

NEW METABOLITES FROM THE MARINE MOLLUSC *SIPHONARIA GRISEA*

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Summary: Four new metabolites **6**, **7**, **8** and **9** possessing a polypropionate carbon skeleton, have been isolated from the marine gastropod mollusc *S. grisea*. Their structures and absolute configurations have been determined by spectroscopical and chemical methods. The absolute configuration of pectinatone **5**, is reassigned on the basis of its chemical degradation and X-ray analysis

Siphonaria are gastropod molluscs found in the high intertidal region. Recent research has led to the isolation of several secondary metabolites with antimicrobial activity which are believed to be employed in chemical defence against predators. These types of compounds are characterized by possessing either a linear or a cyclic polypropionate carbon skeleton with a pyrone, a furanone or a hemiketal moiety. Selected examples of these type of metabolites **1-4** are given in the Fig 1¹⁻⁴. The biosynthetic origin of these compounds has recently been investigated on denticulatins **4** and the results of these experiments confirmed that these compounds arise from the condensation of propionate derived units⁵

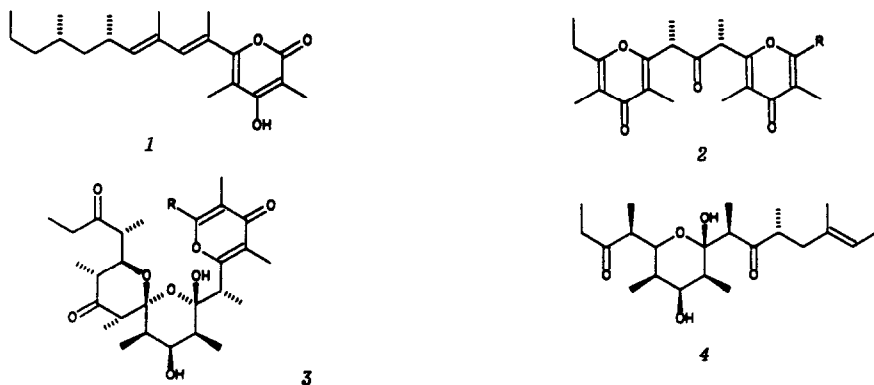
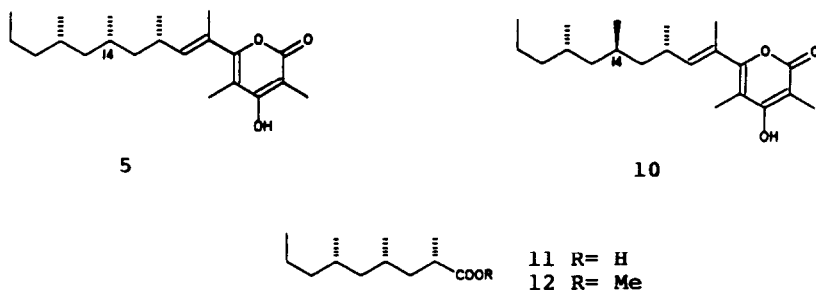


Fig. 1: Examples of metabolites isolated from *Siphonaria* sp: **1** *Diemenensin A*¹⁾; **2** *Maurapyrone*²⁾; **3** *Siphonarins A*³⁾; **4** *Denticulatin A*⁴⁾

The pulmonate *Siphonaria grisea* was collected at Ajuí (Fuerteventura, Canary Islands). The animals were stored in acetone and the ethyl acetate solubles from the acetone extract were combined and chromatographed on medium pressure silica gel and Sephadex LH-20 columns. In a preliminary study,

we have isolated two lineal compounds, the siphonarienedione **7** and the siphonarienolone **8**.⁶ During this work, the presence of other related metabolites was detected, but their amounts prevented structural elucidation. From a new collection of *S. grisea* we have isolated, together with **7** and **8**, another linear compound **9** and two cyclic compounds **5** and **6** having a pyrone and a furanone moiety respectively.

All these compounds showed in their mass spectra the presence of a major fragment ion at m/z 155 caused by α -cleavage to of a double bond resulting in the loss of a $C_{15}H_{23}$ fragment. In order to establish the nature of this alkanyl moiety, these compounds were subjected to oxidative degradation. Instead the ozonolysis, which is the normal method of degradation previously performed on this type of compounds, we used the treatment with $HIO_4/RuCl_3$, as was established by Sharpless,⁷ due to the best yields and reproducibility. On the other hand, the major compound of this collection was the one with a pyrone moiety **5** which showed physical and spectroscopical data identical with those published for the pectinatone **10**, a metabolite previously isolated from *S. pectinata*.⁸ This compound was used as a model in order to establish the optimum experimental conditions for the oxidative degradation to be applied to the minor compounds. Thus, we first isolated 2,4,6 trimethyl nonanoic acid **11**, which was methylated with an ethereal solution of diazomethane to its methyl ester **12**, in accordance with its spectroscopical data. Surprisingly, the optical rotation of **12** was $+21.9^\circ$ which agrees with the value reported by Odham⁹ in the methyl 2,4,6 trimethylnonanoate series by the 2*S*, 4*S*, 6*S* isomer instead the 2*S*, 4*R*, 6*S* as was reported for the pectinatone **10**. Furthermore, when the other metabolites **6-9** were treated in identical conditions, the reactions yielded the same ester establishing the same alkanyl moiety for all of them.



