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TERPENOIDS FROM *PULICARIA GLUTINOSA*

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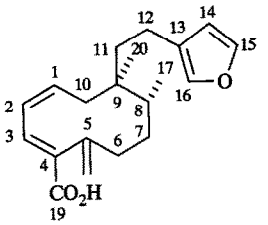
Key Word Index—*Pulicaria glutinosa*; Compositae; sesquiterpenes; bisabolene; diterpenes; puliglutoic acid; strictic acid; pulic acid; 15-deoxypulic acid.

Abstract—The aerial parts of *Pulicaria glutinosa* yielded the diterpene, strictic acid, and its two new derivatives, pulic acid and 15-deoxypulic acid. Their structural assignments were largely based on 1D and 2D NMR spectroscopic data. A fourth compound, the new bisabolene sesquiterpene puliglutoic acid, was also obtained, and its structure determined from its spectroscopic data and by chemical derivatization.

INTRODUCTION

In our previous communications [1, 2], we described the structures of six new sesquiterpenes, namely, the germacranes puliglene and epoxypuliglene, the bisabolenes puliglutol, puliglutal and puliglutone and the guaiane cycloepoxypuliglene, isolated from the aerial parts of *Pulicaria glutinosa* Gaertn. [3]. Examination of several collections of the same source has now led to the isolation and characterization of the three diterpenoids, strictic acid (**1**) and its new derivatives pulic acid (**2**) and 15-deoxypulic acid (**3**), as well as the new bisabolene sesquiterpene, puliglutoic acid (**4**).



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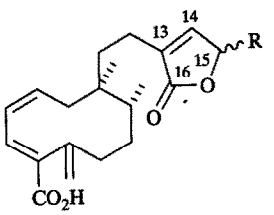

	R
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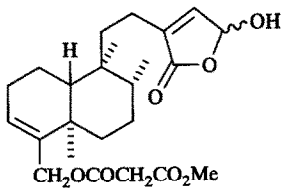
3	H
5	OAc



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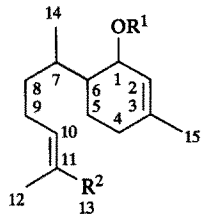

	R^1	R^2
4	H	CO_2H
7	H	CO_2Me
8	Ac	CO_2H
9	H	CHO
10	Ac	CHO
11	Ac	CH_2OAc
12	H	CH_2OH

RESULTS AND DISCUSSION

The acetonitrile fraction of the *n*-hexane extract of *P. glutinosa* was flash chromatographed [4] on silica gel to give, upon crystallization, compounds **1–3** in yields of 0.02, 0.013 and 0.005%, respectively.

Compound **1**, $C_{20}H_{26}O_3$, was identified as strictic acid by comparison of its physical and spectral data with those previously reported [5].

Compound **2**, $C_{20}H_{26}O_5$, possessed ^1H and ^{13}C NMR data (Table 1) that were generally similar to those of strictic acid (**1**), except for the presence of a lactonic hemiacetal group (see Experimental). The presence of the hydroxyl group (ν_{max} 3400 cm^{-1}) was confirmed by acetylation to the corresponding monoacetate (**5**) (δ_H 2.06, 3H, *s*; δ_C 21.3 *q*, 171.3 *s*). The ^1H NMR of **2** showed signals at δ 6.06 (*br s*) and 6.99 (*d*, $J = 1.3$ Hz), due to the hemiacetal proton H-15 and the deshielded olefinic proton H-14, respectively. The latter was assigned β- to the lactone carbonyl as observed in the similar lactonic hemiacetal **6**, isolated from *Baccharis leja* [6]. The 2D-NMR COSY experiment on **2** demonstrated the correlation between δ6.06 (H-15) and 6.99 (H-14), indicating that the olefin methine is indeed adjacent to the hemiacetal group, rather than to the lactone carbonyl group. The ^{13}C NMR spectrum (Table 1) of the monoacetate **5** showed significant shielding of C-14 and C-15 to δ140.7 and 92.5, respectively [cf. δ(MeOH) 146.1 and 99.0 of **2** and $\delta(CHCl_3)$ 142.8 and 96.7 of **6**], confirming the placement of the hydroxyl group at C-15 [6]. The remaining ^{13}C NMR data of **2** (δ138.8, 146.1, 99.0 and 169.6 assigned to C-13–C-16, respectively) also supported the presence of lactonic hemiacetal connected through an ethylene (δ_{C-11} 36.6, δ_{C-12} 21.1) bridge and was in agreement with those previously reported for **6** [6], thus establishing the new diterpenoid as pulic acid (**2**).

