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Predator - Prey Relationship between *Navanax inermis* and *Bulla gouldiana* : a Chemical Approach [1]

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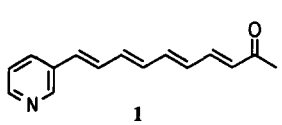
Abstract Two new compounds, 5,6-dehydroaglajne-3 (**9**) and isopulo'upone (**10**), have been isolated and characterized, along with the already known niuhinone-B (**7**), from *Navanax inermis* and its prey *Bulla gouldiana*, firmly establishing on a chemical basis the predator - prey relationship between this pair of cephalaspidean opisthobranchs The peculiarity of the metabolites and their structural analogy with other compounds isolated from other cephalaspidean molluscs assign a taxonomic relevance for them

Opisthobranchs are marine molluscs which, being scarcely protected by the shell, have been object of many chemical studies directed to investigate their defensive strategies [3] The major attention has been devoted to nudibranchs which, being completely devoid of shell, are considered the most evolute opisthobranchs On the contrary, only a few papers have reported chemical studies on the shelled cephalaspideans even though chemical communication seems to play an important role for some species belonging to this order [4-8] The study on the Pacific *Navanax inermis* (Aglajidae)[4] represents the first successful description of alarm pheromones, e g navenone-A (**1**), in a marine mollusc It is quite intriguing that metabolites related to navenones, e g haminol - A (**2**), have been found [5] in some Mediterranean cephalaspideans, *Scaphander lignarius* (Cylichnidae) and



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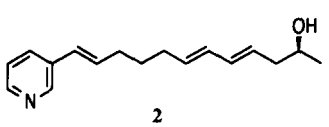

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Haminoea navicula (Haminoeidae), not belonging to the Aglajidae family, whereas other two Aglajidae species, the Pacific *Philinopsis speciosa* and the Mediterranean *Philinopsis depicta*, contain metabolites **3-8** (Fig 1) significantly different from those of *N inermis* The dietary origin of **3-5** in *P depicta* has been supported by the chemical analysis of one of its prey, *Bulla striata* [6] Because of these apparently conflicting data we have reinvestigated the metabolism of *N inermis* and that of one of its prey *Bulla gouldiana*

Populations of *N inermis* and *B gouldiana* were collected in the same habitat (San Diego, California) in

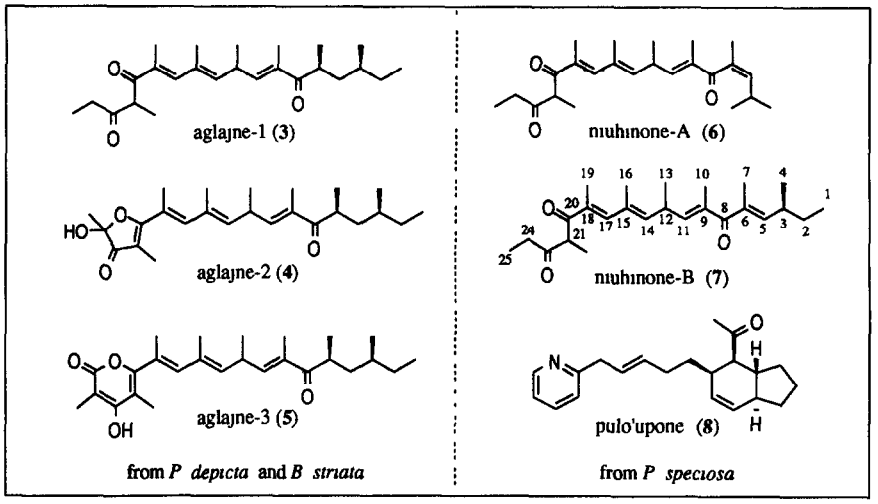
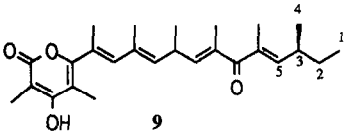
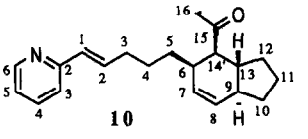


Figure 1 Metabolites from Mediterranean and Pacific *Philinopsis* (Aglajidae)