In view of the contradictory results for pectinatone, we decided to analyse compound **5** by X-ray diffraction of a crystal obtained from a dichloromethane solution of this compound.

The compound crystallizes in the orthorhombic system, space group $P2_12_12_1$, $a = 10.412(6)$, $b = 12.900(5)$, $c = 15.806(4)$ Å, $V = 2122.8(16)$ Å³, $Z = 4$, $D_c = 1.059$ g/cm³, $\mu = 5.03$ cm⁻¹. The intensity of 2458 reflections (including 118 Friedel pairs) was measured up $\theta = 64^\circ$ with a Siemens AED computer controlled four circle diffractometer, using graphite monochromated $CuK\alpha$ ($\lambda = 1.5418$ Å) radiation with $\omega - \theta$ scan and 2035 reflections were judged as observed with $I > 3\sigma(I)$ and corrected for Lorentz and polarization. The structure was solved by direct methods and Fourier recycling methods using the hkl part of the spectrum. The H atoms were placed in calculated positions. An appropriate weighting scheme to normalize $\langle \Delta^2 F \rangle$ vs $\langle F_o \rangle$ and $\langle \sin \theta / \lambda \rangle$ was carried out. A final full-matrix least squares refinement with anisotropic thermal coefficients for hydrogens converged to a con-

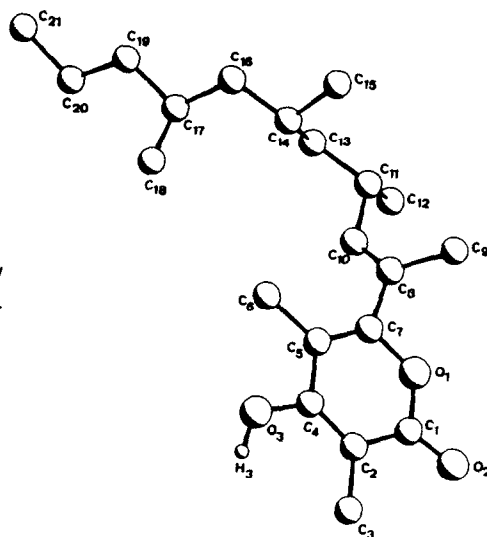


Fig.2 View of the molecule showing the atom-labelling scheme (only H atom of the hydroxy group included)

ventional crystallographic residual of $R = 0.069$ ($R_w = 0.064$) for the right enantiomer

Atomic scattering factors were taken from International Tables for X-Ray Crystallography (1974), maximum shift / $\sigma = 0.15$. The absolute configuration as 11(S), 14(S), 17(S) was determined by comparison of 19 Bijvoet pairs with $F_0 > 10\sigma(F_0)$ which are in the ranges $5 < F_0 < 50$ and $0.08 < \sin \theta / \lambda < 0.45$. The averaged Bijvoet differences are 0.158 for the right enantiomer vs 0.326 for the wrong one. The final atomic coordinates and equivalent isotropic thermal parameters for the non-H atoms are given in Table I. Bond distances and angles are listed in Table II. The molecular structure is shown in Fig. 2

The six-membered ring is planar (r.m.s. displacement of the six atoms from the plane is 0.007 Å). The O2, C3, O3, C6, C8 substituents deviate from the ring plane by 0.004(3), 0.033(3), -0.026(4), 0.009(2), -0.074(3) and -0.005(3) Å respectively.

