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Photochemical reactivity of 6α-hydroxy-7-keto neoclerodane diterpenoids

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Abstract

The photochemical reactivity, in methanol at λ = 254 nm, of two 6α-hydroxy-7-keto neoclerodane, isoeriocephalin (**1**) and teucrolivin B (**2**) was evaluated. From the first compound, two new products were obtained: the 6β-hydroxy epimer (**3**) and the ε-lactone (**4**). The second one yielded exclusively the new spiro γ-lactone (**5**). The formation of these new products can be explained by the well-known radical mechanism Norrish type I.

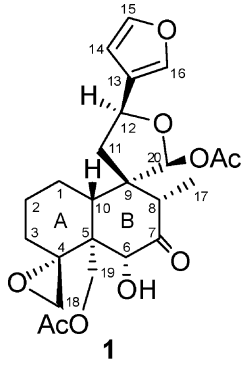
Keywords: Isoeriocephalin; Teucrolivin B; Photochemistry; Neoclerodane; Diterpenes

1. Introduction

The species belonging to the genus *Teucrium* (Lamiaceae) have afforded a great number of diterpenoids and more than 220 neoclerodane diterpenes have been described, all differing in the functional groups on the neoclerodane or 19-nor-neoclerodane skeleton [1]. These secondary metabolites are of interest because of their ecological role as antifeedant against some species of insects [2–5], and potential role in the medicinal properties of the plants [6–8]. As part of our ongoing studies on neo-clerodane diterpenoids, we are interested in obtaining synthetic derivatives of the more abundant compounds in order to investigate how the structural modifications influence the biological activities of these compounds.

2. Results and discussion

In the frame of our works on the photochemical behavior of diterpenoids [9,10], we decided to investigate the reactivity of two neoclerodanes both with a 6α-hydroxy-7-keto structural moiety: isoeriocephalin (**1**) isolated from *Teucrium dunense* [11] and teucrolivin B (**2**) isolated from *Teucrium orientale* and whose biological activity as antifeedant is already known [12].



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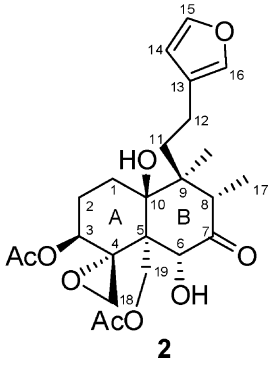

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Isoeriocephalin (**1**) contains in its structure several photochemical active sites such as furan ring, esters and ketone. Irradiation of compound **1** in methanol at 254 nm (1 h) gave a minor product **3** (10% yield) and mainly compound **4** (50% yield), recovering 35% of **1** (Scheme 1). The first product had a molecular formula of $C_{24}H_{30}O_9$ as indicated by its MS (M^{*+} 462). Its ^1H and ^{13}C NMR spectra (Table 1) were quite similar to those of isoeriocephalin (**1**), the main differences being the upfield shifts of the signals of H-6 (δ_H 3.81 br s) and H-19 protons (AB system, δ_H 4.08 d and 4.00 d, J = 12.0 Hz) and the downfield shifts of H-18A (δ_H 3.65 dd), H-8 (δ_H 3.41 q) and H-10 (δ_H 2.73 dd). These data can be justify only by assuming a β-orientation of the hydroxy group at C-6 that deshields H-8 and H-10 due to a 1,3-diaxial relationship. The HMBC spec-