August, 1990 Both molluscs were carefully dissected and the ethylacetate soluble fractions from the acetone extract were separately analyzed by TLC revealing the same metabolic pattern in the extracts from the mantle of *B gouldiana* and from the digestive glands of *N inermis* The specific anatomical compartmentalization of the secondary metabolites clearly supported a predator-prey relationship between *N inermis* and *B gouldiana* The three main components of both the extracts were separated by HPLC yielding in order of decreasing polarity 5,6-dehydroaglajne-3 (**9**), isopulo'upone-B (**10**), and niuhinone-B(**7**) The structures of **7**, **9**, **10** were established mainly on the basis of their spectral properties





The characterization of **7** rapidly led to niuhinone-B, a metabolite already described[7] from the Pacific cephalaspidean *P speciosa* ^{1}H- and ^{13}C-NMR spectra of **7** (Table 1) in $CDCl_3$ showed the presence of nine methyl groups (four singlets, three doublets and two triplets in the ^{1}H-NMR spectrum) over 25 carbon atoms, clearly indicating a polypropionate structure Furthermore, three carbons resonating in the ^{13}C-NMR spectrum at δ 199 4, 201 3 and 207 8, typical of saturated and unsaturated ketones, evidenced a marked analogy with aglajne-1 (**3**), the linear polypropionate compound isolated from the Mediterranean *P depicta - B striata*[6] In fact, niuhinone-B (**7**) can be considered a derivative (5,6-dehydro) of aglajne-1 NMR spectra of **7** in CD_2Cl_2 and C_6D_6 showed δ values in good agreement with those described[7] The same optical rotation ($[\alpha]_D^{20}$ + 72°) indicated the full identity of the two compounds[7] Structure **7** was previously[7] proposed without stereochemical details at the chiral centers 3, 12 and 21 An epimerization at C-21 is now suggested by the observation that the signals at δ 1 02 (H-25) and δ 2 47 (H-24) in the ^{1}H-NMR ($CDCl_3$, 500 MHz) appear doubled according to the presence of a couple of equivalent signals slightly shifted (0 8 Hz and 2 4 Hz respectively) Absolute stereochemistry at C-3 was established to be *S* as follows Ozonolysis of niuhinone-B (**7**) followed by treatment

with $NaBH_4$ afforded a mixture, containing the degradative fragment (2-methyl-1-butanol), which was treated with *R*(-)-α-methoxy-α-trifluoromethylphenylacetic acid chloride Preparative TLC purification of the products obtained from this reaction yielded a MTPA derivative (**13**) characterized by the presence in the ^{1}H-NMR spectrum of the two signals, resonanting at δ 4 25 (dd, *J*= 5 7 and 10 7 Hz) and 4 08 (dd, *J*=6 6 and 10 7 Hz), expected for the *S* stereochemistry at C-3 Model compounds were prepared in the following way Compound **13**

Table 1 ^{1}H (500 13 MHz) and ^{13}C (125 7 MHz) NMR Data for Niuhinone-B (**7**)

	CD_2Cl_2	C_6D_6	$CDCl_3$		$CDCl_3$	
Position	δ^{1}H	δ^{1}H	δ^{1}H	*m, J (Hz)*	δ^{13}C	*m*
1	0 91	0 74	0 87	*t 7 4*	11 8	*q*
2	1 32	1 10	1 28	*m*	29 7	*t*
	1 45	1 30	1 43	*m*		
3	2 50	2 20	2 45	*m*	34 9	*d*
4	1 01	0 82	0 97	*d 6 8*	19 6	*q*
5	5 96	5 94	5 92	*dd 9 9, 1 2*	148 6	*d*
6	-	-	-		143 5	*s*
7	1 84	1 89	1 83	*d 1 2*	13 1	*q*
8	-	-	-		201 3	*s*
9	-	-	-		134 7[a]	*s*
10	1 91	1 92	1 89	*d 1 3*	13 3	*q*
11	6 00	5 97	5 95	*dd 9 3, 1 3*	143 4	*d*
12	3 62	3 34	3 55	*m*	32 9	*d*
13	1 19	0 91	1 14	*d 6 7*	20 4	*q*
14	5 54	5 38	5 48	*dd 9 0, 1 1*	139 1	*d*
15	-	-	-		131 6	*s*
16	1 96	1 59	1 91	*d 1 1*	16 6	*q*
17	6 98	6 86	6 94	*d 1 1*	144 1	*d*
18	-	-	-		134 5[a]	*s*
19	1 96	1 95	1 95	*d 1 1*	13 3	*q*
20	-	-	-		199 4	*s*
21	4 32	3 92	4 25	*q 7 0*	54 6	*d*
22	1 31	1 22	1 33	*d 7 0*	14 0	*q*
23	-	-	-		207 8	*s*
24	2 42	2 00	2 47	*dq 18 0, 7 2*	33 4	*t*
	2 46	2 18	2 33	*dq 18 0, 7 2*		
25	1 02	0 90	1 02	*t 7 2*	7 7	*q*

