllane derivatives **6–8**, previously described by Bohlmann *et al* as components of *P. dysenterica*, were identified by their NMR spectral data [7]. Compounds **9** and **10** were obtained as a mixture (3:2), whose ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed that they are the isobutyryl and 2-methylbutyryl derivatives of **7**.

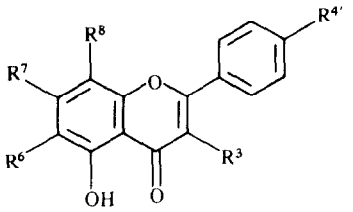
Compounds **11** to **15** formed a complex mixture which, provided it showed no acetate signals in its ^{1}H NMR spectrum, was acetylated to facilitate chromatographic separations. Thus, they were isolated as acetyl derivatives

Compound **11a** was identified as 14-acetoxy-T-cadinol by comparison of its NMR spectra with those of T-cadinol (**4**). As can be seen from the NMR data in Tables 3 and 4 the major differences observed with respect to **4** can readily be explained by the exchange of a hydrogen atom at position 14 by a hydroxyl (**11**) or an acetoxyl (**11a**) group. In fact, compounds **11** and **11a** have no signal of a methyl group on an olefinic carbon in their ^{1}H NMR spectra. By contrast, they do show a deshielded oxygenated methylene which absorbs as a singlet at δ4.46 in the ^{1}H NMR spectrum and at δ68.8 in the ^{13}C NMR spectrum of **11a** (δ3.99 and 67.4, respectively, in the case of **11**).

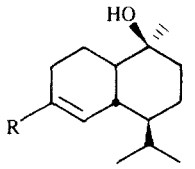
Compounds **12** and **13** obtained by saponification of **12a** and **13a** were identified respectively as oplodiol, described as a component of *Oplopanax japonicus* [8] and isoplodiol described as a component of *Senecio specia* [9] by comparison of their ^{1}H NMR spectral data. Their ^{13}C NMR data are included in Tables 3 and 4.

Compound **14a** showed a molecular ion $[M]^+$ at *m/z* 280, which in combination with the BB and DEPT ^{13}C NMR spectra indicate a molecular formula of $C_{17}H_{28}O_3$ As no signals of olefinic carbons are observed, the substance should contain a tricyclic skeleton with acetate (1740, 1235 cm^{-1} in IR) and tertiary alcohol functions (3605 cm^{-1}). The presence in its ^{1}H NMR spectrum of signals at δ0.13 and 0.66 (Table 5) characteristic of cyclopropane protons and the signal of a quaternary carbon at δ19.2 in the ^{13}C NMR spectrum (Table 4) indicated that the substance would have an aromadendrane skeleton [10], on which the hydroxyl and acetate functions would be located at neighbouring positions 10 and 14. This is because in the diol **14**, obtained by saponification of **14a**, only the signals of the carbon atoms supporting oxygen atoms changed significantly, the rest remaining practically unaltered and also because if the functions were at positions 4 and 15 the chemical shift of H-6 would have to be greater than 0.4 ppm [11]. After carrying out a COSY experiment of homonuclear correlation and several NOE determinations, and comparing the spectral data with those of diasteroisomers globulol, *epi*-globulol, ledol and viridiflorol [12, 13] the structure of 14-acetoxiviridiflorol was assigned to **14a** and correspondingly the structure of *allo*-aromadendrane-10β,14-diol to **14**. For chemical confirmation the diol **14** was transformed into viridiflorol (**14b**) through a sequence of reactions shown in Scheme 1.

