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Structure and Absolute Stereochemistry of Cyclolinteinone a Novel Monocarbocyclic Sesterterpene from *Cacospongia* cf. *linteiformis*

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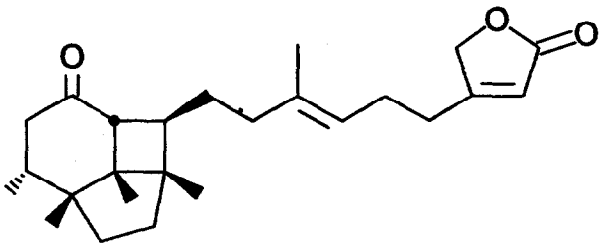
Abstract. A novel sesterterpene, cyclolinteinone (**4**), based on an unprecedented rearranged monocarbocyclic skeleton, has been isolated from *Cacospongia* cf. *linteiformis*. and its structure, complete with absolute stereochemistry, determined by spectral studies and chemical correlations. A plausible pathway for the biogenesis of this compound starting from geranylfarnesol is proposed. Cyclolinteinone has been shown to possess high ichthyotoxicity and antifeedant properties.

The Cacospongia genus is known to be a source of novel sesterterpenes, many of which have been reported to possess an ecological role in preventing predation. The forerunner of this class of compounds is scalarin[1], a metabolite isolated in 1972 from the sponge *Cacospongia scalaris*. Since then a number of similar compounds have been found in the same source and in the closely related species *Cacospongia mollior*.[2]

Recently our attention has been drawn to a Cacospongia species, collected during an expedition of the research vessel Columbus Iselin along the Bahamas Island, and identified as *Cacospongia* cf. *linteiformis*. The alcoholic extract was shown to possess valuable antifeedant properties; on chemical examination this extract was found to be rich in novel sesterterpenes among which we recently isolated lintenone (**1**), a bioactive sesterterpene based on an unprecedented tricarbocyclic skeleton[3] and lintenolide A (**2a,b**) and lintenolide B (**3a,b**), new pentacyclic ichthyotoxic compounds, both of them present as mixtures of epimers .[4]



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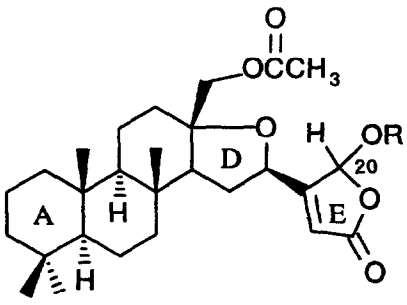
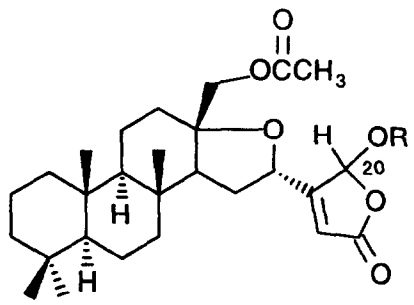


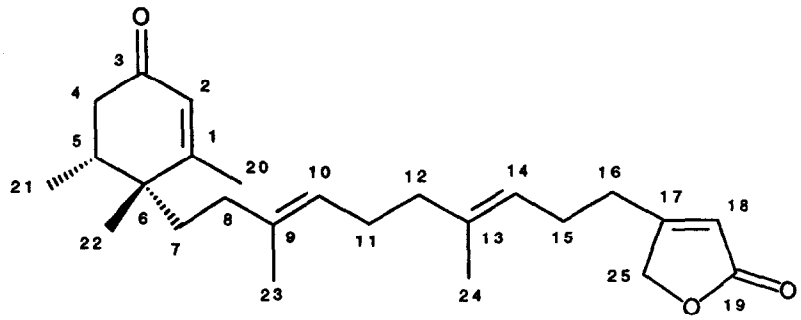
2a, b R=H
2c, d R=$COCH_3$



3a, b R=H
3c, d R=$COCH_3$

a, b and c, d epimers at C20

This report details the isolation from the same source and the structure elucidation of a novel sesterterpene cyclolinteinone (**4**). This compound is characterized by an unprecedented rearranged monocarbocyclic skeleton. The co-occurrence in the same sponge of cyclolinteinone and lintenone allowed us to suggest a possible biogenetic pathway through which both skeletons could be originated.