[a] Values could be interchanged

was obtained by reaction of *S* -2-methyl -1-butanol with *R* (-)-α-methoxy-α-trifluoromethylphenylacetic acid chloride The same reaction conducted on the racemic mixture of *R,S* -2-methyl -1-butanol gave a mixture of diasteromeric esters (**13, 14**) whose ^{1}H-NMR spectrum allowed the assignments of the signals due to product **14** by comparison with the NMR spectrum obtained for **13** Unfortunately *R* -2-methyl -1-butanol was not commercially available and to avoid tedious separation steps, the enantiomer of **14** was used to confirm the assignments made Reaction of *S* -2-methyl -1-butanol with *S* (+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride afforded compound **15** Of course the proton spectrum of **15** confirmed the assignments already made for **14** on the basis of the analysis of the ^{1}H-NMR spectra of the mixture of products **13** and **14** The esters were easily distinguished (Fig 2) mainly by the ^{1}H-NMR chemical shift values of the C-1 methylene protons resonanting at δ 4 25 (dd, *J*= 5 7 and 10 7 Hz) and 4 08 (dd, *J*=6 6 and 10 7 Hz) for **13** , the MTPA derivative

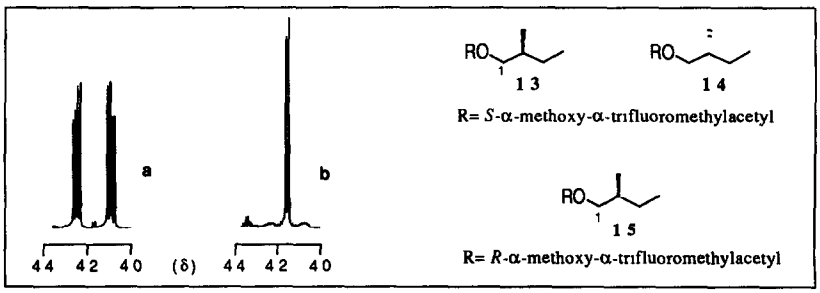
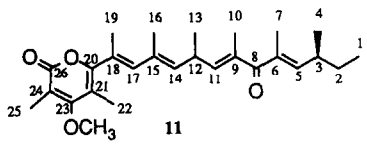


Figure 2 ^{1}H NMR signals (500 13 MHz, $CDCl_3$) due to methylene protons at C-1 for compounds **13** (a) and **15** (b)



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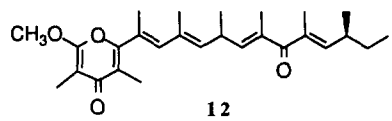

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Table 2 NMR Data [a] for Compounds **9**, **11** and **12**