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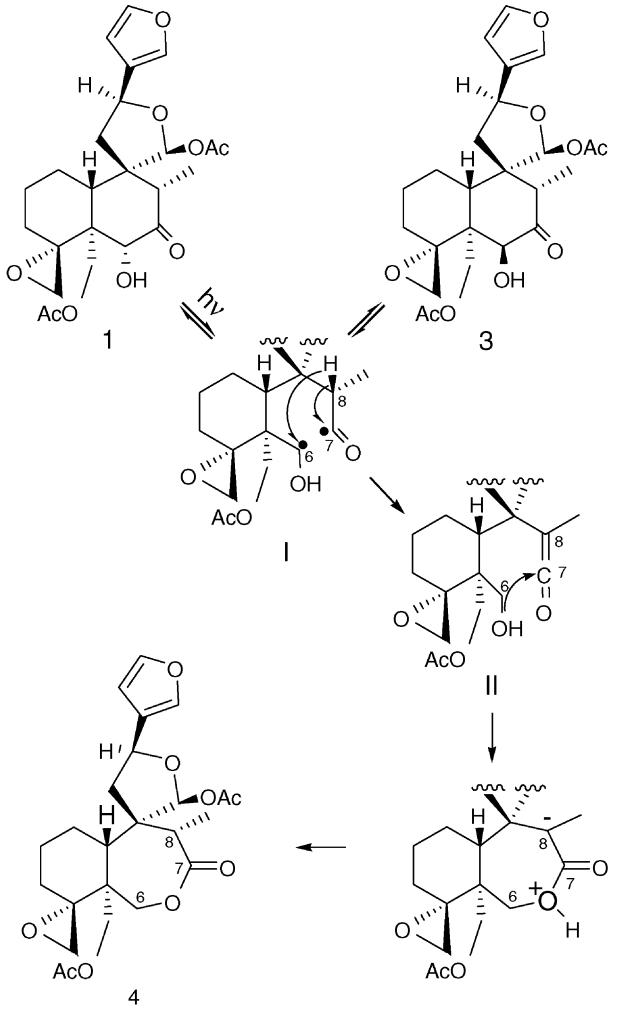


Scheme 1. Photochemical mechanism hypothesized for **1**.

Table 1
NMR spectral data for **3** in $CDCl_3$

C/H no.	δ_H mult. (*J* in Hz)	δ_C[a]	HMBC (H)
1α	2.07[b]	23.15	3β, 10
1β	1.97[b]		
2a	1.92[b]	24.45	
2b	1.58[b]		
3α	2.25 dddd (13.5, 13.5, 4.0, 2.0)	33.39	
3β	1.19 ddd (13.5, 4.0, 3.7)		
4		60.86	3α, 3β, 6α, 18a, 19a, 19b
5		47.05	
6α	3.81 br s	73.89	19a, 19b
7		208.79	6α, 8, 17
8β	3.41 q (6.8)	46.50	6α, 10, 17, 20
9		54.98	8, 11, 17, 20
10β	2.73 dd (12.5, 4.0)	45.06	6α, 11, 19b
11	2.38 d (8.5) (2H)	47.22	8, 10
12	4.84 td (8.5, 0.8)	69.91	11
13		124.78	11, 12, 14, 15, 16
14	6.43 dd (1.8, 0.9)	108.58	12, 15, 16
15	7.39 dd (1.8, 1.7)	143.64	14, 16
16	7.41 ddd (1.7, 0.9, 0.8)	139.88	12, 14, 15
17	1.38 d (6.8) (3H)	9.57	8
18a	3.65 dd (5.0, 2.0)	55.15	
18b	2.46 d (5.0)		
19a	4.08 d (12.0)	63.22	10
19b	4.00 d (12.0)		
20	6.08 s	96.70	8, 10, 11
Ac	2.09 s	170.41	19a, 19b
		20.95	
Ac	2.07 s	169.68	20
		20.84	

[a] Assigned by HMQC.
[b] Assigned by TOCSY 2D.

trum allowed us to assign all the ^1H and ^{13}C signals (Table 1) and consequently the new structure of 6-*epi*-isoeriocephalin (**3**) was given to this product.

The second one had a molecular formula of $C_{24}H_{30}O_9$ as indicated by its MS (M^{*+} 462). ^1H and ^{13}C NMR spectra (Table 2) showed the presence of an epoxide, a furan ring, two acetoxy groups and an acetalic ring, quite similar to those present in the starting material. The absence of the signals of H-6β and ketone at C-7 of isoeriocephalin (**1**) and the presence in the spectra of compound **4** of an additional AB system (δ_H 4.37 d and 4.04 d, $J = 12.7$ Hz) and of a lactone (δ_C 174.72) were the main observed differences. Consequently we proposed for compound **4** the structure depicted in Scheme 1 with a ε-lactone. Our conclusion was confirmed by the correlations between C-7 and H-6α, H-6β, H-8 and Me-17 observed in the HMBC spectrum and by the full attributions of ^1H and ^{13}C NMR signals that were obtained by HMQC and HMBC experiments, as reported in Table 2. Furthermore a ROESY spectrum confirmed the stereochemistry of all the stereogenic centers and identified the signal of H-6β at δ 4.04 due to its clear correlation with H-8, H-10 and H-18A.

