Pergamon

Tetrahedron Vol. 50, No. 3, pp. 849-856, 1994
Elsevier Science Ltd

0040-4020/94 $6.00+0.00

0040-4020(93)E0053-I

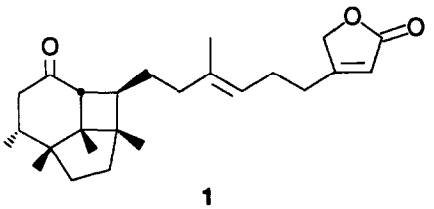
Lintenolides, New Pentacyclic Bioactive Sesterterpenes from the Caribbean Sponge *Cacospongia* cf. *linteiformis*

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

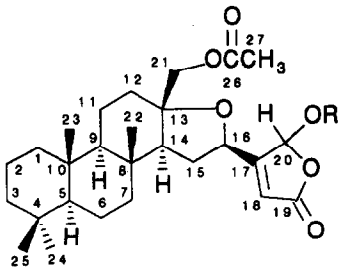
[a] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli, Via D. Montesano, 49, 80131 Napoli, Italy.
[b] Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Via Tiberio 21, 86100 Campobasso, Italy

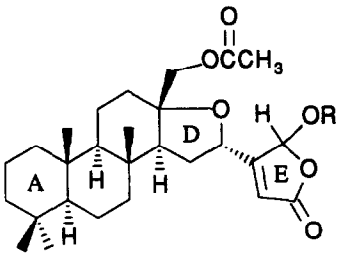
Abstract : New pentacyclic sesterterpenes, lintenolide A (**2 a, b**) and lintenolide B (**3 a, b**), have been characterized from the caribbean sponge *Cacospongia* cf. *linteiformis*. The structure elucidation of these compounds was accomplished by using 2D NMR experiments, including ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (HETCORR and COLOC) and rotating frame NOE (ROESY). Compounds showed high ichthyotoxicity and antifeedant activity, which suggest their potential role as natural feeding deterrents.

A chemical study of *Cacospongia* cf. *linteiformis* was undertaken in the frame of a systematic survey on caribbean sponges. This organism, similarly to other species belonging to the Dyctyoceratida family Thorectidae, turned out to be a rich source of secondary metabolites, among which we have recently isolated



1





2a, b R=H
2c, d R=$COCH_3$ (28 29)

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

a, b and c, d epimers at C20

lintenone (**1**), a biologically active sesterterpene based on a tricarbocyclic skeleton of unprecedented nature[1].

This paper deals with the isolation and the structure elucidation of the two new isomeric sesterterpenes, lintenolide A (**2 a, b**) and lintenolide B (**3 a, b**) both of them present as mixtures of epimers at a hemiacetale carbon.

A collection of *C.* cf. *linteiformis* was made during an expedition at the Bahama Islands. Fresh tissues of the animal were frozen and successively extracted with MeOH/toluene (3:1) in the dark. The EtOAc soluble material was fractionated by silica gel MPLC followed by HPLC on a Lichrosorb Si60 column. Two fractions (**2 a, b** and **3 a, b**), eluted with $CHCl_3$/EtOAc (9:1), subjected to preliminary spectral analyses, were shown to contain almost equimolecular amounts of two compounds each, resistant to any attempt of chromatographic separation into the individual components. The doubling of many resonances in the ^1H and ^{13}C NMR spectra of the two fractions prompted us to hypothesize that the difference between the two terms within each couple of compounds should be confined to the stereochemistry of a chiral center, most likely a hemiacetal carbon whose presence was inferred from the carbon resonances at δ 97.08 and 97.57 for **2 a, b** and δ 97.32 and 97.55 for **3 a, b**. Furthermore, a very close structural relationship also linked the two mixtures to each other, as indicated by the similarity of the ^1H and ^{13}C NMR spectra.

FABMS of the two fractions showed for both fractions a molecular ion at m/z 460, compatible with a molecular formula $C_{27}H_{40}O_6$, deduced also by ^{13}C NMR data.

Acetylation of the two mixtures (**2 a, b** and **3 a, b**) and subsequent HPLC chromatographies of the products resulted in the achievement of pure **2 c**, **2 d**, **3 c** and **3 d**. Each of the four compounds was formulated as $C_{29}H_{42}O_7$ by FABMS and ^{13}C NMR spectroscopy (see Table 1 and 2). Most of the structural work was carried out on compound **2 c**; once its stereostructure had been assigned, those of the three other metabolites was easily derived by comparison of the spectral data.

