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Stereostructures of New Bioactive Sesterterpenes Isolated from the Caribbean Sponge *Cacospongia* cf. *linteiformis*

Alfonso Carotenuto[a], Maria R. Conte[a], Ernesto Fattorusso[a]*, Virginia Lanzotti[b], Silvana Magno[a]

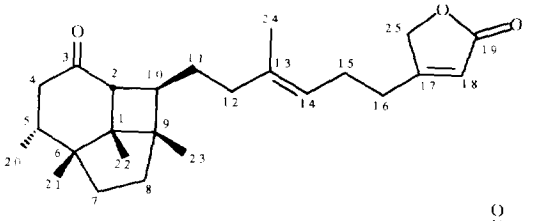
[a]Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli Federico II, Via D. Montesano 49, I-80131 Napoli, Italy.

[b]Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Via Tiberio 21, I-86100 Campobasso, Italy

Abstract: Three new ichthyotoxic and antifeedant sesterterpenes have been isolated from *Cacospongia* cf. *linteiformis* and their stereochemistries determined by chemical evidences and/or spectroscopic methods. The new metabolites are strictly related to previously isolated compounds from the same source.

The marine sponge *Cacospongia* cf. *linteiformis* elaborates a wide variety of biologically active sesterterpenes[1-3] among which we have isolated lintenone (**1**) a novel compound based on an unprecedented skeleton[1]. The finding in the same source of a further metabolite, cyclolinteinone (**2**), also based on a novel rearranged skeleton, allowed us to hypothesize a plausible biogenetic scheme which accounts for the *in vivo* assemblage of both the tri- and mono-carbocyclic frame works starting from a common precursor[2]. With the aim of verifying this hypothesis we are currently examining the other constituents of this organism. In the present paper we report on the isolation and structure elucidation of three further sesterterpenes, two of them were found to be stereoisomers of lintenone (**3** and **4**), whereas the third compound was formulated as the 3-deoxo-derivative of cyclolinteinone (**5**).

The least polar compound **5** was isolated from the EtOAc extract of the sponge as an optically active colourless oil. Its elemental composition, $C_{25}H_{38}O_2$, was established by high-resolution mass measurement on molecular ion at m/z 370 in the EI mass spectrum. The infrared spectrum (ν_{max} 1782, 1749 cm^{-1}) indicated the presence of the same α,β unsaturated γ-lactone functionality already found in **1** and **2**, as confirmed by ^1H- [δ 5.84 (bs, 1H, H-18), 4.73 (bs, 2H, H_2-25)] and ^{13}C [δ 174.13 (C-17), 115.62 (C-18), 170.21 (C-19), 73.14 (C-25)] NMR spectra. Both the last two spectra showed a striking resemblance to those of cyclolinteinone. Particularly, when comparing the NMR spectra of **5** with that of **2**[3], the most evident differences regarded the signals of H_3-20 and H-2 which suffered remarkable upfield shifts in **5** [compound **2** : δ 1.93 (s, 3H, H_3–20), 5.86 (s, 1H, H–2) and δ 20.74 (C-20), 126.44 (C-2), 167.90 (C-1); compound **5** : δ 1.62 (s, 3H, H_3–20),



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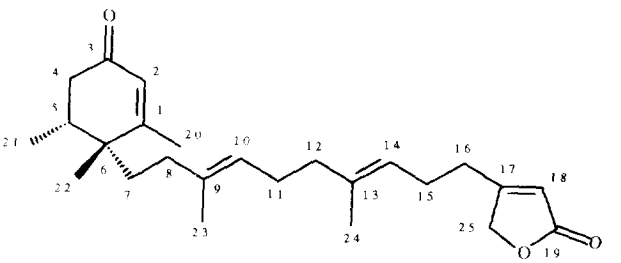

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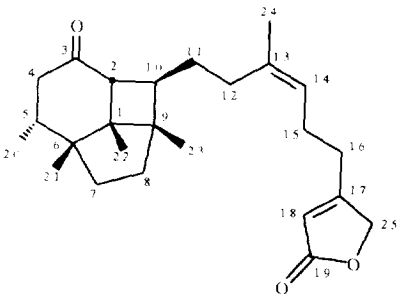

