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The Structure of Laurobtusol, a New Rearranged Sesquiterpenoid from the Mediterranean Red Alga *Laurencia obtusa*

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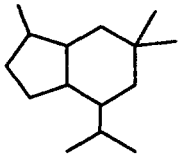
Abstract: *An alcohol with a new tricyclic humulane skeleton, laurobtusol, was isolated from the Mediterranean Red Alga Laurencia obtusa. The structure was established mainly by 2 D NMR methods and the relative configuration was assigned by a quantitative computer simulation of the lanthanide induced shifts in the ^1H NMR spectrum and Molecular Mechanics calculation (MM2).*

The red alga *Laurencia obtusa* continues to be a prolific source of metabolites of diverse nature, particularly terpenes and acetogenins.[1,2] This is due to the dominance of one major metabolite associated with several minor components and to a marked variability of the constituents in the collections from different sites.[3] We recently reported the isolation of several new representatives of the rare brasilane (**1**) class.[4,5] These minor compounds were present with a major acetylenic cyclic ether laurencienyne (**2**), previously characterized.[6]

We now report the isolation and structural elucidation of another very minor component, laurobtusol



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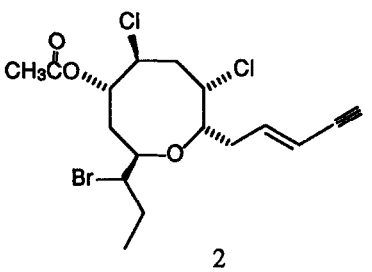

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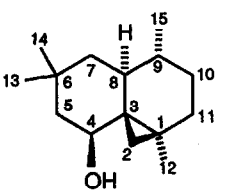


3

(**3**), which possesses an unreported carbon ring system probably derived from the α-humulene skeleton.

Column chromatography and repeated preparative HPLC of selected fractions afforded compound **3** (0.07 % extract, m.p. 121-3 °C) and laurencienyne **2**. The molecular formula of **3** was established as $C_{15}H_{26}O$ (3 unsaturations) on the basis of HREIMS and ^{13}C NMR data. IR absorptions (cm^{-1}) established the hydroxyl (3300, broad) and indicated the absence of a carbonyl functionality. The

^{13}C NMR spectrum of **3** confirmed the presence of a carbon bearing an hydroxyl (δ 72.3) and the absence of sp^2 carbons. These combined data required that **3** possesses a tricyclic skeleton. The ^1H NMR spectrum ($CDCl_3$, Table 1) indicated the presence of a cyclopropane ring (δ 0.10, 1 H and δ 0.79, 1 H). In addition, it showed a hydrogen on a carbon bearing oxygen (δ 3.90, dd, J=2.5, 11.4 Hz), three methyl singlets (δ 0.80, 0.96, 1.23) and a methyl doublet (δ 1.08, J=6.5 Hz). The remaining 11 protons gave multiplet signals congested within the region from 1.00 to 1.90 ppm and also a 2 D COSY experiment showed only a few unequivocal proton-proton correlations, as reported in Table 1. The total assignment of the spectrum was made resorting to clear one-bond HETCOR correlations and by straightforward ^{13}C NMR DEPT signals. HETCOR long-range experiments (Table 1) indicated some connectivities regarding two and three-bond correlations.

Firstly, the cyclopropane H-2 protons (δ 0.79 and 0.10, d), appear to be only geminally coupled (J=4.5 Hz): this methylene is thus bonded to two quaternary carbons. One of them (the C-1 signal at δ 28.1) correlates to both H-2 protons and the other one (the C-3 signal at δ 22.1) correlates with 3H-12, as observed in the long-range HETCOR experiment. Methylene C-11 (δ 35.9) shows a

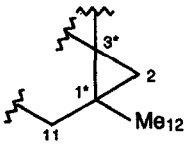
Table 1. ^1H and ^{13}C NMR data for compound **3**.[a]