The remaining diterpenoid, 15-deoxypulic acid (**3**; $C_{20}H_{26}O_4$) was obtained in 0.008% yield. Its ^1H and ^{13}C NMR data (Table 1) were similar to those of **2**, except for the absence of the hydroxyl group at C-15. The ^1H NMR spectrum showed signals at δ4.78 (2H, *br d*, $J = 1.2$ Hz; δ_C70.2, *t*) and 7.13 (1H, *t*, $J = 1.2$ Hz; δ_C143.6, *d*), due to the oxymethylene protons (H-15) and the olefinic proton (H-14), respectively. The ^{13}C NMR data of **3** (Table 1) also supported the presence of 15-dihydro-16-olide group, thus formulating this minor diterpenoid as 15-deoxypulic acid.

The sesquiterpene puliglutoic acid (**4**) was obtained in 0.014% yield. The EI mass spectrum suggested the molecular formula $C_{15}H_{24}O_3$ and the presence of carboxyl (ν_{max} 1680 cm^{-1}; δ_C173.1) and hydroxyl (ν_{max} 3500 cm^{-1}) groups was established by preparing the corresponding methyl ester (**7**; δ3.73, 3H, *s*) and monoacetate (**8**; δ2.06, 3H, *s*; δ_C21.1 *q*, 171.3 *s*), respectively. The ^1H and ^{13}C NMR spectra of **4** (Tables 2 and 3) indicated it to be the corresponding acid of puliglutal (**9**), previously isolated, together with other bisabolenes, from the same source [2]. This new compound is, therefore, now named puliglutoic acid (**4**). The ^{13}C NMR assignments of puliglutal monoacetate (**10**) and puliglutol diacetate (**11**) [2] are reported in Table 3 for the first time.

Compounds **1–4**, **9** and **12** were tested for antimicrobial activity and only strictic acid (**1**) showed moderate

Table 1. ^1H and ^{13}C NMR spectral data and coupling constants (in Hz, in parentheses) for *Pulicaria* diterpenes*