The presence of a β-substituted-γ-acetoxy-butenolide mojety (E) in **2 c** was established by ^{13}C NMR spectrum which displayed resonances for two carbonyl groups [δ 170.80 (C28) and 169.08 (C19)] one hemiacetal [δ 92.11 (C20)] and two sp^2 [δ 116.81 (C18), 168.40 (C17)] carbons in addition to the signal of the $\underline{CH_3}$-COO- group [δ 20.79 (C29)]. IR (ν_{max} 1770, 1757 cm^{-1}) and ^1H NMR [δ 6.18 (1H, s, H18), 2.09 (3H, s, $H_3$29) spectra confirmed the above assignment.

A further five membered heterocyclic substructure (D) was also proposed on the basis of NMR data. Particularly, ^1H-^1H COSY established the connectivities C14-C16 whereas a number of ^{13}C-^1H long range couplings (C13-H14, C13-$H_2$15, C21-H14) observed in a COLOC experiment allowed us to position the oxygen-linked quaternary carbon C13 bearing the acetoxymethylene group. That the -CH_2-OAc group were linked to an oxygenated carbon atom was also evident from the EIMS spectrum of **2 c**, where the peak at m/z 429, deriving from the molecular ion by loss of the above mentioned fragment, was one of the most intense ions. An allylic coupling in the ^1H NMR spectrum of **2 c** between H16 and H18, along with ^{13}C-^1H couplings via ^3J correlating C16 with H18 and C18 with H16 in the COLOC experiment, proved that the two heterocyclic rings were joined through the C16-C17 bond.

The remaining part of the molecule which, as inferred by the molecular formula, possessed three formal unsaturations, had to be comprised of only sp^3 carbon atoms (4 methyls, 7 methylenes, 2 methines and 3 quaternary carbons) as a result of an inspection of ^{13}C NMR spectra and, consequently, it must be tricyclic. Furthermore, since all methyls resonated as singlets in the ^1H NMR spectrum of **2 c**, they should be linked to quaternary carbon atoms.

Table 1 . ^{13}C and ^{1}H NMR assignments for compounds **2 c** and **2 d**[a]

		2 c		**2 d**	
carbon	**DEPT**	^{13}C	^{1}H	^{13}C	^{1}H
1	CH_2	39.82	1.62, 0.83	39.96	1.61, 0.82
2	CH_2	18.32	1.59, 1.41	18.48	1.58, 1.40
3 ax			1.13 dt (13, 3)		1.13 dt (13, 3)
	CH_2	41.89		42.04	
3 eq			1.36		1.36
4	C	33.16[b]		33.27[b]	
5	CH	57.09	0.85 dd (12, 2)	57.17	0.85 dd (12, 2)
6 ax			1.40		1.40
	CH_2	17.87		18.00	
6 eq			1.58		1.57
7 ax			1.16 dt (13, 3)		1.15 dt (13, 3)
	CH_2	41.39		41.49	
7 eq			1.58		1.57
8	C	37.31		37.45	
9	CH	61.28	0.968 dd (11, 2)	61.38	0.95 (11, 2)
10	C	36.59		36.67	
11 ax			1.266		1.26
	CH_2	19.13		19.23	
11 eq			1.76		1.75
12	CH_2	34.77	2.16, 1.33	34.91	1.27, 2,10
13	C	82.08		82.04	
14	CH	61.92	1.81	61.87	1.78
15	CH_2	28.90	1.79. 2.11	28.96	1.88, 2.15
16	CH	73.85	4.80 bt (9)	73.67	4.80 bt (9)
17	C	168.40		168.64	
18	CH	116.81	6.18 s	117.20	6.06 s
19	C	169.08		169.35	
20	CH	92.11	6.88 s	93.07	6.96 s
21	CH_2	65.20	4.51 d (12), 3.61 d (12)	65.15	4.41 d (12), 3.67 d (12)
22	CH_3	16.71	0.85 s	16.76	0.85 s
23	CH_3	15.78	0.83 s	15.97	0.82 s
24	CH_3	33.16[b]	0.84 s	33.27[b]	0.83 s
25	CH_3	21.06	0.81 s	21.25	0.80 s
26	C	168.75		168.95	
27	CH_3	20.44	2.15 s	20.67	2.14 s
28	C	170.80		170.94	
29	CH_3	20.79	2.09 s	20.91	2.08 s

[a] Assignments determined by analogy to model compounds and by the 1D and 2D NMR experiments reported in the text. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$. Proton coupling constants ( ) are given in Hertz.

[b] Values with identical superscript within each column are mutually overlapped.