4

Specimens of *C.* cf. *linteiformis* were collected and extracted as previously reported.[3] Chromatographic purification of the extracts yielded compound **4**, as a major compound, which was isolated as an optically active ($[\alpha]^{25}_D$ = +53°) colorless oil. It gave a molecular ion at m/z 384.2669 in the HREIMS appropriate for a molecular formula of $C_{25}H_{36}O_3$. The distribution of the hydrogens on the 25 carbons was determined by a DEPT NMR analysis, requiring 5 methyls, 8 methylenes, 5 methines and 7 unprotonated carbons. The unsaturated functionalities evident from the ^{13}C-NMR spectra were two carbonyls and four trisubstituted double bonds which accounted for 6 out of the 8 degrees of unsaturation implied by the molecular formula: consequently, a bicyclic structure was apparent for **4**. The nature of the two carbonyls was suggested by the IR bands at 1691 and at 1780 and 1749 cm-1 indicative for an α,β unsaturated ketone and an α,β unsaturated γ-lactone, respectively. The UV spectrum is typical of a β,β-di-dialkylsubstituted enone chromophore.

Consideration of the characteristics of the ^{1}H-NMR spectra and ^{1}H-^{1}H connectivities observed in a COSY 2D experiment established the presence of the fragment (C18-C17-C25-C16-C15-C14-C13-C24). The 4-bonds couplings of H-16 with H-18 and H-25 were particularly useful to define the above part-structure. Further sets of COSY-derived ^{1}H-^{1}H connectivities were consistent with the fragments B (C12-C11-C10-C9-C23), C (C8-C7), D (C4-C5-C21) and E (C2-C1-C20).

Table I. ^{13}C and ^{1}H assignments[a] and long range carbon-proton correlation for compound **4**

carbon	DEPT	^{13}C	^{1}H	^{1}H/^{13}C long range correlation
1	C	167.90		20, 22
2	CH	126.44	5.86 s	20
3	C	198.36		4
4	CH_2	42.02	2.28 ax	21
			2.45 eq	
5	CH	37.44	2.12	21, 22
6	C	40.84		2, 20, 21, 22
7	CH_2	33.71	1.52, 1.65	22
8	CH_2	33.97	1.90, 1.97	23
9	C	134.66		23
10	CH	123.74	5.10 bt	23
11	CH_2	25.91	2.10	12
12	CH_2	38.92	1.98	24
13	C	136.51		12, 24
14	CH	121.67	5.10 bt	24
15	CH_2	25.09	2.31 bq	16
16	CH_2	28.10	2.49 bt	15
17	C	173.59		18
18	CH	114.76	5.83 bs	25
19	C	170.15		16, 25
20	CH_3	20.74	1.93 s	2
21	CH_3	15.34	1.00 d	4
22	CH_3	23.48	1.20 s	
23	CH_3	15.57	1.59 s	
24	CH_3	15.63	1.61 s	
25	CH_2	72.69	4.76 bs	18

[a] J (Hz) 4_{ax}-4_{eq} = 15.0; 4_{ax}-5 = 9.0; 4_{eq}-5 = 4.5; 5-21 = 7.0; 10-11 = 7.5; 14-15 = 7.5; 15-16 = 7.5.

Unambiguous assignment of the proton signals of the segments A-E to the signals for the carbon atoms to which they are attached was established by using a two dimensional shift-correlated heteronuclear NMR experiment (see table I). A long range carbon-proton correlation (COLOC) experiment allowed to define the gross structure of **4** by linking the segments A-E, the CH_3-22 and the unprotonated carbon atoms C6, C3 and C19. Decisive to this purpose were found the correlations involving C1 (H_3-20, H_3 -22), C3 (H-4), C6 (H-2, H_3-20, H_3-21, H_3-22), C7 (H_3-22), C8 (H_3-23), C12 (H_3-24) , C13 (H_3-12) and C19 (H_2-12).

The relative stereochemistry of the molecule at the chiral centers C5 and C22 was suggested by a ROESY experiment, which showed distinct correlations peaks between H-5 and H_3-22 and between H_3-21 and H_2-7. The above interproton contacts, together with the fact that the counterpart of the ROESY spectrum is lacking in the crosspeak correlating H-5 with H_2-7, appeared highly diagnostic in the light of the conformation of the cyclohexenone ring depicted in Figure I as indicated by a molecular mechanics analysis. Indeed, energy minimization calculations on the structure **4** (obtained as described under experimental) revealed that the carbocyclic ring adopts a half-chair-like conformation, with the dihedral angles between H-5 and H_{ax}-4 (-176.2°) and H-5 and H_{eq}-4 (-56.3°) which account for the observed vicinal coupling constants between these two couples of protons (9.0 and 4.5 Hz, repectively).

