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Dactylallene: A Novel Dietary C_{15} Bromoallene from the Atlantic Anaspidean Mollusc *Aplysia dactylomela*‡

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Abstract : A new C_{15} bromoallene, dactylallene (**1**), was isolated from the digestive gland of the anaspidean mollusc *Aplysia dactylomela*. The structure was established by using mainly mono and bidimensional NMR techniques, whereas the absolute stereochemistry was determined by X-ray diffractometric analysis. Ichthyotoxicity and antifeedant-activity suggest a defensive role of **1** against predators. © 1997 Published by Elsevier Science Ltd.

The sea hares (order Anaspidea) are herbivorous opisthobranch molluscs. They sequester and store selected algal metabolites in the digestive gland, from which the metabolites are often released in the mucus secreted to deter potential predators.[2] Among anaspidean molluscs, the family Aplysiidae is the most common and widely distributed. Several chemical studies have been conducted on species belonging to this family, resulting in the finding of a great number of dietary compounds,[3] the majority of which are typical halogenated metabolites from the red algae, generally of the genus *Plocamium* and *Laurencia*, and include mono-, sesqui- and di-terpenes as well as ether C_{15} lipids.

We add here the isolation of a new C_{15} haloether (**1**) displaying a bromoallene function, from an Atlantic *Aplysia dactylomela*, Rang, 1828, population.

Ten *A. dactylomela* specimens, collected off Canary Islands (Spain), during June 1993, were frozen at -20°C and successively transferred to ICMIB. The animals were carefully dissected in parapodia and digestive gland, which were separately extracted by acetone. The ether soluble portion (1.2 g) of the acetone extract of the digestive gland was analyzed by TLC showing the presence of several secondary metabolites. In particular, an intense spot at R_f 0.3 (light petroleum ether/diethyl ether, 4:1) was revealed by spraying with $Ce(SO_4)_2$. An aliquot (70.0 mg) of the ether extract was therefore submitted to a chromatographic silica gel column, which was eluted by light petroleum ether/diethyl ether gradient.

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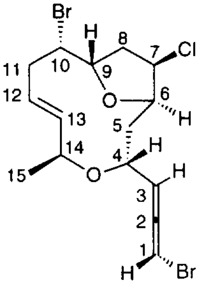
‡ Dedicated to the memory of Professor Luigi Minale, director of ICMIB from 1973 to 1981.

The fraction collected by light petroleum ether/diethyl ether, 4:1 (67.0 mg) was further chromatographed on a silica gel column eluted by benzene/light petroleum ether, 7:3, obtaining 23.5 mg of a pure compound (**1**), which has been named dactylallene.



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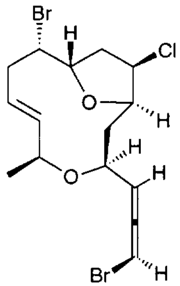