The molecular packing is realized through O-H...O contacts between the carbonyl O2 at C1 and the hydroxyl group at C4. $O2 \cdots O3$ 2.676 (3) Å ($x + 1/2, -y + 3/2, -z + 2$). The molecules are aligned into chains running in the a direction (Fig. 3)

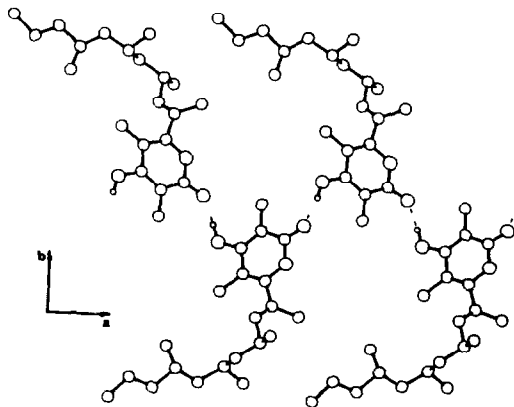


Fig.3 : Molecular packing of structure 5 viewed along c

	X/A	Y/B	Z/C	Ueq
O1	7140(2)	9605(1)	9082(1)	431(6)
O2	8022(2)	8081(2)	9236(2)	666(9)
O3	3835(2)	8791(2)	10266(2)	524(7)
C1	7070(3)	8607(2)	9383(2)	443(8)
C2	5947(3)	8292(2)	9806(2)	437(8)
C3	5889(3)	7205(2)	10150(3)	649(12)
C4	4960(3)	8992(2)	9886(2)	398(8)
C5	5052(3)	10040(2)	9566(2)	385(8)
C6	3955(3)	10776(2)	9723(2)	547(10)
C7	6150(3)	10301(2)	9163(2)	370(7)
C8	6512(3)	11311(2)	8777(2)	401(8)
C9	7829(3)	11718(3)	9009(2)	617(11)
C10	5724(3)	11771(2)	8235(2)	439(8)
C11	5970(3)	12770(2)	7760(2)	508(10)
C12	6449(4)	12511(3)	6861(2)	693(12)
C13	4753(3)	13432(2)	7718(2)	540(10)
C14	4223(4)	13800(2)	8570(2)	552(10)
C15	5212(5)	14439(4)	9072(3)	863(16)
C16	2982(4)	14429(3)	8459(2)	584(11)
C17	1835(3)	13901(3)	8070(2)	581(11)
C18	1398(5)	12981(3)	8603(4)	877(17)
C19	745(4)	14683(3)	7951(3)	715(13)
C20	-404(5)	14294(5)	7470(4)	1018(21)
C21	-1403(7)	15103(6)	7343(6)	1429(35)

Table I.- Atom coordinates ($\text{\AA} \times 10^4$) and equivalent isotropic temperature factors ($\text{\AA}^2 \times 10^4$) of the non-H atoms with e.s.d.'s in parentheses.

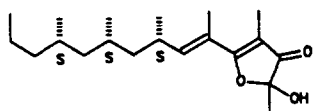
O1 - C1	1.375(3)	C8 - C10	1.325(4)
O1 - C7	1.373(3)	C10 - C11	1.513(4)
O2 - C1	1.223(4)	C11 - C12	1.544(5)
O3 - C4	1.342(4)	C11 - C13	1.529(5)
C1 - C2	1.407(4)	C13 - C14	1.530(5)
C2 - C3	1.505(4)	C14 - C15	1.539(6)
C2 - C4	1.374(4)	C14 - C16	1.536(5)
C4 - C5	1.446(4)	C16 - C17	1.506(5)
C5 - C6	1.505(4)	C17 - C18	1.525(6)
C5 - C7	1.351(4)	C17 - C19	1.530(6)
C7 - C8	1.488(4)	C19 - C20	1.504(7)
C8 - C9	1.513(5)	C20 - C21	1.487(9)
C1 - O1 - C7	122.7(2)	C7 - C8 - C9	115.7(3)
O1 - C1 - O2	114.3(3)	C7 - C8 - C10	120.0(3)
O1 - C1 - C2	118.6(3)	C9 - C8 - C10	124.2(3)
O2 - C1 - C2	127.1(3)	C8 - C10 - C11	126.7(3)
C1 - C2 - C3	118.3(3)	C10 - C11 - C12	109.1(3)
C1 - C2 - C4	123.3(3)	C12 - C11 - C13	110.4(3)
O3 - C4 - C2	124.5(3)	C11 - C13 - C14	115.7(3)
C2 - C4 - C5	122.2(3)	C13 - C14 - C15	112.2(3)
O3 - C4 - C5	113.3(3)	C13 - C14 - C16	111.5(3)
C4 - C5 - C6	118.8(3)	C15 - C14 - C16	109.7(3)
C4 - C5 - C7	117.0(3)	C14 - C16 - C17	118.3(3)
C6 - C5 - C7	124.2(3)	C16 - C17 - C18	111.3(3)
O1 - C7 - C5	121.1(2)	C16 - C17 - C19	109.9(3)
C5 - C7 - C8	128.8(3)	C18 - C17 - C19	111.1(3)
O1 - C7 - C8	110.1(2)	C17 - C19 - C20	115.7(4)
		C19 - C20 - C21	113.0(5)

Table II.- Bond distances (\AA) and angles ($^\circ$) for non-H atoms with e.s.d.'s in parentheses