Pos.	δC	DEPT	δH mult. (J, Hz)	COSY	H/C long-range correlation[b]
1	28.1	C			2H-2, 3H-1
2	22.1	CH_2	H_a 0.79 d (4.5) H_b 0.10 d (4.5)	H_b-2 H_a-2	3H-12
3	38.5	C			3H-12
4	72.3	CH	3.90 dd (11.4, 2.5)		2H-5, H_a-2
5	47.4	CH_2	H_a 1.16 m[c] H_b 1.05 m[c]		3H-13, 3H-14
6	30.9	C			3H-13, 3H-14, H_a-5, H_a-7
7	44.3	CH_2	H_a 1.23 m[c] H_b 1.10 m[c]		3H-13, 3H-14
8	47.1	CH	1.18 m[c]		H_a-2, 3H-15
9	44.7	CH	1.44 m[c]	3H-15	3H-15
10	33.9	CH_2	H_a 1.70 m[c] H_b 1.25 m[c]		3H-15
11	35.9	CH_2	H_a 1.86 m[c] H_b 1.20 m[c]	H_a-2, H_a-10	2H-2, Ha-10, 3H-12
12	20.9	CH_3	1.23 s		2H-2
13	30.0	CH_3	0.80 s		H_a-5, 3H-14
14	29.6	CH_3	0.96 s	3H-13	H_a-5, H_b-7, 3H-13
15	19.2	CH_3	1.08 d (6.5)		H-8, H_b-10

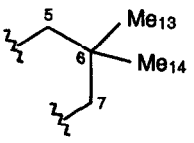
[a]The chemical shifts are given in ppm downfield from tetramethylsilane.
[b]Long-range correlations were obtained with polarization transfer optimized for J=10.0 and 5.0 Hz.
[c]Chemical shift derived by inspection of the relative cross-sections in one-bond HETCOR spectrum F_1-decoupled.

three-bond correlation with 2 H-2 and therefore has to be linked to C-1 or to C-3. But, since the 3 H-12 (δ 1.23) are correlated with C-2 and with C-11, as observed by long-range HETCOR, fragment A can be assembled (the positions 1 and 3 marked by an asterisk are interchangeable). Similarly, fragments B and C are built on the basis of the connectivities elucidated through the long-range HETCOR experiments.

Further assembly of these structural fragments was however not immediate by COSY experiments due to the crowding and partial overlapping of the methylene and methine multiplets C-5, and C-7 through C-11.



A



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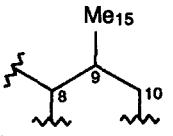


C

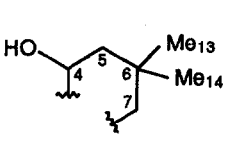
Therefore, we resorted to incremental addition of the lanthanide shift reagent (LSR) Eu(fod)$_3$ to obtain a series of ^{1}H and ^{13}C NMR spectra of compound **3**. A good linear relationship between the observed induced shifts of many proton signals (LIS) and the [LSR] / [**3**] was obtained for the range 0.06-0.6 mole ratio. DEPT experiments were done to assign unequivocally the signals where the ^{13}C resonances intercross each other, due to the differing velocities of their shifts, during successive additions of the LSR.

On the completely resolved doped ^{1}H NMR spectrum ([LSR] / [**3**] molar ratio 0.7) one-bond HETCOR experiment was done to assign unambiguously all proton signals. In addition, a doped COSY experiment afforded the observation of the coupling pathways of all protons. These data are shown in Table 2 where INAPT data confirming the long-range undoped HETCOR experiments are also reported.

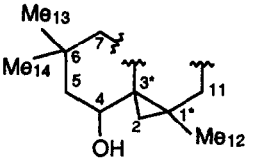
Experimental evidence supporting the C-4/C-5 connectivity is provided by the COSY correlations in Table 2 between H-4 and 2 H-5 and *viceversa*, thus affording the extension from fragment B to fragment D. On the other hand, a long-range HETCOR undoped experiment (Table 1) shows key C-4/H$_a$-2 correlation which affords the combination of structural fragments D and A to give substructure E. This in turn can be linked to fragment C to give substructure F consistently with the detection of a COSY correlation between H-11 and H-10 in the doped spectrum (Table 2) and a weak C-11/H$_a$-10 long-range correlation in the undoped spectrum (Table 1). At this point, formation of the two six-membered rings now gives structure **3** apart from stereochemistry.

