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APLYSINADIENE AND (R,R) 5 [3,5-DIBROMO-4-[(2-OXO-5-OXAZOLIDINYL)] METHOXYPHENYL]-2-OXAZOLIDINONE, TWO NOVEL METABOLITES FROM APLYSINA AEROPHOBA.SYNTHESIS OF APLYSINADIENE.

M. Norte, M.L. Rodriguez, J.J. Fernández, L. Eguren and D.M. Estrada

Centro de Productos Naturales Orgánicos "Antonio González", Instituto Universitario de Química Orgánica, Universidad de La Laguna, Carretera de La Esperanza nº2, La Laguna 38206, Tenerife, Spain.

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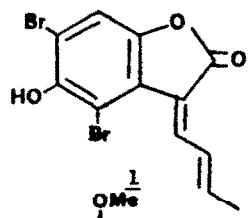
Abstract: Two novel constituents, biogenetically derived from dibromotyrosine, were obtained from a sponge Aplysina aerophoba. The structure of aplysinadiene 1 was established on the basis of its spectral properties and by synthesis of 1 and its isomers 7 and 8. The structure and absolute configuration of the oxazolidinone 5a was established by X-ray diffraction analysis as (R, R) 5 [3, 5-dibromo-4-[(2-oxo-5- oxazolidinyl)] methoxyphenyl]-2-oxazolidinone.

The sponges of the order Verongida, genera Aplysina, Verongula, Psammaplysilla and Iantella, have proved to be a rich source of bromophenolic metabolites derived mainly from dibromotyrosine and some of them from monobromotyrosine, antimicrobial activity being the most common biological property observed for these substances.[1-4]

We have examined the constituents of the sponge Aplysina aerophoba collected near Graciosa Island (Canary Islands) in September 1983, and isolated the dibromoderivatives aplysinadiene, 1, 3,5 dibromo methoxyphenol, 2, aeroplysinin-2, 3, the dienone, 4, the oxazolidinone, 5a and the uranidine, 6.

The fresh sponge was extracted with acetone. The solvent was evaporated "in vacuo" to an aqueous solution which was partitioned between water and ethyl acetate. The ethyl acetate extract (89 gr) was chromatographed on silica gel column using mixtures of n-hexane/ethyl acetate of increasing polarity. Subsequently, separation of the fractions eluted by repeated column chromatography or preparative tlc (with the more polar compound) on silica gel ,



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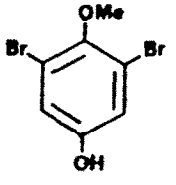

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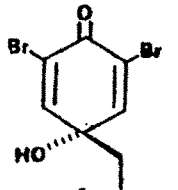

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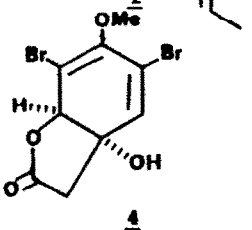

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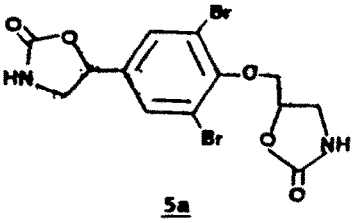
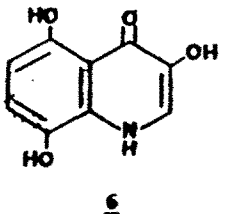


5a



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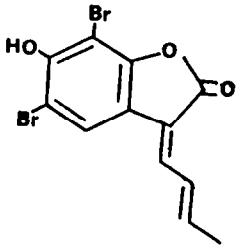
and gel filtration on Sephadex LH-20, afforded, in order of increasing polarity, aplysinadiene, 1 (20 mg), 3,5 dibromo 4 methoxyphenol, 2 (15 mg)[5], aeroplysinin-2, 3 (800 mg)[6], the dienone, 4 (4.3 g)[7], the oxazolidinone, 5a (3.9 g) and the uranidine, 6 (200 mg)[8].

Aplysinadiene 1 : isolation and characterization.