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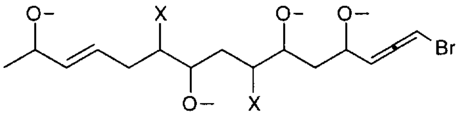
Dactylallene (**1**), m.p. 82-84° (*n*-hexane), $[\alpha]_D$ +242.9° ($CHCl_3$, c=0.7) showed the molecular formula $C_{15}H_{19}Br_2ClO_2$, deduced by both ^{13}C-NMR data and HREIMS on $[M - Br]^+$ (345.0251; required 345.0257). The ^1H-NMR spectrum displayed signals attributable to a secondary methyl at δ 1.33 (H_3-15, d, J = 7.0 Hz), six deshielded methines at δ 4.00 (H-6), δ 4.19 (H-4), δ 4.38 (H-7), δ 4.42 (H-14), δ 4.48 (H-10), δ 4.73 (H-9) and three methylenes at δ 1.59 (H-5a, ddd, J= 14.0, 10.7 and 1.9 Hz) and δ 1.69 (H-5b, m), δ 2.40 (H-8a, m), and δ 2.43 (H-8b, m), δ 2.43 (H-11a, m) and δ 2.89 (H-11b, m), strongly suggesting a non-terpenoid structure containing heteroatoms. The presence of a bromoallene function was indicated both by two long-range coupled methine signals in the ^1H-NMR spectrum at δ 6.01 (H-1, dd, J= 5.8 and 1.7 Hz) and δ 5.35 (H-3, dd, J= 5.8 and 6.0 Hz) and by the resonances in the ^{13}C-NMR spectrum at δ 73.66 (C-1), δ 103.62 (C-3) and δ 200.99 (C-2) and also confirmed by a characteristic band at ν_{max} 1959 cm^{-1} in the infrared spectrum and by a diagnostic loss of C_3H_2Br from the molecular ion (*m/z* 307, M^+ - allene) in the EIMS spectrum. In addition, isotopic peaks at *m/z* 309 [M+2 - allene] and *m/z* 311 [M+4 - allene], in the EIMS spectrum, supported the presence of other two halogen atoms. Finally, a *trans* disubstituted double bond was also present in the structure as indicated by NMR spectra [δ^1H: 5.72 (H-13, m, $w_{1/2}$ 20 Hz) and 5.79 (H-12, m, $w_{1/2}$ 20 Hz); δ ^{13}C: 128.23 (2C)].

A series of mono and bi-dimensional NMR experiments (^1H-^1H homodecoupling, ^1H-^1H COSY, HMQC, HMBC) connected all the proton and carbon resonances (see Table 1) leading to the partial structure **a**.

No evidence revealed the presence of hydroxy groups, therefore the two oxygen atoms were assumed to be involved as ether ring linkages, in a bicyclic structure, accounting also for the remaining two degrees of unsaturation required by the molecular formula.



a

NMR data of **1** reminded the structure, secured by X-ray analysis, of obtusallene II (**2**), previously isolated from the red alga *Laurencia obtusa*,[4,5] suggesting the presence of a similar skeleton which exhibits an ether link between the carbons C-4 and C-14 (a distinctive structural feature for bromoallenes from *L. obtusa*)[6] forming a large size heterocyclic ring, named lauroxadodecane, and an additional oxolane ring by linkage between C-6 and C-9, as indicated in **1**.

However, dactylallene (**1**) differs from **2** mainly in the chemical shifts of C-14 (δ 70.54 in **1**, δ 61.26 in **2**), C-4 (δ 64.68 in **1**, δ 76.34 in **2**) and C-15 (δ 14.05 in **1**, δ 21.19 in **2**), suggesting a different relative stereochemistry for C-4 or C-14. Anyway the too large difference between the values of C-4 and C-14 in **1** and **2** is not justified only by a different relative stereochemistry at the chiral center C-4 or C-14, most likely these assignments in **2** should be reversed. In addition, the sign of $[\alpha]_D$ of **1**, opposite of that of **2**, indicated a different absolute stereochemistry of the allene residue, which could be predicted as *S* according to the Lowe-Brewster's rule.[7]

Table 1 - ^{1}H and ^{13}C-NMR Data[a,b] for Dactylallene (**1**)

Position	δ ^{1}H	m	*J* (Hz)	δ ^{13}C	m[c]	long-range connectivities[d]
1	6.01	dd	5.8 and 1.7	73.66	d	H-3
2				200.99	s	H-1, H-3, H-4
3	5.35	dd	5.8 and 6.0	103.62	d	H-1, H-4, H-5a
4	4.19	m		64.68	d	H-1, H-3, H-5a, H-14
5	1.59	ddd	14.0, 10.7, 1.9	37.05	t	H-4
	1.69	m				
6	4.00	m		76.69	d	H-5b, H-7, H-8, H-9
7	4.38	m		62.51	d	H-6, H-8
8	2.40	m		38.61	t	
	2.43	m				
9	4.73	m		78.26	d	H-7, H-11
10	4.48	m		49.96	d	H-8, H-11
11	2.43	m		37.84	t	H-9
	2.89	m				
12	5.79	m		128.23	d	H-11, H-14, H-15
13	5.72	m		128.23	d	H-11, H-14, H-15
14	4.42	m		70.54	d	H-4, H-12, H-13, H-15
15	1.33	d	7.0	14.05	q	

