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A TRITERPENE EPOXIDE AND A GUAIANOLIDE FROM *PTILOSTEMMON GNAPHALOIDES**

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Key Word Index—*Ptilostemmon gnaphaloides*; Compositae; lignan; sesquiterpene lactones; triterpene epoxide; taraxasterol derivative; ptiloepoxide; 2D NMR.

Abstract—A guaianolide and a triterpene epoxide with a taraxasterene skeleton (ptiloepoxide) have been isolated from the aerial parts of *Ptilostemmon gnaphaloides* together with known compounds. The structure and relative stereochemistry of the triterpene epoxide were determined by high-field two-dimensional spectroscopy and DIFNOE experiments.

INTRODUCTION

Previous phytochemical studies on the *Ptilostemmon* genus have shown the presence of acetylenes, triterpenes, sesquiterpenes and lignans [1–3]. As part of an investigation of the flora of Calabria [4], we describe the structural determination of a novel triterpene epoxide and a guaianolide, isolated from *P. gnaphaloides*, an endemic species from southern Italy [5].

RESULTS AND DISCUSSION

Upon repeated column chromatography, an extract of the aerial parts yielded mixtures of lupeol and β-amyrin, and of the respective acetyl derivatives, as well as a novel triterpene epoxide, a lignan and two sesquiterpene lactones. The lignan and one of the sesquiterpene lactones were identified as 3″-desoxy-γ-thujaplicaten-4′-*O*-methyl (**1**) and 8-desacyloxy-8α-(2-methylacryloxy)-subluteolide (**2**), respectively, previously isolated from *P. afer* [2]. The ^{13}C NMR data of **2** are reported for the first time (see Experimental).

The second guaianolide was assigned the structure **3** and the name gnaphaloide. In the ^{1}H NMR spectrum, most of the signals of **3** showed similar chemical shifts and the same complexity as those of **2**. The major differences were the absence of the signals caused by the epoxide protons substituted by two one-proton doublets (J = 11.7 Hz) at δ 4.33 and δ 3.95, respectively, attributable to a CH_2–OH. Accordingly, in the ^{13}C NMR spectrum, the C-4 signal was shifted from 68.2 to 84.4 ppm. Gnaphaloide may be generated from **2** by ring opening of the epoxide.

The new triterpene (**4**), named ptiloepoxide, had mass spectral and NMR data consistent with the molecular formula $C_{30}H_{48}O_2$, indicating seven double-bond equivalents in the molecule. A HECTOR [6] experiment was used to establish connectivities between carbons and their directly attached protons in the NMR spectra, while two- or three-bond correlations were determined by long-range HECTOR [7] and INEPT [8] measurements. The complete ^{1}H and ^{13}C assignments are shown in Tables 1 and 2. The NMR spectra displayed resonances for an exocyclic double bond, while, in accordance with IR spectrum, no signal attributable to a carbonyl was evident. This makes necessary the presence of a further cycle in the pentacyclic triterpene framework.

Acetylation of **3** gave the mono-acetate **4**, suggesting that the second oxygen was in form of an epoxide. In addition, the NMR spectra of **3** exhibited signals for three oxymethine groups, only one of which was shifted by acetylation; the methine signals at 64.0 and 56.1 ppm in the ^{13}C NMR spectrum were therefore assigned to an epoxyl group. Because the methine protons at δ 2.90 and 3.47 appended to these carbons were not coupled to any other, the epoxyl function had to be located on the D or E rings of an ursane or taraxasterane skeleton. A comparison of the ^{13}C NMR data with those of taraxasterol and taraxasteryl acetate [9] showed that, except for the ring E carbons, the chemical shifts are almost identical, indicating that the exocyclic double bond and the epoxide in **3** must be located on ring E. The definitive location of the epoxide on C-21 and C-22 was indicated by the long-range connectivities of C-21/C-22 (Table 1), and

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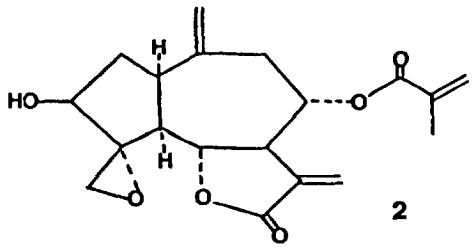

