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Novel 2(5H)-Furanones from the Red Marine Alga *Delisea elegans* (Lamouroux).

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ABSTRACT - Six novel 2(5H)-furanones, all related to the previously reported 'fimbrolide' *3-butyl-4-bromo-5-(dibromomethylidine)-2(5H)-furanone*, have been isolated from the red marine alga *Delisea elegans* (family Bonnemaisoniaceae). Three of the six compounds, characterised by spectroscopic and single crystal X-ray structure analyses, contain unusual poly-brominated cyclobutane functions.

INTRODUCTION

Red marine algae from the family *Bonnemaisoniaceae* have been shown to produce a wide range of halogenated metabolites,[1] including butenones, acetones, acrylic and acetic acids,[2,3,4] pyranones, octenones,[5] and from the genus *Delisea*, halogenated 2(5H)-furanones.

A series of 2(5H)-furanones, named fimbrolides, with the structures (1) through (4)[6] were isolated from the dichloromethane soluble material of extracts of *D. fimbriata* , collected on the east coast of Australia. A similar study of *D. fimbriata* collected from Antarctica described the isolation of 2(5H)-furanones (1a) - (1d), (1f) and (1g),[7] together with a series of oct-1-en-3-ones (5a) - (5e).[8]

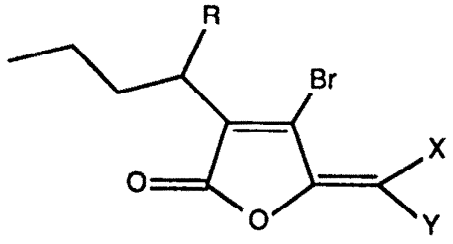
In both cases interest in the species was due to observations of biological activity. In the first instance high *in vitro* antimicrobial activity was found,[6] while in the second, plants were observed to be remarkably free of epibionts.[7,8]

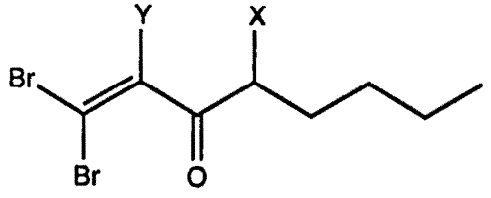
In this present study six new metabolites were isolated from *Delisea elegans* collected at Kaikoura, New Zealand. The previously reported furanone (4) was identified as the major component of the mixtures, and six novel halogenated furanones, (6), (7), (9) - (12), all related to (4), were found.

DISCUSSION

Extraction of air dried *Delisea elegans*, collected from Kaikoura in April 1981, afforded a 5% organic extract (extract A). Initial open-column chromatography on silica gel, and subsequent HPLC resulted in the isolation of compounds (4), (7), (10), (11) and (12). Using similar procedures the metabolites (6) and (9) were obtained from an extract (extract B) of the alga collected from the same site in April 1983.

Comparison of the crude organic soluble extracts A and B by HPLC showed that they both contained all of the metabolites identified, although in slightly differing amounts.





		X	Y
(1) R = -OAc	(a)	Br	H
	(b)	H	Br
	(c)	I	H
	(d)	H	I
	(e)	Cl	H
	(f)	H	Cl
	(g)	Br	Br
(3) R = -H	(a)	Br	H
	(b)	H	Br

		X	Y
(2) R = -OH	(a)	Br	H
	(b)	H	Br
	(c)	I	H
	(d)	H	I
	(e)	Cl	H
	(f)	H	Cl
	(g)	Br	Br
(4) R = -H		Br	Br

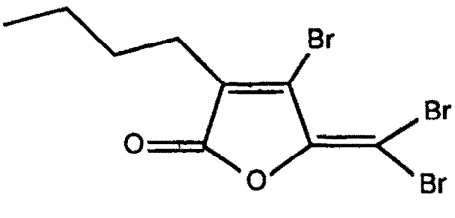
		X	Y
(5)	(a)	Br	H
	(b)	Br	Br
	(c)	Br	Cl
	(d)	I	H
	(e)	I	Cl

The least polar compound in the extracts was heptadecane, which was identified by GC and ^{13}C-NMR comparisons with authentic samples.

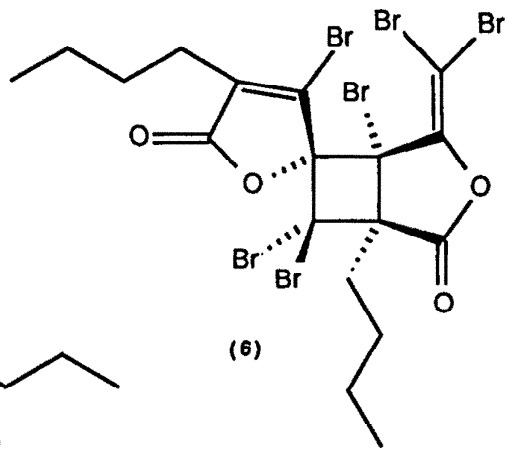
The major component of the extracts, also among the least polar of the compounds, was the previously reported furanone (4). This compound was estimated to represent 80% of the organic soluble material in each of the extracts.

A more polar compound *(±)-trans-4,10,11,11,12,12-hexabromo-3,9-dibutyl-1,7-dioxa-dispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione* (7) was obtained from a silica gel column fraction of extract A by HPLC. The spectroscopic data indicated a strong structural similarity to (4). The ^{13}C-NMR spectrum of (7) contained nine resonances, an n-butyl group (δ_c 28.43, 25.69, 22.06, 13.59), a carbonyl group (δ_c 165.56), two olefinic carbon atoms (δ_c 140.09, 136.66), and two further quaternary resonances (δ_c 89.79, 69.88). The only resonances in the ^{1}H-NMR spectrum were associated with the n-butyl group. The strong carbonyl absorption (1800 cm^{-1}) was characteristic of the α,β-unsaturated furanone system in compounds (1) - (4), while the UV absorption maximum (249nm) suggested a singly conjugated enone system. Finally the observation of a single quintet (Br_4) observed in the low resolution FAB mass spectrum at *m/z* 615 ($C_{18}H_{19}Br_4O_4$) suggested a dimeric structure for compound (7), with a requirement for a high degree of symmetry. The compound was eventually crystallized as colourless needles, and the structure solved using single crystal X-ray methods.