a) Bruker AMX 500 MHz, $CDCl_3$; δ values are reported in ppm referred to $C\underline{H}Cl_3$ (δ ^{1}H 7.26) and to $\underline{C}DCl_3$ (δ ^{13}C 77.0).

b) Assignments determined by ^{1}H-^{13}C-HETCOR, ^{1}H-^{1}H COSY, ^{1}H-^{1}H decoupling experiments.

c) Determined by DEPT sequence.

d) By HMBC (*J* = 10 Hz).

All structural features of **1**, including the absolute stereochemistry, were clarified by a X-ray diffraction study on crystal samples obtained by careful crystallization from *n*-hexane solution. The structure refinement led to a discrepancy index *R* = 0.058 on 1051 observed reflections and 180 variables. The final positional and equivalent isotropic thermal parameters for non hydrogen atoms are reported in Table 2.

Dactylallene (**1**) proves to be a polyhalogenated bicyclic system characterized by a 12-membered ether ring which has a $\Delta^{12,13}$ *trans* double bond. The cyclododecene system is further bridged, between C6 and C9 positions, by a second oxygen forming an oxolane ring. The bicyclic system presents a bromoallene side chain at C4 carbon and in addition Cl, Br and methyl substituents at C7, C10 and C14 atoms, respectively.

Table 2 - Dactylallene (**1**): Fractional atomic coordinates and equivalent isotropic displacement parameters (Å2): $B\mathrm{eq} = \frac{1}{3} \sum_i \sum_j B_{ij}\, a_i^*\, a_j^*\, \mathbf{a}_i\, \mathbf{a}_j$

atom	*x*	*y*	*z*	*Beq*
Br1	0.9258(2)	-0.0197(2)	0.500*	11.50(5)
Br2	-0.0336(2)	0.6556(3)	0.6220(2)	16.42(6)
Cl1	0.5310(5)	0.7334(4)	0.5303(3)	12.7(1)
O1	0.4890(9)	0.1639(8)	0.5528(4)	7.4(2)
O2	0.3669(7)	0.5034(8)	0.6152(4)	6.7(2)
C1	0.809(2)	-0.010(2)	0.5804(8)	10.7(4)
C2	0.749(1)	0.103(2)	0.5987(7)	10.6(4)
C3	0.692(1)	0.217(2)	0.6201(8)	10.6(4)
C4	0.551(1)	0.268(1)	0.5970(6)	7.4(3)
C5	0.560(1)	0.406(1)	0.5555(7)	7.6(3)
C6	0.421(1)	0.470(1)	0.5473(6)	6.6(3)
C7	0.404(2)	0.608(1)	0.5077(7)	9.2(4)
C8	0.255(2)	0.642(1)	0.5286(7)	9.6(4)
C9	0.253(1)	0.594(1)	0.6077(6)	7.6(3)
C10	0.123(1)	0.523(2)	0.6334(7)	9.9(4)
C11	0.080(1)	0.387(2)	0.5987(7)	9.5(4)
C12	0.186(1)	0.276(1)	0.6143(7)	8.7(3)
C13	0.244(1)	0.194(2)	0.5679(6)	8.4(3)
C14	0.364(2)	0.096(1)	0.5784(7)	8.5(4)
C15	0.379(2)	0.035(2)	0.6519(9)	11.8(5)

