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(+)-LEPIDOZENE, A NEW BICYCLIC SESQUITERPENE FROM THE GORGONIA *LOPHOGORGIA RUBERRIMA*

Manuel Norte, José J. Fernández, Rafael González, Matias L. Rodríguez,
Catalina Ruiz-Pérez and Victor Rodríguez-Romero

C.P.N.O. "Antonio González", Instituto Universitario de Bio-Organica, Universidad de La Laguna,
La Laguna 38206, Tenerife, SPAIN

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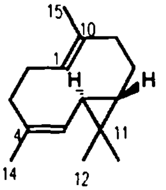
Summary: (+)-Lepidozene 1, a new sesquiterpene possessing a trans-fused (8,1,0) undecane system, has been isolated from the gorgonia **Lophogorgia ruberrima**. Its structure and absolute configuration have been determined by spectroscopical methods and X-ray diffraction analysis.

The chemical examination of the **Alcyonaceae** and **Gorgonaceae** has proved them to be a rich source of natural products yielding interesting sesquiterpenes, diterpenes, sterols and other compounds[1]. We wish to report here on the isolation and structural elucidation of a new sesquiterpene from the gorgonia **Lophogorgia ruberrima**.

The gorgonia **L. ruberrima** was collected near La Graciosa (Canary Islands) at a depth of 35 meters. The fresh material was frozen and extracted with acetone. The diethyl ether soluble fraction from the acetone extract was chromatographed through a series of silica gel medium pressure and Sephadex LH-20 chromatographies which led to the pure compound 1.

The molecular formula of $C_{15}H_{24}$ (based on high resolution mass measurement of the parent ion) together with other spectral evidence suggested for 1 a bicyclic sesquiterpenoid skeleton containing two trisubstituted double bonds and a dimethylcyclopropane ring. Thus, the analysis of the NMR spectral data (see experimental part) placed one methyl group on each double bond (δ, 1.63 and 1.75) and established the geometry of these double bonds as E and Z (^{13}C δ : 133.37, 132.95, 126.28, 125.52, 24.24 and 15.59), the position being established at C(10)-C(1) and C(4)-C(5) respectively through the observed COSY correlations. Moreover, the correlations observed in the COSY spectrum between the proton at δ 5.21 and the cyclopropyl methine at δ 0.7 fixed the position of the cyclopropane ring at C(6)-C(7). From these data we concluded that compound 1 has a bicyclo (8.1.0) undecane system as in isobicyclogermacrene 2.[2] However, comparison of the spectral data of 1 and 2 showed clear differences in the chemical shifts of the cyclopropyl protons as well as in those of the vinylic protons. Futhermore, the NOESY experiment established the presence of nOes indicating that the cyclopropyl methyl group Me-13 and the protons H-5 and



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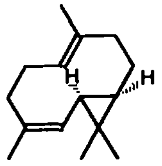

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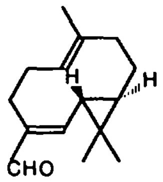

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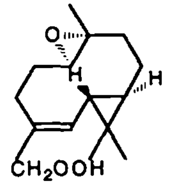

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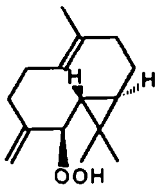

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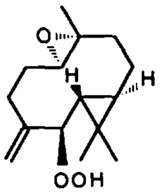

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H-7 have a β-orientation, while the vinylic methyl group Me-15 and H-6 are α. We also observed nOe correlations between H-6 and H-3 (α) as well as between H-7 and H-9 (β), establishing in the molecular structure a trans-fused bicyclo (8.1.0) undecane system instead of the cis-fused system present in 2. This type of system was also present in the sesquiterpene lepidozenal 3, which was isolated from the terrestial leafy liverwort **Lepidoza vitrea** Steph[3] and very recently, together with the related hydroperoxides 4-6, from the marine actinia **Antopheura pacifica**[4]. On the other hand, although lepidozene had not been isolated from natural sources, it has recently been synthetized as a racemic mixture by intramolecular carbonyl coupling[5]. Comparison of the NMR spectral data of compound 1 with those reported for the synthetic (±)-lepidozene showed them to be identical.