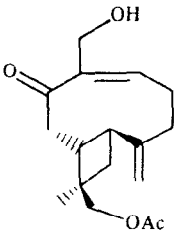
Compound **15a** displayed bands of acetate and ether groups (1735, 1260, 1040, 1005 cm^{-1}) in its IR spectrum. Its molecular formula $C_{17}H_{28}O_3$ and the absence of



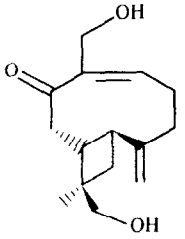
	R^3	R^4	R^7	R^8	$R^{4'}$
1	OMe	OMe	OH	H	OH
2	H	OH	OMe	H	OMe
3	H	OH	OMe	OH	OMe



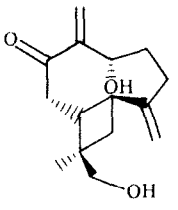
4 R = Me
11 R = CH_2OH
11a R = CH_2OAc



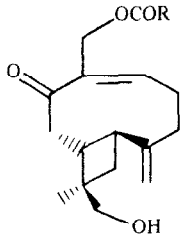
6



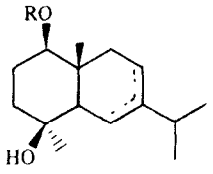
7



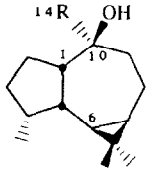
8



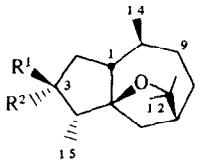
9 R = *i*Pr
10 R = *sec*-Bu



12 R = H, Δ^7
12a R = Ac, Δ^7
13 R = H, Δ^6
13a R = Ac, Δ^6



14 R = CH_2OH
14a R = CH_2OAc
14b R = Me

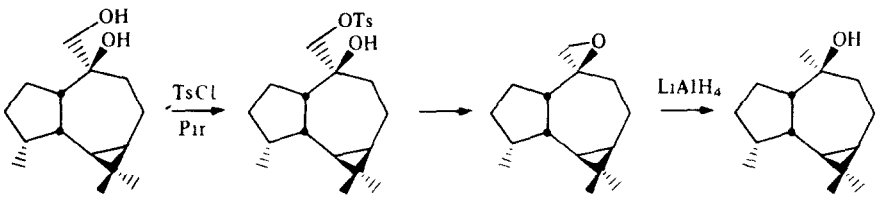


15 R^1 = OH, R^2 = H
15a R^1 = OAc, R^2 = H
15b R^1 = R^2 = O

Table 1 ^{1}H NMR (200 MHz) data for compounds **9**, **10**, **4**, **11** and **11a** (in $CDCl_3$, TMS as int standard)

H	**9**	**10**	**4**	**11**	**11a**
5	5.85 *dd* (11.3, 5.5)	5.82 *dd* (10.5, 4.5)	5.58 *br s*	5.83 *br s*	5.58 *sa*
12, 12′	4.87, 4.94 *br s*	4.87, 4.94 *br s*	0.80 *d* (6.9)	0.80 *d* (6.9)	0.80 *d* (6.9)
13, 13′	4.53, 4.75 AB (12.0)	4.53, 4.75 AB (12.0)	0.91 *d* (6.9)	0.90 *d* (6.9)	0.92 *d* (6.9)
14, 14′	3.63, 3.64 AB (11.0)	3.63, 3.64 AB (11.0)	1.65 *s*	3.99 *s*	4.46 *s*
15	1.07 *s*	1.07 *s*	1.20 *s*	1.22 *s*	1.23 *s*
Others	0.94 *d* (6.0)	0.91 *d* (6.0) 0.90 *t* (6.4)	—	—	2.07 *s*

(*J* in Hz)



14 **14b**

Scheme 1

Table 2 ^{13}C NMR (50 3 MHz) data for compounds **9**, **10**, **4**, **11** and **11a** (in $CDCl_3$, TMS as int. standard)