*fixed to define the origin

Table 3 - Dactylallene (**1**): Molecular geometry with e.s.d.'s in parentheses

bond lengths (Å)							
Br1 - C1	1.90(2)	O2 - C9	1.40(1)	C5 - C6	1.48(2)	C10 - C11	1.52(2)
Br2 - C10	1.98(1)	C1 - C2	1.27(2)	C6 - C7	1.53(2)	C11 - C12	1.51(2)
Cl1 - C7	1.76(2)	C2 - C3	1.29(2)	C7 - C8	1.51(2)	C12 - C13	1.30(2)
O1 - C4	1.43(1)	C3 - C4	1.50(2)	C8 - C9	1.57(2)	C13 - C14	1.49(2)
O1 - C14	1.45(2)	C4 - C5	1.55(2)	C9 - C10	1.50(2)	C14 - C15	1.52(2)
O2 - C6	1.43(1)						
bond angles (°)							
C4 - O1 - C14	117.4(9)	O2 - C6 - C7	102.(1)	Br2 - C10 - C9	108.(1)		
C6 - O2 - C9	109.3(8)	C5 - C6 - C7	121.(1)	Br2 - C10 - C11	107.(1)		
Br1 - C1 - C2	122.(2)	Cl1 - C7 - C6	113.(1)	C9 - C10 - C11	118.(1)		
C1 - C2 - C3	177.(2)	Cl1 - C7 - C8	116.(1)	C10 - C11 - C12	110.(1)		
C2 - C3 - C4	124.(1)	C6 - C7 - C8	99.(1)	C11 - C12 - C13	126.(1)		
O1 - C4 - C3	109.(1)	C7 - C8 - C9	101.(1)	C12 - C13 - C14	128.(1)		
O1 - C4 - C5	108.8(9)	O2 - C9 - C8	106.(1)	O1 - C14 - C13	108.(1)		
C3 - C4 - C5	112.(1)	O2 - C9 - C10	110.(1)	O1 - C14 - C15	114.(2)		
C4 - C5 - C6	111.(1)	C8 - C9 - C10	117.(1)	C13 - C14 - C15	116.(1)		
O2 - C6 - C5	109.(1)						
selected torsion angles (°)							
C14 - O1 - C4 - C5	-123.(1)	C5 - C6 - C7 - Cl1	-44.(1)				
C4 - O1 - C14 - C13	78.(1)	C5 - C6 - C7 - C8	-168.(1)				
C4 - O1 - C14 - C15	-53.(1)	C6 - C7 - C8 - C9	39.(1)				
C9 - O2 - C6 - C7	37.(1)	C7 - C8 - C9 - O2	-19.(1)				
C6 - O2 - C9 - C8	-11.(1)	C7 - C8 - C9 - C10	-141.(1)				
Br1 - C1 - C3 - C4	90.(1)	C8 - C9 - C10 - Br2	-60.(1)				
C2 - C3 - C4 - O1	5.(2)	C8 - C9 - C10 - C11	62.(2)				
O1 - C4 - C5 - C6	73.(1)	C9 - C10 - C11 - C12	66.(1)				
C3 - C4 - C5 - C6	-167.(1)	C10 - C11 - C12 - C13	-131.(1)				
C4 - C5 - C6 - C7	180.(1)	C11 - C12 - C13 - C14	170.(1)				
O2 - C6 - C7 - C8	-47.(1)	C12 - C13 - C14 - O1	-99.(2)				

Fig. 1 shows a perspective drawing of the final crystallographic model for the dactylallene molecule (**1**). The shown absolute configuration has been established by inclusion of the anomalous dispersion contributions in the atomic scattering factors. The six chiral centres display C4*R*, C6*R*, C7*R*, C9*S* C10*S* C14*S* absolute stereochemistry and the unsymmetrically substituted allene moiety, present at C4 carbon, is in the enantiomeric *S* form.[7] Dactylallene (**1**) is a diastereoisomer of obtusallene II (**2**), the compound previously isolated[4,5] from different varieties of the widespread red alga *Laurencia obtusa* that is part of the diet of *Aplysia dactylomela* molluscs. The two compounds differ in the absolute configurations at C4 carbon and at bromoallene residue that in the algal metabolite **2** are *S* and *R*, respectively.