In order to establish its absolute configuration, we have prepared its diepoxy derivative 7 by treatment of 1 with two equivalents of MCPBA in dichloromethane. This compound crystallized in the orthorhombic system, space group $P2_12_12_1$, a = 6.617 (1), b = 12.159 (2), c = 17.854 (1) Å; v = 1436.5 (3) $Å^3$, z = 4, Dc = 1.093 $gr.cm^{-3}$, μ = 5.19 cm^{-1}. The intensity of 1268 reflections (including 230 Friedel pairs) was measured up to θ = 61° with a Siemens AED computer-controlled four circle diffractometer, using graphite monochromated CuKα (λ = 1.5418 Å) radiation and ω:θ scan. 1184 reflections were judged as observed with $I > 2\sigma$ (I) and corrected for Lorentz and polarization.

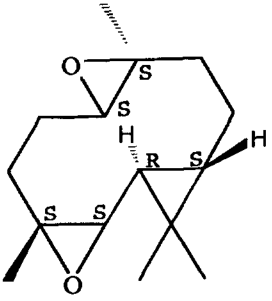
The structure was solved by direct methods with SHELXS 86[6] using the hkl part of the spectrum. After isotropic full-matrix least-squares refinement using F magnitudes with SHELX 76[7], an empirical absorption correction was applied using the program DIFABS[8]. Correction factors were between 0.400 and 1.983. The hydrogen atoms were placed in calculated positions (C-H 1.08 Å). Refinement with anisotropic thermal parameters for non-H atoms and the H-atoms as fixed isotropic contribution converged to R = 0.072, wR = 0.076, w = 2.414/ [σ^2(F) + 0.00005

Atom	x/a	y/b	z/c	Beq
O (1)	0.1820(6)	1.0683(2)	0.2966(2)	5.5(2)
O (2)	0.4167(6)	0.6903(3)	0.4613(2)	5.5(2)
C (1)	0.3321(8)	0.7869(3)	0.4263(3)	4.4(2)
C (2)	0.4022(9)	0.8970(4)	0.4570(3)	5.2(2)
C (3)	0.3923(9)	0.9888(4)	0.3991(3)	4.9(2)
C (4)	0.1810(8)	1.0042(3)	0.3655(3)	4.6(2)
C (5)	0.1254(8)	0.9529(3)	0.2941(3)	4.7(2)
C (6)	0.2558(8)	0.8781(3)	0.2489(2)	4.5(2)
C (7)	0.1904(7)	0.7601(3)	0.2388(3)	4.5(2)
C (8)	0.3378(9)	0.6638(4)	0.2500(3)	5.4(2)
C (9)	0.3540(9)	0.6278(4)	0.3315(3)	5.5(2)
C (10)	0.4582(8)	0.7109(4)	0.3823(3)	4.6(2)
C (11)	0.1935(9)	0.8357(4)	0.1727(3)	5.4(2)
C (12)	0.3629(13)	0.8246(5)	0.1146(3)	7.5(4)
C (13)	-0.0121(12)	0.8699(5)	0.1396(4)	7.5(4)
C (14)	0.0150(10)	1.0354(4)	0.4197(3)	5.8(3)
C (15)	0.6799(8)	0.7369(5)	0.3650(3)	6.0(3)

Table 1.- Final fractional atomic coordinates with their e.s.d.s. and equivalent isotropic thermal parameters (Å) for non-H atoms.

F^2], s = 0.0796 $(\Delta/\sigma)_{max} \leq 0.185$, $\Delta\rho_{max} = 0.21$ eÅ^{-3} for the right enantiomer, shown in Fig. 1.

The absolute configuration was determined by comparison of the 33 more relevant Bijvoet pairs with Fo > 5σ(Fo), which are in the range $5 \leq Fo \leq 50$ and $0.15 \leq$ sen $\theta/\lambda \leq 0.40$. The



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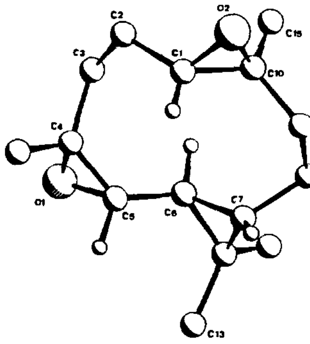

Fig.1

averaged Bijvoet differences are 0.470 for the right enantiomer vs 0.508 for the wrong one. Final