The aplysinadiene was isolated as an amorphous, optically non-active yellow compound (m.p. 218-220 ºC.). The high resolution mass spectrum of this compound indicated an elemental composition of $C_{12}H_8O_3Br_2$. The IR and UV showed the presence of hydroxyl and conjugated lactone groups (IR 3500, 1775 and 1630 cm^{-1}; UV 335 and 209 nm.) The ^{1}H-NMR ($CDCl_3$) spectrum contained a singlet at δ 7.3 and signals assigned to a butenylide moiety at δ 2.07 (dd, 3H, J=7.4 and 1.6 Hz); 6.59 (dq, 1H, J=14.3 and 7.4 Hz); 7.74 (ddq, 1H, J=14.3, 11.4 and 1.6 Hz) and 8.21 (d, 1H, J=11.4 Hz). The ^{13}C-NMR spectrum showed the presence of a methyl at 19.79 ppm; four methines at 109.12, 128.12, 144.6 and 148.85 ppm and five fully substituted carbon atoms at 103.75, 113.68, 146.35, 147.16 and 165.91 ppm.

From the spectroscopic data three structures 7, 8 and 1 were considered for this compound, the isomer 9 being precluded due to the interaction of the bromine atom with the butenylide side chain. The structure of the natural compound was established by total synthesis of its isomers.



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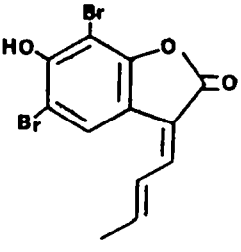

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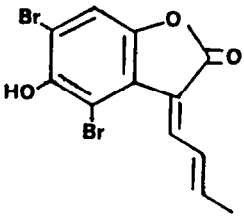

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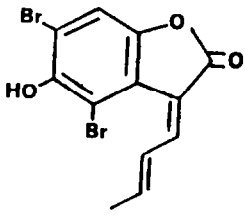

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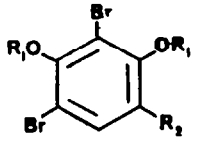
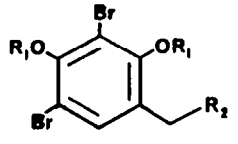
Synthesis of compounds 7 and 8.

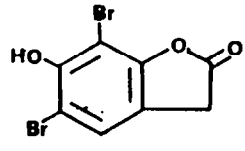
The starting material, 3,5 dibromoresorcylic acid 10, was converted via sequential reactions with CH_2N_2 in diethyl ether and then with K_2CO_3/Me_2SO_4 in acetone to the methyl ester 11 (88 % overall yield). Side chain homologation was accomplished by the following reactions. Reduction of 11 with DIBAL in diethyl ether to obtain the alcohol 12 (90 %) and mesylation of this alcohol gave 13 (90 %); nucleophilic subtitution of the mesyl derivative 13 with KCN in DMSO gave 14 (90 %) and the acidic hydrolysis of this compound with HCl yielded 15 (90 %). Exposure of this compound to F_3B, CH_2Cl_2 provided the lactone 16 (85 %) which was converted into the mixture (4:1) of 7 and 8 by treatment with E-crotonaldehyde and HNa in THF (60 %). Both compounds were isolated by preparative tlc.



10 R_1= H R_2= COOH
11 R_1= Me R_2= COOMe
12 R_1= Me R_2= CH_2OH
13 R_1= Me R_2= CH_2OMs



14 R_1= Me R_2 = CN
15 R_1= Me R_2 = COOH



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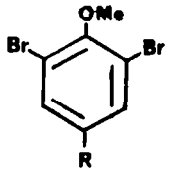
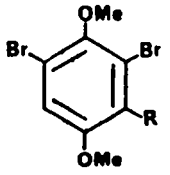
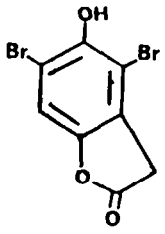


17 R = Me
18 R = CHO
19 R = OH
20 R = OMe



21 R = CH_2Cl
22 R = CH_2CN
23 R = CH_2COOH



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Synthesis of compounds 1 and 9.

Treatment of 3,5 dibromo 4 methoxytoluene 17 with CrO_3/Ac_2O and acidic hydrolysis of the resulting compound yielded the 3,5 dibromo 4 methoxybenzaldehyde 18 (80 % overall yield). Baeyer-Villiger oxidation of this compound 18 with m-chloroperbenzoic acid in dichloromethane, as described in the literature,[9] yielded almost exclusively, 3,5 dibromo 4 methoxybenzoic acid. However, using sulphuric acid as catalyst,[10] the correspondig formate was obtained and its aqueous hydrolysis gave the phenol 19. The crude compound was treated with Me_2SO_4 in acetone to obtain the dimethoxy derivative 20 (55 % from 18). Chloromethylation of this compound using formaldehyde and hydrolchloric acid, and treatment of the resulting compound 21 with KCN in DMSO gave 22 (90 %). This compound was treated as in the above synthesis, to give sequentially the acid 23, the lactone 24 and, finally, aplysinadiene 1 and traces of 9 (42 % overall yield from 22).

