BISABOLENE AND GUAIANE SESQUITERPENES FROM PULICARIA GLUTINOSA

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Abstract—Aerial parts of *Pulicaria glutinosa* yielded three bisabolene sesquiterpenes, puliglutol, puliglutal and puliglutone; structural assignments were based largely on their 2D NMR spectral data. A fourth sesquiterpene, the guaiane cyclo-epoxypuliglene, was also obtained, and its structure and relative stereochemistry were established by X-ray crystallographic analysis.

INTRODUCTION

Pulicaria glutinosa Gaertn. is a perennial herb which is widely distributed throughout Saudi Arabia and adjacent countries [1]. Earlier work on the aerial parts of this plant [2] yielded the two germacrane sesquiterpenes puliglene (1) and epoxypuliglene (2). We now report on the isolation and characterization of four additional sesquiterpenes from this source, namely, the bisabolenes puliglutol (3), puliglutal (4), and puliglutone (5), as well as the guaiane cyclo-epoxypuliglene (6).

RESULTS AND DISCUSSION

A n-hexane extract of the aerial parts of P. glutinosa was partitioned between *n*-hexane and acetonitrile. The latter fraction was flash chromatographed [3] on silica gel to give puliglutol (3) and puliglutal (4) as the major constituents in yields of 0.07 and 0.06%, respectively. Both compounds were obtained as oils that were homogeneous on TLC. Upon acetylation, puliglutol (3) yielded the diacetate 7, while puliglutal (4) gave the monoacetate 8. Their bisabolane carbon skeleton was suggested on the basis of their ¹³CNMR data [4, 5] (Table 1). The gross structure of 3 was substantiated by extensive 2D NMR experiments involving the determination of its COSY, HETCOR and 2D-INADEQUATE spectra, which unambiguously demonstrated the key connectivities between the signals at δ 69.1, 125.7 and 46.2, ascribed to C-1, C-2 and C-6, respectively. The remaining assignments were consistent with structure 3. The structural relationship between puliglutol (3) and puliglutal (4) was readily established by manganese dioxide oxidation of 3 to 4 or, conversely, by sodium borohydride reduction of 4 to 3.

12

6

OH OH .,Н . "н 2 1 13 сно 5 $R_1 = CH_2OH$, $R_2 = OH$ = CHO, $R_2 = OH$ = CH₂OAc, R₂ = OAc OH 10 $R_1 = CHO_1$ $R_2 = OAc$

	3		4	
Atom	¹³ C	¹ H	¹³ C	¹ H
1	69.10 (1)*	4.0 d (1.3, 6.3)	69.03 (1)	4.03 d (1.6, 3.5)
2	125.65 (1)	5.36 d (1.2)	125.58 (1)	5.38 d (1.2)
3	137.33 (0)		137.48 (0)	
4	30.41 (2)	1.89–1.95 m	30.38 (2)	1.96-2.02 m
5	20.79 (2)	1.58–1.62 m	20.89 (2)	1.62 m
6	46.16 (1)	1.23–1.31 m	46.45 (1)	1.31–1.35 m
7	30.63 (1)	1.95–2.0 m	31.28 (1)	1.94 m
8	34.94 (2)	1.31–1.39 m	33.95 (2)	1.50 m
9	25.51 (2)	2.0–2.09 m	27.21 (2)	2.37 m
10	126.38 (1)	5.41 dq (1.3, 7.1)	154.90 (1)	6.50 dq (1.3, 7.4)
11	134.63 (0)		139.24 (0)	
12	13.68 (3)	1.66 br s	9.16 (3)	1.74 br s
13	68.86 (2)	3.96 s	195.32 (2)	9.38 s
14	14.35 (3)	0.80 d (6.9)	14.33 (3)	0.85 d (7.0)
15	25.51 (3)	1.65 br s	23.02 (3)	1.68 br s

Table 1. ¹H and ¹³C NMR chemical shifts and coupling constants (in Hz, in parentheses) for compounds 3 and 4

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

In addition, two minor sesquiterpenes were isolated. One of these, namely, puliglutone (5) was obtained as an oil in 0.004% yield. Its structure was readily determined from its spectral data (see Experimental) and by chemical correlation with 3. Thus, pyridinium chlorochromate oxidation of the latter gave a product indistinguishable from puliglutone (5). Although bisabolene sesquiterpenes have been previously reported [4, 6, 7] in genera belonging to the Compositae, one unique feature in 3 is the presence of the same side chain as that of sirenin [8], which is the sperm attractant produced by the female gametes of the water mould Allomyces.