Geometrical parameters involving non-hydrogen atoms are given in the Table 3. The higher than usual estimated standard deviations are probably a consequence of the strong degradation of the crystals in X-ray beams (see experimental section). Anyhow bond lengths and bond angles are in good agreement with the values observed in structurally correlated molecules.[5,8-10] The torsion angle around C12-C13 double bond is lightly distorted (170°) with respect to the ideal *trans* conformation. Like distortion is present in obtusallene II (**2**).[5]

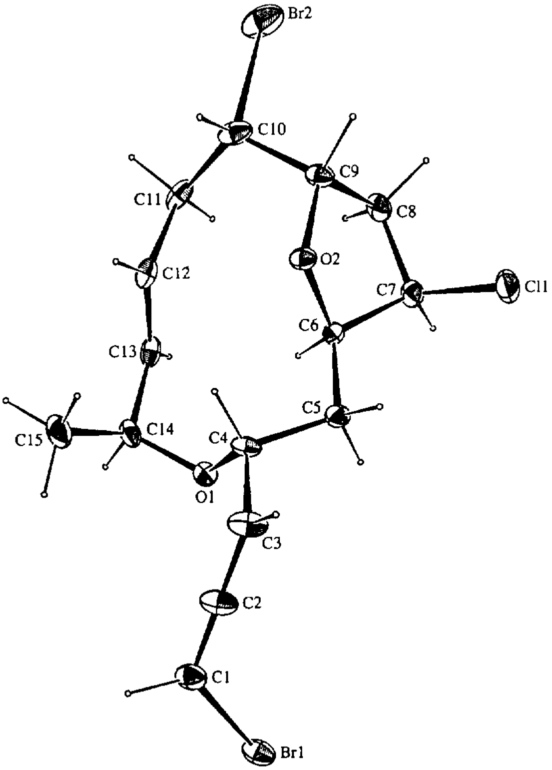


Fig 1. Perspective view of the final X-ray model of dactylallene **1** in the absolute configuration established by X-ray study. Labels of hydrogen were omitted for clarity. Displacement ellipsoids are plotted at the 30% probability level.

The oxolane ring adopts a distorted twist conformation with C6 and C7 atoms displaced 0.26(1) and 0.48(1) Å in opposite sides of the plane passing through O2, C8 and C9 atoms. The distortion toward the envelope form, with C7 0.68(1) Å out of the best plane through the remaining ring atoms, can be evaluated by the $\Delta C_2[C9]$ = 5.6° and $\Delta C_s[C7]$ = 13.8° parameters.[11] As found in similar ring-size unsaturated systems,[5,8,10] the dioxaundecene ring (O1-C4-C5-C6-O2-C9-C10·······C14) presents an appreciably short transannular separation, the shortest contact is O2·······C12 = 2.79(2) Å.

A view of the molecular packing in the *ab* plane is shown in Fig 2. Dactylallene molecule is arranged in the crystal with the best molecular plane practically parallel to the crystallographic *ab* plane. As a consequence, thin layers formed by translated molecules extend perpendicularly to the crystallographic 4-fold screw axis. Adjacent molecular layers along the *c* direction (thin and thick lines in Fig. 2) are each other related by rotation of 90° (4-fold screw symmetry). Only weak van der Waals interactions connect molecules, both in the same layer and in the adjacent layers. The shortest bromine contact is 3.90(1) Å between Br1 and Br2 belonging to molecules translated along the *a,-b* direction.