The assignment of the relative stereochemistry to structure **3** was a challenging task. The signals of H-8 and H-9 in the NMR spectrum were buried in the δ 1.1-1.4 envelope, thus a NOESY experiment was not attempted. We resorted instead to a detailed study of the Eu(fod)$_3$ doped ^{1}H

NMR spectra to obtain the observed LIS and to calculate them from the geometrical factors (GF) of eleven protons that give identifiable signals during the incremental addition of the LSR. Since the GF are related to relative stereochemistry it was possible to select the most probable configuration among the eight diastereomeric structures (of course the cyclopropane attachment sites 1 and 3 are *cis* each other and the enantiomeric pairs are not considered). The procedure considers both the angle O-Eu-H (χ) and the Eu-O distance (r) according



D



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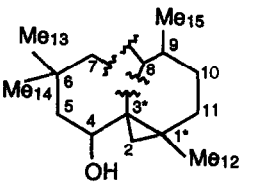


F

to the McConnell-Robertson equation $\Delta v=K[(3\ \cos^2\chi-1)\ r^{-3}]$ where K is the pseudocontact constant, Δv is the observed chemical shift and GF is in brackets. For all eight configurations the position of the Europium atom with respect to the lone pair of the coordinating Oxygen was optimized analogously to a brasilane sequiterpenoid whose structure was elucidated by us.[5] Thus we defined the angle ϕ

Table 2. ^1H and ^{13}C NMR data for compound 3 in the presence of Eu(fod)$_3$.[a]

Pos.	δ_C	DEPT	δ_H mult. (J, Hz)		COSY	INAPT[b]
1	33.0	C				
2	28.1	CH_2	H_a	8.11 d (4.0)	H_b-2	C-1
			H_b	5.06 d (4.0)	H_a-2	
3	41.6	C				
4	104.0	CH		20.44 dd (11.0, c)	2H-5	
5	56.7	CH_2	H_a	12.89 dd (13.0, c)	H-4, H_b-5	
			H_b	10.61 dd (13.0, 11.0)	H-4, H_a-5	
6	34.4	C				
7	51.4	CH_2	H_a	4.74 dd (13.2, 11.4)	H_b-7, H-8	C-6
			H_b	3.25 dd (13.2, 6.6)	H_a-7, H-8	
8	51.1	CH		4.89 ddd (11.4, 6.6, c)	2H-7, H-9	C-1, C-3, C-6, C-7, C-9, C-15
9	46.7	CH		3.30 m H-8, 2H-10, 3H-15		
10	35.4	CH_2		3.09 m H-9, 2H-11	C-15	
11	37.7	CH_2	H_a	4.43 ddd (11.7, 8.0, 7.6)	2H-10, H_b-11	C-1, C-2, C-3, C-9
			H_b	3.86 ddd (11.7, 3.0, c)	2H-10, H_a-11	
12	29.3	CH_3		7.74 s		
13	32.1	CH_3		2.66 s	C-6, C-7	
14	31.6	CH_3		2.24 s	C-6, C-7	
15	20.6	CH_3		2.37 d (6.5)	H-9	C-8, C-9

[a][Eu(fod)$_3$]/[3] = 0.8 molar ratio.
[b]Carbons observed upon irradiation of the indicated proton.
[c]Unresolved by broadening.