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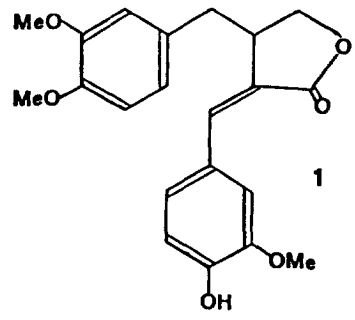

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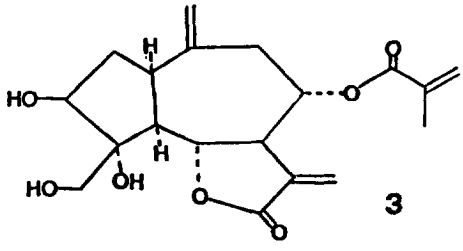

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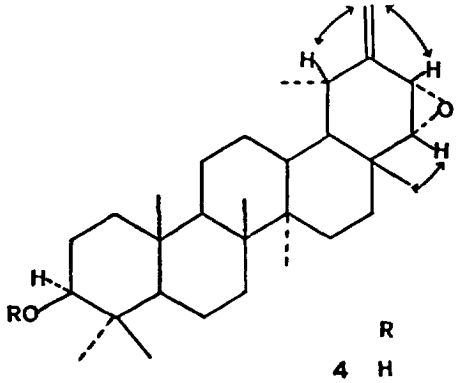

R
4 H
5 Ac

confirmed by INEPT [10] experiments (Table 2). Finally, the configuration of the ring E substituents was assigned by difference NOE [10] enhancements as summarized by the arrows on **4**.

EXPERIMENTAL

Plant material. P. gnaphaloides (Cyr.) Sojak (tribe Cinereae, subtribe Caudineae) was collected in Stilo, Reggio Calabria (southern Italy) and identified by Dr D. Puntillo (University of Calabria). A voucher sample has been deposited at the Botanical Garden of the same University.

Extraction and identification. Air-dried aerial parts (800 g) were extracted with cold Me_2CO and the residue partitioned between hexane and MeOH–H_2O (9:1). The hydroalcoholic portion was chromatographed on a silica gel column. Elution with C_6H_6 containing increasing amount of EtOAc (up to 20%) yielded 5 frs: PG1 (500 mg), PG2 (450 mg), PG3 (800 mg), PG4 (730 mg), and PG5 (400 mg). Repeated chromatography (silica gel, C_6H_6) of PG2 gave a mixture of lupeol and β-amyrin (110 mg) and ptiloepoxide (80 mg). Purification (silica gel; C_6H_6–EtOAc, 9:1) of PG4 yielded gnaphaloide (70 mg) and 3″-deoxy-γ-thujaplicaten-4′-*O*-methylester (100 mg). 8-Desacyloxy-8α-(2-methylacryloxy)-11β, 13-dihydrosubluteolide (110 mg) was obtained from PG5 (silica gel, C_6H_6–EtOAc, 4:1).

Identification of the known compounds. 3″-Deoxy-γ-thujaplicaten-4′-*O*-methyl (**1**) and 8-desacyloxy-8α-(2-methyl-acryloxy)-subluteolide (**2**) were identified by ^{1}H NMR [2]. **2**; ^{13}C NMR (75 MHz, $CDCl_3$), δ: 168.9 (C-12), 166.4 (C-1′), 141.6 (C-10), 137.3 (C-11), 136.0 (C-2′), 126.5 (C-4′), 122.5 (C-13), 118.4 (C-14), 76.8 (C-6), 76.2 (C-8), 74.0 (C-3), 68.2 (C-4), 53.1 (C-5), 48.5 (C-15), 48.1, 45.7 (C-7, C-1), 37.7, 36.6 (C-9, C-2), 18.1 (C-3′).