	9			**11**				**12**			
	δ ^{1}H	*m, J (Hz)*		δ^{1}H	*m, J (Hz)*	δ^{13}C	*m*	δ ^{1}H	*m, J (Hz)*	δ^{13}C	*m*
1	0 87	*t 7 4*		0 87	*t 7 4*	11 8	*q*	0 88	*t 7 4*	11 9[e]	*q*
2	1 28	*m*		1 28	*m*	29 8	*t*	1 28	*m*	29 8	*t*
	1 43	*m*		1 41	*m*			1 42	*m*		
3	2 46	*m*		2 43	*m*	34 9	*d*	2 47	*m*	35 0	*d*
4	0 98	*d 6 7*		0 98	*d 6 7*	19 7	*q*	0 98	*d 6 7*	19 7	*q*
5	5 92	*dd 1 0, 9 6*		5 92	*dd 1 0, 9 7*	148 4	*d*	5 93	*dd 9 8, 1 5*	148 5	*d*
6	-			-		134 2[d]	*s*	-		134 4[f]	*s*
7	1 83[b]	*d 1 0*		1 83	*d 1 0*	12 9	*q*	1 84	*d 1 5*	13 2	*q*
8	-			-		201 5	*s*	-		201 5	*s*
9	-			-		134 7[d]	*s*	-		134 7[f]	*s*
10	1 84[b]	*d 1 2*		1 90	*d 0 9*	13 1	*q*	1 91	*d 1 5*	12 9	*q*
11	5 97	*dd 1 2, 10 0*		5 97	*dd 0 9,9 1*	144 4	*d*	5 97	*dd 9 5, 1 5*	144 2	*d*
12	3 53	*m*		3 52	*m*	32 8	*d*	3 54	*m*	32 9	*d*
13	1 13	*d 6 7*		1 13	*d 6 7*	20 6	*q*	1 15	*d 6 7*	20 6	*q*
14	5 30	*bd 10 3*		5 31	*bd 8 9*	135 3	*d*	5 34	*dd 6 7, 1 5*	135 7	*d*
15	-			-		131 3	*s*	-		131 2	*s*
16	1 90[c]	*bs*		1 85	*bs*	16 8	*q*	1 88	*d 1 5*	16 9	*q*
17	6 00	*bs*		6 01	*d 1 0*	138 0	*d*	6 08	*d 1 6*	138 7	*d*
18	-			-		127 7	*s*	-		126 6	*s*
19	2 02[c]	*bs*		2 02	*d 1 0*	16 5	*q*	2 05	*d 1 6*	16 2	*q*
20	-			-		159 2	*s*	-		158 7	*s*
21	-			-		110 0	*s*	-		117 9	*s*
22	2 03[c]	*bs*		2 05	*s*	10 2	*q*	2 00	*s*	11 8[e]	*q*
23	-			-		165 4	*s*	-		181 4	*s*
24	-			-		108 9	*s*	-		99 5	*s*
25	2 01[b]	*bs*		1 99	*s*	11 8	*q*	1 86	*s*	6 9	*q*
26	-			-		168 4	*s*	-		161 9	*s*
OCH_3				3 83	*s*	60 1	*q*	3 95	*s*	55 2	*q*

[a] WM 500 Bruker instrument,$CDCl_3$

[b,c,d,e,f] Values with identical superscript in the same column could be interchanged

obtained from *S*-2-methyl-1-butanol, while **14** , the MTPA derivative obtained from the *R* alcohol, and its enantioner **15** showed the corresponding signals at δ 4 17 (2H, d, *J*= 6 0)

Analogously with *B striata*, **7** co-occurs with the corresponding γ-hydroxy-α-pyrone **9** In fact, the NMR spectra of **9** displayed signals indicative of a partial structure similar to the C-1/C-13 portion of niuhinone-B (**7**) However, because of the instability of **9** the structure elucidation was conducted on the two methyl ether derivatives, **11** and **12**, obtained after treatment of **9** with diazomethane Structures **11** and **12** were assigned by comparison of their spectral properties with those of similar compounds [6] In particular, all the ^{1}H- and ^{13}C-NMR resonances were assigned (Table 2) by a series of 1D and 2D experiments In this way **9** showed to be the 5,6-dehydroderivative of aglajne-3 (**5**) Analogously with **7**, ozonolysis of **11** followed by reduction and esterification afforded the same compound **13** indicating that also **9** has an *S* absolute stereochemistry at C-3

Table 3 ^{1}H- and ^{13}C-NMR Data for Isopulo'upone (**10**).[a]