C4 - O1 - C5	60.5(3)	C1 - O2 - C10	61.1(3)
O2 - C1 - C10	60.0(3)	O2 - C1 - C2	116.3(4)
C2 - C1 - C10	125.0(4)	C1 - C2 - C3	112.8(4)
C2 - C3 - C4	113.2(4)	O1 - C4 - C3	113.0(4)
C3 - C4 - C14	116.4(4)	C3 - C4 - C5	121.1(4)
O1 - C4 - C14	114.2(4)	O1 - C4 - C5	59.5(2)
C5 - C4 - C14	118.7(4)	O1 - C5 - C4	59.9(3)
C4 - C5 - C6	125.7(4)	O1 - C5 - C6	117.1(3)
C5 - C6 - C11	122.4(4)	C5 - C6 - C7	118.6(4)
C7 - C6 - C11	59.3(3)	C6 - C7 - C11	60.5(3)
C6 - C7 - C8	121.7(4)	C8 - C7 - C11	124.3(4)
C7 - C8 - C9	112.9(4)	C8 - C9 - C10	114.2(4)
C1 - C10 - C9	118.6(4)	O2 - C10 - C9	112.1(4)
O2 - C10 - C1	58.9(3)	C9 - C10 - C15	116.9(4)

O1 - C4	1.459(6)	C5 - C6	1.493(6)
O1 - C5	1.453(5)	C6 - C7	1.509(5)
O2 - C1	1.442(5)	C6 - C11	1.512(7)
O2 - C10	1.458(6)	C7 - C8	1.537(7)
C1 - C2	1.518(6)	C7 - C11	1.494(7)
C1 - C10	1.473(7)	C8 - C9	1.525(7)
C2 - C3	1.525(7)	C9 - C10	1.522(7)
C3 - C4	1.532(7)	C10 - C15	1.532(7)
C4 - C5	1.468(6)	C11 - C13	1.541(10)
C4 - C14	1.511(8)	C11 - C12	1.533(9)

Table 2.- Angles (º) and interatomic distances (A) of compound 7 with e.s.d.s. in ( ).

O1 - C7^{a}	3.451 (5)	O2 - C1^{c}	3.414 (6)
O1 - C8^{b}	3.483 (7)	O2 - C14^{c}	3.531 (6)
O1 - C15^{b}	3.656 (6)	O2 - C15^{d}	3.582 (6)

symmetry code:

a) -x, y+1/2, -z+1/2
b) -x+1, y+1/2, -z+1/2
c) x+1/2, -Y+3/2, -z+1
d) x-1/2, -y+3/2, -z+1

Table 3.- Van der Waals contacts (Å).

averaged Bijvoet differences are 0.470 for the right enantiomer vs 0.508 for the wrong one. Final positional coordinates with e.s.d.s in parentheses are listed in Table 1. Atomic coordinates and bond angles are given in Table 2.

Crystal cohesion is ensured by a dense three dimensional network of Van der Waals interactions listed in Table 3. The result established the absolute configuration of (+)-lepidozene 1 at the carbons C(6) and C(7) as S. This analysis is the first carried out on lepidozene-type compounds, and it is of biological interest because it has been observed that the structures of the sesquiterpenes isolated from coelenterates as well as those from liverworts tend to belong to the same enantiomeric form as that observed for (-)-lepidozenal. Moreover, the generalization has been made by other workers that they possess structures antipodal to those from terrestrial plants[10]. Thus, although lepidozene has not yet been isolated from this source, it is worthy of note that the absolute configuration observed for (+)-lepidozene is antipodal to that established for (-)-lepidozenal, which has been chemically transformed into (-)-lepidozene[3].

EXPERIMENTAL PART

NMR spectra were recorded on a Bruker Mod. 200 SY spectrometer (200 MHz), chemical shifts are reported relative to Me_4Si (δ,0) and coupling constants are given in hertz. The 2D-NMR spectra were obtained using a Bruker microprogram. Samples for nOe experiments were degassed by bubbling Ar through the solutions. IR spectra were recorded on a Perkin-Elmer Mod. 257 spectrometer. Optical rotation was determined for solution in $HCCl_3$ with a Perkin-Elmer Mod. 241 polarimeter. Low and high resolution mass spectra were obtained from a VG Micromass ZAB-2F spectrometer. Mps were determined on a Kofler block and are uncorr. Silica gel column chromatography, tlc and plc were perfomed on silica gel 60 G. Sephadex LH 20 obtained from Pharmacia was used for gel filtration chromatographies. The tlc plates were developed by spraying with 6N sulphuric acid and heating. All solvents were purified by standard techniques. Anhydrous magnesium sulphate was used for drying solutions.