Atom	**1** ^{13}C	^{13}C	**2** ^1H	^{13}C	**3** ^1H	^{13}C	**5** ^1H
1	127.8(1)†	128.2	5.48 *br dt* (1.8, 10.3)	127.5	5.47 *ddd* (2.0, 4.8, 12.0)	127.2	5.41 *dt* (1.4, 12.1)
2	127.30 (1)	129.1	5.98 *ddd* (1.2, 2.5, 11.5)	127.5	5.94 *dd* (1.8, 12.0)	127.7	5.94 *br dd* (1.9, 11.4)
3	143.6(1)	141.8	7.23 *dd* (2.4, 4.7)	143.7	7.40 *dd* (2.2, 4.4)	143.4	7.38 *br d* (2.2)
4	144.7(0)	146.8	—	144.7	—	144.6	—
5	136.4(0)	138.8[a]	—	136.2	—	136.2	—
6	33.8(2)	35.0	2.66 *dd* (1.6, 13.8)	33.8	2.66 *d br* (13.0)	33.7	2.67 *br dd* (1.2, 13.2)
			2.08 *dt* (2.5, 13.7)		2.03 *dd* (1.8, 12.8)		2.07 *br dd* (1.8, 13.7)
7	29.1(2)	30.3	1.61 *dt* (1.7, 13.7)	29.1	1.55 *d br* (13.2)	29.1	1.57 *br dd* (1.5, 13.3)
			0.86 *dt* (2.6, 13.9)		0.82–0.92 *m*		0.86 *ddd* (1.6, 6.4, 13.9)
8	35.7(1)	36.8	1.40–1.45 *m*[c]	35.6	1.30–1.42 *m*	35.5	1.38 *br q* (6.8)
9	37.7(0)	38.9	—	37.9	—	37.9	—
10	35.9(2)	36.7[b]	2.24–2.27 *m*[d]	35.9	2.21 *dd* (1.6, 13.6)	35.8	2.18–2.21 *m*
			1.82 *dd* (2.1, 12.2)		1.80 *d br* (13.2)		1.77 *dd* (1.6, 13.6)
11	37.9(2)	36.6[b]	1.46–1.52 *m*[c]	35.1	1.44–1.52 *m*	34.8	1.47–1.51 *m*
12	19.6(2)	21.1	2.27–2.33 *m*[d]	20.4	2.26–2.34 *m*	20.4	2.25–2.34 *m*
13	125.7(0)	138.8[a]	—	135.1	—	139.5	—
14	111.0(1)	146.1	6.99 *d* (1.2)	143.6	7.13 *t* (1.2)	140.7	6.88 *br s*
15	142.7(1)	99.0	6.06 *s br*	70.2(2)	4.78 *d br* (1.2)	92.5	6.88 *br s*
16	138.4(1)	169.4	—	171.4	—	169.1	—
17	13.8(3)	14.1	0.79 *d* (6.8)	13.8	0.77 *d* (6.4)	13.8	0.77 *d* (6.7)
18	118.2(2)	118.1	5.02 *br t* (2.0)	118.3	5.09 *d br* (2.2)	118.3	5.08 *br s*
			4.82 *br dd* (1.1, 2.1)		4.86 *d br* (2.2)		4.86 *br s*
19	171.7(0)	174.1	—	174.4	—	171.1	—
20	18.6(3)	18.9	0.75 *s*	18.5	0.73 *s*	18.5	0.72 *s*
OAc	—	—				20.7(3)	2.15 *s*
						170.7 (0)	—

*Spectra for **1**, **2** (in MeOH-d_4) and **5** were taken at 300 MHz (^1H) and 75 MHz (^{13}C); for **3** at 200 MHz (^1H) and 50 MHz (^{13}C). Assignments for carbons 1,2,3,6,11 and 12 of **1** were incorrectly reported in ref. [5].

†The number in parentheses indicates the number of attached protons as determined by APT and/or DEPTGL. Assignments are aided by COSY and HETCOR experiments for **1**, **2** and **5**.

[a,b]Interchangeable signals.

[c,d]Overlapped signals, *J*-unresolved.

Table 2. ^{1}H NMR spectral data and coupling constants (in Hz, in parentheses) for compounds **4**, **7** and **8**

H	4	7	8
1	4.05 *dd* (1.3, 6.3)	4.10 *br d* (6.0)	5.32 *br*[a] *s*
2	5.38 *d br* (1.1)	5.39 *br d* (1.2)	5.32 *br*[a] *s*
4	1.92–1.94 *m*	1.85–1.90 *m*	1.95–2.0 *m*
5	1.58–1.64 *m*	1.50–1.65 *m*	1.55–1.65 *m*
6	1.28–1.35 *m*	1.22–1.35 *m*	1.35–1.45 *m*
7	1.96–1.99 *m*	1.85–1.90 *m*	1.95–2.0 *m*
8	1.38–1.47 *m*	1.40–1.50 *m*	—
9	2.17–2.27 *m*	1.15–1.25 *m*	2.10–2.25 *m*
10	6.90 *dq* (1.4, 7.4)	6.80 *br t* (6.9)	6.90 *br t* (7.0)
12	1.83 *br s*	1.83 *d* (1.0)	1.84 *d* (1.0)
14	0.83 *d* (6.9)	0.83 *d* (6.8)	0.83 *d* (6.6)
15	1.67 *br s*	1.68 *br s*	1.69 *br s*
–COMe	—	3.73 *s*	—
OAc	—		2.06 *s*

Spectra for **7** and **8** were measured at 200 MHz and for **4** at 300 MHz.

[a]Signals superimposed on each other, *J*-unresolved.