	CD_2Cl_2			*$CDCl_3$*			*$CDCl_3$*	
Position	δ^{1}H	*m*,	*J (Hz)*	δ^{1}H	*m*	*J (Hz)*	δ^{13}C	*m*
2	-	-	-	-	-	-	155 9	*s*
3	7 23	*bd*	*7 9*	7 23	*d*	*7 9*	121 0	*d*
4	7 60	*ddd*	*1 8, 7 9, 6 8*	7 60	*bdd*	*7 9, 6 8*	136 4	*d*
5	7 08	*bdd*	*4 9, 6 8*	7 08	*bdd*	*4 9, 6 8*	121 6	*d*
6	8 48	*bd*	*4 9*	8 51	*bd*	*4 9*	149 3	*d*
1'	6 45	*d*	*15 5*	6 45	*d*	*15 5*	130 1	*d*
2'	6 69	*dt*	*15.5, 7 1*	6 69	*dt*	*15 5, 7 1*	135 4	*d*
3'	2 23	*m*		2 20	*m*		33 0	*t*
4'	1 65	*m*		1 65	*m*		26 8	*t*
	1 45	*m*		1 45	*m*			
5'	1 35	*m*		1 38	*m*		32 2	*t*
	1 22	*m*		1 28	*m*			
6'	2 71	*m*		2 68	*m*		37 7	*d*
7'	5 72	*m*		5 68	*ddd*	*10 2, 4 0, 2 0*	129 4	*d*
8'	5 88	*bd*	*10 2*	5 87	*bd*	*10 2*	130 1	*d*
9'	1 75[b]	*m*		1 75[b]	*m*		45 4	*d*
10'	1 78	*m*		1 78	*m*		28 6	*t*
	1 15	*m*		1 18	*m*			
11'	1 68	*m*		1 68	*m*		22 2	*t*
12'	2 00	*m*		2 05	*m*		28 1	*t*
	0 95	*m*		0 95	*m*			
13'	1 56	*dddd*	*6 2,10 6,11 2,9*	1 59	*m*		40 5	*d*
14'	2 79	*dd*	*6 3, 11 2*	2 79	*dd*	*11 2, 6 3*	57 6	*d*
15'	-	-	-	-	-	-	210 6	*s*
16'	2 10	*s*		2 13	*s*		29 7	*q*

[a] WM 500, AMX 500 and WP 200 Bruker instruments, assignments were made by 2D-NMR (^{1}H -^{1}H and ^{1}H-^{13}C) COSY experiments

[b] Assigned by coupling with protons H-7' and H-8' observed in 2D ^{1}H-^{1}H COSY and ^{1}H-^{1}H decoupling NMR spectra

Finally, the minor components from the pair *Navanax* - *Bulla* displayed strong analogies with pulo'upone (**8**) Four downfield signals in the ^{1}H-NMR spectrum of **10** (CD_2Cl_2, 500 MHz, δ 8 48 , 7 60 , 7 23, 7 08) suggested the presence of an α-substituted pyridine Extensive use of 1D and 2D NMR experiments disclosed the presence of an hydrindene system similar to that one described for pulo'upone (**8**) [8] Comparison of the NMR data of **10** with those reported for **8** showed the lack of the 2',3' double bond while a downfield doublet at δ 6 45 (H-1', *J* =15 5 Hz) coupled with a signal at δ 6 69 (H-2', dt) supported the presence of a *trans* double

bond conjugated with the pyridine system The double bond was linked with the hydrindene system by three methylenes The values of coupling costants of protons 6',14',13',9' measured in CD_2Cl_2 supported relative stereochemistry identical to that one of pulo'upone (**8**) [8] In particular, the shape (dddd , J= 10 6,11 2,9 0,6 2 Hz) of the signal at δ 1 56 (H-13') suggested a *trans* relationship with H-14' and H-9' In fact, ^1H-^1H decoupling experiments at δ 2 00 (H-12') simplified the signal at δ 1 56 eliminating the smallest (6 2 Hz) coupling The negative optical rotation , $[\alpha]_D^{20}$ (-119°), suggests that most likely isopulo'upone (**10**) possesses the same absolute stereochemistry of pulo'upone-B (**8**, $[\alpha]_D^{20}$ -156 2°) [9]

5,6-Dehydroaglajne-3 (**9**) and isopulo'upone (**10**) were both toxic in the assays conducted against the mosquito fish *Gambusia affinis* [10] and the brine shrimp *Artemia salina* [11] (Table 4)

Table 4 Toxicity Bioassays on *B gouldiana* Metabolites

Metabolite	Ichthyotoxicity to mosquito fish *G affinis*	Toxicity to brine shrimp (*A salina*) LD_{50} (ppm)
niuhinone -B (**7**)	not toxic	24 6
isopulo'upone (**10**)	very toxic at 10 ppm	2 2
dehydroaglajne-3 (**9**)	very toxic at 10 ppm	0 88