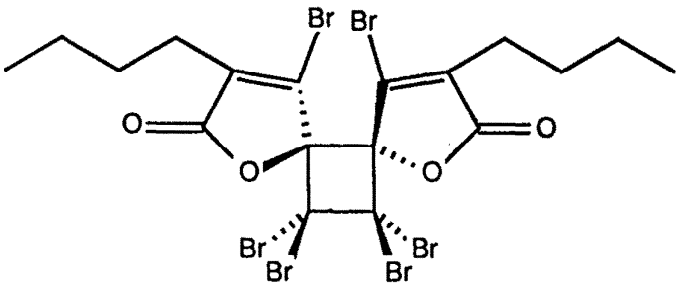
A more polar column fraction from extract A contained two major components, in addition to some minor contaminants. The minor components were removed by HPLC, and fractional recrystallisation from pentane yielded crystals of two distinct types, one with needle-like crystals, and the other with plate-like ones.



(4)



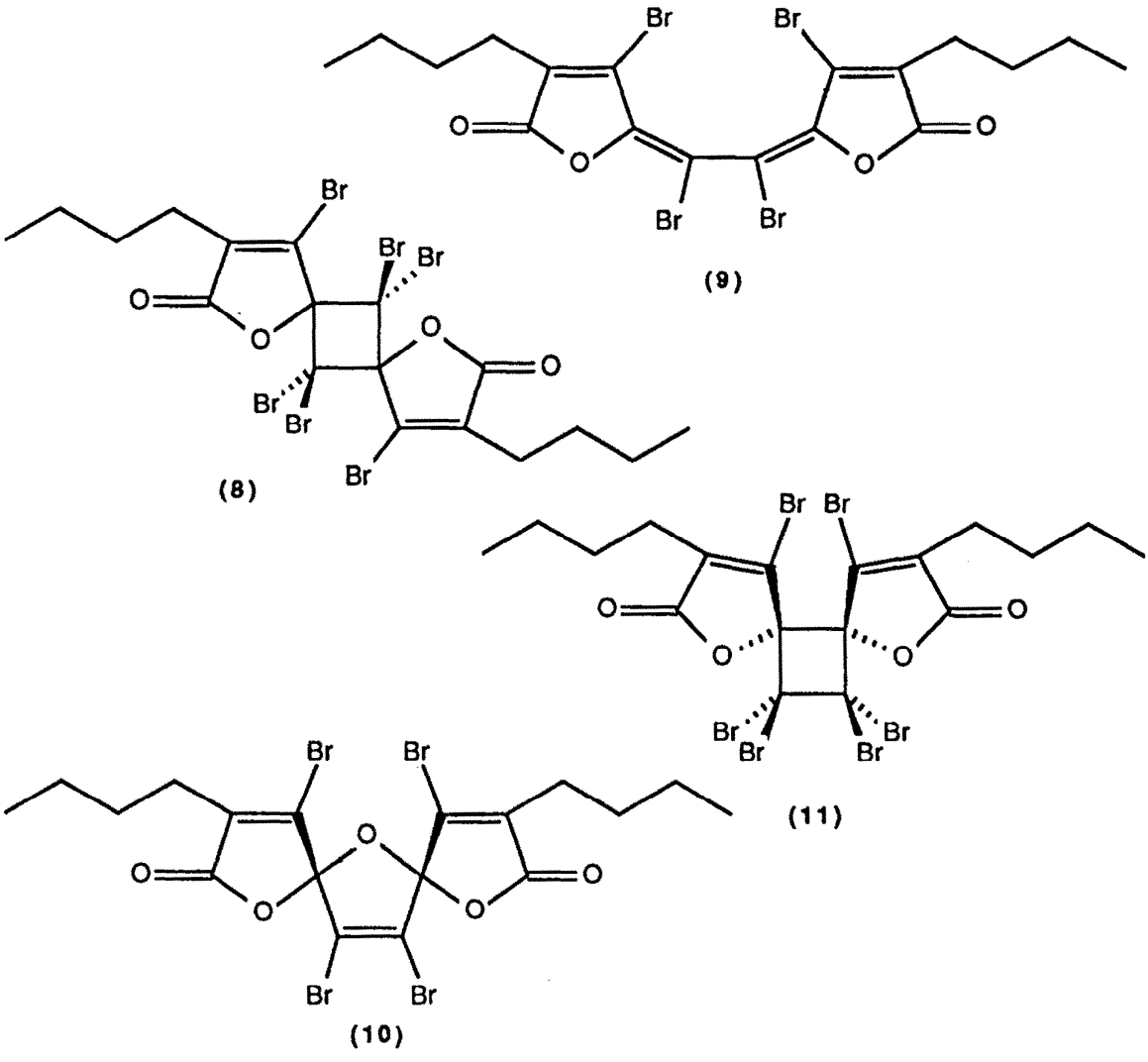
(6)



(7)

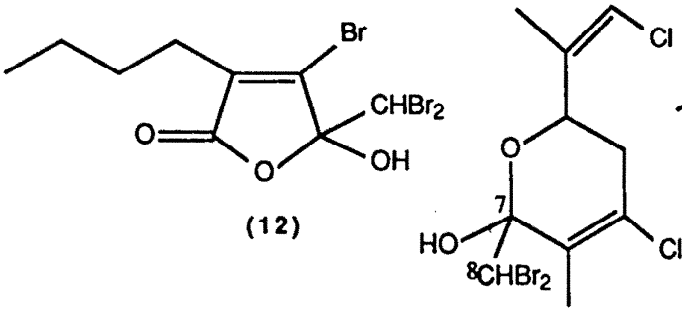
The compound with needle-like crystals was shown to be *cis-4,11,12,13-tetrabromo-3,10-dibutyl-1,6,8-trioxadispiro[4.1.4.2]trideca-3,10,12-triene-2,9-dione* (**10**) by single crystal X-ray structure analysis. High thermal motion, or disorder, in the terminal n-butyl groups was indicated by the very large temperature factors for these atoms. Many attempts were made, using disordered models, to account more satisfactorily for the geometry of the C7', C8' and C9' moiety. None were better than that implicit in table 1, and the extreme difficulties involved in obtaining more material precluded recollection of the X-ray data at low temperature. No useful spectroscopic data could be obtained from the small amount of material available.