Our investigation was extended to the other neoclerodane, teucrolivin B (**2**), with the same 6α-hydroxy-7-keto structural moiety. Irradiation of compound **2** in methanol at 254 nm (1 h) only gave one product (**5**) with a 70% yield. Its MS (M^{*+} 464) indicated a molecular formula of $C_{24}H_{32}O_9$ (Scheme 2). The ^1H and ^{13}C NMR spectra run at room temperature showed broad signals due to a conformational equilibrium. Therefore the spectra were run at different temperatures and the one's run at 333 K showed well defined signals. Some structural moieties such as an epoxide, a furan ring, also present in the starting material, were clearly identified (Table 3). On the other hand, the absence of the signals of H-6β and ketone at C-7 of teucrolivin B (**2**) and the presence of an additional AB system (δ_H 3.65 and 3.51 d, $J = 13.5$ Hz) and of a lactone (δ_C 179.01) showed that the irradiation changed the structure of ring B. The absence of the correlations between C-7 and H-6a, H-6b in the HMBC spectrum, and the downfield shift of C-10 (δ_C 92.06) with respect to the starting material clearly indicated the presence of a γ-lactone involving C-7 and C-10. In order to determinate the stereochemistry of all the stereogenic centers a ROESY experiment was carried out. Clear correlation peaks were observed between H-8 at δ_H 3.21 and methyl group (C-17) and, unexpectedly, with H-6b at δ_H 3.51 and methyl group (C-20) at δ_H 1.35. Furthermore, methyl group (C-20) at δ_H 1.35 showed a NOE correlation with proton H-8 but not with methyl C-17. These facts

Table 2
NMR spectral data for **4** in $CDCl_3$

C/H no.	δ_H mult. (*J* in Hz)	ROESY (H)	δ_C[a]	HMBC (H)
1a	1.87[b]		23.90	3β, 10
1b	1.87[b]			
2a	2.10[b]	2b	25.34	3α
2b	1.50[b]	1, 2a, 3β		
3α	2.38 dddd (13.5, 13.5, 4.5, 2.4)	3β, 19a	31.07	1
3β	1.06 ddd (13.5, 4.5, 2.3)	2b, 3α		
4			61.79	2a, 3α, 3β, 6α, 10, 19a, 19b
5			45.78	3β, 6α, 10, 19a, 19b
6α	4.37 d (12.7)	6β, 18a	66.29	19a
6β	4.04 d (12.7)	6α, 8, 10, 18a		
7			174.72	6α, 6β, 8, 17
8β	3.16 q (6.7)	6β, 10, 17	40.73	11a, 17
9			55.24	8, 10, 11a, 11b, 17, 20
10β	1.87[b]	6β, 8, 18a,	58.49	6α, 6β, 8, 11a, 11b, 20
11a	2.71 dd (14.0, 7.0)	11b, 12, 17	42.66	10, 12, 20
11b	2.17 dd (14.0, 8.5)	10, 11a		
12	5.19 dd (8.5, 7.0)	1, 11a, 11b	74.17	11a, 20
13			127.56	11a, 11b, 12, 14, 15, 16
14	6.42 dd (1.8, 0.8)	11a, 17	108.70	12, 15, 16
15	7.40 dd (1.8, 1.7)	14	143.67	14, 16
16	7.36 dd (1.7, 0.8)	12, 11a	139.57	15, 14, 12
17	1.48 d (6.7) (3H)	8, 11a	17.13	8
18a	2.61 dd (4.0, 2.4)	6β, 6α, 18b	47.32	3α
18b	2.34 d (4.0)	18a		
19a	5.10 d (11.4)	1, 3α, 19b, 20	59.61	10
19b	4.23 d (11.4)	19a, 20		
20	6.24 s	1, 19a, 19b	97.88	
Ac19	2.12 s (3H)		170.55	19a, 19b, Ac(19)
			21.00	
Ac20	2.08 s (3H)		169.48	20, Ac(20)
			20.92	