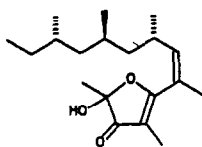
The X-ray analysis agrees with the conclusion obtained from the chemical degradation of compound 5 and, consequently, they establish the nature and absolute configuration of the alkanyl moiety not only for compound 5 but also for the other metabolites isolated 6-9. The nature of the non-alkanyl parts of

Table III.- NMR chemical shifts from the furanone moiety of 6 and the mixture of 13 and 14

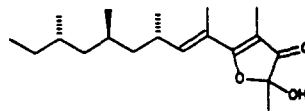
carbon	^{13}C -RMN		^1H -RMN	
	6	13-14	6	13-14
1	22.3	22.3	1.53	1.54
2	101.78/101.84	101.3	----	----
3	204.2	203.6	----	----
4	106.2	106.2	----	----
5	7.6	7.7	1.80	1.82
6	182.1/182.5	181.8/182.0	----	----
7	126.0	125.8	----	----
8	13.4	13.4	1.97	1.96
9	147.6	147.7	6.10	6.10



6



13



14

these metabolites was established as follows

The second cyclic compound, the siphonarienfuranone 6, was a mixture of isomers. This mixture could not be separated by different chromatographies, although the presence of two isomers was indicated by the NMR spectral data of the mixture. Thus, the ^1H -NMR spectrum showed two olefinic signals at δ 6.07 (d, 0.5 H, 10.2 Hz) and at δ 6.13 (d, 0.5 H, 10.2 Hz) and methyl signals at δ 1.53, 1.80, 1.97 and 1.00 as duplicate signals. Bands at 1690 and 3650 cm^{-1} in the IR spectrum and absorptions at 305 and 240 nm in the UV spectrum together with the ^{13}C -NMR spectral data, evidenced the presence of a 2-hydroxy-2,3-dihydro-2,4-dimethylfuran-3-one system in the molecule. Comparison of the NMR chemical shifts of the furanone moiety of 6 with those published for the E,Z mixture of furanones 13 and 14, previously isolated from *S. lessona*,¹⁰ showed good correlations between them (Table III) and we had to conclude that we had the same kind of mixture. Consequently we must have two different chemical shifts in the ^{13}C -NMR spectrum for the methyl group, Me-8, at around δ 13 for the E isomer and at δ 20 for the Z isomer. However the COSY (HETCOR) ^1H - ^{13}C experiment (Fig 4), showed only the correlation between the methyl signal at δ 1.96 (Me-8) in the ^1H -NMR spectrum and the carbon signal at δ 13.4 in the ^{13}C -NMR spectrum, which precluded the presence of a mixture of geometrical isomers.

On the other hand, the siphonarienfuranone 6, slowly isomerized on standing to another compound, and the ^1H -NMR spectrum of the resultant mixture showed, in addition to the signals from 6, new signals at δ 5.4, 2.35 and 1.64 which were also duplicates. Moreover, the COSY (HETCOR) (^1H - ^{13}C) map