In conclusion, dactylallene (**1**), apart from differences in the stereochemistry, shows the same skeleton of obtusallene II (**2**). Most likely, **1** derives from the algal diet of the *A. dactylomela* mollusc and in particular from a red alga of the genus *Laurencia* from which several bromoallenes have been until now isolated.[3]

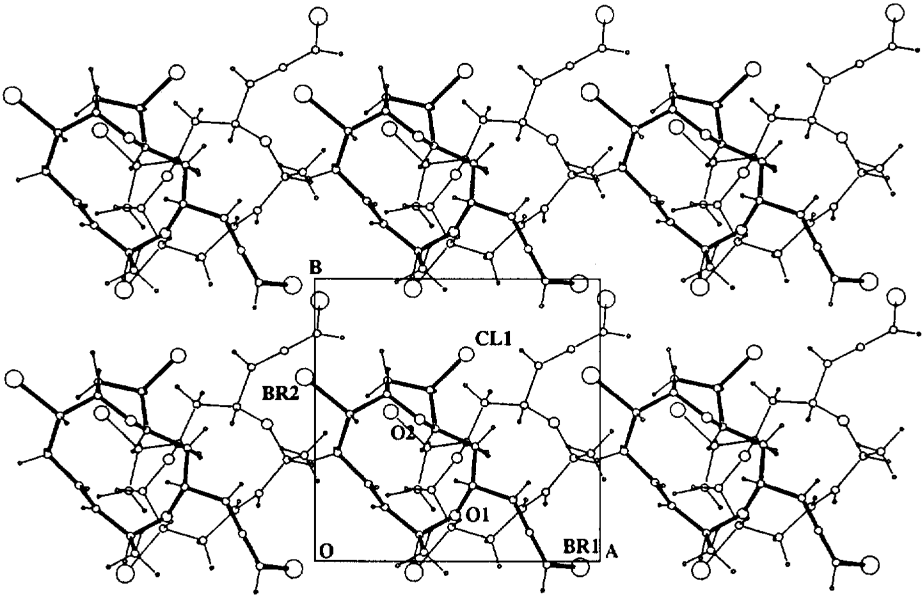


Fig 2. Crystal packing of **1** projected onto *ab* plane. Thin and thich lines indicate two adjacent molecular layers along the 4-fold screw axis of tetragonal P43 space group.

Dactylallene (**1**) was found to be very toxic to the mosquito fish *Gambusia affinis*[12] at 10 ppm and to deter the golden fish *Carassius auratus*[13] at a concentration of 100 µg/cm^2 of food pellets. These activities suggest a potential defensive role against predators.

EXPERIMENTAL SECTION

General Experimental Procedures. Precoated TLC plates Merck Si gel 60 F254 were used for analytical TLC and Merck Kieselgel 60 powder was used for preparative column chromatography. HREIMS spectrum was recorded on a VG Autospec spectrometer with peak matching technique, using PFK as calibrant; EIMS was recorded on a C. Erba TRIO VG 2000 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 500 (500 MHz) spectrometer. Optical rotation was measured on a Jasco DIP 370 polarimeter. IR spectrum was obtained on a BIORAD FTS 7 FTIR.

Biological Material. Ten specimens of *A. dactylomela* (dry weight) were collected off Canary Islands, at a depth of 0.5 meters, and classified by J. Ortea. The individuals were immediately frozen and kept at -20°C until extraction. A voucher specimen of *Aplysia dactylomela* is deposited at Departamento de Biologia de Organismos y Sistemas, in Oviedo.

Extraction and isolation procedures. After a careful dissection in parapodia and viscera, the Et_2O soluble material (1.2g) from the CH_3COCH_3 (100x3 mL) extract of the viscera was fractionated on Si gel column, eluting with light petroleum ether and increasing amounts of diethyl ether.

The fractions containing dactylallene (67.0 mg) were submitted to a further Si gel column eluted with benzene/light petroleum ether 7:3, yielding pure dactylallene (23.5 mg).