The compound which crystallised as plate-like crystals was identified as *(±)- cis-4,10,11,11,12,12-hexabromo-3,9-dibutyl-1,7-dioxadispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione* (**11**). The infrared spectrum of the compound contained a strong carbonyl band (1800cm^{-1}) characteristic of the 2(5H)-furanone function, while the ^{1}H-NMR spectrum on comparison with (**4**) indicated the presence of an n-butyl chain. The carbonyl (δ_c 165.57) and the olefinic resonances (δ_c 142.57, 136.37) were very similar to those of the cyclobutane dimer (**7**), indicating a closely related structure. Comparison of the two remaining singlet resonances (δ_c 86.42, 59.08) with those of the dimer (**7**) suggested that the structure was either the *cis* diastereomer (**11**) of (**7**), or the [4.1.4.1] (**8**) structural isomer. A room temperature single crystal X-ray structure analysis showed unambiguously that the structure was the diastereomer (**11**). The only small single crystal sample was of very poor quality, and could not be improved with the limited material available. The present refinement level of R=0.11, (owing to disorder in the n-butyl chains) is not yet good enough to warrant publication of further detail in this paper.



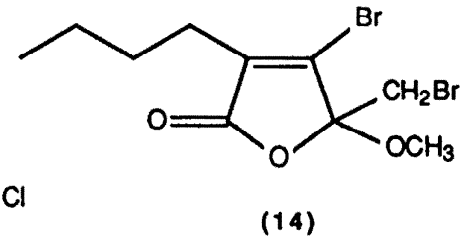
Preparative HPLC of the most polar silica gel column fractions, from extract A, yielded the *3-butyl-4-bromo-5-hydroxy-5-(dibromomethyl)-2(5H)-furanone* (**12**). High resolution mass spectrometry indicated a molecular formula of $C_9H_{11}Br_3O_3$ (*m/z* 404, Br_3) which allowed for three double bond equivalents. The observed loss of 18 AMU is characteristic of an alcohol function, while the resulting ion cluster at *m/z* 387 (Br_3) suggested that the compound was closely related to (**4**). The presence of an alcohol function was confirmed by the infrared spectrum which showed a sharp absorption band at 3525 cm^{-1}. A strong carbonyl band at 1790 cm^{-1} was further evidence for the similarity of structure to (**4**). The UV absorption maximum (235nm) was consistent with a 3,4–unsaturated 2-furanone. Comparison of the ^{1}H-NMR spectrum with that of (**4**) showed that nine of the eleven protons were associated with an n-butyl chain. The hydroxyl proton was observed as a broad singlet at δ_H 4.65. The remaining proton, a sharp singlet at δ_H 5.85, was assigned by comparison with costatone (**13**)[9], δ_H 5.80, as belonging to a dibromomethyl group. The ^{13}C-NMR multiplicities were consistent with the eleven proton integral. Comparison of the chemical shifts of the n-butyl and olefinic resonances with those of (**4**) ruled out the possibility of the 3 or 4 position being substituted by either the hydroxyl or dibromomethyl groups. This together with the other spectroscopic data indicated the presence of a 3-butyl-4-bromo-2(5H)-furanone moiety. This assignment of the substitution of the olefin implied that both the hydroxyl and dibromomethyl groups

were placed γ to the carbonyl group, i.e. structure (12). ORD measurements showed that the compound was a racemic mixture.



(12)

(13)



(14)

Further proof of the identity of this compound was obtained by reaction of (4) with aqueous potassium hydroxide in tetrahydrofuran. Analysis by HPLC of the reaction mixture indicated a product with an identical retention volume to (12). This reaction is analogous to that that reported by Pettus *et al* [7] in which 3-butyl-4-bromo-5-(bromomethylidine)-2(5H)-furanone (3a) or (3b) was reacted with methanolic potassium hydroxide to give the corresponding methoxyl derivative (14).

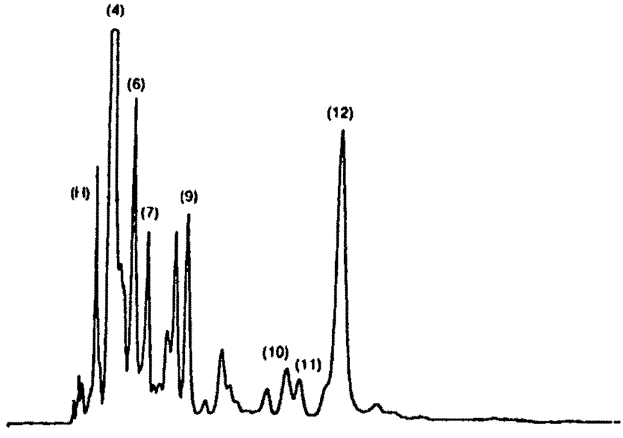


Figure 1. HPLC chromatogram of extract B (cyanopropyl, 1% propan-2-ol/hexane, 210nm). The peaks are marked with compound numbers (H - heptadecane).

The two new compounds isolated from extract B of *D. elegans*, collected in April 1983, were characterised by single crystal X-ray structure determinations as *3,5',7',7',-tetrabromo-1',4-dibutyl-4'-(dibromomethylene) spiro[furan-2(5H),6'-[3]oxabicyclo[3.2.0]heptane]-2',5-dione* (6) and *(Z,Z)-5,5'-(1,2-dibromo-1,2-ethanediylidene)bis[4-bromo-3-butyl-2(5H)-furanone]* (9).

A number of structural and stereoisomers of the compounds described here are possible. Several other compounds were isolated whose ^{13}C-NMR spectra indicated they were closely related to the compounds described here, but lack of crystals and poor spectroscopic data prevented their structural elucidation. The compounds isolated are shown in an HPLC chromatogram of extract B (figure 1).