[a] Assigned by HMQC.
[b] Overlapped signal.

show that methyl C-20 and proton H-8, in the γ-lactone, have a *cis* relationship and, then, the C-8 configuration was reversed with respect to the starting material.

The photochemical reactive site in both molecules (**1** and **2**), at 254 nm, was only the 6α-hydroxy-7-keto moiety undergoing to a Norrish type I cleavage [13], with formation of biradical intermediate **I** which loses the stereochemistry of carbon 6. This photochemical mechanism is widely accepted for this kind of products [14,15]. In this condition the furan chromophore remained unchanged. The reformation of the C(6)–C(7) bond in intermediate **I** can give the starting material and its epimer (**3**) at C-6 (Scheme 1). Although the formation of compound **4** could be explained by a direct rearrangement of biradical **I**, it is more probable that both products **4** and **5** could be justified through a common pathway since their very similar structural features. This mechanism also explains the reversed stereochemistry at C-8 in compound **5** with respect to the starting material **2**. In fact the intermediate **I** can evolve in the ketene **II** with a planar arrangement of carbons C-7 and C-8. This ketene can react with the only available hydroxyl group at C-6 of intermediate **I** forming compound **4** (Scheme 1) with the same configuration at C-8 of starting material **1**. This is a preferred configuration because the methyl group has a pseudoequatorial arrangement.

The analogue ketene **III**, deriving from compound **2**, prefers to react with the hydroxyl group at C-10 rather than with the hydroxyl group at C-6 to form the γ-lactone **5** (Scheme 2) more stable than the ε-lactone. In this case, the rearrangement of zwitterion **IV**, through a probable intramolecular proton transfer from hydroxy group in C-6 or a solvent protonation from the less sterically hindered face, gives compound **5** in which the C-17 methyl group has a *trans* relationship with C-20 methyl group and therefore a reversed stereochemistry at C-8. On the other hand, in compound **4**, the C-17 methyl group assumes an α pseudoequatorial stereochemistry, identical with respect to starting material.

It is quite interesting to note that these photoreactions show an unusual behaviour since they are extremely selective and no side products were detected with common analytic methods.

We performed then the same reactions in different conditions: in the presence of oxygen, we observed that the formation of photoproducts was quenched (clearly indicating the presence of radical intermediate species); at different wavelength irradiations (λ = 313 and 360 nm) no significant reactivity was observed.

Although compounds **3**–**5** have not been isolated yet as natural products it is possible to assume that they could be identified

Table 3
NMR spectral data for **5** in $CDCl_3$

C/H no.	δ_H mult. (*J* in Hz)	ROESY (H)	δ_C[a]	HMBC (H)
1α	2.13[b]		21.03	
1β	2.25 ddd (15.0, 4.5, 4.0)	2β, 11a, 11b, 12a, 19b		
2α	2.08[b]		26.43	
2β	1.93 dddd (12.0, 12.0, 12.0, 4.5)			
3α	5.39 dd (12.0, 5.0)	19b, 2α	65.98	Ac(3), 2β
4			60.55	18a
5			53.60	
6a	3.65 d (13.5)	6b, 18b	56.77	19b
6b	3.51 d (13.5)	6a, 8, 18b, 20		
7			179.01	17
8	3.21 q (7.5)	6b, 17, 20	41.87	17, 20
9			50.41	11a, 11b, 17, 20
10			92.06	11, 19b, 20
11a	1.72 ddd (15.0, 12.5, 4.3)	1β, 11b, 12a, 12b, 17, 20	37.39	20
11b	1.56 ddd (15.0, 12.5, 4.8)	1β, 11a, 12a, 12b, 17, 20		
12a	2.46 ddd (15.0, 12.5, 4.3)	1β, 11a, 11b	21.16	14
12b	2.37 ddd (15.0, 12.5, 4.8)	11a, 11b, 17		
13			123.98	12, 14, 15, 16
14	6.20 br d (1.9)	15	110.35	15, 16
15	7.32 dd (1.9, 1.8)	14	143.15	14
16	7.17 m		138.39	12a, 12b, 14, 15
17	1.19 d (7.5) (3H)	8, 11a, 11b, 12b	9.20	
18a	2.75 d (3.7)	18b, Ac(3)	43.46	
18b	2.52 d (3.7)	6a, 6b, 18a		
19a	5.02 br d (12.5)	19b, 20	61.43	Ac(19)
19b	4.30 d (12.5)	1β, 3α, 19a, 20, Ac(19)		
20	1.35 s (3H)	6b, 8, 11a, 11b, 19a, 19b	21.63	
Ac3	2.01 s (3H)		169.74	
			20.94	
Ac19	2.12 s (3H)		172.86	19b
			20.82	