Table 3. ^{13}C NMR spectral data for compounds **4**, **8**, **10** and **11**

C	4	8	10	11
1	69.1(1)*	72.1	71.9	72.1
2	125.5(1)	121.3	121.2	121.3
3	137.5(0)	139.5	139.4[a]	139.5
4	30.4(2)	30.0	29.9	30.0
5	20.9(2)	20.9	20.9	21.0
6	46.4(1)	42.1	42.2	42.3
7	31.3(1)	31.3	31.4	31.3
8	34.0(2)	33.5	33.5	34.5
9	27.1(2)	26.9	27.0	25.8
10	145.1(1)	145.1	154.7	129.9
11	126.9(0)	127.0	139.6[a]	130.0
12	12.0(3)	12.1	9.0	14.0
13	173.1(0)	173.1	195.3	70.3(2)
14	14.3(3)	14.7	14.8	14.7
15	23.1(3)	23.1	23.1	23.1
OAc(s)	—	21.3(3)	21.4(3)	21.4 (3)
		171.3(0)	171.3(0)	21.0 (0)
				171.2 (0)
				171.0 (0)

Spectra for **8**, **10** and **11** were measured at 50 MHz and for **4** at 75 MHz.

*The number in parentheses indicates the number of attached protons as determined by APT and/or DEPTGL.

[a]Interchangeable signals.

activity against Gram-positive bacteria and the yeast *Candida albicans*,* using a two-fold serial dilution assay [7].

EXPERIMENTAL

Mp: uncorr; IR: KBr and neat; ^{1}H and ^{13}C NMR: 300 (or 200) and 75 (or 50) MHz, respectively, in $CDCl_3$ (unless otherwise stated), TMS as int. standard, standard Varian pulse sequences were used for COSY, HETCOR, DEPTGL (or DEPT) and APT, which aided structural assignments; HR and LRMS: 70 eV; TLC: Et_2O–*n*-hexane (1:1), with visualization by anisaldehyde–H_2SO_4 spray reagent [8]. The plant material was collected in Abha, Saudi Arabia in October 1989. A voucher specimen is deposited in the herbarium of the MAPPRC, College of Pharmacy, KSU, Riyadh 11451, Saudi Arabia.

Isolation of terpenoids. Ground aerial parts of *P. glutinosa* (440 g) were continuously extracted with *n*-hexane in a Soxhlet for 48 hr. The oily residue (15.0 g), obtained after evapn of the solvent in *vacuo*, was partitioned between *n*-hexane (300 ml) and MeCN (4 × 75 ml) presatd with each other. The MeCN fr. (9 g) was subjected to flash CC over silica gel using Me_2CO–*n*-hexane (1:10) to yield crude **1** which upon silica gel CC using 30% Et_2O–*n*-hexane as solvent gave **1** {90 mg R_f 0.50 (Et_2O–*n*-hexane, 1:1), mp 168–169°, $[\alpha]_D^{25}$ −188° ($CHCl_3$; *c* 0.045); lit. mp 160° and $[\alpha]_D$ −182° [5], $[M]^+$, *m/z* 314.1861 by HRMS}. Further elution with Me_2CO–*n*-hexane (1:4) afforded **4**, which was purified by additional silica gel CC (Et_2O–*n*-hexane, 2:3) to yield **3** as a transparent gum (65 mg, R_f 0.42). Finally, elution with Me_2CO–*n*-hexane (3:10) provided a crude mixture of **2** and **3** which was subsequently purified on a short silica gel column using Et_2O–*n*-hexane (1:1) to give **3** (37 mg; R_f 0.40), followed by **2** (60 mg; 0.38).

Pulic acid (**2**). Plates from EtOAc–*n*-hexane, mp 90–91°. $[\alpha]_D^{25}$ −157° ($CHCl_3$; *c* 0.44). IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1740 (lactone), 1670 (CO_2H), 1605, 1435, 1265, 1200 and 1000; UV λ_{max}^{MeOH} (nm): 215 and 245 sh (log ε 3.92); UV $\lambda_{max}^{CHCl_3}$ (nm): 243 (log ε 3.96); EIMS *m/z*: 346 $[M]^+$, $C_{20}H_{26}O_5$ (17%); ^{1}H and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 346 $[M]^+$ (17), 329 $[M-OH]^+$ (72), 311 $[329-H_2O]^+$ (22), 301 $[329-CO]^+$ (7), 283 $[329-CO_2H]^+$ (15), 219 $[M-C_6H_7O_3]^+$ (20), 201 $[329-C_6H_7O_3]^+$ (45), 199 (65), 173 (70), 145 (75), 131 (80), 105 (95) and 91 (100).