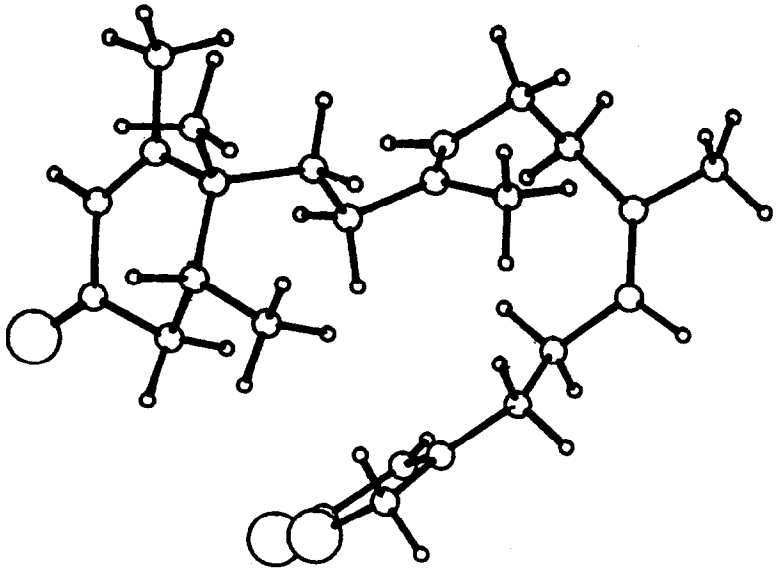
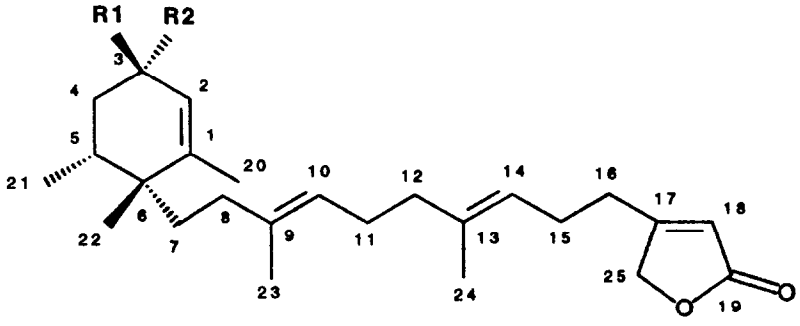


Figure I. CHARMm lowest energy conformation of model compound **4**.

The absolute configuration of **4** was established through the application of the modified Mosher method suggested by Kakisawa[5] performed on the two epimeric alcohols **5** and **6**, obtained by the selective reduction[6] of cyclolinteinone (**4**).

Two aliquots of each of the two epimers were separately treated with R(+) and S(-) α-methoxy-α-(trifluoromethyl) phenylacetyl (MTPA) chloride in pyridine solution at room temperature for 2 h to give the four esters **7**, **8**, **9** and **10**. The stereochemical determination were based on the chemical shifts differences of the protons located around the chiral center C3.



	R1	R2
5	H	OH
6	OH	H
7	H	O-(*R*)-MTPA
8	H	O-(*S*)-MTPA
9	O-(*R*)-MTPA	H
10	O-(*S*)-MTPA	H

The whole of the data (see table II) are fully consistent with the Mosher model and allowed us to confidently assign to C3 the S configuration in **5** and the R configuration in **6**.

Table II. Selected ^{1}H NMR data of (R)-MTPA (**7** and **9**) and (S)-MTPA (**8** and **10**) esters.

	7	**8**	δΔ	**9**	**10**	δΔ
H-3	5.48	5.50		5.47	5.40	
H-2	5.37	5.46	+0.09	5.65	5.60	-0.05
H_{ax}-4	1.64	1.58	-0.06	1.68	1.75	+0.07
H_{eq}-4	2.02	1.98	-0.04	1.80	1.90	+0.10
H-5	1.70	1.66	-0.04	1.72	1.78	+0.06
H_3-20	1.66	1.70	+0.04	1.78	1.70	-0.08
H_3-21	1.00	0.95	-0.05	0.93	0.97	+0.05
H_3-22	1.00	1.02		1.00	0.98	

Once determined the chirality of C3 in **5** and **6** that of C5 (and consequently that of C6) followed from its relative configuration to C3 in the two molecules, which was assigned as follows. The large vicinal coupling constant of H-5 with H-4_{ax} in both **5** and **6** indicated that this proton, upon reduction of the carbonyl function, retained its initial quasi-axial orientation in either epimers. On the other hand, NOE experiments revealed that H-5 is *cis* related to H-3 in **5**, thus pointing to a quasi-equatorial disposition of the -OH group in **5** and quasi-axial in **6**. This conclusion was corroborated by the J values of H-3 with the vicinal protons H_2-4 in **5** (and **6**) (3-4_{ax}=9.5 Hz, 3-4_{eq}=4.0 Hz and 3-4_{ax}=5.0 Hz, 3-4_{eq}=3,5 Hz respectively).