Eu-C4-O 80°, the dihedral angle Θ Eu-O-C4-H4 55° at the distance Eu-O 3.5 Å. The constant K was derived by a least squares minimization of the observed Δν the GF for eleven protons. Hence, the theoretical shift for each proton was calculated. The difference between observed and calculated LIS for the *i* protons was then expressed *via* the Hamilton agreement factor (AF)[7] where the LIS_{obsd} was the slope of the relationship Δν *vs* [LSR] / [3]. For calculation to optimize ϕ and Θ we applied a simulation computer method successfully used by one of us to study a number of structurally rigid and flexible α,ß-unsaturated carbonyl systems.[8,9] This method uses as input data the atomic coordinates and the LIS_{obsd} for each studied proton. The procedure is repeated for the molecular geometry corresponding to each diastereomer. The minimum value of AF defines the correct relative configuration. The observed and simulated LIS and the AF for the eight possible diastereomeric structures are given in Table 3.

$$AF = \left(\frac{\sum_i (LIS_{obsd\ i} - LIS_{calcd\ i})^2}{\sum_i (LIS_{obsd\ i})^2} \right)^{1/2}$$

The flexibility of both six-membered rings introduced a complexity in the calculation of the

Table 3. Measured and simulated LIS for the eight possible diastereomeric structures of compound 3[a,b]

Proton	LIS^{c}_{obsd}		ttc 1[d]	tcc 2[d]	ctc 3[e]	ccc 4[f]	ttt 5[g]	tct 6[g]	cct 7[h]	ctt 8[h]
			LIS_{calcd}	LiS_{calcd}	LIS_{calcd}	LIS_{calcd}	LIS_{calcd}	LIS_{calcd}	LIS_{calcd}	LIS_{calcd}
H_a-2	5.87	(0.11)	6.90	7.05	7.47	6.74	2.65	2.64	2.50	2.50
H_b-2	4.13	(0.06)	3.49	3.56	3.96	3.54	3.31	3.23	2.90	3.06
H-4	16.20	(0.90)	16.01	16.03	15.50	14.07	17.72	17.66	16.44	15.99
H_a-5	9.42	(0.09)	9.13	9.14	9.82	1.96	3.60	3.56	3.02	2.94
H_b-5	7.98	(0.09)	7.92	7.88	7.95	12..13	6.88	7.04	8.78	8.93
H-8	2.90	(0.02)	2.65	2.75	3.46	3.21	3.47	3.51	6.78	6.22
H-9	2.00	(0.04)	1.84	1.15	2..13	1.46	4.07	2.58	2..27	2.41
H_a-11	2.20	(0.04)	2.30	2.27	2.14	1.65	3.41	3.14	2.50	3.90
H_b-11	2.13	(0.03)	2..31	2..25	1.77	1.46	2.31	2.20	2.08	2.46
H-12	5.58	(0.08)	5.77	5.66	4.33	3.12	4.87	4.59	3.78	4.72
H-15	1.14	(0.01)	0.76	1.30	0.96	0.98	1.86	3.93	1.89	1.46
AF^{i}			0.0603	0.0713	0.101	0.406	0.326	0.337	0.375	0.368
K^{c}			721(14)	720(16)	711(23)	634(89)	727(88)	794(90)	734(94)	721(30)

[a]Data reported in the 1st column indicate the observed molar induced shifts (LIS_{obsd}); data in the following columns indicate calculated molar induced shifts (LIS_{calcd}) for each diastereomeric structure. [b]For each diastereomer (1-8) first letter indicates a *trans* (t) or *cis* (c) relationship between hydroxyl (equatorial "up") and H-8, second letter indicates the relationship between hydroxyl and Me-15, third letter indicate the relationship between hydroxyl and C-2. [c]In parenthesis estimated standard deviation. [d]Ring A chair, ring B half-chair, OH eq "up". [e] Ring A boat, ring B boat, OH eq "up". [f]Ring A chair, ring B half-chair, OH ax "up". [g]Ring A boat, ring B half-chair, OH ax "up". [h]Ring A chair, ring B boat, OH ax "up". [i]Agreement factor.