The remaining minor product, cyclo-epoxypuliglene (6) was obtained in 0.004% yield as crystals. Its NMR spectral data pointed to a cyclised form of epoxypuliglene (2). Unfortunately, treatment of 2 with *p*-toluene sulphonic acid or acidic ion exchange resins did not lead to the formation of 6. The structure and relative stereochemistry* of 6 were unambiguously determined by X-ray crystallographic analysis.

The crystal structure of **6** was determined by direct methods. A view of the solid-state conformation is provided in Fig. 1. Bond lengths are, in general, close to expected values [9], although those involving the C-3 methylene centre [1.556 (3) Å, 1.569 (3) Å] are somewhat elongated indicated strain in this region. Endocyclic

torsion angles in the cyclopentane ring are related by an approximate C_2 axis of symmetry passing through C-3 and the mid-point of the C-1–C-5 bond, and, thus, this ring approximates to a half-chair form. The tetrahydropyran ring has an envelope conformation with C-6 as the out-of-plane atom while the tetrahydrofuran ring is best described as a distorted boat form. Molecules of **6** are associated in the solid state by two O–H... O hydrogen bonds which link molecules related by the 2_1 screw axes along the *b*- and *c*-directions.[†]

EXPERIMENTAL

Mp: uncorr. IR: KBr and neat. ¹H and ¹³C NMR: 300 and 75 MHz, respectively, in CDCl₃ (unless otherwise stated), TMS as int. standard, standard Varian pulse sequences were used for COSY, HETCOR, DEPTGL and APT, which aided structural assignments, 2D-INADEQUATE (CCC2DQ): spectral width (F_1 and F_2), 4470.3 Hz; aquisition time, 0.115 sec, relaxation delay, 3 sec., J_{ec} , 35 Hz, no of increments 32 and no of repetitions 1024 per increment. EIMS: 70 eV. TLC: Et₂O-*n*-hexane (1:1) unless otherwise indicated with visualization by anisaldehyde spray reagent [10]. For collection of plant material and other experimental details see ref. [2].

Isolation of puliglutol (3), puliglutal (4), puliglutone (5) and cyclo-epoxypuliglene (6). Ground aerial parts of P. glutinosa (480 g) was continuously extracted with n-hexane in a Soxhlet for 48 hr. The oily residue (15.2 g), obtained after evapn in vacuo, was partitioned between *n*-hexane (300 ml) and MeCN $(4 \times 75 \text{ ml})$ presatd with each other. The MeCN partition (9 g) was subjected to flash CC over silica gel using Et₂O-n-hexane (1:1) yielded a mixt. (1.2 g) which, upon silica gel CC using CHCl₃ as solvent, gave the minor compound 5 (20 mg; R_f 0.43), followed by an oily material 4 (290 mg: R_f 0.45). Further elution with Et₂O-nhexane (1:1) afforded 3, which was purified by additional silica gel CC (CHCl₃-MeOH, 7:3) to yield a transparent oil, 3 (325 mg, R_f 0.25). Finally, elution with Et₂O-*n*-hexane (4:1) provided crude compound 6 that was subsequently purified on a short silica gel column using THF-Et₂O (1:4) to give 6 (37 mg: R_{c} 0.12).