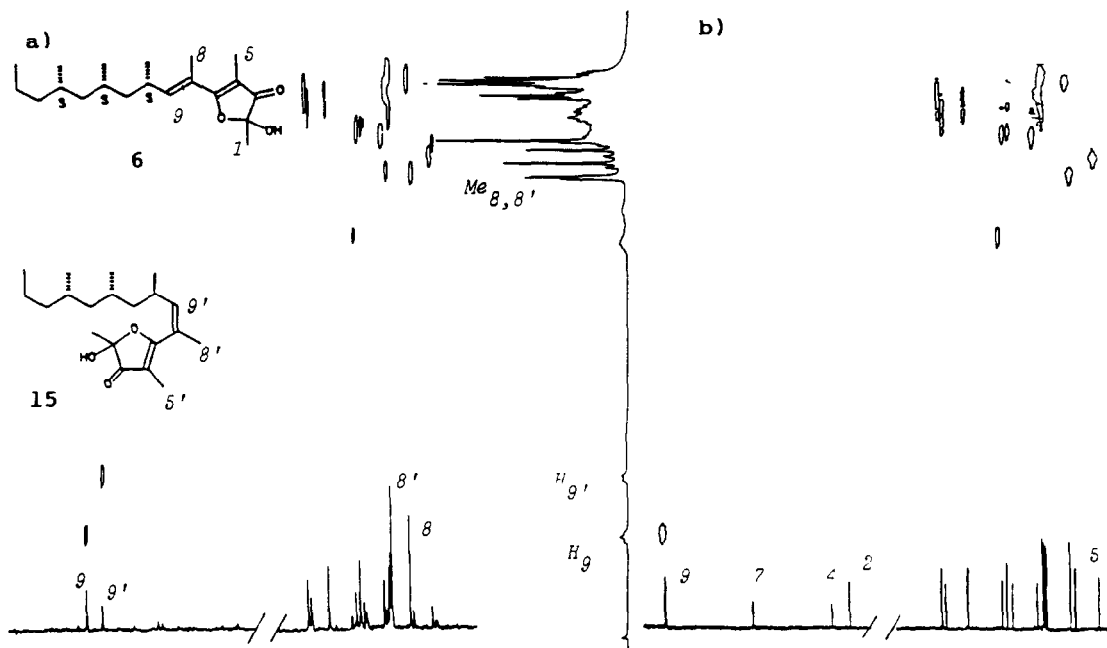


Fig. 4 ^1H - ^{13}C heteronuclear COSY one-bond correlations of the mixture of 6 and 15 (a) and of 6 (b)

(Fig 4), showed the correlation between the methyl signal at δ 196 and the ^{13}C -NMR signals at δ 13.4 and 20.4, which agrees with the presence of geometrical isomers. This mixture was chromatographed and the *Z* isomer, 15, was isolated and fully identified on the basis of its spectroscopical data. Accordingly, the only satisfactory explanation of all the slight differences in spectral data is a mixture of epimers at the carbon C-2.

Siphonarienedione 7, (α_D) = +32.5°, has a molecular formula of $\text{C}_{20}\text{H}_{36}\text{O}_2$ obtained from its high resolution mass spectrum M^+ at m/z 308.2709 ($\text{C}_{20}\text{H}_{36}\text{O}_2$, calc 308.2715). Bands at 1655 and 1720 cm^{-1} in the IR spectrum together with the maximum at 335 nm (ϵ = 12781) in the UV spectrum showed the presence of saturated and unsaturated carbonyl groups. The ions formed by simple fragmentations of the molecular ion in the EIMS are as follows: m/z 308 \rightarrow m/z 279 loss of an ethyl radical; m/z 308 \rightarrow m/z 251 loss of a propionyl radical, m/z 308 \rightarrow 223 loss of a 2-methyl-3-keto-pentanoyl radical. The ^1H -NMR spectrum showed, in addition to the signals from the alkanyl moiety, the presence of three methyl groups at δ 1.00, 1.31 and 1.80 together with three proton signals at δ 2.42, 4.26 and 6.41. These spectroscopical data suggested the presence of a β -diketone moiety in the molecule, which was confirmed by using 2D-NMR techniques. The proton signals which were found to be connected in the COSY (^1H - ^1H) spectrum of 7, are linked by underline in the following sequence: 6.41(H-9) - 2.73(H-10), 6.41(H-9) - 1.80(Me-8), 2.42(H-2) - 1.00(Me-1), 4.26(H-4) - 1.31(Me-5), and no further couplings of these signals, except those at δ 2.73, were observed. The DEPT and HETCOR (^1H - ^{13}C) experiments led to carbon as well as proton assignments that had already been made (Table IV). The long-range ^1H - ^{13}C correlations (Fig 5) between the carbon signals at δ 207.5 (C-3) and 199 (C-6) and the proton signals

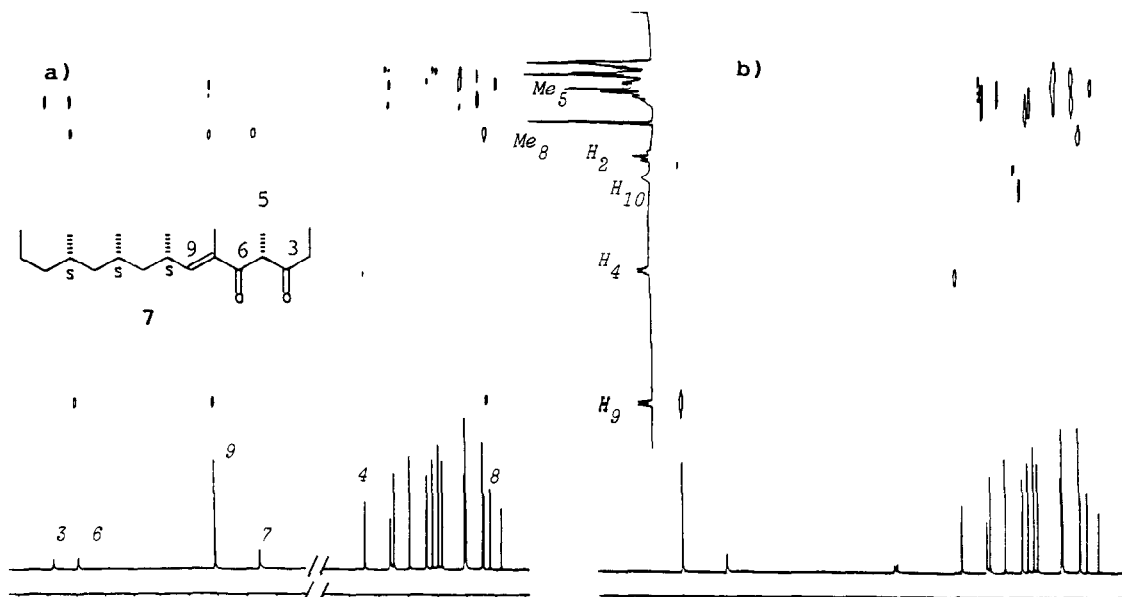
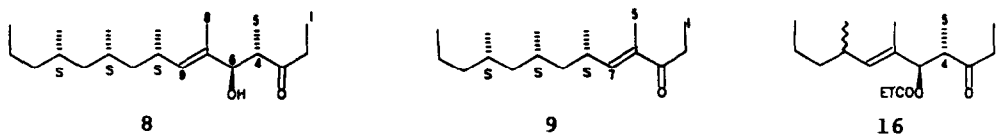