Navanax inermis, like the Mediterranean species *P depicta*, feeds generally on *Haminoea* and *Bulla* species, particularly on *H virescens* and *B gouldiana* [4] This work firmly establishes on a chemical basis the predator-prey relationship between *N inermis* and *B gouldiana* Analogously with the Mediterranean pair *P depicta* and *B striata*, polypropionates seem to be characteristic metabolites of *Bulla* species There is a strong parallelism between Pacific and Mediterranean *Bulla* species in spite of their very different environments Furthermore, it is interesting to note that niuhinone-B (**7**) and isopulo'upone (**10**) are respectively identical and very similar to the metabolites isolated from *P speciosa* (Fig 1) indicating, at least, a taxonomic relevance for them Likely more general conclusions can be drawn In fact, the parallelism of the metabolic pattern between *P speciosa* and *B gouldiana* suggests that the origin of the metabolites in *P speciosa* may be due to the predation on *Bulla* species Finally, most likely, the polypropionate metabolites are biosynthetized *de novo* by *Bulla* species In fact, their anatomical localization only in the mantle should exclude a dietary origin

EXPERIMENTAL

General Experimental Procedures IR spectra were measured with a Nicolet FT 5DXB spectrophotometer UV spectra were recorded on a Varian DMS 90 spectrophotometer Optical rotations were measured on a Jasco DIP-370 polarimeter ^1H-^{13}C NMR spectra were recorded on WP-200, WM 500 and AMX 500 Bruker spectrometers, chemical shifts are reported in parts per million referred to TMS as internal standard Mass spectra were obtained on AEI MS30 and MS50 Kratos instruments Merck Kieselgel 60 (70-230 mesh) was used for silica gel chromatography and precoated Kieselgel 60 F_{254} plates were used for analytical and preparative TLC

Extraction of the Biological Samples and Isolation of the Metabolites. Specimens of *B gouldiana* and *N inermis* were collected in the flood control channel of the San Diego River (Mission Bay, San Diego, California) in August, 1990 Freshly collected specimens (40) of *B gouldiana* were dissected in order to separate

the skin (mantle) from the internal organs The mantles and the internal organs were separately extracted with acetone. The filtered acetone solutions were concentrated and then, after dilution with water, extracted with ethyl acetate Preliminary TLC analysis on silica gel (Merck silica gel 60 UV 254, 0 25 mm precoated plates, visualization ceric sulphate, eluant isooctane/ethyl acetate, 1 1) demonstrated the presence only in the mantle extract of products **7, 9** and **10**. After evaporation of the solvent under reduced pressure, the mantle extract yielded 729 mg of an oil residue while from the internal organ extract 1259 mg of oil residue was obtained The mantle extract was chromatographed by HPLC over a normal phase column employing isooctane/ethylacetate 75 25 as eluant to afford **7** (42 mg), **10** (16 mg) and **9** (73 mg) The same extraction procedure was used for the digestive glands of 7 specimens of *N inermis* TLC and NMR comparison with the extract obtained from the mantle of *B gouldiana* showed the presence in both of the same compounds **7, 9** and **10**

Niuhinone-B (7) $[\alpha]_D^{20}$ + 72° (c 2 3 , *n*-hexane), UV (EtOH) λ_{max} 282 (ε =9800), 236 (ε = 10800) nm, IR (film) ν_{max} 3406, 2962, 1724, 1639 cm^{-1}, MS, *m/z* 329 (M^+ - 57 , 0 4 %), 301 (M^+ - 85, 1 %), 273 (M^+ - 113, 0 8 %), 233 (M^+ - 153, 2 %), 221 (M^+ - 165, 3 %), 193 (M^+ - 193, 100 %), 165 (8 %), 153 (7 %) ^1H- and ^{13}C-NMR data,Table 1

5,6-Dehydroaglajne-3 (9) $[\alpha]_D^{20}$ + 53° (c 0 1 , $CHCl_3$) , UV (MeOH) λ_{max} 310 (ε=7700), 240 (ε= 19000) nm, IR (film) ν_{max} 3356, 2962, 1795, 1680, 1632 cm^{-1}, MS, *m/z* 412 2608 (M^+, $C_{26}H_{36}O_4$ requires 412 2613), 355 (M^+ - 57, 5 %), 287 (M^+ - 125, 2 %), 247 (M^+ - 165, 10 %), 219 (M^+ - 193, 23 %), 163 (51 %), 137 (54 %), 83 (100 %), ^1H-NMR data, Table 2