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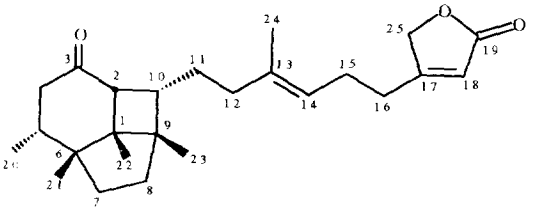

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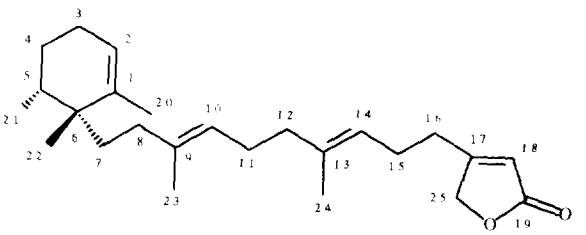

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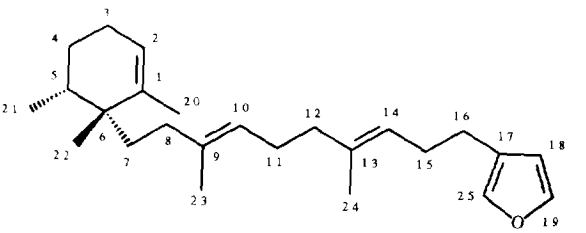

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5.39 (m, 1H, H-2) and δ 19.62 (C-20), 122.66 (C-2)]. These data along with the upfield shift of the C-1 signal (δ 139.43 in **5** vs δ 167.90 in **2**) in the ^{13}C NMR spectrum of **5** which, in contrast with the spectrum of **2**, displaced a CH_2 resonance at δ 1.95 in place of the C=O signal of C-3, strongly suggested that the difference between these two metabolites was the absence of the carbonyl function in **5**. Two dimensional homo- and hetero-nuclear (direct and long-range) shift correlated NMR spectra of **5**, which allowed to assign all the proton and carbon resonances (see Table I), fully confirmed this hypothesis.

Table I. ^{13}C and ^{1}H assignments[a] and long range carbon-proton correlation for compound **5**

carbon	DEPT	^{13}C	^{1}H	$^{1}H/^{13}C$ long range correlation
1	C	139.43		20, 22
2	CH	122.66	5.39 m	20
3	CH_2	23.98	1.95 m	4
4	CH_2	27.44	1.60[b]	3, 21
5	CH	37.59	1.55[b]	21, 22
6	C	39.50		2, 20, 21, 22
7	CH_2	34.71	1.35 m, 1.48 m	22
8	CH_2	35.66	1.85 m, 1.95 m	23
9	C	136.66		23
10	CH	123.23	5.07 bt	23
11	CH_2	26.50	2.08 m	12
12	CH_2	39.62	2.00 m	24
13	C	137.60		24
14	CH	121.89	5.08 bt	24
15	CH_2	25.74	2.30 bq	16
16	CH_2	28.78	2.45 bt	15
17	C	174.13		18
18	CH	115.62	5.84 bs	16, 25
19	C	170.21		16, 25
20	CH_3	19.62	1.62 s	2
21	CH_3	15.92	0.94 d	4
22	CH_3	26.43	1.02 s	
23	CH_3	16.14	1.59 s	
24	CH_3	16.19	1.62 s	
25	CH_2	73.14	4.73 bs	18

[a] J (Hz) 5-21 = 7.0; 10-11 = 7.5; 14-15 = 7.5; 15-16 = 7.5.

[b] superimposed by other signals.

Conclusive proof was obtained by LAH/$AlCl_3$ reduction of **2** which yielded the furan-containing compound **6**, identified by spectroscopic data (see Experimental), identical in all respect to that obtained from **5** under the same experimental contitions. This result also interrelated cyclolinteinone and the new compound at the two chiral centers C-5 and C-6.