Fig. 5. ^1H - ^{13}C heteronuclear COSY Long-range ($J = 10\text{Hz}$) (a) and one-bond (b) correlations of siphonarienedione 7.

at δ 131 (Me-5) and at δ 6.41 (H-9), 1.80 (Me-8) and 1.31 (Me-5) respectively, together with the other correlations showed in the Fig 5 established unambiguously the presence of a 4,6 dimethyl hept-6-en-3,5-dione fragment in the molecule and, consequently, the structure of the compound 7

Siphonarienolone 8, oil, (α_D) = +19.6°, showed in the IR spectrum bands at 3600 and 1700 cm^{-1} according with the presence of a carbonyl and hydroxyl groups. The HMRS led to an M^+ at m/z 310.2888 ($\text{C}_{20}\text{H}_{38}\text{O}_2$, calc 310.2872). The similarity between this compound and the siphonarienedione 7 was obvious from the ^1H and ^{13}C -NMR spectral data (Table IV). These data indicated the presence of a β -hydroxy ketone moiety in 8, instead of the β -diketone present in 7, the secondary hydroxyl group being at the carbon C-6. The analysis of the ^1H -NMR and the COSY (^1H - ^1H) spectra of 8, indicated the connectivities between the proton signals from this moiety centered at δ 0.99 \rightarrow 2.77 \rightarrow 4.10, the coupling constants being identical with those reported for the ester 16, previously isolated from the mollusc *S. australis*. Obviously, both compounds 8 and 16 have the same relative configurations at the carbons C-4 and C-6. On the other hand, siphonarienolone 8 was oxidized with PCC to obtain siphonarienedione 7, which confirmed the structure proposed for 8.



Siphonarienone 9, oil, (α_D) = +13.3°, was shown to be an α - β unsaturated ketone (IR 1650 cm^{-1} and UV 229 nm). The ^{13}C -NMR (DEPT) and the HMRS spectra established a molecular formula of

Table IV.- NMR Spectral data of 6,7,8 and 9

C	¹³ C				¹ H			
	6 ^a	7 ^a	8 ^b	9 ^c	6 ^d	7 ^d	8 ^d	9 ^d
1	22 3	7 7	7 7	9 2	1 53 s	1 00 t, 7 2	1 03 t, 7 3	1 09 t, 7 3
2	101 78/101 84	33 4	36 5	30 5	---	2 42 m	2 54 m	2 66 m
3	204 2	207 5	216 0	200 0	---	---	---	---
4	106 2	54 3	48 6	134 0	---	4 26 q, 7 0	2 77 dq, 10 1, 7 2	---
5	7 6	13 8	14 3	11 7	1 80 s	1 31 d, 7 0	0 90 t, 7 2	1 80 s
6	182 1/182 5	199 0	80 9	148 4	---	---	4 10 d, 10 1	6 30 d, 9 8
7	126 0	135 2	133 7	31 4	---	---	---	2 67 m
8	13 4	11 6	10 8	20 6	1 96 s	1 80 s	1 63 s	1 01 d, 6 7
9	147 6	150 5	136 7	45 7	6 07 d, ¹ H, 10 2 6 13 d, ¹ H, 10 2	6 41 d, 9 8	5 13 d 9 8	1 10 m
10	30 8	31 5	29 8	29 8	2 73 m	2 73 m	2 52 m	1 54 m
11	20 9	20 5	20 4	20 9	1 00 d, 6 6	0 99 d, 6 5	0 92 d, 6 3	0 80 d, 6 3
12	45 7	45 5	46	44 6	1 07 m	1 10 m	1 10 m	1 40 m
13	29 7	29 5	29 8	28 5	1 54 m	1 54 m	1 54 m	1 53 m
14	20 4	20 4	20 3	20 2	0 80 d, 6 5	0 80 d, 6 4	0 79 d, 6 5	0 85 d, 6 5
15	44 6	44 4	45 1	39 5	1 40 m	1 40 m	1 38 m	1 28 m
16	28 3	28 3	28 3	20 1	1 52 m	1 53 m	1 53	1 30 m
17	20 1	20 2	21 8	14 5	0 84 d, 7 0	0 83 d, 7 0	0 85 d, 6 3	0 85 t, 6 3
18	39 3	39 1	39 4		1 31 m	1 30 m	1 28 m	
19	19 9	19 9	20 0		1 30 m	1 30 m	1 30 m	
20	14 4	14 3	14 3		0 85 t, 6 9	0 79 t, 6 3	0 90 t, 7 2	