^{*}Attempts to define the absolute stereochemistry of **6** using the anomalous scattering of X-rays were not conclusive. The reference C-7 isopropyl group is assigned the usual β -orientation. Endocyclic torsion angles $[\omega_{ij}, \sigma \pm 0.1^{\circ}-0.2^{\circ})$ about the bonds between atoms *i* and *j* follow: $\omega_{1,2} - 41.1^{\circ}$, $\omega_{2,3} 17.7^{\circ}$, $\omega_{3,4} 12.5^{\circ}$, $\omega_{4,5} - 38.7^{\circ}$, $\omega_{5,1} 51.7^{\circ}$ in the cyclopentane ring; $\omega_{1,5} - 31.8^{\circ}$, $\omega_{5,6} 51.9^{\circ}$, $\omega_{6,16} - 53.7^{\circ}$, $\omega_{16,10} 33.7^{\circ}$, $\omega_{10,1} 0.1^{\circ}$ in the tetrahydrofuran ring; $\omega_{6,7} - 1.6^{\circ}$, $\omega_{7,8} - 50.5^{\circ}$, $\omega_{8,9} 36.4^{\circ}$, $\omega_{9,10} 27.0^{\circ}$, $\omega_{10,16} - 81.1^{\circ}$, $\omega_{16,6} 66.3^{\circ}$ in the tetrahydropyran ring.

[†]Hydrogen-bonded donor... acceptor distances follow: O-17...O-18 (at -x, -1/2 + y, 1/2 - z) = 2.892(2) Å, and O-18...O-16 (at 1/2 - x, 1 - y, -1/2 + z) = 2.864(1) Å.



Fig. 1. Structure and solid-state conformation of cyclo-epoxypuliglene (6); small circles represent hydrogen atoms.

Puliglutol (3). Oil. $[\alpha]_D^{25} + 17^{\circ}$ (CHCl₃; c 0.051). IR ν_{max}^{neat} (cm⁻¹): 3300 (OH), 1635 (C=C) and 1425. UV λ_{max}^{MeoH} (nm): 226 (log ε 3.80). HRMS (*m*/*z* 220.1821 ([M-H₂O]⁺, 9.9%) with no [M]⁺ observed. ¹H and ¹³C NMR in Table 1. MS (*m*/*z*, % rel. int.): 220 (9.9), 202 (7.6), 187 (7.4), 137 (52.8), 132 (33.1), 119 (48.1), 111 ([C₇H₁₁O]⁺, 8.8), 110 (62.7), 109 (42.9), 93 (55), 84 (100) and 55 (64.3).

Puliglutal (4). Oil. $[\alpha]_{D}^{25} + 15^{\circ}$ (CHCl₃; c 0.059). IR ν_{meat}^{neat} (cm⁻¹): 3350 (OH), 1660 (CHO), 1620 (C=C) and 1425. UV λ_{max}^{meOH} (nm): 226 (log ε 3.87). EIMS (m/z) 236 ([M]⁺, 2.4%). ¹H and ¹³C NMR in Table 1. MS (m/z, % rel. int.): 236 (2.4), 218 (10.9), 179 (11.7), 167 (19.8), 166 (22.7), 161 (12), 148 (24.8), 137 (95.8), 124 (72.6), 111 (28.6), 109 (100), 93 (58.8) and 83 (80.7).

Puliglutone (5). Oil. $[\alpha]_{D}^{25} - 15^{\circ}$ (CHCl₃; c 0.056). IR ν_{max}^{max} (cm⁻¹): 1660–1650 (br, CHO and α, β-unsaturated C=O) and 1420. UV λ_{max}^{MeOH} (nm) 225 (log ε 3.78). ¹H NMR (80 MHz): δ 0.86 (3H, d, J = 6.8 Hz, Me-14), 1.75 (3H, br s, Me-12), 1.95 (3H, s, Me-15), 5.87 (1H, br s, H-2), 6.50 (1H, br t, J = 6.8 Hz, H-10) and 9.39 (1H, s, H-13). GC/MS (m/z): 234 ([M]⁺, 0.5%).