Metabolites **3** and **4** occurred in the organic extract of *C.* cf. *linteiformis* in remarkably different relative amounts (0.00013 and 0.0021 % of dry weight, after extraction, respectively). Data from HRMS and ^{13}C NMR spectroscopy (Experimental and Table II) established a molecular formula of $C_{25}H_{36}O_3$ for these compounds, thus indicating them to be isomers of lintenone. Their IR spectra were almost superimposible to each other and to that of lintenone thus suggesting the presence of the same functionalities (ν_{max} 1783, 1749 cm^{-1} for α,β-unsaturated γ-lactone and 1714 cm^{-1} for ketone). Also ^1H and ^{13}C NMR and CD spectra of **3** and **4** were very similar and strongly reminiscent of those of lintenone. Having in mind the NMR data of lintenone, the chemical shifts, multiplicities and coupling constants for all protons of **3** and **4** were readily defined by homo- and hetero-nuclear NMR experiments. Similarly all the resonances in the ^{13}C NMR spectra of **3** and **4** were assigned to the pertinent carbons (see Table II).

The whole of the data led us to conclude that both compounds possessed the same gross structure as lintenone and, therefore, a more detailed analysis of ^1H and ^{13}C NMR spectra needed to define the stereochemical details which made different the three sesterterpenes from each other.

An accurate comparison of ^1H and ^{13}C NMR spectra of **3** with those of **1** revealed that the main differences of chemical shifts were confined to the proton and carbon atoms close to the Δ^{13} double bond [compound **3** : δ 1.78 and 2.07 (H_2-12), 1.66 (H_3-24) and δ 29.73 (C-12), 122.34 (C-14), 23.22 (C-24); compound **1** : see Table II]. That the two compounds were geometric isomers about this double bond was confirmed by nOe difference experiments which indicated a spatial proximity between H_3-24 and H-14, thus pointing to a Z-configuration for the Δ^{13} double bond in **3**. The remainder of nOe data showed the same interproton contact found in lintenone and this was indicative that the two compounds were characterized by the same relative stereochemistry at the six stereogenic chiral centers present in the molecules.

As for compound **4**, NMR data [^1H : δ 1.59 (H_3-24); ^{13}C : δ 38.51 (C-12), 121.68 (C-14), 16.15 (C-24)] already showed that it possessed the E configuration at C-13 double bond as lintenone. This was also confirmed by nOed's experiments which showed the nOe proximity of H_2-15 with H_3-24.

Therefore, the difference between lintenone and **4** must regard some of the six stereogenic centers contained in the tricarbocyclic part structure. As already discussed for lintenone, the fusion of the three rings required a unique, well defined relative configuration for chiral carbons C-1, C-2, C-6 and C-9 and this limited our investigation to C-5 and C-10. On the other hand the inversion of configuration at C-5 could be ruled out by CD spectrum of **4**, which is almost superimposable to that of **1**, thus also pointing to the same absolute stereochemistry around the C=O chromophore in the two molecules. A comparative analysis of ^{13}C NMR chemical shift of **4** and lintenone showed that the most significant variations were found for the atoms surrounding the chiral centers C-10 thus confirming this carbon as the responsible for the difference between the two stereoisomers. This was definitely substantiated by nOed's experiment. Particularly, a cis relationship as in the case of lintenone, was found among H-5, H_3-21 and H_3-22 whereas the inversion of configuration at C-10 was already indicated by intense nOe effects among H-10, H_3-23, H_3-22 and H-2 (Figure I).

Table II. ^{13}C and ^{1}H assignments for compounds **4**[a] and **3**[b] in comparison with that of lintenone (**1**)[1]