a) Assignments made by ¹H-¹³C heteronuclear COSY. b) Assignments made by comparison with 6, 7 and 16 c) Assignments made by comparison with 7. d) Assignments made by homonuclear COSY

C₁₇H₃₂O The major differences between the ¹³C-NMR spectra of 7 and 9 can be ascribed to the loss of the terminal propionyl group [δ 207 5 (s), 33.4 (t) and 7.7 (q)] and the replacement of a methine at δ 54.3 by a methylene at δ 30.53. This was confirmed by the COSY correlations between the proton signal at δ 2.67 and the methyl signal at δ 1.09 without further coupling for these protons.

All these compounds showed antimicrobial activity against gram (+) bacteria, *S. aureus*, *M. luteus*, *B. subtilis*, pectinatone 5 and siphonarienefuranone 6 being the most potent. Compounds 5 and 6 were also active against yeast *C. albicans* and *S. pombe*.

EXPERIMENTAL PART.

IR and UV spectra were recorded on a Perkin-Elmer Mod 257 and Mod 402 spectrophotometers respectively. Optical rotation were determined for solution in CHCl₃ with a Perkin-Elmer Mod. 241 polarimeter. NMR spectra were recorded on a Bruker Mod. WP-200 SY (200 MHz), chemical shifts are reported relative to Me₄Si (δ 0) and coupling constants are given in hertz. The 2D-NMR spectra were obtained using Bruker's microprograms. Low and high resolution mass spectra were obtained from a VG micromass ZAB-2F spectrometer. Silica gel chromatography was performed on silica gel 60 G, TLC and PLC obtained from Merck products. The TLC plates were developed by spraying with 6N sulphuric acid and heating. Sephadex LH-20 obtained from Pharmacia was used for gel filtration chromatography. All solvents were purified by standard techniques. Anhydrous sodium sulfate was used for drying solution.

Collection, extraction and chromatographic separation.

Specimens (200) of *Siphonaria grisea* were collected at Ajui (Fuerteventura, Canary Islands) in September 1986. The animals were stored in acetone. The acetone solution was filtered, the shells discarded and the extraction with acetone repeated on a Soxhlet apparatus. The acetone extracts were combined, the resultant solution concentrated, diluted with H₂O and extracted with ethyl acetate (3 x 250 ml). The ethyl acetate extracts were combined and evaporated to yield an oil (18 gr) which was chromatographed on SiO₂ using mixtures of n-hexane/ethyl acetate of increasing polarity as eluent, and 50 fractions of 500 ml each, were collected.

The fractions eluted with n-hexane:ethyl acetate (95.5) and (70:30) containing siphonarienone **9** and the mixture of siphonarienfuranone **6** and pectinatone **5**, respectively, were rechromatographed on Sephadex LH-20 column with CHCl₃:MeOH:n-Hex. (1:1:2) to afford pure **9** (11 mg), **6** (150 mg) and **5** (250 mg). The fraction containing the mixture of siphonarienedione **7** and siphonarienolone **8** was rechromatographed on SiO₂ (flash chromatography), yielding pure siphonarienedione **7** (200 mg) and siphonarienolone **8** (21 mg).

Pectinatone 5 · (α_D) = + 64° (c, 0.11, CHCl₃), m.p. 131° C. The spectral data of this compound were identical with those published for pectinatone **10**⁸.

Siphonarienfuranone 6 (α_D) = + 101.5° (c, 0.14, CHCl₃). UV λ_{max}(EtOH): 305 nm (ε = 10,717), 240 nm (ε = 5201). IR ν_{max} cm⁻¹: 3560, 2950, 1690, 1570, 1450, 1370, 1055. MS m/z: 322, 2509 (C₂₀H₃₄O₃, requires 322, 2507), 304, 279, 223, 195, 167, 155, 125, 109. NMR spectral data: see Table II.