Table 2. ^{13}C and ^{1}H NMR assignments for compounds **3 c** and **3 d**[a]

		3 c		**3 d**	
carbon	**DEPT**	^{13}C	^{1}H	^{13}C	^{1}H
1	CH_2	39.73	1.61, 0.84	39.73	0.847, 1.623
2	CH_2	18.27	1.57, 1.41	18.28	1.413, 1.571
3 ax			1.11 dt (13, 3)		1.133[b]
	CH_2	41.75		41.76	
3 eq			1.35		1.36
4	C	33.06[b]		33.03[b]	
5	CH	56.92	0.83 dd (12, 2)	56.81	0.85 dd (12, 2)
6 ax			1.41		1.41
	CH_2	17.63		17.64	
6 eq			1.55		1.56
7 ax			1.06 dt (13, 3)		1.13[b]
	CH_2	41.11		40.99	
7 eq			1.51		1.52
8	C	37.27		37.27	
9	CH	61.27	0.93 dd (11, 2)	61.12	0.96 dd (11, 2)
10	C	36.32		36.32	
11ax			1.26		
	CH_2	18.75		18.73	1.26, 1.78
11 eq			1.77		
12	CH_2	34.58	2.26, 1.26	34.53	1.25, 2.23
13	C	82.35		82.19	
14	CH	59.44	1.54	59.78	1.56
15	CH_2	28.74	1.67. 2.20	28.19	1.83, 2.17
16	CH	71.82	4.89 bd (6.5)	72.00	4.88 bd (6.5)
17	C	168.32		168.01	
18	CH	118.24	6.08 s	117.88	6.03 s
19	C	169.01		168.99	
20	CH	92.20	6.87 s	92.76	6.93 s
21	CH_2	62.69	4.29 d (12), 4.04 d (12)	62.64	3.95 d (12), 4.37 d (12)
22	CH_3	16.45	0.83 s	16.35	0.83 s
23	CH_3	15.66	0.82 s	15.67	0.82 s
24	CH_3	33.06[b]	0.82 s	33.03[b]	0.82 s
25	CH_3	21.00	0.79 s	21.02	0.79 s
26	C	168.79		168.37	
27	CH_3	20.45	2.17 s	20.41	2.14 s
28	C	170.96		170.84	
29	CH_3	20.80	2.09 s	20.76	2.06 s

[a] Assignments determined by analogy to model compounds and by the 1D and 2D NMR experiments reported in the text. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$. Proton coupling constants ( ) are given in Hertz.

[b] Values with identical superscript within each column are mutually overlapped.

Homonuclear shift correlated 2D-experiment (COSY) provided evidence for the presence in the molecule of segments C1-C3, C5-C7 and C9-C12.

The building up of the whole basic carbon skeleton from the above subunits was achieved on the basis of a series of data arising from 2D ^{13}C-^{1}H hetero-correlations both via ^{1}J and long-range (COLOC), which also allowed to assign all the resonances in the ^{13}C NMR spectrum to the pertinent carbons (Table 1). A list of the most informative correlations via ^{2}J and ^{3}J is reported in Table 3.

Table 3. Long range carbon-proton correlations (COLOC) of compounds **2 c**, **2 d**, **3 c** and **3 d**.

carbon	**protons**			
	2 c	**2 d**	**3 c**	**3 d**
1	2, 23	2, 3, 23	2, 3, 23	2, 3
2		1	1	
3	24	2, 24	24, 25	1, 2, 24, 25
4		6		2, 6
5	6, 7, 23, 24	6, 7, 23, 24, 25	6, 7, 23, 24	6, 7, 23, 24, 25
6		5		5
7	5, 22	5, 6, 22	6, 22	6
8	6, 7, 22	6, 7, 14, 22	6, 7, 9, 22	6, 7, 9, 14, 22
9	7, 12, 23	11, 12, 22	12, 22, 23	7, 11, 12, 22, 23
10	6, 11, 23	6, 9, 11, 23	1, 5, 6, 9, 23	1, 5, 6, 9, 11, 23
11	9			9
12	9	9, 11		
13	12, 14, 15	12, 14, 15	12, 15	11, 12, 14, 15
14	12, 15, 22	12 ,22	12, 15, 22	12, 22
15	14	14	14, 16	
16	18	18	18	
17	16	15, 16	15, 16	18
18	16, 20	16, 20	16, 20	
19	18, 20	18, 20	18, 20	18, 20
20	18	18	18	18
21	14	12, 14	12, 14	12, 14
22	14	7, 14	14	7
23	9	1, 9	1, 9	9
24	3, 25	3, 25	3, 5, 25	3, 5
25	3	24	3, 24	3, 5, 24
26	21, 27	21, 27	21, 27	21, 27
27				
28	20, 29	20, 29	20, 29	20, 29
29				