protons atomic coordinates. Thus, it was necessary to calculate the most preferred conformation of the rings. To this end we carried out a computational analysis using the MacroModel program [10,11] (MM2). The rigidity of the bridge C-3–C-8 involves some restraint to the conformations that can be built; in fact, when the H-8 is *trans* to the cyclopropane C-2, the ring A which bears the hydroxyl group can assume only chair conformation with equatorial OH. To have chair conformation with axial OH, H-8 and C-2 must be *cis* each other. The preferred conformation for each possible diastereomer is given in Table 3. An examination of the results reveals that the largest deviations between observed and calculated LIS appear for the stereogenic centers of the molecule or for protons connected to them. The AF resulting for the *t,t,c* configuration (0.0603, see Table 2 for the symbols used) is lower with respect to the resulting AF for the seven other stereochemistries. On this basis, laurobtusol possesses the configuration depicted in formula 3. In particular, from Table 3 we find that the ratio R of the AF between the *t,t,c* and the *t,c,c* configurations is 1.18. Since our monodimensional hypothesis has nine degrees of freedom (11 observed protons-2 varying angles ϕ and Θ of the Eu position) the significance Tables in ref. 7 give us the experimental R value at an 8 % level, that is the level at which the second hypothesis (*t,c,c* configuration) cannot be rejected. Moreover, the most stable conformation for the correct diastereomer *t,t,c* has a much lower energy (38.5 Kcal mol^{-1}) with respect to other conformations that are thermodynamically strongly unfavored (A boat, B boat 41.4; A chair, B boat 41.1; A boat, B half-chair 40.6, in Kcal mol^{-1}). A computer-obtained stereoview of it is shown in Figure 1. The AF calculated for configurations possessing rings in higher energy conformations resulted in any case significantly higher than those reported in Table 3.

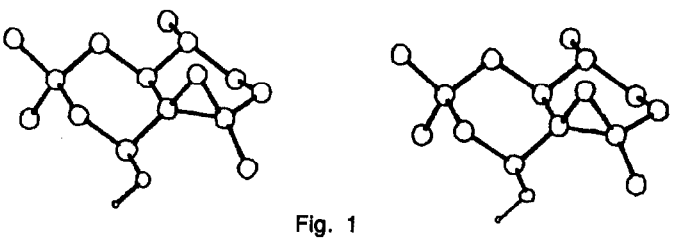
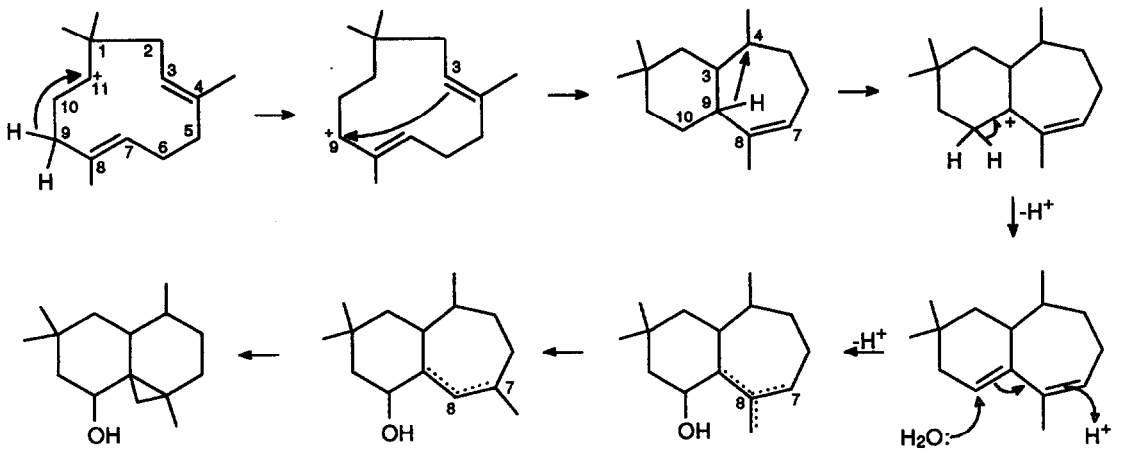


Fig. 1

Laurobtusol is presumably derived from humulene according to the following Scheme starting with the α-humulyl cation which undergoes consecutive 1,3-hydride shifts to give, after a proton elimination, a stabilized diene. This undergoes hydration and further double bond delocalization with a plausible cyclopropane formation.