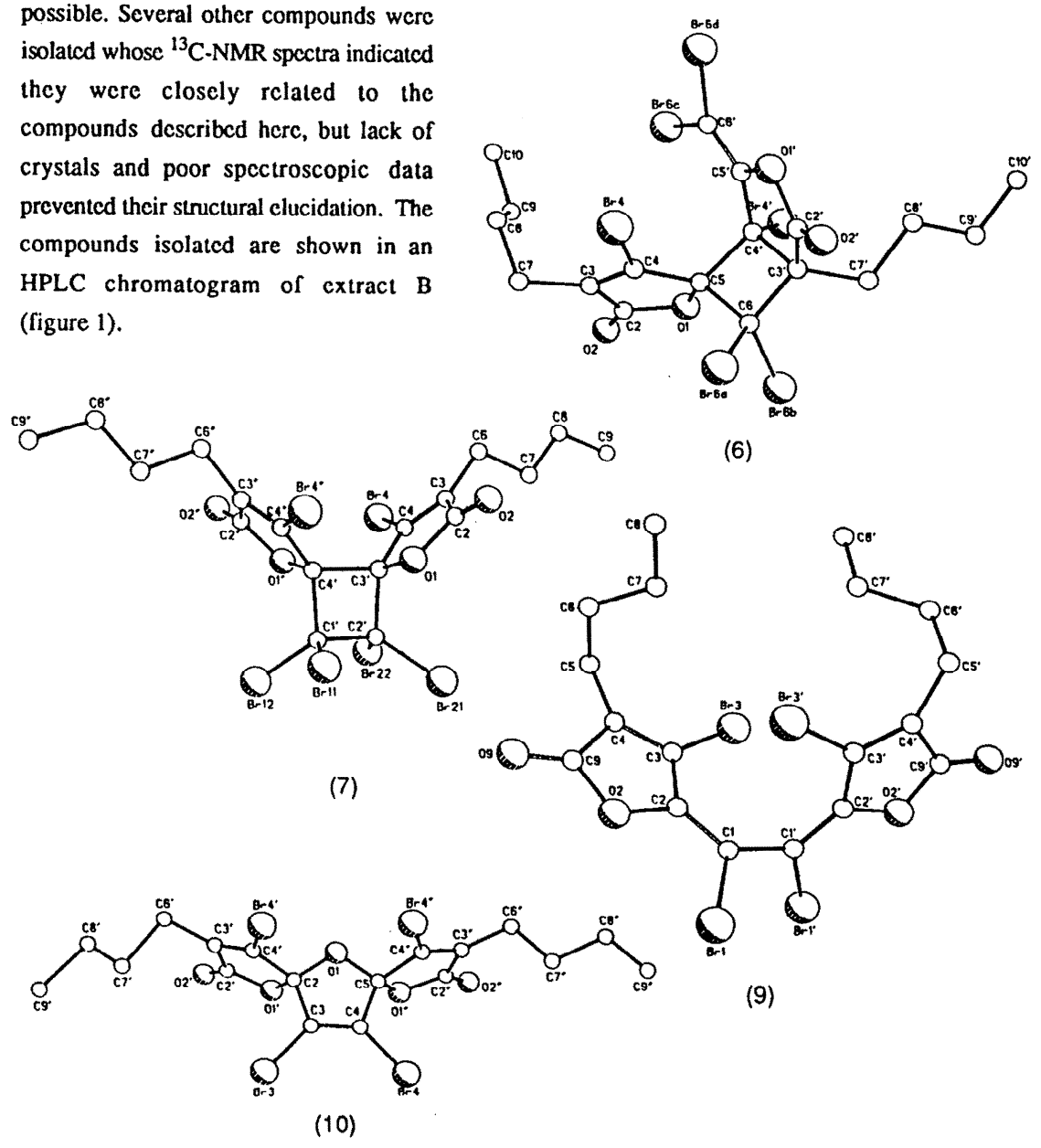


Figure 2. Perspective Drawings of compounds (6), (7), (9) and (10). Note (7) lies on a crystallographic two-fold axis.

From a spectroscopic view point these compounds presented considerable problems. In spite of using FAB and FD ionisation techniques reliable mass spectra proved almost impossible to obtain. The only molecular ions observed were for the compounds (10) and (12). The highly substituted nature of these structures meant the ^{1}H-NMR spectra contained little useful information, while the long relaxation times for many of the carbon atoms made it difficult to record adequate ^{13}C-NMR spectra with the limited quantities of compound available.

Computer generated perspective drawings of (6), (7), (9) and (10) are shown in figure 2. Only non-hydrogen atoms are shown. Table 1 shows the fractional atomic coordinates for these compounds. A crystallographically convenient system of atom numbering has been used for these diagrams and tables and in the deposited material.

Comparison of collections.

A total of seven collections of *D. elegans* were made, at times which spanned most of the observed growing season at Kaikoura (mid October to late April). To check whether there was a geographical or seasonal variation in the types of compounds found, extracts of samples from each collection were made using a standard technique, and compared by HPLC.

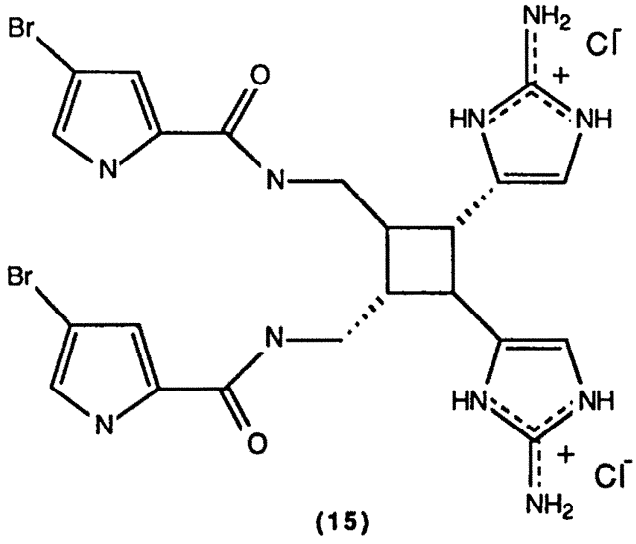
Dried, powdered samples of the alga were extracted with dichloromethane, and filtered through short "Florisil" columns in ether to remove pigments and other polar materials. The resulting oils were analysed by HPLC on a cyanopropyl column. Examination of the chromatograms revealed variations of the relative concentrations of compounds, but no significant qualitative differences were found.