The relative configuration of the chiral centres C5, C8, C9 and C10 was easily established taking into account the J values for H5 and H9, indicative of their axial disposition and from a series of interproton contacts observed in a ROESY experiment, particularly those correlating H9 with H5, Me-10 with $H_{ax}6$ and Me-8 with $H_{ax}11$. These data, taken togheter, pointed to a *trans-anti-trans* nature for the perhydrophenantrene mojety of the molecule, as depicted in formula **2 c**. This conclusion was corroborated by the chemical shifts of the carbon atoms of rings A and B which matched those of the related sesterterpene acetate recently described in the literature[2].

Going on, the assignment of the chirality of carbon 13 was mainly based on the nOe effect of Me-8 with $H_2$21 protons which, in turn were found to be within nOe proximity with $H_{ax}11$, whereas the ROESY cross peak correlating H9 and H14 was diagnostically important in order to fix the stereochemistry of C14. Finally, the nuclear Overhauser effect observed between $H_2$21 and H18 was the key contact to determine the relative configuration of the chiral centre C16.

Unfortunately, the data deriving from the ROESY experiment did not allow us to assign the stereochemistry at C20. On the other hand, since all NMR data (including ROESY) of compound **2 d**, perfectly matched those of **2 c**, we reasonably concluded that the two compounds were epimer at the hemiacetal carbon C20.

As far as compounds **3 c**, **3 d** are concerned, the whole of spectroscopic data (see Table 2 and 3) led to gross structures identical to those of **2 c**, **2 d**.

Likewise, the ROESY experiment provided evidence for assigning all the relative configurations except C20. Since these nOe measurements did not enable to distinguish **3 c** from **3 d**, the logical deduction was that, also in this case, the difference between the two compounds had to be confined to C20. A clear distinction, on the contrary, could be drawn from the ROESY spectra of **3 c**, **3 d** when compared with those of **2 c**, **2 d**. In fact the contour plots of the ROESY spectra of **3 c**, **3 d** were lacking in the cross peaks correlating $H_2$21 and H18, thus indicating that the chirality at C16 had to be different. This was confirmed by the concomitant presence of nOe effects between $H_2$21 and H16 for compounds **3 c**, **3 d**. No further significative difference could be noticed by comparing the ROESY spectra of **2 c**, **d** and **3 c**, **d** and, therefore, we were led to conclude that the chirality at C16 was the only structural feature differentiating the two couples of compounds.

It is to be noted that formulae **2 a, b** and **3 a, b** show the stereostructure of lintenolides without regard to their absolute configuration.

Ichthyotoxicity tests on the mosquito fish *Gambusia affinis*[3] showed that lintenolide A (**2 a, b**) and lintenolide B (**3 a, b**) were toxic at a concentration of 10 ppm. Antifeedant assays conducted with the fish *Carassius auratus*[4] showed that both compounds possessed a high feeding deterrence at a concentration of 30 μg per cm^2 of food pellets. The biological activity exhibited by lintenolides A and B points to a potential role of these compounds as natural feeding deterrents.

EXPERIMENTAL SECTION

General methods. Optical rotations were measured on a Perkin-Elmer 243-B polarimeter in $CHCl_3$ solution at 20-22°C. FT-IR spectra were determined on a Perkin-Elmer 1600 spectrophotometer ($CHCl_3$ solution). Electron impact mass spectra (EIMS) were obtained at 70 eV on a Kratos MS-50 mass spectrometer. Fast atom bombardment mass spectra (FABMS) were obtained on a VG-ZAB instrument equipped with a FAB source. ^1H and ^{13}C NMR spectra were determined on a Bruker AMX-500 spectrometer in $CDCl_3$, using the solvent signal as an internal standard. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear ^1H connectivities were determined by using COSY experiments. One bond heteronuclear ^1H-^{13}C connectivities were determined with a HXCORR pulse sequence optimized for $^1J_{CH}$ of 135 Hz. Two and three bond ^1H-^{13}C connectivities were determined by COLOC experiments, optimized for $^{2,3}J_{CH}$ of 8 Hz. Nuclear Overhauser effect (nOe) measurements were performed by 2D ROESY experiments. Medium pressure liquid cromatography (MPLC) was performed on a Buchi 861 apparatus using a SiO_2 (230-400 mesh) column. High performance liquid chromatogaphy (HPLC) separations were performed on a Varian 5000 apparatus equipped with a RI-3 refractive index detector using a Hibar Si60 LiChrospher 10 mm column.