The finding of metabolite **4** in the extractives of *C.* cf *linteiformis* could throw light on the biogenetic pathway involved in the formation of the unprecedented sesterterpene skeletons elaborated by this marine source. The new compound, indeed, although not immediately reminiscent of the previously isolated sesterterpene lintenone (**1**), can be easily related to a common biogenetic precursor. In fact a plausible biogenetic scheme, which accounts for the two different skeletons both carrying an oxygenated function at C3, may be hypothesized (Figure II). The key intermediate of this biogenetic hypothesis is the monocyclic epoxide **11**, which could form the carbon skeleton of **1** or, alternatively, that of **4** through two pathways initiated by the protonation of the oxirane ring, both of them involving a single methyl migration.

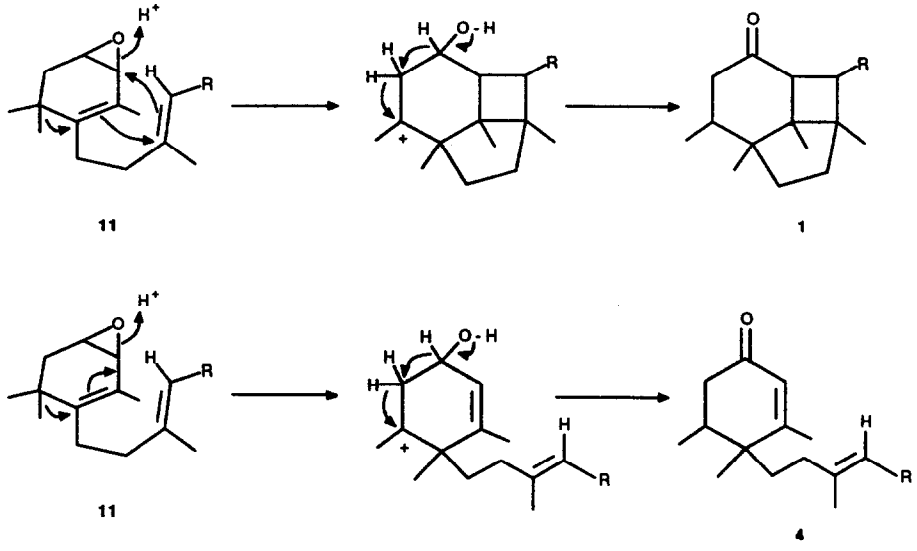


Figure II. Biogenetic pathway as proposed by the authors

Ichthyotoxicity tests on the mosquito fish *Gambusia affinis*[7] showed that cyclolinteinone **4** was toxic at a concentration of 10 ppm. Antifeedant assays conducted with the fish *Carassius auratus*[8] showed that the compound possessed a high feeding deterrence at a concentration of 30 μg per cm^2 of food pellets.

Experimental Section

General methods. 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 rotation 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 Bruker IFS-48 spectrometer. High resolution mass spectra

electron impact at 70 eV on a Kratos MS-50 mass spectrometer. 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 5000 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 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 voucher 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 EtOAc were purified by HPLC on a Hibar LiChrospher Si60 column with a mobile phase of $CHCl_3$/EtOAc (9:1) to give pure compound **4**.

Compound 4: Yield 600 mg; $[\alpha]^{25}_D = +53°$ (c 0.04, $CHCl_3$); IR = 1780, 1749, 1691 cm^{-1} (KBr); ^1H and ^{13}C NMR spectra see Table I; HREIMS (70 eV) obsd. m/z 384.2669, $C_{25}H_{36}O_2$, calcd m/z 384.2666.

Selective reduction of compound 4 to give compounds 5 and 6. The selective reduction of the α,β unsaturated ketone to the corresponding alcohol was obtained using 9-BBN (9-borabicyclo(3.3.1.)nonane) following a procedure previously described.[6] Compound **4** (30 mg) was dissolved in THF (0.5 ml) under a dry nitrogen atmosphere. To this solution mantained at 0° C, 0.5 ml of 0.5 M solution of 9-BBN in THF was added dropwise. The resulting mixture was stirred at 0° C for 2 h and at 25° C for 1 h. Then MeOH was added to destroy excess of 9-BBN. THF was removed under reduced pressure and dry Et_2O (1 mL) was introduced followed by 5 μl of 2-aminoethanol. Immediately the ethanolamine derivative of 9-BBN starts to precipitate. Removal of Et_2O and vacuum distillation yielded a mixture of epimeric allylic alcohols, which were separated by HPLC (SiO_2 column, *n*-hexane/EtOAc 6:47 to give **5** (14 mg) and **6** (8 mg).