C	9	10	4	11	11a
1	49 9	49 9	46 6	46 6	46.4
2	150.6	150 6	22 4*	22 3*	22 2*
3	32 5	32 5	30 8	26 0	26 8
4	26 9	26 9	133 7	137.8	133 1
5	136 7	136 7	122 6	124 3	127 9
6	136 7	136 7	47.9	48.3	47 9
7	206 8	206.8	37 5	37 6	37.7
8	45 4	45.4	19 9*	20 0*	20.0*
9	42 1	42.1	40 3	40 4	40 4
10	38 5	38 5	70 1	70 7	70.5
11	37.7	37 7	26 0	26 3	26.4
12	110.4	110 4	23 5	28.5	28 5
13	67.3*	67 3*	21 2	21.4	21 4
14	65.8*	65.8*	28 3	67.4	68.8
15	24 9	24.9	15 1	15.3	15.3
Others					
	172 0 (COO).	172.0 (COO)	—	—	170 9 (AcO)
	22 5 (Me)	19 0 (Me)			20 9
	45.4 (CH)	20 9 (Me)			
		34 5 (CH_2)			
		43 2 (CH)			

*Assignments may be interchanged.

Table 3 ^{1}H NMR (200 MHz) data for compounds **12**, **12a**, **13** and **13a** (in $CDCl_3$, TMS as int. standard)

H	12	12a	13	13a
1	3.25, 3.35 *dd* (1.6, 4.0)	4 52, 4 57 *dd* (11 5, 3 8)	3.30; 3.35 *dd* (11.6; 4 0)	4.56, 4.61 *dd* (11 3; 4.6)
8	5 35 *dd* (5 2, 3 2)	5 28 *m*	5 45 *br s*	5.45 *br s*
12	1 03 *d* (6 8)	1 02 *d* (7 1)	1 03 *d* (6 8)	1.03 *d* (6.9)
13	1 03 *d* (6.8)	1 02 *d* (7 1)	1.02 *d* (6 8)	1 03 *d* (6.9)
14	1 18 *s*	1 19 *s*	1.23 *s*	1 24 *s*
15	0.96 *s*	1 04 *s*	0 97 *s*	1 05 *s*
AcO-	—	2.06 *s*	—	2 06 *s*

(*J* in Hz)

unsaturations different from that of the acetate carbonyl, showed that we were dealing with a tricylic sesquiterpenoid with an ether bridge between two non-protonated carbons absorbing at 81.2 and 90.4 ppm (Table 4). The ^{1}H NMR spectrum (Table 5) displayed signals of two methyl doublets (0.86; $J = 6.5$ and 0.97, $J = 6.9$ Hz), of a *gem*-dimethyl group bearing an oxygen (1.16 *s*, 1 32 *s*) and of a secondary alcohol acetate (2.04 *s* and 4.47; 1H; *ddd*). These data and the analysis of the ^{13}C DEPT spectra led to a structural proposal based on the guaioxide system [14]. The stereochemical assignment of **15a** has been achieved by the study of coupling constants and some NOEs (Table 6) observed upon irradiation of each methyl group and the signal corresponding to the proton geminal to the acetate group.

An alternate structure with the acetate function at position 9 was ruled out because the oxidation of the natural alcohol with pyridine dichromate yielded a cyclopentanone derivative **15b** with absorption in the IR spectrum at 1745 cm^{-1}. The absolute stereochemistry of these compounds was deduced from the positive Cotton effect shown by **15b** at 301 nm $\Delta\varepsilon = +0.6$. Although **15** is described for the first time as a component of higher plants it has been previously produced by hydroxylation of liguloxide by *Mucor parasitis* [15].

EXPERIMENTAL

General. Mps: uncorr Optical rotations were measured in $CHCl_3$. UV spectra were recorded in EtOH; λ_{max} values are expressed in nm. IR spectra were obtained in $CHCl_3$. ν_{max} values are expressed in cm^{-1}. ^{1}H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz) spectra were measured in $CDCl_3$ with TMS as int. std; δ values are expressed in ppm. EIMS were obtained at 70 eV. *M/z* values followed by rel int. (%) are stated. Flash chromatography was carried out on silica gel (Merck No. 9385).