Figure 1 shows the partial ^{1}H-NMR of compounds 7, 8 and 1, which displayed very distinctive spectra. The natural compound was identical with 1 in all its spectral data (IR, UV, ^{13}C-NMR, tlc, etc.).

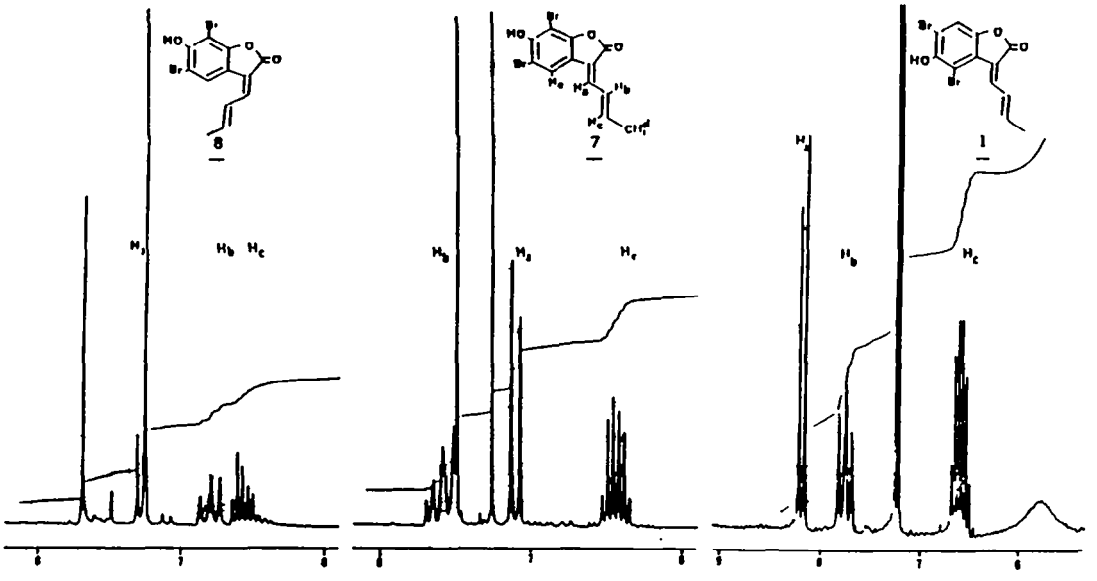
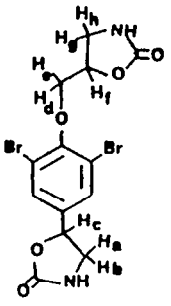


Fig.1: 200 MHz NMR of aplysinadiene 1 and its synthetic isomers. 2D NMR data support the chemical shifts assignements.

Oxazolidinone 5a; isolation and characterization

The oxazolidinone 5a was isolated from the more polar fractions of the extracts and purified by chromatography on silica gel using ethyl acetate as eluent and then by gel filtration with Sephadex LH-20 using Cl_3CH:MeOH:n-hexane (1:4:1) as mobile phase. The compound was crystalline, m.p. 220-222 °C, and

optically active $[\alpha]_D$ =-33 º (c, 1.1, MeOH). A preliminary analysis of the spectroscopical data of this compound showed that this oxazolidinone was closely related with the other previously isolated from sponges of this order 5b and 5c. Compound 5b was isolated by Border from the sponge Verongia lacunosa[11] as the (+) enantiomer $[\alpha]_D$ =+8.9 º. Makarieva et al., later published the isolation of the (-) enantiomer 5c $[\alpha]_D$ =-6.5 º and the racemic from Aplisina sp collected in Cuba. Careful comparison of the ^{1}H-NMR of our compound and the published by Border et al.[12], showed slight differences between the chemical shifts of the oxazolidinone moiety (Table 1), which suggest that our compound must be a diastereomer of the previously reported oxazolidinone. In order to establish the absolute configuration of our compound, this was crystallized by standing in EtOAc to obtain crystals suitable for X-ray analysis.