Methylation of 9 with CH_2N_2 An ethereal solution of CH_2N_2 was added to a solution containing **9** (58 mg) dissolved in diethyl ether (10 ml) After 15 min at room temperature, the excess of CH_2N_2 and the solvent were removed by evaporation under reduced pressure and the residue was chromatographed on silica gel using light petroleum ether/diethyl ether (1 1) as eluant, affording 25 mg of the methoxy pyrone **11** and 18 mg of the methoxy pyrone **12**

Pyrone 11. $[\alpha]_D^{20}$ + 81 2° (c 2 5 , $CHCl_3$) , UV (MeOH) λ_{max} 320 (ε =12800), 237 (ε = 18500) nm, IR (film) ν_{max} 2960, 1706, 1634 cm^{-1}, MS, *m/z* 426 2761 (M^+, $C_{27}H_{38}O_4$ requires 426 2770), 369 (25 %), 261 (100 %), 233 (96 %), 153 (64 %), ^1H- and ^{13}C-NMR data, Table 2

Pyrone 12. $[\alpha]_D^{20}$ + 60 7° (c 0 9 , $CHCl_3$) , UV (MeOH) λ_{max} 265 (ε =19000) , 238 (ε = 24000) nm, IR (film) ν_{max} 1642 cm^{-1}, MS, *m/z* 426 2763(M^+, $C_{27}H_{38}O_4$ requires 426 2770), 369 (12 %), 233 (100%), 163 (50 %), ^1H- and ^{13}C-NMR data, Table 2

Isopulo'upone (10) $[\alpha]_D^{20}$ - 119 ° (c 0 4 , *n*-hexane), UV (MeOH) λ_{max} 281 (ε =5400), 242 (ε = 11400) nm, IR (film) ν_{max} 1709 cm^{-1}, MS *m/z* 309 2090 (M^+, $C_{21}H_{27}NO$ requires 309 2093), 266 (M^+ -43, 100%) 132 (73%) NMR data , Table 3

Ozonolysis of niuhinone-B and MTPA derivatives preparation . A solution of **7** (40 mg), in CH_2Cl_2 (30 ml) at the temperature of -50 °C, was treated with a stream of ozone for 5 min After stirring for 10 min, a solution (2 ml) of 50% aqueous ethanol containing $NaBH_4$ (1 0 mg) was added and the mixture was stirred at room temperature for 12 hours The usual work-up afforded an oily crude product This mixture was dissolved in anhydrous pyridine and *R*(-)-α-methoxy-α-trifluoromethylphenylacetic acid chloride (*R*-MTPA cloride)(0 010 ml) was added After three hours the solvent was evaporated and preparative TLC purification allowed the isolation of compound **13** (7 mg, silica-gel, petroleum ether/ diethyl ether 9 1, Rf 0 9)

Ozonolysis of 11 and MTPA derivatives preparation **11** (25 mg) was treated as described above affording the same compound **13** (4 mg)

Model MTPA derivatives preparation Model compounds **13** and **14** were prepared respectively by reaction of *S*- and *S,R*-2-methyl-1-butanol with *R*(-)-α-methoxy-α-trifluoromethylphenylacetic acid chloride Model compound **15** was prepared in the same way by reaction of *S*-2-methyl-1-butanol with *S*(+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride

Compound 13 ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7 52 (m, 2H), 7 41 (m, 3H), 4 25 (dd, *J*=5 7 and 10 7 Hz, H-1a), 4 08 (dd, *J*=6.6 and 10 7 Hz, H-1b), 3.55 (s, CH_3O-), 1 78 (m, H-2), 1 42 (m, H-3a), 1 21 (m, H-3b), 0 91 (d, *J*=6 8 Hz, 3H), 0.90 (t, *J*= 7 5 Hz, 3H)

Compounds 14 and 15 ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7 52 (m, 2H), 7 41 (m, 3H), 4 17 (d, *J*=6 0 Hz, 2H), 3 55 (s, CH_3O-), 1 78 (m, H-2), 1 42 (m, H-3a), 1 21 (m, H-3b), 0 92 (d, *J*=6 8 Hz, 3H), 0 89 (t, *J*= 7 6 Hz, 3H)

Biological assays Ichthyotoxicity tests (*Gambusia affinis*)[10] and brine shrimp assays (*Artemia salina*)[11] were conducted as described in the references The results are reported in table 4

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