Plant material, extraction and isolation. Material was collected in July and September 1985, at Parada de Rubiales, Salamanca (Spain). Voucher specimens are deposited in the Botany Department, Faculty of Pharmacy, Salamanca (register number

Table 4 ^{13}C NMR (50.3 MHz) for compounds **12**, **12a**, **13a**, **14**, **14a**, **14b**, **15**, **15a** and **15b** (in $CDCl_3$, TMS as int standard)

C	12	12a	13a	14	14a	14b	15	15a	15b
1	80 0	81 5	80 3	53 6	54 0	58 4	54 3	54 5	55 1
2	40 7	40 6	39 4	32 3*	32 7*	37 9	38 8	36 2	44 0
3	39 7	39 4	38 7	24 7	24 7	25 9	74 8	77 0	215 2
4	71 0	70 9	71 0	38 3	38.2	38 5	50 9	47 5	52 8
5	46 5	46 8	50 3	40 2	40 2	39 8	90 8	90 4	90 4
6	26 9	23 5	115 3	29 3	29 1	28 7	30 6	30 6	30 4
7	142 1	142 1	146 2	22 7	22.6	22 5	45 4	45 6	46 0
8	116 2	116 1	23 7	18 4	18 2	18 9	33 3	33 3	33 2
9	23 2	23 1	22 9	29 3*	29 2*	29 3	30 6	30 7	30 7
10	37 9	36 9	37 4	76 4	75 0	74 6	38.8	38 9	38 2
11	35 1	35 1	35 3	19 2	19 2	18 4	81 0	81 2	81 8
12	21 9	21 9	21 8	16 2†	16 2†	16 3	31 4	31 5	31 4
13	21 3	21 3	21 3	28 8	28 7	28 7	22 8	23 0	23 0
14	29 9	29 9	29 5	70 8	72 2	32 1	10 9	11 1	7 3
15	11 8	12 9	12 8	16 1†	16 1†	16 1	22 8	22 8	23 0
AcO	—	170 9	170 9	-	171 1	—	-	171 2	—
		21.3	21 3		20 9			21 2	

*,†Assignments may be interchanged

Table 5 ^1H NMR (200 MHz) for compounds **14**, **14a**, **14b**, **15**, **15a** and **15b** (in $CDCl_3$, TMS as int standard)

H	14	14a	14b	15	15a	15b
3	—	—	—	3 50 *ddd* (10 0, 8 5, 5 6)	4 47 *ddd* (10 7, 8.7, 5 6)	–
6	0 12 *dd* (9 0)	0 13 *dd* (9 0)	0 11 *dd* (9.1)	—	α 1 52 *d* (12 2)	–
					ß 2 13 *ddd* (12 2 7 0, 1 6)	
7	0 66 *m*	0 66 *m*	0 61 *m*	—	– –	-
12	1 01 *s*	1 01 *s*	1.01 *s*	1 31 s	1 32 s	1 35 *s*
13	1 03 *s*	1 03 s	1 03 s	1 16 *s*	1 16 s	1 19 s
14	3 41, 3 28 *d* (10 9)	3 88 s	1 11 *s*	1 02 *d* (6 8)	0 97 *d* (6 9)	1 06 *d* (6 9)
15	0 93 *d* (6 6)	0 94 *d* (6 4)	0 93 *d* (6 6)	0 88 *d* (6 5)	0 86 *d* (6 5)	0 96 *d* (6 6)
AcO-	—	2 08 *s*	—	– –	2 04 s	

(*J* in Hz)

Table 6 NOEs observed for **15a***

H	Enhanced protons
3	6α, (6β), 2α
13	9β,
12	6β, 7
14	– –
15	3, 6β

*Results from NOE difference experiments localization of the hindered signals for protons 2α, 7 and 9 was previously performed by a heteronuclear 2D-NMR correlation