15-Deoxypulic acid (**3**). Plates from EtOAc–*n*-hexane; mp 188–190°, $[\alpha]_D^{25}$ −131° ($CHCl_3$; *c* 0.06). IR ν_{max}^{KBr} cm^{-1}: 1740 (lactone), 1680 (CO_2H), 1600 (C=C), 1270 (br), 1200, 1075, 1060, 1040 and 900; UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 242 (3.86); EIMS *m/z*: 330 $([M]^+$; $C_{20}H_{26}O_4$, 5%); ^{1}H and ^{13}C NMR: Table 1; MS *m/z* (rel. int): 330 $[M]^+$ (5), 312 (45), 297 $[312-Me]^+$ (25), 286 $[M-CO_2]^+$ (15), 255 (15), 215 (40), 201 $[312-C_6H_7O_2]^+$ (60), 173 (60), 153 (65), 145 (55), 131 (65), 119 (45), 105 (65) and 91 (100).

Puliglutoic acid (**4**). Gum. $[\alpha]_D^{25}$ −6.8° ($CHCl_3$; *c* 0.08). IR ν_{max}^{neat} cm^{-1}: 3300–3400 (OH), 1680 (CO_2H), 1650, 1450, 1370 and 1280; UV λ_{max}^{MeOH} (nm): end absorption; EIMS *m/z*: 252 ($[M]^+$, $C_{15}H_{24}O_3$, 5%); ^{1}H and ^{13}C NMR: Tables 2 and 3, respectively; EIMS *m/z* (rel. int.): 252 $[M]^+$ (5), 251 (8), 235 (63), 217 $[235-H_2O]^+$ (12), 206 (8), 189 $[235-CO_2H]^+$ (50), 163 (13), 151 (19), 137 (100), 122 (82) and 109 (72).

Acetylation of compound **2**. Compound **2** (25 mg) dissolved in pyridine was treated with Ac_2O at room temp for 24 hr. Usual work-up [9] gave **5** (22 mg) as a gum, $[\alpha]_D^{25}$ −115° ($CHCl_3$; *c* 0.134); IR ν_{max}^{neat} cm^{-1}: 1780 (br, lactone and OAc), 1695 (CO_2H), 1610, 1220, 1200, 1030 and 1000; ^{1}H and ^{13}C NMR: Table 1; FABMS *m/z* (rel. int.): 388 $[M]^+$, $C_{22}H_{28}O_6$ (18).

Methylation of compound **4** *with* CH_2N_2. Compound **4** (10 mg) was treated with ethereal CH_2N_2 at room temp. for 3 hr. The reaction mixt. was dried *in vacuo* to afford an oily residue (**7**; 8 mg), $[\alpha]_D^{25}$ +5.5 ($CHCl_3$; *c* 0.06). IR ν_{max}^{neat} cm^{-1}: 3450 (br, OH), 1705 (CO_2Me), 1645, 1430, 1370, and 1265; ^{1}H NMR: Table 2; EIMS *m/z* (rel. int.): 266 $[M]^+$ (5).

Acetylation of compound **4**. Compound **4** (10 mg) was acetylated as described for **2** to give **8** (8 mg) as a gum, $[\alpha]_D^{25}$ +22° ($CHCl_3$, *c* 0.086). IR ν_{max}^{neat} cm^{-1}: 1740 (OAc), 1690 (COOH) 1460, 1385 and 1250; ^{1}H and ^{13}C NMR: Tables 2 and 3,

*Compound **1** gave MIC values of 10, 20 and 50 μg ml^{-1} against *Bacillus subtilis*, *Staphylococcus aureus* and *C. albicans* (NCTC # 10400, # 6571 and ATCC # 10231), respectively.

respectively; EIMS m/z (rel. int.): 294 $[M]^+$ (2) and 234 $[M-60]^+$ (50).

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