	Chemical shifts			Multiplicity, J(Hz)	
	V. lacunosa	A. aerophoba		V. lacunosa	A. aerophoba
H_a	3.38	3.71	J_{ab} =	9.3	11.5
H_b	3.89	4.3	J_{ac} =	7.1	8.5
H_c	5.60	5.65	J_{bc} =	8.7	9.2
H_d,H_e	4.15	4.15	$J_{(d,e)f}$ =	4.5	4.2
H_f	4.96	4.96	J_{fg} =	7.1	7.1
H_g	3.61	3.52	J_{hg} =	9	8.5
H_h	3.61	3.65	J_{hf} =	8.7	8.5

Table 1: Comparison of ^{1}H-NMR resonances (ppm) of oxazolidinones 5a from Aplisina aerophoba and 5b from Verongia lacunosa.

X-ray analysis of the oxazolidinone 5a.

Compound 5a, $C_{13}H_{12}Br_2N_2O_5$ crystallizes in the monoclinic system, space group P 2_1, a= 9.684 (9), b= 6.578(5), c= 12.824(8) Å ; V= 769(1) Å^3, z= 2, Dc= 1.8 g cm^{-3}, = 69.8 cm^{-1}. The intensity of 1051 reflections (including 384 Friedel pairs) was measure up to θ = 55 with a Siemens AED computer controlled, four circle diffractometer, using graphite monochromated CuK α (λ= 1.5418 Å) radiation and ω: θ sacn. and 1047 reflections were judged as observed with I > 3 σ(I) and corrected for Lorentz and polarization. The structure was solved by standard Patterson and Fourier recycling methods[13], using the hkl part of the spectrum. Most of the hydrogen atoms were located in a difference synthesis map and the remainder placed in calculated positions.

A final full-matrix least squares refinement with anisotropic thermal coefficients for halogens, isotropic for light atoms, and a fixed isotropic contribution for hydrogens converged to a conventional crystallographic residual of R= 0.066 for the right enantiomer, show in Figure 2.

The absolute configuration[14] as (R). (R) was determined by comparison of the 16 more relevant Bijvoet pairs with Fc >1.5, which are in the ranges 15 ≤ Fo ≤ 50 and .2 ≤ sen θ/λ ≤.6. The averaged Bijvoet differences are 2.15 for the right anantiomer vs 4.08 for the wrong one. Final atomic positional coordinates with e.s.d.'s in parentheses are listed in Table 2.

The Altona[15] conformatinal parameters, Table 3, indicate that both 2-oxazolidinone rings are in the half-chair conformation.

TABLE 2

Non-hydrogen Atom Fractional Coordinates ($\times 10^4$) and Equivalent Isotopic Temperature Factor for **5a**.

Atom	x	y *	z	U(iso) or U(equiv)
Br_1	1339(3)	2500(0)	1383(2)	66(1)
Br_2	3910(3)	9101(6)	- 64(2)	48(1)
O_1	2997(14)	6378(20)	1546(10)	27(4)
O_2	993(16)	1368(24)	- 2969(12)	45(4)
O_3	- 256(16)	-1115(29)	-4116(12)	58(4)
O_4	4517(16)	8398(21)	3473(12)	44(4)
O_5	5999(17)	11085(27)	4218(12)	56(5)
N_1	-1275(16)	2009(27)	-4064(13)	32(5)
N_2	6807(18)	7740(31)	4412(13)	35(5)
C_1	2540(22)	5708(34)	468(15)	18(5)
C_2	2735(19)	6639(27)	-396(14)	13(5)
C_3	2251(20)	5965(31)	-1468(15)	19(5)
C_4	1458(19)	4162(39)	-1673(15)	26(5)
C_5	1186(22)	3207(32)	-832(17)	24(5)
C_6	1734(19)	3787(34)	231(15)	23(5)
C_7	789(23)	3526(32)	-2878(18)	35(6)
C_8	-875(23)	3795(40)	-3401(17)	42(6)
C_9	-237(24)	576(37)	-3769(17)	36(6)
C_{10}	4468(21)	5736(32)	2194(16)	27(5)
C_{11}	4653(21)	6195(31)	3363(16)	21(5)
C_{12}	6252(23)	5724(36)	4149(17)	34(6)
C_{13}	5849(23)	9199(44)	4056(16)	36(6)

* The y coordinate of Br_1 was held constant throughout the analysis to define the origin in this direction

TABLE 3

Altona conformational parameters* for 2-oxazolidone rings

Ring	Δ (°)	φ (°)
A	642	22
B	630	10

* Taking τ_0 opposite to the O atoms and in the senses $C_8 \rightarrow N_1$ and $C_{12} \rightarrow N_2$