SALAF No 13230) Extn and fractionation have been reported previously [2] From the weakly acid fraction (1 4% of ext) by CC were obtained (mg). **1** (8), **2** (70) and **3** (80) The neutral part (87 g. 31 5%) was chromatographed over silica gel with hexane–EtOAc mixts of increasing polarity, yielding (mg): **4** (120), **5** (90), **6** (235), **7** (100), **8** (50), **9**+**10** (94), and after previous acetylation **11a** (128), **12a** (35), **13a** (18), **14a** (212) and **15a** (289)

(Z)-13,14-Dihydroxy-7-oxo-caryophylla-2(12),5-dien-13-iso-butyrate (**9**) *and 2-methylbutyrate* (**10**) (3 2) Oil, $[\alpha]^{23}$ (λ) = −119 3° (589), −134 5° (578), −143 3° (546), UV ν_{max} = 227 (ε = 2250) EIMS *m/z* 320 (1), 326 (1), 317 (1), 289 (2), 263 (2), 232 (4), 201 (8), 173 (8), 160 (11), 147 (50), 131 (12), 120 (21), 105 (24), 93 (64), 85 (63), 79 (38), 58 (100) IR 3610, 3460, 2940, 1730, 1680, 1640, 1240, 1100, 1040, 900 ^1H NMR (Table 1). ^{13}C NMR (Table 2)

14-Acetoxy-T-cadinol (**11a**) $[\alpha]^{23}$ (λ) = +6 6° (589), +7 4° (578), +8 4° (546). EIMS. *m/z* 280 (1), 262 (3), 232 (3), 202 (23), 177 (7), 159 (93), 145 (9), 132 (22), 118 (25), 107 (12), 91 (30), 79 (20), 43 (100) IR 3500, 2920, 1780, 1680, 1380, 1250, 1150, 890 ^1H NMR (Table 1) ^{13}C NMR (Table 2) Saponification of **11a** (60 mg) yielded, after chromatography, **11** (20 mg) Oil, $[\alpha]^{23}$ (λ) = +3 2° (589), +3 4° (578), +3 7° (546), +6 1° (436). IR 3600, 3460, 2920, 2860, 1470, 1380, 1030, 1000, 910, 880 ^1H NMR (Table 1) ^{13}C NMR (Table 2)

14-Acetoxy-viridiflorol (**14a**) $[\alpha]^{23}$ (λ) = +6.9° (589), +5 7° (578), +6.6° (546), +9 2° (476) EIMS *m/z* 280 (5), 220 (3), 202 (49), 187 (31), 159 (86), 147 (2), 120 (31), 107 (56), 105 (38), 91 (45), 81 (45), 69 (41), 43 (100) IR 3600, 2890, 1740, 1380, 1235, 1040 ^1H NMR (Table 5) ^{13}C NMR (Table 4) Saponification of **14a**

(30 mg) gave *allo*-aromadendrane-10β,14-diol **14** (18 mg) $[\alpha]^{23}$ (λ) = +2.6° (589), +2.8° (578), +3.1° (546), +5.0° (476), +7.1° (365) EIMS. *m/z* 238 (1), 207 (6), 189 (14), 159 (3), 147 (4), 133 (6), 121 (4), 119 (5), 109 (8), 95 (10), 85 (61), 83 (100), 47 (21) IR 3600, 2940, 2870, 1460, 1380, 1070, 1030, 890 ^{1}H NMR (Table 5) ^{13}C NMR (Table 4) **14** treated with TsCl (400 mg) in pyridine followed by $LiAlH_4$ reduction yielded viridiflorol **14b** (17 mg) mp 74° $[\alpha]^{23}$ (λ) = +2.0° (589), +2.2° (578), +2.9° (546), +5.3° (436) IR 3365, 1375, 1255, 1166, 1120, 1105, 1065, 1045, 1015, 985, 930 ^{1}H NMR (Table 5) ^{13}C NMR (Table 4)