Origin of the compounds.

To ensure that the compounds described here were not artifacts, especially of photochemical origin, a collection of *D. elegans* was made in March 1982. The alga was placed in an opaque plastic bag before being brought to the surface. The subsequent extraction and analysis procedures were performed in total darkness to prevent any possible photo-dimerisation. To eliminate the possibility of artifacts being caused by the extraction and column chromatographic methods the alga was simply homogenised with dichloromethane, and the centrifuged extract immediately analysed by HPLC. Comparison of the chromatogram obtained by this method with those of extracts from other collections revealed no significant qualitative difference, supporting the view that the compounds described here are of natural origin.

The biochemical origin of the compounds described is unclear. The cyclobutane containing dimers (6), (7) and (11) could all be derived from (4) by $[_{\pi}2_{s}+_{\pi}2_{s}]$ cycloaddition reactions. Compound (10) can be derived from a condensation between (4) and (12), while the dimer (9) could have arisen from the appropriate vinyl radicals. Compound (12) may be formed by the addition of water across the exocyclic double bond of (4) or alternatively (12) could the precursor of (4).

Molecules which are formed by enzyme mediated reactions are almost invariably optically active.[10] Examples of cyclobutane-containing dimers are known. Optically active sceptrin (15) is related to the achiral debromooroidin (oroidin (16) by a $[_{\pi}2_{s}+_{\pi}2_{s}]$ cycloaddition reaction. Although this cycloaddition reaction is photochemically allowed attempts to dimerise oroidin failed and a biosynthetic origin was postulated.[11] In contrast all of the compounds isolated here were either *meso* or racemic mixtures. This lack of stereospecificity in the biosynthesis of these compounds suggests they may be formed photochemically *in situ*.



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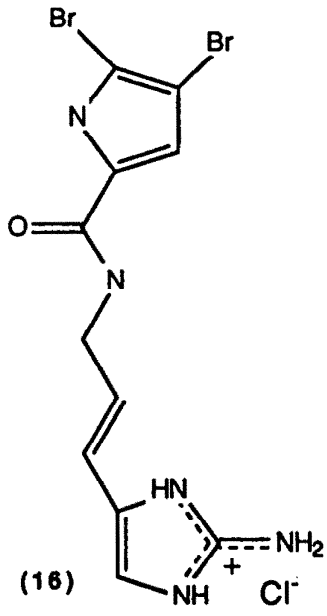

(16)

Table 1. Fractional coordinates for atoms in compounds (6), (7), (9) and (10). The equivalent isotropic temperature factor in this table is defined as one third of the trace of the orthogonalised U tensor.

Atom	10^4 X/a	10^4 Y/b	10^4 Z/c	10^3U	Atom	10^4 X/a	10^4 Y/b	10^4 Z/c	10^3U
3,5',7',7',-Tetrabromo-1',4-dibutyl-4'-(dibromomethylene)spiro[furan-2(5H),6'-[3]oxabicyclo[3.2.0]heptane]-2',5-dione (6).									
Br(4)	-943(2)	6700(2)	7954(1)	47(1)	C(6)	2421(15)	7088(14)	6860(6)	37(4)
Br(4')	4684(2)	3655(2)	7293(1)	48(1)	C(7)	1940(21)	8773(17)	9373(7)	58(6)
Br(6a)	845(2)	8662(2)	6681(1)	63(1)	C(8)	1688(17)	7553(19)	9855(7)	57(6)
Br(6b)	4820(2)	8120(2)	6643(1)	62(1)	C(9)	3401(26)	6937(19)	10177(8)	83(8)
Br(6c)	2210(2)	2819(2)	8604(1)	66(1)	C(10)	2870(20)	5563(19)	10625(8)	70(6)
Br(6d)	-1724(2)	1367(2)	7756(1)	58(1)	C(2')	-123(15)	4612(14)	6317(6)	36(4)
O(1)	4610(11)	7172(10)	7982(4)	45(3)	C(3')	1963(14)	5255(14)	6492(6)	31(4)
O(1')	-546(11)	3497(10)	6740(4)	46(3)	C(4')	2555(14)	4717(13)	7201(6)	29(4)
O(2)	5893(12)	8705(12)	9034(5)	60(4)	C(5')	871(13)	3543(13)	7290(6)	34(4)
O(2')	-1179(11)	4895(11)	5860(4)	56(4)	C(6')	556(19)	2716(14)	7797(6)	47(5)
C(2)	4525(22)	8071(15)	8644(7)	51(6)	C(7')	2764(20)	4593(14)	5856(6)	49(5)
C(3)	2628(17)	8003(14)	8714(6)	39(5)	C(8')	2116(17)	2726(15)	5540(6)	46(5)
C(4)	1573(18)	7149(14)	8120(6)	41(5)	C(9')	3249(20)	2089(15)	4997(7)	55(6)
C(5)	2791(13)	6598(14)	7599(6)	30(4)	C(10')	2644(19)	222(16)	4697(7)	56(6)
(±)-trans-4,10,11,11,12,12-Hexabromo-3,9-dibutyl-1,7-dioxadispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione (7).									
Br(21)	1184(2)	-608(2)	7126(2)	73(2)	C(4)	125(12)	2362(15)	6272(14)	42(12)
Br(22)	-876(2)	-172(2)	5678(2)	66(2)	C(6)	1027(15)	3853(17)	5826(17)	66(16)
Br(4)	-1024(1)	2603(2)	5109(2)	61(2)	C(7)	1319(20)	3400(21)	4978(20)	95(21)
O(1)	1240(1)	1720(10)	7860(9)	50(9)	C(8)	1400(22)	4294(23)	4253(22)	105(28)
O(2)	2370(10)	2810(10)	7930(10)	72(12)	C(9)	1675(34)	3898(32)	3359(28)	181(53)
C(2)	1605(20)	2530(18)	7435(20)	57(15)	C(2')	116(13)	225(15)	7006(15)	51(13)
C(3)	876(10)	2901(20)	6450(15)	51(14)	C(3')	279(12)	1544(14)	7161(13)	44(13)
(Z,Z)-5,5'-(1,2-Dibromo-1,2-ethanediylidene)bis[4-bromo-3-butyl-2(5H)-furanone] (9)									
Br(1)	9108(2)	824(1)	7332(1)	53(1)	C(6)	1339(15)	2013(11)	9729(7)	61(4)
Br(3)	3280(1)	2173(1)	6700(1)	50(1)	C(7)	2257(18)	3270(11)	9394(8)	73(5)
Br(1')	7492(2)	295(1)	5026(1)	57(1)	C(8)	2353(22)	4123(14)	10195(10)	102(7)
Br(3')	7404(2)	3035(1)	8073(1)	54(1)	C(9)	3962(14)	-482(10)	9076(6)	46(4)
O(2)	5606(9)	-667(6)	8523(4)	48(2)	C(1')	7428(12)	1189(9)	6131(6)	38(3)