Compound 5: $[\alpha]^{25}_D = +47°$ (c 0.02, $CHCl_3$); ^1H NMR : 5.82 (1H, bs, H-18), 5.45 (1H, bs, H-2), 5.06 (1H, t, J=7.5 Hz, H-14), 5.02 (1H, t, J=7.5 Hz, H-10), 4.67 (2H, bs, H_2-25), 4.17 (1H, dd, J=9.5 and 4.0 Hz, H-3), 2.45 (2H, bt, J=7.5 Hz, H_2-16), 2.29 (2H, bq, J=7.5 Hz, H_2-15), 1.83 (1H, ddd, J=15.0, 4.5 and 4.0 Hz, H-4_{eq}), 1.68 (3H, s, H_3-20), 1.65 (1H, H-5), 1.62 (3H, s, H_3-24), 1.57 (3H, s, H_3-23), 1.45 (1H, ddd, J=15.0, 9.5 and 9.0 Hz, H-4_{ax}), 1.01 (3H, d, J=7.0 Hz, H_3-21), 0.97 (3H, s, H_3-22).

Compound 6: $[\alpha]^{25}_D = +34°$ (c 0.02, $CHCl_3$); ^1H NMR : 5.84 (1H, bs, H-18), 5.56 (1H, bs, H-2), 5.07 (1H, t, J=7.5 Hz, H-14), 5.03 (1H, t, J=7.5 Hz, H-10), 4.71 (2H, bs, H_2-25), 4.20 (1H, dd, J=5.0 and 3.5 Hz, H-3), 2.44 (2H, bt, J=7.5 Hz, H_2-16), 2.29 (2H, bq, J=7.5 Hz, H_2-15), 1.80 (1H, ddd, J=15.0, 4.5 and 3.5 Hz, H-4_{eq}), 1.69 (3H, s, H_3-20), 1.64 (1H, H-5), 1.61 (3H, s, H_3-24), 1.58 (3H, s, H_3-23), 1.44 (1H, ddd, J=15.0, 9.0 and 5.0 Hz, H-4_{ax}), 1.07 (3H, s, H_3-22), 0.98 (3H, d, J=7.0 Hz, H_3-21).

Synthesis of the (R)- and (S)-MTPA esters of alcohol 5 (7 and 8). To compound **5** (5 mg) in 0.6 ml of anhydrous pyridine, 60 μl of (R)-MTPA (α-methoxy-α-(trifluoromethyl) phenylacetyl) chloride were added, and the mixture was heated in a sealed tube at room temperarure for 2 h. After cooling, 5 μl of water and solid K_2CO_3 were addedd, and the solution was extracted with $CHCl_3$ (5 ml). The organic phase was evaporated to dryness, and partioned between MeOH and *n*-hexane. The *n*-hexane phase, after evaporation of the solvent , yielded 5 mg of the (R)-MTPA ester **7**. The use of (S)-MTPA chloride in the same procedure led to 5 mg of the (S)-MTPA ester **8**.

Synthesis of the (R)- and (S)-MTPA esters of alcohol 6 (9 and 10). The procedure was similar to that described for **5**. Starting from 3 mg of **6**, 2.5 mg of the (R)-MTPA ester **9** were obtained by reaction with (R)-MTPA chloride. The use of (S)-MTPA chloride in the same procedure led to 3 mg of the (S)-MTPA ester **10**.

Molecular Modeling. Molecular modeling studies were performed using the Quanta/CharmM9 3.2 program on a Personal Iris 4D-35G computer. The effect of the solvent was approximated by using a dielectric constant of 4.806 ($CHCl_3$), and all the energy terms were calculated. Molecular dynamics simulations involved a heating period of 1.2 ps, followed by a 1.2 ps equilibration period and then 100 ps of dynamics simulation. The time step of integration was 1 fs. Bond lengths involving hydrogen atoms were kept fix using SHAKE[9] algorithm. The coordinate produced by the simulation were saved every 0.2 ps, giving 500 structures. Each of them was subjected to energy minimization using the conjugated gradient protocoll. All energies are relative to the lowest energy conformer (E = 89.135 Kcal/mol for compound **4**).

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