3β-Acetoxy-liguloxide (**15a**) $[\alpha]^{23}$ (λ) = +7.0° (589), +7.7° (578), +8.3° (546), +14.7° (436), +24.5° (365) IR 2940, 1735, 1470, 1375, 1260, 1040, 1005, 890. ^{1}H NMR (Table 5). ^{13}C NMR (Table 4) Saponification of **15a** gave liguloxide-3β-ol **15** (30 mg): $[\alpha]^{23}$ (λ) = −22.2° (589), −23.3° (578), −26.4° (546), −43.4° (436), −62.6° (365) IR 3590, 3400, 2860, 1450, 1380, 1240, 1130, 1030, 880 ^{1}H NMR (Table 5) ^{13}C NMR (Table 4) **15** (30 mg) treated with PDC (70 mg) in CH_2Cl_2 yielded **15b** (20 mg) liguloxide-3-one Mp 47–49° $[\alpha]^{23}$ (λ) = +26.0° (589), +27.0° (578), +34.0° (546), +105.0° (476) EIMS *m/z* 236 (6), 221 (13), 218 (32), 207 (9), 190 (3), 175 (10), 163 (17), 149 (30), 135 (11), 123 (16), 117 (5), 110 (27), 107 (15), 95 (17), 83 (100), 59 (81), 43 (55) IR 2870, 1740, 1460, 1375, 1130, 1050, 890 ^{1}H NMR (Table 5) ^{13}C NMR (Table 4)

Acknowledgements—We thank Prof Dr M. Ladero (Universidad de Salamanca) for plant identification Dr A Gutiérrez Ravelo (Instituto de Química Orgánica, La Laguna, Tenerife) and Dr A Virgili (Universidad Autónoma de Barcelona) for MS measurements

REFERENCES

1 San Feliciano, A., Medarde, M., Gordaliza, M., Olmo, E del and Miguel del Corral, J M (1987) *An. Quim*, **83C**, 283.
2 San Feliciano, A., Medarde, M., Gordaliza, M., Olmo, E del and Miguel del Corral, J. M (1988) *J Nat Prod* **51**, 1153.
3 Sachdev, K. and Kulshreshtha, D K (1983) *Phytochemistry* **22**, 1253
4. Sastri, V. D N and Seshadri, T R (1946) *Proc. Indian Acad. Sci* **24A**, 248
5 Pascual Teresa, J., de Barrero, A F., Caballero, M C and San Feliciano, A. (1978) *An Quím.* **74**, 966
6 San Feliciano, A., Medarde, M., López, J. L., Miguel del Corral, J M., Puebla, P and Barrero, A. F (1988) *Phytochemistry* **27**, 2248
7. Bohlmann, F. and Zdero, C. (1981) *Phytochemistry* **20**, 2529
8 Minato, H and Ishikawa, M (1967) *J. Chem Soc. (C)*, 423
9 Bohlmann, F., Knoll, K., Zdero, C. Mahanta, P K., Grenz, M., Suwita, A., Ehlers, D., Le Van, N., Abraham, W and Natu, A. A. (1977) *Phytochemistry* **16**, 965
10 Streith, J., Pesnelle, P. and Ourisson, G (1963) *Bull Soc. Chim Fr* 518
11 Beecham, C M., Djerassi, C. and Eggert, H (1978) *Tetrahedron* **34**, 2503.
12. Naves, Y. R. (1959) *Helv Chim Acta* **42**, 1996.
13. Dolejs, L., Motl, O., Soucek, M., Herout, V and Sorm, F. (1960) *Coll. Czech Chem Comm.* **25**, 1483
14. Ishii, H., Tozyo, T., Nakamura, M and Minato, H. (1970) *Tetrahedron* **26**, 2751
15 Funke, B E., Tozyo, T., Ishii, H. and Takeda, K (1970) *J Chem Soc (C)*, 2548