Table 1. continued.

Atom	10^4 X/a	10^4 Y/b	10^4 Z/c	10^3U	Atom	10^4 X/a	10^4 Y/b	10^4 Z/c	10^3U
O(9)	3606(10)	-1140(8)	9838(5)	67(3)	C(2')	7601(13)	2492(10)	6055(6)	42(3)
O(2')	7796(9)	3216(6)	5128(4)	53(3)	C(3')	7567(12)	3478(9)	6729(6)	40(3)
O(9')	8007(12)	5389(8)	4529(6)	81(4)	C(4')	7651(14)	4710(10)	6264(7)	52(4)
C(1)	7139(13)	300(8)	7046(6)	37(3)	C(5')	7601(16)	6081(10)	6592(9)	70(5)
C(2)	5663(13)	218(9)	7674(6)	40(3)	C(6')	5778(20)	6745(11)	6635(10)	84(6)
C(3)	3980(13)	926(9)	7690(6)	40(3)	C(7')	4445(22)	6115(14)	7404(10)	96(7)
C(4)	2976(13)	589(10)	8536(6)	44(3)	C(8')	2656(18)	6838(14)	7463(10)	90(6)
C(5)	1223(13)	1102(11)	8953(7)	51(4)	C(9')	7819(15)	4567(10)	5232(8)	57(4)
cis-4,11,12,13-Tetrabromo-3,10-dibutyl-1,6,8-trioxadispiro[4.1.4.2]trideca-3,10,12-triene-2,9-dione (10).									
Br(3)	6112(1)	8579(1)	697(2)	75(1)	C(3')	6604(8)	10927(9)	1314(22)	91(15)
Br(4)	7838(1)	7466(1)	-998(2)	90(1)	C(4')	7099(7)	10334(8)	1544(16)	67(10)
Br(4')	7581(1)	9996(1)	3514(2)	84(1)	C(6')	6250(10)	11528(12)	2497(24)	132(18)
Br(4")	9395(1)	8941(1)	1738(2)	67(1)	C(7')	5357(14)	1043(15)	3175(33)	178(29)
O(1)	8064(5)	9944(5)	-493(11)	68(6)	C(8')	5134(20)	11637(15)	3635(46)	404(87)
O(1')	6758(6)	10285(6)	-1187(12)	90(8)	C(9')	4205(12)	11194(13)	4383(27)	152(20)
O(2')	5922(7)	11358(7)	-1175(16)	136(2)	C(2")	9210(9)	9062(9)	-3248(17)	76(11)
O(1")	8437(5)	9215(5)	-2840(9)	74(7)	C(3")	9703(8)	8934(7)	-1733(14)	60(9)
O(2")	9428(6)	9031(7)	-4633(13)	103(10)	C(4")	9198(8)	9023(7)	-518(15)	66(10)
C(2)	7263(7)	9869(7)	-20(16)	65(9)	C(6")	10563(7)	8749(8)	-1736(16)	72(10)
C(3)	7090(7)	8976(9)	-28(16)	67(10)	C(7")	10725(7)	7834(8)	-1814(15)	72(10)
C(4)	7697(8)	8576(8)	-639(15)	66(8)	C(8")	11618(9)	7636(9)	-1761(18)	89(11)
C(5)	8336(7)	9182(8)	-1075(13)	63(9)	C(9")	11780(10)	6742(10)	-1771(19)	111(16)
C(2')	6373(9)	10922(11)	-452(21)	90(14)					

EXPERIMENTAL

General Experimental. Preparative and analytical high pressure liquid chromatography (HPLC) was conducted using a Varian 5020 Liquid Chromatograph, a Varian UV-50 UV/Visible detector, and an Hewlett Packard 3390A Integrating Recorder. All chromatograms were obtained using Du Pont Zorbax bonded phase micro-particle columns. The solvents used were either Waters chromatographic quality, or carefully purified[12] and dried prior to use. Pet. ether refers to light petroleum (boiling range 50-70°C), and ether to diethyl ether. A Varian 3770 Gas Chromatograph with an FID and an Hewlett Packard 3390A integrating recorder were used for the gas chromatography. Redistilled and dried solvents were used for column chromatography. Thin layer chromatograms were obtained using deactivated silica gel G (Stahl), 0.5mm thick on glass plates. The plates were visualised by either spraying with iodine solution (1% w/v, methanol), or by baking at 100° after spraying with phosphomolybdic acid solution (10% w/v, ethanol), or 2,4-dinitrophenylhydrazine solution (0.1g 2,4-DNP, 100ml ethanol, 1ml HCl). Fast atom bombardment mass spectroscopy (FAB) was performed on an MS-80 mass spectrometer using xenon as the ionisation gas, and glycerol as the dispersion matrix. Field desorption spectra (FD) were obtained using a Varian MAT 720 mass spectrometer. The high resolution EI spectrum of (12) was obtained with a VG 7070F spectrometer. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, and proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a Varian CFT-20 instrument. The multiplicities of ^{13}C-NMR spectra were established using either single frequency offset resonance decoupling (SFORD) or 'attached proton test' (APT) techniques.[13] Infrared spectra were recorded using

either a Pye Unicam SP3-300 Infrared Spectrophotometer, or a Shimadzu IR27G spectrophotometer. Samples were run as carbon tetrachloride solutions in a 10mm sodium chloride cell. Ultraviolet spectra were recorded on a Varian Super Scan 3 Ultraviolet-visible spectrophotometer. Melting points were obtained using a Reichiert 'Kofler' hot stage microscope and are uncorrected. Optical rotatory dispersion (ORD) spectra were recorded on a JaSCo Model ORD/UV-5 spectrophotometer.