[a] Assigned by HMQC.
[b] Assigned by TOCSY 2D.

in the future in the extract of some plant species. In fact this is the case of the rearranged neoclerodane fruticolide, isolated as natural product from *Teucrium fruticans* [16], and successively synthesized by photochemical means starting from fruticolone co-occurring in the same species [14].

3. Experimental

3.1. General

Ir spectra were determined with a Perkin-Elmer 257 instrument. ^1H and ^{13}C NMR spectra were obtained on Bruker AMX-600 operating at 600.13 and 150.9 MHz for proton and carbon, respectively. DEPT experiments were acquired on a Bruker AMX-300 spectrometer. Measurements were made on solution in $CDCl_3$, chemical shifts were referred to TMS set at 0 ppm, and coupling constants are given in Hz. Heteronuclear two-dimensional ^1H-^{13}C correlations, one-bond HMQC (heteronuclear multiple quantum correlation) [17] and long-range HMBC (heteronuclear multiple bond correlation) [18], were carried out in the ^1H-detected mode with broad-band decoupling in the ^{13}C domain. ROESY [19] experiments were obtained using as spin-lock a continuous low power transmitter pulse and mixing time of 0.2 and 0.4 s, using standard BRUKER pulse sequence. MS were recorded on a Finnigan TSQ70 instrument (70 eV, direct inlet). Elemental analysis was carried out with a Perkin-Elmer 240 apparatus. Flash chromatography was performed by using silica gel (Merck, 0.040–0.063 mesh) and mixtures of EtOAc and light petroleum (fraction boiling in the range 40–60 °C) in varying ratios. Anhydrous methanol (from Romil Pure Chemicals) were used as received.

3.1.1. Compound *1*

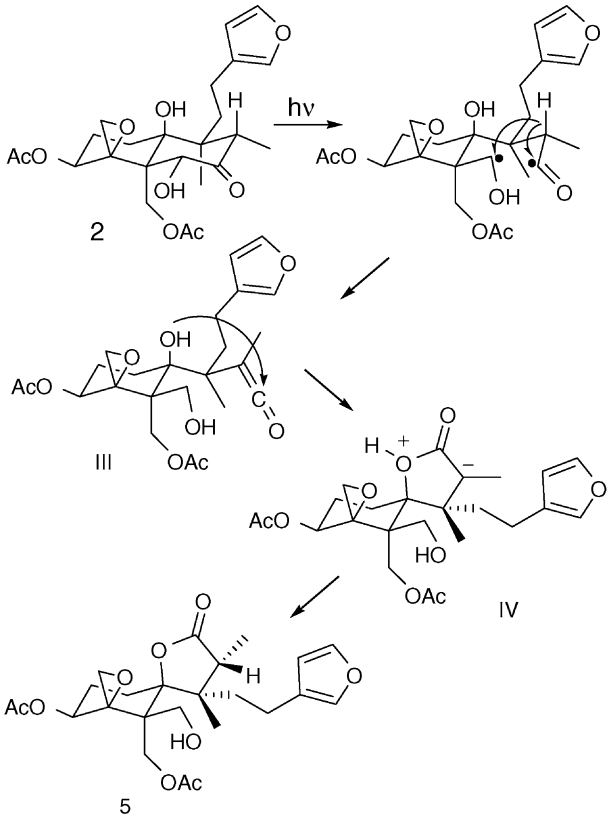
Isoeriocephalin (**1**) was isolated from *Teucrium dunense* as previously reported [11].