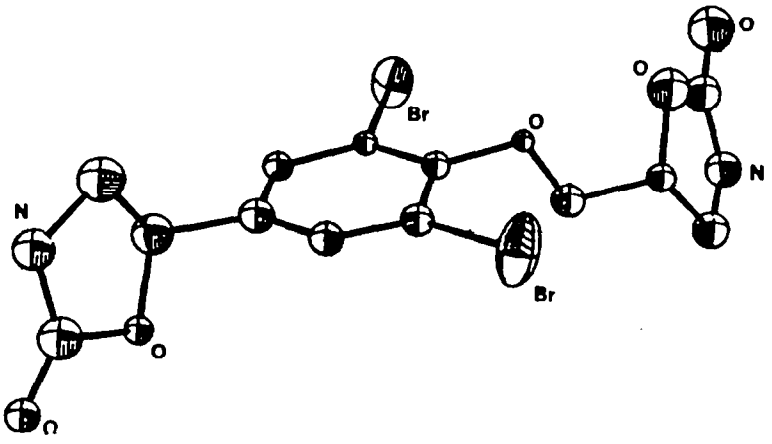


Fig. 2: ORTEP drawing of 5a showing the rigth enantiomer.

EXPERIMENTAL PART

Mps were determined on a Kofler block and are uncorr. Infrared spectra were recorded on a Perkin-Elmer Mod. 257 and ultraviolet spectra recorded on a Perkin-Elmer Mod. 402 spectrophotometers. Optical rotations were determined for solution in chloroform or methanol with a Perkin-Elmer Mod. 241 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Mod.WP-200 SY (200 MHz.), chemical shifts are reported relative to Me_4Si (δ0) and coupling constants are given in hertz. Low and high resolution mass spectra were obtained from a VG Micromass ZAB-2F spectrophotometer. Column and dry column chromatography were performed on silica gel 0.2 - 0.5 and 0.005 - 0.2 mm respectively, and TLC and FLC on silica gel G, all Merck products. The TLC plates were developed by spraying with 6N-sulphuric acid and heating. All solvents were purified by standard techniques. Anhydrous sodium sulphate was used for drying solutions.

Isolation of 1, 2, 3, 4, 5a and 6. The fresh sponge (A. aerophoba, 6 kg), collected in September 1983 at La Graciosa (Canary Islands), was extracted with acetone (6 l) at 20-25ºC. After filtration, the solvent was removed under reduced pressure to give an aqueous residue, which was extracted with ethyl acetate (2 x 1l). The combined extracts were dried and concentrated to yield a dark green oil (89 g). The crude extract was applied to a silica gel column (75 x 12 cm), eluted with a mixture of n-hexane/ethyl acetate of increasing polarity and 50 fractions of 1l each were collected. The earlier fractions (fractions nº 5-10) eluted with h-hexane/ethyl acetate (90:10) afforded, on evaporation of the solvent, aplysinadiene 1 and 3,5 dibromo 4 methoxyphenol 2 in the crude extract. This was chromatographed on a medium pressure silica gel column eluted with a 95:5 mixture of n-hexane/ethyl acetate and then on a Sephadex LH-20 column with $CHCl_3$:MeOH:n-hexane (1:1:2) as eluent to give pure aplysinadiene 1 (20 mg) and 3,5 dibromo 4 methoxyphenol 2 (15 mg). The middle fractions (nº 15-30) eluted with a mixture of n-hexane/ethyl acetate (60:40) give a crude extract which was chromatographed on a Sephadex LH-20 column using $CHCl_3$:MeOH:n-hexane (1:1:2) as eluent to afford aeroplysin-2 3 (800 mg). The later fractions (fractions nº 36-50) eluted with mixtures of n-hexane/ethyl acetate (20:80) and ethyl acetate afforded a crude extract (9 g) which contained the dienone 4, the oxazolidinone 5a and the uranidine 6. These compounds were purified by chromatography on Sephadex LH-20 using CHCl$_3$:MeOH:n-hexane (1:4:1), affording the dienone 4 (4.3 g), the oxazolidinone 5a (3.9 g) and the uranidine 6 (200 mg).

Aplysinadiene 1. m.p.: 218 - 220 ºC. I.R. ($CHCl_3$): 3090, 2910, 1175, 1630, 1610, 1430, 1420, 1080 and 900 cm^{-1}. U.V. λ_{max}^{EtOH} = 335 nm (ϵ= 47222) and 209 nm (ϵ= 64000). ^{1}H-NMR ($CHCl_3$) and ^{13}C-NMR are listed in the text. HRMS found: 361.8794 (M+). $C_{12}H_8O_3Br_2$ requires: 361.8796 MS at m/z 358, 360, 362 (M+); 343, 345, 347; 329, 331, 333.