Isolation of Metabolites from Extract A . Delisea elegans was collected sublittorally (-15m) from St Kilda Rocks, Kaikoura, 160km north of Christchurch on the 2nd of April 1981. The alga was frozen and subsequently air dried, ground and extracted with dichloromethane in a Soxhlet apparatus. The extraction yielded a dark green-brown odiferous oil (3.8g; 4.6% of 82g dry weight).

An analysis by HPLC (cyanopropyl 4.5 x 250mm; heptane/propan-2-ol (5%), 210nm) resolved more than eleven components.

The organic soluble oil (2.5g) was chromatographed on "Florisil" (Sigma 100-200 mesh) (120g; 25 x 500mm column). Fifty fractions of 25ml were collected with increasing solvent polarity (fractions 1 to 4, pentane; 5 to 6, pet. ether; 7 to 10, 0.25% dichloromethane/pet.ether; 11 to 15, 1.0%; 16 to 17, 2%; 10 to 20, 5%; 21 to 25, 10.0%; 26 to 31, 20%; 32 to 39, 50%; 40 to 45, 100%; 46 to 50, ether).

Each fraction was analysed by TLC and fractions with similar composition were combined as follows: 1 to 4 (21mg), 5 to 6 (15mg), 7 to 10 (23mg), 11 to 17 (600mg), 18 to 29 (358mg), 30 to 35 (52mg), 36 to 37 (13mg), 38 to 42 (95mg), 43 to 45 (41mg), 46 to 47 (468mg), 48 to 50 (24mg).

Isolation of 3-butyl-4-bromo-5-(dibromomethylidine)-2-(5H)-furanone (4). Fractions "11" to "17" (585mg) all showed one major component by TLC (R_f = 0.65; pet. ether/ether 10%). This component (200mg) was subjected to preparative HPLC (cyanopropyl 9.5 x 250mm; heptane/propan-2-ol (1%); 220nm), which yielded a very pale yellow, sweet smelling oil (160mg; > 98% pure) which was identified as (4).[14] (4): IR ($CHCl_4$) 1790 cm^{-1}; EIMS *m/z* 385 ($C_9H_9Br_3O_2$); ^{1}H-NMR ($CDCl_3$) δ_H 2.4 (t,J=7Hz,2H), 1.1 to 1.7 (m,4H), 0.92 (t,J=7Hz,3H); ^{13}C-NMR δ_c 164.78 (s), 144.71 (s), 138.07 (s), 128.35 (s), 81.44 (s), 28.80 (t), 25.82 (t), 22.46 (t), 13.71 (q); UV λ_{max} cyclohexane (ε) 308nm (16000).

Isolation of (±)- trans-4,10,11,11,12,12-Hexabromo-3,9-dibutyl-1,7-dioxadispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione (7). High pressure liquid chromatography (cyanopropyl 4.5 x 250mm; heptane/propan-2-ol (2%); 220nm) of the combined fractions "18" to "29" (358mg) revealed five major components. These five components were separated from the mixture (190mg) using preparative HPLC (cyanopropyl 9.5 x 250mm; heptane/propan-2-ol (1%); 270nm). Colourless crystals (44mg) were obtained from the second fraction eluted. (7) ; m.p. 102-104°C; IR 1800cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H 2.42 (t,J=7Hz,4H), 1.1 to 1.7 (m,8H), 0.93 (t,J=7Hz,6H); ^{13}C-NMR ($CDCl_3$) δ_c 165.56 (s), 140.09 (s), 136.66 (s), 89.79(s), 69.88 (s), 28.43 (t); 25.69 (t), 22.06 (t), 13.59 (q); UV λ_{max} cyclohexane (ε) 249nm (15400).

Preparative High Liquid Chromatography of Combined Fractions "38" to "42". This fraction contained two major components of similar HPLC retention volumes, contaminated with several minor components. Initial purification was by HPLC (cyanopropyl 4.5 x 250mm; hexane/propan-2-ol (5%),

220nm), and fractional recrystallisation from pentane yielded two compounds, one with needle-shaped crystals (**10**), and the other with plate-like crystals (**11**).

cis-4,11,12,13-Tetrabromo-3,10-dibutyl-1,6,8-trioxadispiro[4.1.4.2]trideca-3,10,12-triene-2,9-dione (**10**). The compound with needle-like crystals was identified by X-ray crystallography as (**10**). FABMS *m/z* 630 ($C_{18}H_{18}O_5Br_4$).

(±)-cis-4,10,11,11,12,12-Hexabromo-3,9-dibutyl-1,7-dioxadispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione (**11**). The compound with plate-like crystals was identified as the furanone dimer (**11**): m.p. 153-154°C; IR 1800 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H 2.0 (t,J=7Hz,4H), 1.1 to 1.8 (m,8H), 0.95 (t,J=7Hz,6H); ^{13}C-NMR δ_c 165.57 (s), 142.57 (s), 136.37 (s), 86.42 (s), 59.08 (s), 28.65 (t), 26.41 (t), 22.35 (t), 13.7 (q); UV λ_{max} cyclohexane (ε) 246nm (8750).