3.1.2. Compound *2*

Teucrolivin B (**2**) was isolated from *Teucrium orientale* as previously reported [12].

3.1.3. General procedure for photochemical reactions

A solution of 100 mg of compounds **1** or **2** (0.22 mmol) in anhydrous methanol (150 ml) was partitioned into six quartz tubes, purged by nitrogen bubbling (10 min). The solution was then irradiated by using a Rayonet RPR-100 photoreactor equipped with 16 Hg lamps at λ = 254 nm (RPR-2573A) and a merry-go-round apparatus. The photoreaction was followed by TLC and irradiation was stopped after 1 h to avoid a deep degra-



Scheme 2. Photochemical mechanism hypothesized for **2**.

dation. The solvent was evaporated to dryness under red. pres. at low temperature (25°) yielding a residue which was subjected to column chromatography eluting with light petroleum/EtOAc at various ratios. Yields are referred to reacted product.

*3.1.4. Irradiation of compound **1***

Irradiation of compound **1** gave compound **3** (10 mg, 10%), compound **4** (50 mg, 50%) and starting material **1** (35 mg, 35%).

Compound **3**: amorphous solid. $[\alpha]_D^{20} = -31.5$ (*c* 0.13, $CHCl_3$). IR (film) $\nu_{max} = 3460, 3070, 1760, 1740, 1715, 1505, 1250, 1020, 915, 875\,cm^{-1}$. ^1H NMR (600.13 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (150.9 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (%): 462 $[M]^+$ (1), 433 (1), 403 (10), 373 (15), 360 (30), 332 (18), 255 (15), 163 (38), 145 (30), 111 (37), 94 (55), 43 (100). $C_{24}H_{30}O_9$ (462.49): calculated: C, 62.33; H, 6.54; found: C, 62.29; H, 6.57.

Compound **4**: amorphous solid. $[\alpha]_D^{20} = +2.7$ (*c* 1.54, $CHCl_3$). IR (film) $\nu_{max} = 3080, 2953, 2928, 1740, 1448, 1373, 1232, 1165, 1026, 955, 876, 704\,cm^{-1}$. ^1H NMR (600.13 MHz, $CDCl_3$): see Table 2. ^{13}C NMR (150.9 MHz, $CDCl_3$): see Table 2. EIMS *m/z* (%): 462 $[M]^+$ (1), 420 (15), 403 (10), 361 (22), 343 (15), 286 (10), 255 (18), 205 (25), 190 (30), 153 (50), 121 (58), 111 (100), 81 (89), 79 (82). $C_{24}H_{30}O_9$ (462.49): calculated: C, 62.33; H, 6.54; found: C, 62.38; H, 6.50.

*3.1.5. Irradiation of compound **2***

Irradiation of compound **2** gave compound **5** (70 mg, 70%) and starting material **2** (25 mg, 25%).

Compound **5**: amorphous solid. $[\alpha]_D^{20} = -26.6$ (*c* 0.79, $CHCl_3$). IR (film) $\nu_{max} = 3480, 2953, 2880, 1774, 1760, 1480, 1390, 1267, 1064, 975, 892\,cm^{-1}$. ^1H NMR (600.13 MHz, $CDCl_3$): see Table 3. ^{13}C NMR (150.9 MHz, $CDCl_3$): see Table 3. EIMS *m/z* (%): 464 $[M]^+$ (1), 405 (1), 344 (2), 253 (6), 232 (10), 205 (18), 177 (20), 150 (62), 108 (58), 81 (100), 69 (30). $C_{24}H_{32}O_9$ (464.51): calculated: C, 62.06; H, 6.94; found: C, 62.10; H, 6.99.

Acknowledgments

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