3,5 Dibromo 4 methoxyphenol 2. solid, m.p.: 124-126ºC. The physical and espectroscopic data (tlc, glc, IR, ^{1}H-NMR, MS) were identical with those reported for 3,5 dibromo 4 methoxyphenol.

Aeroplysin-2 3 solid m.p.: 106-108 ºC, $[\alpha]_D$= + 22º (c, 0.4, MeOH). The physical and spectroscopic data (tlc, glc, IR, ^{1}H-NMR, MS) were identical with those reported for aeroplysin-2.

Dienone 4. solid m.p.: 193-195ºC. The physical and spectroscopic data (tlc, glc, IR, H-NMR, MS) were identical with those reported for the dienone 4.

(R, R) 5 [3,5 - dibromo - 4 - [(2 - oxo - 5 -oxazolidinyl)] methoxyphenyl] - 2 - oxazolidinone 5a. solid m.p. 220-222 C, $[\alpha]_D$ =-33 º (c, 1.1, MeOH); IR ($CHCl_3$): 3670, 3000, 1760 and 1595 cm^{-1}. UV (EtOH): 274 nm (ε=524) and 282 nm ε =491). ^{1}H-NMR ($CDCl_3$) δ : 7.75 (s, 2H); 5.65 (dd, 1H, J= 8.5 and 9.2 Hz); 5.65 (dd, 1H, J= 8.5 and 9.2 Hz); 4.96 (dddd, 1H, J= 8.5, 7.1, 4.2 and 4.2 Hz); 4.15 (d, 2H, J= 4.2 Hz); 3.71 (dd, 1H, J= 11.5 and 8.5 Hz); 3.65 (t, 1H, J= 8.5 Hz), 3.52 (dd, J= 7.1 and 8.5 Hz). ^{13}C-NMR ($CDCl_3$) δ : 41.47 (t); 47.0 (t); 53.0 (t); 53.55 (d); 54.13 (d); 130.8 (d); 118.2 (s); 140.0 (s); 142.3 (s); 157.8 (s) and 158.4 (s). HRMS found 433.9094 (M^+) $C_{13}H_{12}Br_2N_2O_5$ requires 433.9102. MS at m/z : 434, 436, 438 (M^+); 348, 350, 352 ($M^+ - C_3H_4O_2N$); 318, 320, 322 ($M^+ - C_4H_6O_3N$).

Synthesis of compounds 7 and 8

Methyl 3,5 dibromo 2,4 dimethoxy benzoate 11. To a solution of 3,5 dibromoresorcylic acid 10 (1g, 3.2 mmol) in diethyl ether (100 ml) at 0ºC. was added excess of N_2CH_2 in diethyl ether and the mixture was stirred overnight. After addition of a few drops of acetic acid, the solvent was removed, the resulting extract was dissolved in acetone (100 ml) and K_2CO_3 (662 mg, 4 8 mmol) and Me_2SO_4 (580 mg, 4.8 mmol) were added. The mixture was heated under reflux for two houfs, cooled to room temperature, poured into a 1% aqueous solution of KOH (100 ml), extracted with diethyl ether (3x150 ml), shed with H_2O, dried and the solvent removed to afford 11 (998 mg, 88%). m.p.: 88ºC., I.R. ($CHCl_3$): 3020, 3000, 2920, 1720, 1595, 1315 and 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.72 (s, 3H); 3.83 (s, 3H), 3.88 (s, 3H), 7.42 (s, 1H). M.S. at m/z 350, 352, 354 (M+), 320, 322, 324