		4		**3**		**1**	
carbon	**DEPT**	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	C	51.17		50.71		50.71	
2	CH	51.17	2.35 d	57.11	2.02 d	56.68	2.05 d
3	C	214.88		214.81		214.76	
4	CH_2	44.66	2.32 m, 1.94 m	43.85	2.27[c]	43.78	2.21[c]
5	CH	35.52	1.90 m	35.65	1.94 m	35.73	1.89 m
6	C	45.18		45.61		45.57	
7	CH_2	31.17	1.35 m, 1.50 m	30.39	1.40 m, 1.98 m	30.42	1.38 m, 1.92 m
8	CH_2	32.24	1.39 m, 1.71 m	38.83	1.52 m	38.76	1.48 m
9	C	47.39		47.39		47.33	
10	CH	42.37	2.20 m	43.16	1.80 m	42.42	1.79 m
11	CH_2	24.66	1.16 m, 1.62 m	31.25	1.33 m, 1.57 m	30.89	1.40, 1.55[c]
12	CH_2	38.51	1.88 m	29.73	1.78 m, 2.07 m	37.16	1.80 m, 1.97 m
13	C	137.73		137.87		137.38	
14	CH	121.68	5.06 bt	122.34	5.05 bt	121.90	5.05 bt
15	CH_2	25.57	2.25 bq	25.55	2.27 bq	25.58	2.26 bq
16	CH_2	28.68	2.42 bt	28.96	2.43 bt	28.68	2.42 bt
17	C	174.17		174.04		174.08	
18	CH	115.43	5.79 bs	115.70	5.86 bs	115.40	5.82 bs
19	C	170.38		170.45		170.21	
20	CH_3	17.67	0.89 d	17.59	0.96 d	17.57	0.93 d
21	CH_3	22.11	0.84 s	23.45	0.89 s	23.41	0.87 s
22	CH_3	17.96	1.06 s	19.47	0.98 s	19.38	0.95 s
23	CH_3	25.87	1.06 s	16.95	1.02 s	16.94	1.00 s
24	CH_3	16.15	1.59 s	23.29	1.66 s	16.13	1.56 s
25	CH_2	73.19	4.69 bs	73.17	4.75 bs	73.10	4.72 bs

[a] J (Hz) : 7a - 7b = 15; 5 - 20 = 7; 4ax - 4eq = 16; 4ax - 5 = 12; 4eq - 5 = 6; 2 - 10 = 8; 11a - 11b = 16; 14 - 15 = 7.5; 15 - 16 = 7.5.

[b] J (Hz) : 7a - 7b = 15; 5 - 20 = 7; 4ax - 4eq = 16; 4ax - 5 = 11; 4eq - 5 = 5.5; 2 - 10 = 7.5; 11a - 11b = 15; 14 - 15 = 7.5; 15 - 16 = 7.5.

[c] further coupled AB system.

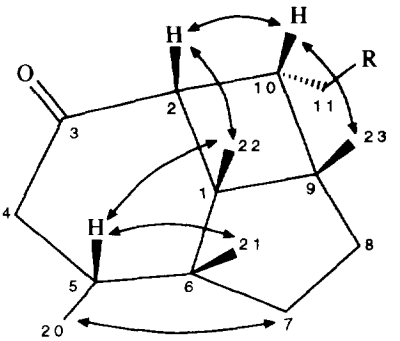
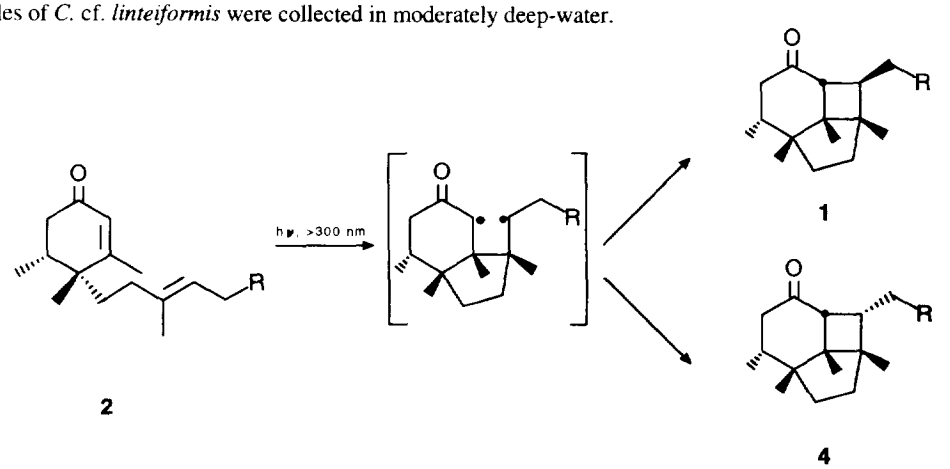


Figure I. Most diagnostic nOe observed for **4** in $CDCl_3$.