Siphonarienedione 7 oil, (α_D) = + 32.5° (c, 0.52, CHCl₃). UV λ_{max}(EtOH): 234 nm (ε = 12,781). IR ν_{max} cm⁻¹: 3020, 2950, 1720, 1655, 1450, 1370. MS: m/z 308, 2709 (C₂₀H₃₆O₂, requires 308, 2715); 279, 251, 223, 155, 153, 125, 109, 69. NMR spectral data: see Table II.

Siphonarienolone 8: oil, (α_D) = + 19.6° (c, 0.11, CHCl₃). UV: λ_{max}(EtOH): 203 nm (ε = 7,218). IR ν_{max} cm⁻¹: 3600, 3450, 2980, 1700, 1450, 1370, 1009, 970. MS m/z: 310, 2888 (C₂₀H₃₈O₂, requires 310, 2870); 293, 225, 207, 155, 153, 151, 140, 129, 123, 69. NMR spectral data: see Table II.

Siphonarienone 9 oil, (α_D) = + 13.3° (c, 0.7, CHCl₃). UV λ_{max}(EtOH): 229 nm (ε = 5,288). IR ν_{max} cm⁻¹: 2960, 2940, 1700, 1650, 1450, 1360. MS m/z: 252, 2451 (C₁₇H₃₂O, requires 252, 2453), 223, 166, 155, 139, 123. NMR spectral data: see Table II.

Degradative oxidation of compounds 5-9. Although the method is only described for siphonarienfuranone **6**, was used as a general procedure. To a solution of siphonarienfuranone **6** (24 mg, 0.075 mmol) in 1.75 ml of a mixture CCl₄:CH₃CN:H₂O (1.1:1.5) at r.t. were added with stirring 139 mg (8.2 mmol) of peryoic acid and a catalytic amount of RuCl₃·3H₂O. The mixture was stirred for 3 hr, concentrated and extracted with diethyl ether. After chromatography on SiO₂, the reaction yield 10.3 mg of pure 2,4,6-trimethyl nonanoic acid **11**.

Methylation of 11 with CH₃N₂. To a solution of **11** (10 mg) in diethyl ether (5 ml) was added an excess of an ethereal solution of diazomethane and stirred for 1 hr. The solvent was evaporated in vacuo and the residue was flash chromatographed on silica gel to yield 9 mg of pure methyl 2,4,6-trimethyl nonanoate **12**.

(2S,4S,6S) Trimethyl nonanoic acid **11**. oil (α_D) = + 15.3° (c, 0.6, CHCl₃). ¹H-NMR δ: 0.83 (t, 3H, J = 6.5 Hz), 0.85 (d, 3H, J = 6.5 Hz), 0.88 (d, 3H, J = 6.5 Hz), 1.18 (d, 3H, J = 7 Hz), 2.57 (m, 1H). MS m/z: 200, 157, 127, 87.

Methyl (2S,4S,6S) trimethyl nonanoate 12 oil (α_D) = + 21.9° (c, 0.7, CHCl₃) ¹H-NMR δ , 0.84 (t, 3H, J = 6.5 Hz), 0.85 (d, 6H, J = 6.5 Hz), 1.16 (d, 3H, J = 7 Hz), 2.55 (m, 1H), 3.67 (s, 3H). MS m/z 214, 183, 171, 101, 88.

Compound 15. ¹H-NMR δ , 5.43 (H-9, d, J = 10.6 Hz); 2.35 (H-10, m); 1.96 (Me-8, s), 1.64 (Me-5, s), 1.54 (Me-1, s); 0.98 (Me-11, d, J = 6.5 Hz); 0.86 (Me-20, d, J = 6.9 Hz), 0.79 (Me-17, d, J = 6.5 Hz), 0.72 (Me-14, d, J = 6.5 Hz) ¹³C-NMR δ , 6.7 (C-5), 14.5 (C-20), 20.1 (C-19); 20.4 (C-8); 21.0 (C-17), 21.5 (C-14); 22.2 (C-1), 22.2 (C-11), 28 (C-16); 29.7 (C-13), 32.4 (C-10), 39.5 (C-18); 44.8 (C-15), 45.7 (C-12); 101.9 (C-2), 108.2 (C-4); 124.7 (C-7), 143.2 (C-9); 183.5 (C-6), 203.0 (C-3). MS m/z 322, 304, 279, 223, 167, 155

Oxidation of 8. To a solution of 9.2 mg of **8** in 5 ml of dry dichloromethane at 0° C, was added an excess of PCC (20 mg) and the mixture was stirred for 2 hr. After usual work-up the reaction yielded 8 mg of pure siphonarienedione **7**.

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