Detailed biosynthetic studies on 1,3 hydride shifts to give tricyclic humulane were reported.[12] The 8 to 7 methyl migration is similar to that depicted in the trasformation of aromadendranes in poitanes.[1] For this reason, the numbering system shown for compound 3 was chosen to coincide with that used for humulene.[13]



Recently, humulene and a monocyclic dihydroxyhumulene derivative were isolated from japanese *Laurencia obtusa*.[14] The presence of humulane derivative from this species collected in different geographical areas stimulates the search for other representatives of this class in this prolific alga.

EXPERIMENTAL

General. Low and high-resolution EIMS (probe) 18 eV were obtained on a VG ZAB 2SE instrument. IR and UV spectra were recorded on a Perkin-Elmer mod. 684 and on a Perkin-Elmer mod. 330 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC-250 instrument operating at 250.13 MHz and 62.9 MHz using $CDCl_3$ as solvent. Chemical shifts are quoted in ppm (δ) relative to TMS. DEPT, COSY and HETCOR experiments were performed using standard Bruker microprograms. Long-Range HETCOR was performed using the pulse sequence described in the literature.[15] INAPT experiments were performed using the pulse sequence described in well known reports[16,17] with standard Bruker microprograms using delays $\Delta_1 = \Delta_2 = 36$ ms corresponding to J=7 Hz. Optical rotation was determined with a Perkin-Elmer 141 polarimeter. Preparative Liquid Chromatography (PLC) was carried out on a Jobin-Yvon LC Miniprep instrument, using LiChroprep Si-60, 15-40 μm, (Merck) as stationary phase.

Molecular Modeling and Force Field calculations (MM2) were performed on a Digital Vax-Station 3100/38 computer using MacroModel Version 2.5 program. Simulation of LIS spectra was performed using a computational program written in Fortran 77 language.[5] The program runned on a Digital Vax-Station 2000 computer, using as atomic input coordinates the data obtained from MacroModel program.

Collection, extraction and chromatographic separation. Laurencia obtusa was collected at

Castelluccio, 40 Kms South of Catania, eastern Sicily, in October 1988, in littoral zones. The fresh alga (ca. 15 kg wet wt.) was immediately soaked in *iso*-PrOH and left steeping for three months. The material was then filtered and the algal residue was repeatedly homogenized with *iso*-PrOH in a Waring blender and filtered. The exhausted dried powder residue weighed 720 g. The *iso*-PrOH/water solution was concentrated *in vacuum* and partitioned with Et_2O and $NaNO_3$. The ether layer was dried with Na_2SO_4, and evaporated to give 30 g. of dark green oil. The extract was applied to an open column (3x100 cm) of Silica gel and eluted with increasing concentrations of Et_2O in petrol. Fractions of 250 ml were collected and those exhibiting similar TLC profiles combined.

Fraction 13 (440 mg) was subjected to PLC (gradient of CH_2Cl_2/C_6H_{14} from 1:1 to 3:2) to give laurencienyne (2) and impure laurobtusol (3) which was further purified (CH_2Cl_2/C_6H_{14} 3:2) to give pure laurobtusol (21 mg, 0.07% extract).

Laurobtusol (3) was obtained as a white optically active powder; m.p. 121-123 °C; $[\alpha]^{25}$ (λ) 28.7 (589), 28.9 (578), 33.1 (546), 57.2 (436), 90.6 (365) (*c*=0.9, EtOH); IR ν_{max} (liquid film) cm^{-1}: 3300, 1455, 1360, 1010; HRMS $[M]^{+\cdot}$ 222.1986 (calcd for $C_{15}H_{26}O$ 222.1983); MS *m/z* (rel. int.) 222 (10), 209 (21), 207 (34), 204 (28), 189 (42), 180 (63), 165 (23), 162 (22), 161 (30), 153 (33), 150 (37), 139 (49), 136 (58), 125 (60), 109 (74), 95 (100); ^1H and ^{13}C NMR see Table 1.

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