Cyclo-epoxypuliglene (6). Plates from EtOH-Et₂O, mp 166–168°. $[\alpha]_D^{25} + 25^\circ$ (CHCl₃; c 0.056). IR ν_{max}^{KBr} (cm⁻¹): 3520 and 3420 (OH), 1490 and 1180. UV λ_{max}^{MeOH} (nm): 220 (log ε 3.92). HRMS (m/z) 254.1871 (C₁₅H₂₆O₃; [M]⁺, 30%). ¹H NMR (pyridine- d_5 : $\delta 0.88$ (6H, d, J = 6.12 Hz, Me-12 and Me-13), 1.48 (3H, s, Me-15), 1.58 (3H, s, Me-14), 1.50-1.56 (2H, m, H-3), 1.61 (1H, m, H-11), 1.76 (1H, m, H-7), 1.76 and 2.16 (1H each, m, H-8), 2.19 (1H, m, H-1), 2.21 (1H, m, H-2) and 2.51 (1H, dd, br, J = 9.81 and 13.62 Hz, H-2), 2.80 (1H, dd, J=4.11 and 13.89 Hz, H-5), 3.74 (1H, dd, J = 6.81 and 12.87 Hz, H-9) 4.16 (1H, dd, J = 1.5 and 12.87 Hz)4.02 Hz, H-6), 5.57 and 6.12 (1H each, s, D₂O exch). ¹³C NMR (# attached H; pyridine-d₅): δ 17.45 (3, C-15), 19.83 (3, C-13), 20.57 (3, C-12), 23.96 (2, C-3), 26.73 (3, C-14), 32.13 (2, C-8), 33.05 (1, C-11), 38.15 (1, C-7) 48.89 (2, C-2), 53.57 (1, C-1), 67.75 (1, C-5), 73.43 (0, C-10), 74.79 (1, C-9), 75.76 (1, C-6) and 78.20 (0, C-4). MS (m/z, % rel. int.): 254 ([M]⁺, 30), 236 (5), 210 (30), 193 (25), 167 (25), 149(95), 139 (45), 128 (95), 109 (55), 95 (65), 81 (100) and 71 (60).

Acetylation of compound 3. Compound 3 (35 mg) dissolved in pyridine was treated with Ac₂O at room temp. for 24 hr. Usual work-up gave 7 (30 mg) as an oil, $[\alpha]_D^{25} + 66^{\circ}$ (CHCl₃; c 0.05). IR ν_{max}^{nax} (cm⁻¹): 1730 (OAc), 1440, 1370, 1240 and 1020. ¹H NMR (80 MHz): 0.81 (3H, d, J = 6.8 Hz, Me-14), 1.65 (6H, s, Me-12 and Me-15), 2.05 (6H, s, 2 × OAc), 4.43 (2H, s, H-13), 5.31 (2H, br s, H-1 and H-2) and 5.38 (1H, br t, J = 6.8 Hz, H-10). GC-MS (m/z): 262 ($[M - 60]^+$, 9%).

Acetylation of compound 4. Compound 4 (35 mg) was acetylated as described for 3 to give 8 (30 mg), obtained as an oil, $[\alpha]_D^{2s}$ + 78° (CHCl₃; c 0.045). IR v_{max}^{neat} (cm⁻¹): 1730 (OAc), 1685 (CHO), 1640 (C=C), 1440, 1370, 1240 and 1015. ¹H NMR (80 MHz): $\delta 0.86$ (3H, d, J = 6.5 Hz, Me-14), 1.68 (3H, br s, Me-15), 1.75 (3H, br s, Me-12), 2.03 (3H, s, OAc), 5.32 (2H, br s, H-1 and H-2), 6.40 (1H, br t, J = 7.0 Hz, H-10) and 9.38 (1H, s, H-13). GC-MS (m/z): 218 ([M-60]⁺, 25%).

Preparation of compound 5 from 3. Compound 3 (35 mg) and pyridinium chlorochronate (65 mg) were stirred in EtOH-free CH₂Cl₂ (5 ml) at room temp for 4 hr. The reaction mixt. was dild with CHCl₂, filtered and washed with H₂O (3 × 10 ml). The dried CH₂Cl₂ residue was flash chromatographed on a silica gel column eluting with *n*-hexane-Me₂CO (19:1), which gave 5 (17 mg). $[\alpha]_D^{25}$ -15.5° (CHCl₃; c 0.05). UV, IR, ¹H NMR and TLC indistinguishable from those of puliglutone.