Collection, extraction and chomatrographic separation.

Lophogorgia ruberrima was collected in September 1985 at La Graciosa (Canary Islands) using SCUBA (-35 m). The gorgonia was frozen until removed to the laboratory, where it was air-dried and ground in a Wiley mill to a 1 mm particle size (1.8 kg) and extracted with acetone. The solvent was evaporated in vacuo to afford 28 g of crude extract and the diethyl ether soluble fraction was chromatographed on a silica gel column eluted with n-hexane and mixtures of n-hexane/EtOAc of increasing polarity. Fractions on n-hexane (305 mg) were rechromatographed on Sephadex LH 20 (using as eluent $HCCl_3$:n-hexane:methanol 1:2:1) to afford pure 1 (30 mg).

Compound 1: oil, $[\alpha]_D^{25} = +223.13$ (c, 0.86, $HCCl_3$). IR ν_{max} ($HCCl_3$): 3000, 2960, 2920, 2860, 1450 and 1370 cm^{-1}. ^1H-NMR ($DCCl_3$) δ : 5.21 (H1, bt, J= 6.8 Hz); 5.02 (H5, d, J= 9.4 Hz); 2.50 (H3, m); 2.40 (H2, m); 2.21 (H9, ddd, J= 3.1, 3.5 and 12.3 Hz); 2.03 (H2, ddd, J= 3.6, 6.8 and 12.8 Hz); 1.90 (H3, m); 1.80 (H9, m); 1.76 (3 H14, s); 1.64 (3 H15, s); 1.09 (3 H13, s); 1.05 (3 H12, s); 0.90 (H8, m); 0.72 (H6, dd, J= 9.4 and 5.3 Hz) and 0.02 (H7, ddd, J= 3, 5.3 and 11.5 Hz) ^{13}C-

NMR (DCCl$_3$) δ: 133.37 (s), 132.95 (s), 126.28 (d), 125.52 (d), 40.58 (t), 34.13 (d), 32.14 (d), 31.30 (t), 26.20 (t), 25.00 (t), 24.24 (q), 22.50 (q), 21.99 (q), 19.05 (s), 15.59 (q). HRMS (m/z 204.1895, $C_{15}H_{24}$ calc. 204.1878) MS m/z (rel int %) 204 (18), 189 (6), 175 (5), 161 (25).

Compound 7: To a solution of 1 (20 mg, 0.1 mmol) in H_2CCl_2 was added MCPBA (36 mg, 2.1 mmol). The mixture was stirred for 2 h at 0° C and then KF (10 mg) was added. After 50 min, the mixture was filtered and the filtrate concentrated in vacuo and purified on microflash silica gel column. Crystallization in n-hexane afforded pure 2: colourless crystals, mp 77 ºC, $[\alpha]_D^{25}$= + 64.0 (c, 0.7, HCCl$_3$). IR ν_{max} (HCCl$_3$): 3000, 2960, 2920, 2860, 1450, 1380, 1170 and 1150 cm^{-1}. ^{1}H-NMR (DCCl$_3$) δ : 2.89 (1H, dd, J= 4.1 and 10.2 Hz); 2.35 (1H, d, J= 9.6 Hz); 2.26 - 1.93 (4H, m); 1.37 (3H, s); 1.21 (3H, s); 1.15 (3H, s); 1.09 (3H, s); 0.32 (1H, ddd, J= 5.5, 5.4 and 10.8 Hz) and 0.13 (1H, dd, J= 5.5 and 9.6 Hz). ^{13}C-NMR (DCCl$_3$) δ : 64.50 (d), 61.81 (d), 59.97 (s), 59.17 (s), 40.39 (t), 29.65 (t), 28.87 (d), 28.78 (d), 25.86 (t), 24.41 (t), 21.78 (q), 21.73 (q), 18.75 (s) and 15. 52 (q). MS m/z (rel int %) 236 (1), 221 (1), 203 (2) ,193 (2) and 176 (19).

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