3,5 Dibromo 2,4 dimethoxyphenylacetonitrile 14. To a stirred solution of 11 (998.4 mg, 2.82 mmol) in diethyl ether (10 ml) at -40ºC. was added dropwise a 1M solution of DIBAL in toluene (5.6 mmol) under argon. After 20 min, a 5% aqueous solution of HCl (5 ml) was added to the mixture and then extracted with diethyl ether (3 x 5 ml). The ethereal extracts were combined, washed with H20, dried and concentrated to give 12 (874 mg, 2.68 mmol) which was dissolved in pyridine (1 ml) and MsCl (522 mg, 5.3 mmol) was slowly added and the mixture stirred for 20 min. The mixture was extracted with diethyl ether (3 x 5 ml), washed with a 5% aqueous HCl solution (3 x 5 ml) and H_2O (4 x 5 ml), dried and concentrated to yield 13 (974 mg, 90%). The crude compound 13 (974 mg, 2.4 mmol) was dissolved in DMSO (2 ml) and KCN (234 mg, 3.6 mmol) was added. The mixture was vigorously stirred at room temperature for 15 min, diluted with H_2O (2 ml), extracted with diethyl ether (4 x 10 ml), washed with H_2O and dried. The solvent was removed to afford 14 (763 mg, 81% overall yield from 11): m.p.: 118-120ºC., I.R. ($CHCl_3$): 3000, 2970, 2235, 1708 and 1470 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3 72 (s, 2H), 3.89 (s, 2H), 3 91 (s, 3H), 7.57 (s, 1H); M.S. at m/z 227, 339, 341 (M+), 277, 279, 281.

3,5 Dibromo 4 hydroxy 2(3H) benzofuranone 16. A mixture of 10 ml a 35% HCl and 763 mg (2.28 mmol) of 14 was heated under reflux for 2 hr After cooling, the mixture was extracted with diethyl ether (3 x 10 ml), drieded and the solvent evaporated to yield 15 (725 mg, 90%). m.p.: 179-180 ºC; I.R.(THF) 3200, 2600, 1730, 1590 and 1470 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ: 3.63 (s, 2H), 3.83 (s, 3H), 3.88 (s, 3H), 7 42 (s, 1H), 10.5 (bs, 1H); M.S. at m/z 352, 354, 356 (M+); 337, 339, 341.

A solution of 15 (725 mg, 2.05 mmol) and 6.15 ml of a 1M solution of F B in CH_2Cl_2 at 0º C. was stirred for 60 hr, diluted with H_2O (6 ml), extracted with diethyl ether (3 x 10 ml), dried and concentrated. Purification of the crude compound by crystallization in n-hexane afforded pure 16 (536 mg, 85%) m p · 145-147 ºC.; I R. ($CHCl_3$): 3500, 3000, 1820, 1730, 1615 and 1440 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ : 3.95 (s, 2H), 7.52 (s, 1H), 8.79 (s, 1H). M.S. at m/z 306, 308, 310 (M+), 278, 280, 282.

Compounds 7 and 8. A solution of 16 (536 mg, 1.74 mmol) in THF (5 ml) and 487 mg (6.96 mmol) of E-crotonaldehyde under argon was cooled to -70 ºC. and HNa (87 mg, 3.65 mmol) was added. The mixture was stirred for 2 hr and few drops of acetic acid were then added, and the solution extracted with diethyl ether. The ethereal extract was washed with H_2O, dried and concentrated to give a (4:1) mixture of 7 and 8. Pure 7 (288 mg) and 8 (72 mg) were obtained by preparative tlc, using n-hexane/ethyl acetate (85:15) as eluent. Compound 7 m.p · 208-210 ºC.; I.R. (CHCl): 3500, 3000, 1780, 1630, 1600, 1420, 1115 and 965 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ : 2.03 (dd, 3H, J= 7.02 and 16 Hz.), 6.43 (dq, 1H, J =14 2 and 7.02 Hz), 7.1 (d, 1H, J= 11.6 Hz.), 7.49 (s, 1H), 7.57 (ddq, 1H, J= 14.2, 11.6 and 1.6 Hz.); M.S. at m/z 358, 360, 362 (M+), 343, 345, 347. Compound 8· m.p.: 198 ºC.: I.R. ($CHCl_3$): 3495, 3050, 1775, 1705, 1630, 1600, 1420, 1115 and 965 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ : 2.08 (dd, 3H, J= 6.7 and 1.51 Hz.), 6.56 (dq, 1H, J= 13.67 and 6.7 Hz.), 6.8 (ddq, 1H, J= 13.67, 10.1 and 1.51 Hz.), 7.28 (d, 1H, J= 10.1 Hz.), 7.69 (s, 1H); M.S. at m/z 358, 360, 362 (M+), 343, 345, 347.