Reduction of 4 with NaBH₄. Compound 4 (20 mg) in EtOH (5 ml) was stirred with NaBH₄ (15 mg) at room temp for 3 hr. The reaction mixt. was dild with H₂O (10 ml) and extracted into Et₂O from which compound 3 was obtained as an oil (15 mg). $[\alpha]_D^{25} + 16.8^{\circ}$ (CHCl₃; c 0.05). IR, ¹H NMR and TLC indistinguishable from those of natural product 3.

Oxidation of 3 with MnO₂. Compound 3 (35 mg) and MnO₂ (65 mg) were stirred in *n*-hexane (5 ml) at room temp for 7 days. The reaction proceeded up to 50% completion and showed a single product on TLC. Flash chromatography on silica gel using Me₂CO-*n*-hexane (1:10) gave 4 (17 mg). $[\alpha]_0^{25} + 15.2^{\circ}$ (CHCl₃; c 0.05). IR, ¹H NMR and TLC indistinguishable from those of puliglutal (4).

X-Ray crystal structure analysis of compound 6. $C_{15}H_{26}O_3$, M_r 254.37, orthorhombic, a = 9.633(1), b = 15.963(1), c = 9.230(1) Å (from 25 orientation reflections, $45^\circ < \theta < 48^\circ$), V = 1419.3(4) Å³, Z = 4, $D_{calcd} = 1.190 \text{ g cm}^{-3}$, $\mu(CuK_{\alpha} \text{ radiation}, \lambda = 1.5418 \text{ Å})$ = 6.1 cm⁻¹; space group $P2_12_12_1(D_2^4)$ uniquely from the systematic absences: h00 when $h \neq 2n$, 0k0 when $k \neq 2n$, 00 $l \neq 2n$; sample dimensions: $0.20 \times 0.26 \times 0.50$ mm. Intensity data (+h, +k, +l, 1682 reflections) [CuK_{α} radiation, graphite monochromator; ω -2 θ scans; scanwidth $(1.00 + 0.14 \tan \theta)^{\circ}$; θ_{max} $=75^{\circ}$], were corrected for the usual Lorentz and polarization effects. Those 1578 reflections with $I > 3.0\sigma$ (I) were retained for structural analysis. The crystal structure was solved by direct methods (MULTAN-11/82). Initial carbon and oxygen atom positions were derived from an E-map. Hydrogen atoms were all located in a difference Fourier synthesis evaluated following several rounds of full-matrix least-squares adjustment of the non-hydrogen atom positional and thermal (at first isotropic, then anisotropic) parameters. In the subsequent least-squares iterations, hydrogen atom positional and isotropic thermal parameters, and latterly an extinction correction (g), were included as variables. The refinement converged (max. at $R = \Sigma ||F_{o}| - |F_{c}|| / \Sigma |F_{o}| = 0.033$ shift:esd = 0.02) $\{R_w$ = $[\Sigma w(|F_o| - |F_c|)^2 / \Sigma w|F_o|^2]^{\frac{1}{2}} = 0.046$, GOF = $[\Sigma w(F_o| - |F_c|)^2 / \Sigma w|F_o|^2]^{\frac{1}{2}}$ $N_{\text{observations}} - N_{\text{parameters}}$]¹ = 2.01, g = 9.0 (4) × 10⁻⁶}. A final difference Fourier synthesis was essentially featureless (max. 0.16 eÅ⁻³). Anisotropic temperature factor parameters, hydrogen atom parameters, torsion angles, and a list of observed and calculated structure amplitudes have been deposited at the Cambridge Crystallographic Data Centre. Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from ref. [11]. In the least-squares iterations, $\Sigma w \Delta^2 [w = 1/\sigma^2 (|F_o|), \Delta = (|F_o| - F_c|]$ was minimized.

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