The finding of the two lintenone epimeric at C-10 in the same natural source support an alternative pathway through which their very peculiar tricarbocyclic skeleton can arise. As a matter of fact both compounds could derive from a [2+2] intramolecular photocycloaddition starting from cyclolinteinone (**2**). In fact, this reaction proceeds through a biradicalic intermediate from which the two epimers at C-10 (**1** and **4**) could be obtained, as reported in Scheme I[4]. To confirm this hypothesis we performed a photochemical cycloaddition on cyclolinteinone (**2**). Thus, irradiation of a solution of **2** with a UV lamp ($\lambda > 300$ nm) yielded a mixture of **1** and **4** in almost quantitative yields in a ratio of 6 : 4, which parallels the occurrence of natural **1** and **4**. In the light of these results we wondered whether a photocycloaddition could occurr during the isolation procedure. This was ruled out by repeating the extraction and isolation in the darkness on specimens of sponge, freeze-dried immediately after collection. We could not notice any significant difference in the yields of cyclolinteinone (**2**) as well as in those of **1** and **4**, which therefore must be considered true metabolites of the sponge. It cannot be excluded, however, that a light-induced [2+2] cycloaddition might occurr *in vivo* taking into account that our samples of *C.* cf. *linteiformis* were collected in moderately deep-water.



Scheme I

Ichthyotoxicity tests on the mosquito fish *Gambusia affinis*[5] showed that compounds **3-5** were toxic at a concentration of 10 ppm. Antifeedant assays conducted with the fish *Carassius auratus*[6] showed that the compounds possessed a high feeding deterrence at a concentration of 30 μg per cm^2 of food pellets.

Experimental Section

General procedures. All NMR measurements were performed in $CDCl_3$ on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. Proton and carbon chemical shifts were referenced to the residual solvent signals. Methyl, methylene and methine carbons were distinguished by DEPT experiments. One-bond heteronuclear ^1H-^{13}C connectivities were determined with an XHCORR experiment, optimized for an average of coupling of 125 Hz. Two- and three-bond heteronuclear ^1H-^{13}C connectivities were determined by COLOC experiments, optimized for 7 and 9 Hz. Optical rotations were measured on a Perkin-Elmer 243-B polarimeter, using a sodium lamp operating at 589 nm in $CHCl_3$ solution. Infrared spectra (KBr) were recorded on a Perkin-Elmer 1600 spectrometer. CD spectra were obtained on a JASCO J710 spectropolarimeter in EtOH solution. Mass spectra were obtained by electron impact at 70 eV on a Fisons VG ProSpec instrument. Medium-pressure liquid chromatography (MPLC) was performed on a Buchi 861 apparatus using a SiO_2 (230-400 mesh) column. High-performance liquid chromatography (HPLC) was performed on a Varian apparatus equipped with a RI-3 refractive index detector using a Hibar Si-60 LiChrospher column.

Extraction and isolation. Specimens of *C.* cf. *linteiformis* were collected during an expedition of the research vessel Columbus Iselin at a depth of 9 m off Grand Bahama Island. They were frozen when still alive at -18° C and then dispatched to the laboratory. A reference specimen is deposited at the Istituto di Zoologia, University of Genova, Italy. The sponge (103 g, dry weight after extraction) was next five times extracted with MeOH/toluene (3:1) at room temperature. The extracts were pooled and evaporated *under vacuum* to give an aqueous phase, which was extracted with EtOAc. Evaporation of the combined EtOAc extracts afforded 23 g of a crude organic extract which was chromatographed by MPLC on a SiO_2 column using sequential mixture of increasing polarities from petroleum ether to EtOAc as eluants. Fractions eluted with petroleum ether/EtOAc (7.5 : 2.5) were purified by HPLC on a Hibar LiChrospher Si60 column with a mobile phase of *n*-hexane/EtOAc (8:2) to give compound **5**. Fractions eluted with petroleum ether/EtOAc (4:6) were purified by HPLC on a Hibar LiChrospher Si60 column with a mobile phase of *n*-hexane/EtOAc (7:3) to give pure **4** and **3**. Purification of the latter compound was performed by reversed-phase HPLC on a Hibar LiChrospher RP-18 column with a mobile phase of methanol.