Gnaphaloide (**3**). Oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 6.20 (*br s*, H-13a, H-4′a), 5.69 (*br s*, H-4′b), 5.61 (*d*, J = 3 Hz; H-13b), 5.14 (*br s*, H-14a), 5.08 (*m*, H-8), 4.82 (*br s*, H-14b), 4.77 (*dd*, J = 11 and 9 Hz, H-6), 4.33 (*d*, J = 11.7 Hz; H-15a), 4.17 (*br d*, J = 6 Hz; H-3), 3.95 (*d*, J = 11.7 Hz; H-15b), 3.60 (*br q*, H-1), 3.17 (*m*, H-7), 2.66 (*dd*, J = 15 and 5.3 Hz; H-9a), 2.53 (*m*, H-2a), 2.43 (*d*, J = 15 Hz; H-9b), 2.33 (*dd*, J = 11 and 10 Hz; H-5), 1.99 (*s*, Me-3′), 1.60 (*dd*, J = 15 and 8 Hz; H-2b); ^{13}C NMR (75 MHz, $CDCl_3$), δ: 169.0 (C-12), 166.5 (C-1′), 142.3 (C-10), 136.9 (C-11), 135.9 (C-2′), 126.7 (C-4′), 122.8 (C-13), 117.8 (C-14), 84.5 (C-4), 76.9 (C-6), 76.5 (C-8), 73.9 (C-3), 57.5 (C-5), 49.8 (C-15), 47.1, 46.5 (C-7, C-1), 37.8, 35.2 (C-9, C-2), 18.2 (C-3′).

Ptiloepoxide (**4**). $C_{30}H_{48}O_2$, mp 219–221° (CH_2Cl_2–heptane), $[\alpha]_D$ + 68 (c = 0.2, $CHCl_3$). IR, ν_{max} cm^{-1}: 3025, 2845, 1205, 780, 650; NMR: Tables 1 and 2; EI-MS (probe) 70 eV, *m/z* (rel. int.): 440 $[M]^+$ (30), 422 (30), 407 (15), 357 (37), 339 (22), 271 (10), 243 (15), 231 (10), 217 (18),

Table 1. NMR data of compounds **4** and **5**

Position	**4**			**5**
	δ_H^*	δ_C	C–H connectivities	δ_C
1	1.73, 0.90	38.7		38.3
2	1.65	27.4		23.6
3	3.20 *dd* (10.7, 5.4)	78.9		80.9
4	—	38.9	0.97, 0.77	37.7
5	0.71	55.3		55.3
6	1.45	18.3		18.1
7	1.37	34.1	1.02	34.0
8	—	41.0	0.95	40.9
9	1.30	50.4		50.2
10	—	37.1		37.0
11	1.58	21.4		21.4
12	1.55	26.2		26.1
13	1.63	37.9		37.9
14	—	42.2		42.2
15	1.75, 1.05	26.5	0.95	26.5
16	1.72, 1.20	33.6		33.6
17	—	36.3		36.3
18	1.40	42.1		42.1
19	2.00 *q* (7.9, 6.7)	36.2	0.81	36.1
20	—	151.2	1.05	151.3
21	3.47 *d* (4.6)	56.1	5.06	56.0
22	2.90 *d* (4.6)	64.0	0.81	64.0
23	0.97 *s*	28.0	0.77	27.9
24	0.77 *s*	15.4		16.5
25	0.84 *s*	16.2		16.3
26	1.02 *s*	16.0		15.9
27	0.95 *s*	14.8		14.7
28	0.81 *s*	15.1		15.1
29	1.05 *d* (6.7)	27.2		27.2
30	5.06, 4.87 *br s*	112.0		112.0
Ac	—	—		171.0, 21.3

*The coupling constants in Hertz are given in parentheses.

Table 2. INEPT experiments on compound **4**

Irradiated resonance (δ)	Proton	Connected carbon	
		^3J	^2J
5.06	H-30 *a*	C-19, C-21	
4.87	H-30 *b*	C-19	
3.47	H-21	C-19	C-20
2.90	H-22	C-16	
2.00	H-19	C-21	C-20
1.05	Me-29	C-20,C-18	

207 (100), 203 (33), 189 (95), 187 (33): Acetylation (pyridine–Ac_2O) gave the mono-acetyl derivative **5**: mp 246–248° (CH_2Cl_2–heptane); ^1H NMR δ: 5.06, 4.87 (*br s*, H_2-30), 4.48 (*dd*, H-3), 3.47 (*d*, H-21), 2.91 (*d*, H-22), 2.05 (*s*, MeAc), 1.99 (*quintet*, H-19), 1.05 (*d*, Me-29), 1.02 (*s*, Me-26), 0.95 (*s*, Me-27), 0.87 (*s*, Me-25), 0.85 (*s*, Me-23), 0.84 (*s*, Me-24), 0.81 (*s*, Me-28).

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