Synthesis of aplysinadiene 1

3,5 Dibromo 4 methoxy phenol 19. To a solution of 3,5 dibromo 4 methoxytoluene 17 (1 g, 3.57 mmol) in acetic acid (4 ml) at 0 ºC. were cautiously added 0.8 ml of sulphuric acid and then a solution of CrO_3 (1 g, 10 mmol) in 5 ml of acetic anhydride. The mixture was stirred at 0º C. for 1 hr, poured into ice water, filtered and washed with cold water. The residue was dissolved in MeOH and 500 mg of K_2CO_3 were added and the mixture stirred. After 2 hr the basic solution was neutralized by addittion of a 5% soln. of HCl and the compound was precipitated. Purification of the crude compound by crystallization in n-hexane /diethyl ether afforded 834 mg (80%) of 18: m.p. 92 ºC., I.R. ($CHCl_3$): 3010, 1690, 1580 cm^{-1}; ^1H-NMR ($CDCl_3$) δ : 3,96 (s, 1H), 8.03 (s, 1H), 9.86 (s, 1H); M.S. at m/z 292, 294, 296 (M+). A mixture of aldehyde 18 (834 mg, 2.85 mmol) H_2SO_4 (0.05 ml) and m-chloroperbenzoic acid (538 mg, 3.1 mmol) in 15 ml of CH_2Cl_2 was stirred for 2 hr, diluted with H_2O (5 ml), stirred at rt for 6 hr, extracted with diethyl ether (3 x 10 ml), washed with H_2O (3 x 10 ml), dried and concentrated. The crude compound was purified by crystallization in n-hexane to give 485 mg (60%) of 19. m.p.: 124-126 ºC; I.R. ($CHCl_3$): 3590, 3020, 2975, 1590, 1470 cm^{-1}; ^1H-NMR ($CDCl_3$) δ : 3.82 (s, 3H), 5.50 (bs, 1H), 7.01 (s, 2H). M.S. at m/z 280, 282, 284 (M+), 265, 267, 269.

2,4 Dibromo 3,6 dimethoxybenzyl chloride 21. Compound 19 was converted into 21 under the same conditions employed to transform 10 into 11. To a suspension of 19 (453 mg, 1.53 mmol) in 35% HCl (10 ml) heated under reflux, was added paraformaldehyde (68 mg). Heating and stirring were continued until the tlc indicated that the substance had been consumed (4 hr). The mixture was diluted with H_2O (3 x 25 ml) dried and concentrated to afford 21 (447 mg, 85%) m.p.: 66 ºC.; ^1H-NMR ($CDCl_3$) δ : 3.84 (s, 3H), 3.87 (s, 3H), 4.78 (s, 2H), 7.05 (s, 1H); M.S. at m/z 342, 344, 346 (M+), 327, 329, 331.

2,4 Dibromo 3,6 dimethoxyphenyl acetonitrile 22. A mixture of 21 (447 mg, 1.3 mmol) and KCN (93 mg, 1.4 mmol) in DMSO (2 ml) was stirred at room temperature for 3 hr, diluted with H_2O (5 ml), extracted with diethyl ether (3 x 10 ml), washed with H_2O (3 x 15 ml), dried and concentrated to give 22 (397 mg, 90%) m.p.: 116-118 ºC.; I.R. ($CHCl_3$): 2970, 2860, 2235, 1708, 1470 cm^{-1}; ^1H-NMR ($CDCl_3$) δ : 3.83 (s, 3H), 3.85 (s, 2H), 3.87 (s, 3H), 7.07 (s, 1H); M.S. at m/z 337, 339, 341 (M+).

Compounds 1 and 9. Aplysinadiene 1 and traces of its isomer 9 were obtained from 2,4 dibromo 3,6 dimethoxyphenyl acetonitrile 22 (46% overall yield) under the same conditions employed to transform 14 into the mixture of 7 and 8. During this synthesis the following compounds were obtained:

2,4 Dibromo 3,6 dimethoxyphenyl acetic acid 23 m.p.: 182-184 ºC. I.R.(THF): 3200, 2600, 1730, 1685, 1590, 1470, 1435, 1315, 1235, 1160 and 855 cm^{-1}; ^1H-NMR ($CDCl_3$) δ : 3.82 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 7.07 (s, 1H), 10.8 (bs, 1H); M.S. at m/z 352, 354, 356 (M+), 337, 339, 341.

4,6 Dibromo 5 hydroxy 2 (3H) benzofuranone 24 m.p. 150-152 ºC.; I.R. ($CHCl_3$) : 3500, 3000, 1800, 1720, 1615, 1440 cm^{-1}; ^1H-NMR ($CDCl_3$) δ: 3.81 (s, 2H), 7.34 (s, 1H), 8.5 (bs, 1H). M.S. at m/z 306, 308, 310 (M+), 278, 280, 282.

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