Compound 3 : Yield 14 mg; $[\alpha]^{25}_D$ = - 75° (c 0.03, $CHCl_3$); $[\theta]_{301}$ = -9494 (EtOH); IR = 1783, 1749, 1714 cm^{-1} (KBr); ^1H and ^{13}C NMR spectra see Table II; HREIMS (70 eV) obsd. m/z 384.2672, $C_{25}H_{36}O_3$, calcd m/z 384.2666.

Compound 4: Yield 220 mg; $[\alpha]^{25}_D$ = - 70° (c 0.04, $CHCl_3$); $[\theta]_{302}$ = -9530 (EtOH); IR = 1783, 1749, 1714 cm^{-1} (KBr); ^1H and ^{13}C NMR spectra see Table II; HREIMS (70 eV) obsd. m/z 384.2670, $C_{25}H_{36}O_3$, calcd m/z 384.2666.

Compound 5: Yield 30 mg; $[\alpha]^{25}_D$ = + 89° (c 0.03, $CHCl_3$); IR = 1782, 1749 cm^{-1} (KBr); ^1H and ^{13}C NMR spectra see Table I; HREIMS (70 eV) obsd. m/z 370.2878, $C_{25}H_{38}O_2$, calcd m/z 370.2873.

Compound 6: $[\alpha]^{25}_D$ = + 82° (c 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$) : 7.32 (1H, bs, H-19), 7.20 (1H, s, H-25), 6.28 (1H, bs, H-18), 5.39 (1H, m, H-2), 5.16 (1H, t, J = 7.5 Hz, H-14), 5.08 (1H, t, J = 7.5 Hz, H-10), 2.43 (2H, t, J = 7.5 Hz, H_2-16), 2.22 (2H, q, J = 7.5 Hz, H_2-15) 1.60 (6H, s, H_3-24 and H_3-20), 1.58 (3H, s, H_3-23), 1.03 (3H, s, H_3-22), 0.95 (3H, d, J = 7.0 Hz, H_3-21); ^1H NMR (acetone-d_6) 7.38 (1H, bs, H-19), 7.27 (1H, s, H-25), 6.29 (1H, bs, H-18), 5.35 (1H, m, H-2), 5.14 (1H, t, J = 7.5 Hz, H-14), 5.07 (1H, t, J = 7.5 Hz, H-10), 2.39 (2H, t, J = 7.5 Hz, H_2-16), 2.20 (2H, q, J = 7.5 Hz, H_2-15) 1.55 (6H, s, H_3-24 and H_3-20), 1.54 (3H, s, H_3-23), 0.99 (3H, s, H_3-22), 0.90 (3H, d, J = 7.0 Hz, H_3-21) ; EIMS (70eV) M^+ at m/z 354.

Selective reduction of cyclolinteinone (2) and 5 to give compound 6. The selective reduction of carbonyl of the α,β unsaturated ketone to the corresponding hydrocarbon was obtained using 3:1 mixture of aluminum hydride following a procedure previously described[7]. Compound **2** (20 mg, 0.05 mmoles) was added dropwise under nitrogen to a cooled solution prepared from 50 µl of an ether solution of $LiAlH_4$ (0.05 mmoles) and a solution of 18 mg (0.15 mmoles) of aluminum chloride in 0.5 ml of ether. The mixture was then let stand for 2h at room temperature. Unchanged hydride was decomposed by dropwise addition of ethyl acetate and then water to the cooled solution. The layers were separated and the aqueous layer was extracted with ether. Evaporation of the combined ether layer afforded a mixture which was separated on HPLC (*n*-hexane:EtOAc 6:4 as eluant) thus obtaining 8 mg of compound **6**. The same compound was obtained using the same procedure on compound **5**.

Cycloaddition of cyclolinteinone (2) to give compounds 1 and 4. In 10 mL of cyclohexane was placed **2** (14 mg), and the solution was purged with N_2 for 30 min. The container was sealed and exposed to a UV lamp irradiating at $\lambda > 300$ nm for 45 min. The solvent was removed *in vacuo* and the residue analysed by TLC and NMR. Successive purification by HPLC (*n*-hexane/EtOAc 7:3) gave 7 mg of **1** and 5 mg of **4**.

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