Extraction and Isolation . Specimens of *C.* cf. *linteiformis* were collected by scuba in the Summer of 1990 along the coast of Grand Bahama Island (Bahamas) and identified by Dr. M. Pansini (Università di Genova). They were stored frozen when still alive at -18°C and dispatched to the laboratory. Reference specimens are deposited at the Istituto di Zoologia, Università di Genova (Italy). The collected material (103 g, dry wt after extraction) was homogenized and successively extracted with MeOH/Toluene 3:1 (500 ml x 5) at room temperature. After evaporation of the solvent, the aqueous phase was extracted with EtOAc. The combined EtOAc extracts were dried (Na_2SO_4) and concentrated in vacuo to afford 23 g of a brown residue, which was chromatographed by MPLC using a solvent gradient system from *n*-hexane to EtOAc and then to MeOH. Fractions eluted with EtOAc were rechromatographed by HPLC with a mobile phase of $CHCl_3$/EtOAc (9:1) to yield two inseparable mixtures : **2 a, b** (400 mg) and **3 a, b** (160 mg). Mixture of **2 a, b** was acetylated with Ac_2O and pyridine (1:1) overnight at room temperature, concentrated under reduced pressure, and subjected to HPLC (eluent : *n*-hexane/EtOAc 7:3) which afforded compounds **2 c** and **2 d**. Mixture of **3 a, b** subjected to the same treatment gave compounds **3 c** and **3 d**.

Lintenolide A acetate 2 c. $[\alpha]^{25}_D$ = + 52° ($CHCl_3$); ^1H and ^{13}C NMR spectra see Table 1; FABMS m/z 502 (M^+); EIMS m/z (%) 430 (10), 429 (68), 369 (8), 237 (13), 177 (29), 149 (30), 137 (94), 123 (100).

Lintenolide A acetate 2 d. $[\alpha]^{25}_D$ = + 6° ($CHCl_3$); ^1H and ^{13}C NMR spectra see Table 1; FABMS m/z 502 (M^+); EIMS m/z (%) 430 (18), 429 (70), 369 (10), 237 (12), 177 (35), 149 (25), 137 (85), 123 (100).

Lintenolide B acetate 3 c. $[\alpha]^{25}_D$ = - 3° ($CHCl_3$); ^1H and ^{13}C NMR spectra see Table 2; FABMS m/z 502 (M^+); EIMS m/z (%) 430 (16), 429 (65), 369 (13), 237 (14), 177 (29), 149 (26), 137 (90), 123 (100).

Lintenolide B acetate 3 d. $[\alpha]^{25}_D$ = - 24° ($CHCl_3$); ^1H and ^{13}C NMR spectra see Table 2; FABMS m/z 502 (M^+); EIMS m/z (%) 430 (20), 429 (71), 369 (14), 237 (19), 177 (28), 149 (29), 137 (88), 123 (100).

Acknowledgements

This work is a result of research supported by CNR, contr. 91-3242, and by MURST Rome, Italy. We wish to thank Prof. W. Fenical for giving us the opportunity to partecipate in an expedition to the Caribbean Sea, during which the sponge *C.* cf. *linteiformis* was collected. We thank Dr. G. Villani, Istituto per la Chimica di Molecole di Interesse Biologico, CNR, Arco Felice, Italy, for the antifeedant and ichthyotoxicity tests. Mass spectral data were provided by Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli. The assistance of the staff is greatly appreciated.

REFERENCES

1. Fattorusso, E.; Lanzotti, V.; Magno, S.; Mayol, L.; Pansini, M., *J. Org. Chem.* **1992**, 57, 6921.
2. Crews, P.; Jimenez, C.; O'Neil-Johnson, M., *Tetrahedron* **1991**, 47, 3585.
3. Gunthorpe, L.; Cameron, A. M., Mar. Biol. **1987**, 94, 39. Coll, J. C.; La Barre, S.; Sammarco, P. W.; Williams, W. T.; Bakus, G., *J. Mar. Ecol. Prog. Ser.* **1982**, 8, 271.
4. Cimino, G.; De Rosa, G.; De Stefano, S.; Sodano, G., *Comp. Biochem. Physiol.* **1982** 1982, 73B, 471.

(*Received in UK* 31 *May* 1993; *revised* 19 *October* 1993; *accepted* 21 *October* 1993)