Dactylallene (**1**) : obtained as pale yellow crystals from *n*-hexane m.p. 82-84°C; $[\alpha]_D = +242°$ ($CHCl_3$, c 0.7); IR (ν_{max}): 2917, 1959, 1448, 1090, 1062 cm^{-1}; HREIMS found 345.0251 (calculated 345.0257 for $C_{15}H_{19}O_2ClBr$); EIMS at *m/z* 349, 347, 345 (1.5:6:4.5) [M- Br]$^+$, 311, 309, 307 (10:30:23) [M - C_3H_2Br]$^+$, 293, 291, 289 (0.5:4.5:3.8) [M - C_3H_2Br - H_2O]$^{+\cdot}$ 267, 265, 263 (3:7:4), 229, 227 (30:80), 191 (28), 147 (40), 119 (50), 81 (100).

^{1}H- and ^{13}C-NMR data see Table 1.

Crystal data and structure refinement of dactylallene (**1**). Single crystals of **1** were obtained by slow evaporation of a *n*-hexane solution in form of yellow prisms which darken rather speedy.

Crystal Data: $a = b = 9.584(3)$, $c = 18.996(3)$ Å, tetragonal system, space group $P4_3$, $Z = 4$, $V = 1745(1)$ Å^3, $C_{15}H_{19}Br_2ClO_2$, $M_w = 426.59$, $D_c = 1.636$ g·cm^{-3}, $\mu = 74.5$ cm^{-1}.

Accurate cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections at medium θ ($25° < \theta < 31°$), using Cu $K\alpha$ radiation at room temperature and an Enraf-Nonius CAD4-F diffractometer on line with a MicroVAX 3100 computer. Using both Cu and Mo radiations, the crystals presented a severe degradation in the X-ray beam. As a consequence, many samples were required for a three dimensional data collection and high scansion speeds were utilized: the maximum scanning time for reflection was 90". The intensities of three check reflections were monitored every hour and showed a crystal decay close to 50% during 10 hr. The trends of the decay were roughly linear. The strategy for the data collections was chosen to increase the peack-background ratio of the non-strong reflections at high θ. The intensities of five different crystals were scaled,[14] after the corrections for linear decay and for absorption effects using a semi-empirical method.[15] A total of 4412 reflections with $\theta_{max} = 65°$ were collected and averaged giving 1537 unique reflections. 1051 reflections with $I \geq 2.5\ \sigma(I)$ were considered observed.

The structure was solved by SIR92 package.[16] The refinement was carried out on the positional and anisotropic displacement parameters of all 20 non-hydrogen atoms using full-matrix least squares method. Hydrogens were placed on the basis of geometric considerations and ΔF map suggestions for the methyl group. H-atoms were included as fixed atoms in the final refinement with isotropic displacement parameters set equal to *B*eq of the parent atoms.

The final discrepancy index *R* (on *F*) was 0.058 on 1051 observed reflections and 180 variables. *R*w = 0.062 with w = $1/[\sigma^2(Fo)+(0.02Fo)^2+1]$.[17]

The absolute configuration was determined by inclusion of the anomalous dispersion terms in the atomic scattering factors. The opposite chirality, refined in the $P4_1$ space group, gave a higher *R* value: the *R*-factor ratio between the two enantiomorph structures was 1.053, with the Rogers η factor = 0.925(3).[18] All that supported the correctness of the absolute configuration shown in the Fig. 1. For a last check on the absolute configuration, a new single crystal was employed in a very accurate recollection of the intensity of 216 Friedel-pairs at medium θ, appropriately chosen. Each Friedel-pair was collected in direct sequence in order to minimize the decay effects. Using the previously refined molecular models of the two enantiomers, the ratio between the respective discrepancy *R*-factors increased to 1.118, further confirming the chosen absolute configuration.

All calculations were performed on a MicroVAX3100 computer using the Enraf-Nonius *SDP* software.[19] Structure factors, anisotropic displacement parameters and hydrogen atoms coordinates have been deposited.

Biological assays. Ichthyotoxicity test (*Gambusia affinis*)[12,20] and antifeedant assay (*Carassius auratus*)[13] were conducted as described in literature.

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