3-Butyl-4-bromo-5-dibromomethyl-5-hydroxy-2-(5H)-furanone (**12**). Column fractions "46" to "50" were shown to contain one major component by TLC (silica; pet. ether/ether 30%) and analytical HPLC (cyanopropyl 4.5mm x 250mm; heptane/propan-2-ol (20%); 220nm). Preparative HPLC (cyanopropyl 9.5 x 250mm; heptane/propan-2-ol; 220nm) of the combined fractions (180mg) gave the hydroxy-furanone (**12**) as a viscous pale brown-yellow oil (102mg). (**12**): IR 3525, 1790 cm^{-1}; the ORD spectrum indicated no optical activity; ^{1}H-NMR ($CDCl_3$) δ_H 5.85 (s,1H), 4.65 (bs,1H), 2.38 (t,J=7Hz,2H); 1.1 to 1.7 (m,4H), 0.93 (t, J=7Hz,3H); ^{13}C-NMR δ_c 168.03(s), 139.27 (s), 137.41 (s), 102.59 (s), 44.44 (d), 28.57 (t), 24.81 (t), 22.32 (t), 13.63 (q); HREIMS *m/z* 405.82202 ($C_9H_{11}O_3{}^{79}Br_2{}^{81}Br$) requires 405.8257; UV λ_{max} cyclohexane (ε) 235nm (10000).

To 1.5mg of (**4**), dissolved in tetrahydrofuran, three drops of concentrated aqueous potassium hydroxide were added. The reaction mixture was neutralised with HCl, and extracted with dichloromethane (3x3ml). The concentrated extract was compared with the original crude extract by HPLC (cyanopropyl column 4.5 x 250mm; hexane/propan-2-ol 5%; 220nm;). The reaction showed one major component whose retention volume corresponded to the hydroxy-furanone (**12**), and several other minor components.

Isolation of Compounds from Extract B. A second substantial collection of *Delisea elegans* was made from St Kilda Rocks, in April 1983, and stored by freezing. The frozen alga (4kg) was air dried in darkness (300g dry weight), ground and extracted with ether, for 24 hours, in a Soxhlet apparatus. The green brown odiferous oil (10g) was chromatographed on a silica gel filtration column (50mm deep, 150mm dia., 350g Grace 923 silica gel), eluting with solvent mixtures ranging from pet. ether through to ether: pet. ether 1.5l; 0.5%, 5%, 10%, 20%, 50%, 100% ether, 1.0l each.

The three initial fractions (39mg). were shown to be heptadecane. Identification was by ^{13}C-NMR. δ_c 32.04, 29.78, 22.70, 14.02;[15] HREIMS *m/z* 240.2819 ($C_{17}H_{36}$) requires 240.2817. The identification was confirmed by capillary GC comparison with standard n-alkanes, using a semilog plot of retention time against chain length; column, DB-1 (J&W); C_{16}, 3.69 min; C_{18} 6.13 min; C_{19} 8.26 min; C_{20} 11.40 min; C_{22} 22.77 min; unknown 4.67 min. A calculated retention time for $C_{17}H_{36}$ was 4.69 min.

The fourth and fifth fractions (3.4g) were mixtures of 3-butyl-4-bromo-5-(dibromomethylidine)-2(5H)-furanone (**4**) and more polar compounds. The two fractions were chromatographed by HPLC

(cyanopropyl; 0.1% propan-2-ol/hexane) to yield pure (4) (440mg) and a mixture of more polar compounds (85mg). Further HPLC of the mixture of polar compounds, under similar conditions, afforded two pure crystalline compounds (30mg, 15mg) . Crystallisation was achieved by dissolving the material in pet. ether/ether and allowing the solvents to evaporate slowly at 4° C. The structures of both compounds were determined by X-ray crystallography.

3,5',7',7',-Tetrabromo-1',4-dibutyl-4'-(dibromomethylene) spiro[furan-2(5H),6'-[3]oxabicyclo-[3.2.0]heptane]-2',5-dione (6): m.p. 105.5-106° C; IR (CCl_4) 1830, 1800 cm^{-1};^{13}C-NMR δ_c 165.46, 138.62, 137.59, 131.32, 81.33, 63.79, 61.70, 60.48, 37.55, 28.54, 26.28, 25.85, 22.60, 13.68. A number of resonances were not observed due to the small quantity of material available. UV λ_{max} acetonitrile (ε) 238nm (14900).

(Z,Z)-5,5'-(1,2-Dibromo-1,2-ethanediylidene)bis[4-bromo-3-butyl-2(5H)-furanone] (9): IR (CCl_4) 1790 cm^{-1}; ^{13}C-NMR ($CDCl_3$) δ_c 164.85, 148.06, 138.29, 127.58, 102.16, 28.93, 25.56, 22.39, 13.66; UV λ_{max} acetonitrile (ε) 292nm (26000).

Crystallography.

The data for compounds (6) and (9) were collected with a Nicolet XRD P3 single crystal four circle diffractometer, using Mo Kα (λ 0.71069 Å) radiation from a crystal monochromator, while those for compounds (7), (10) and (11) were collected with a Hilger and Watts four circle diffractometer using Ni-filtered Cu Kα (λ 1.5418 Å) radiation. Data were collected at 298 K, except for (6) and (9) which were collected at 173 K. The space group was, in each case, determined unambiguously as a result of the structure analyses reported below, but initially indicated by systematic absences of the appropriate reflections. The cell parameters were determined by a least-squares refinement of the setting angles of 12 or 25 accurately centred high angle reflections for the Hilger and Picker diffractometers respectively.

The structures were solved by Patterson and difference Fourier syntheses,except for (6) and (9) which were solved by direct methods and difference Fourier syntheses. Blocked cascade least-squares (SHELXTL) and full-matrix (SHELX76)[16] refinements were employed, the reflection weights being $1/[\sigma^2(F)+g(F^2)]$. The function minimized was $\Sigma w (|F_o| - |F_c|)^2$. Anomalous dispersion corrections were from Cromer and Liberman.[17] Hydrogen atoms were included as rigid groups pivoting about their carbon atoms, and all non-hydrogen atoms were assigned anisotropic thermal parameters. Numerical absorption corrections were applied in each case. The final electron density maps showed no significant residual electron density, and there were no abnormal discrepancies between observed and calculated structure factors.[18]

Crystal Data.

3,5',7',7',-Tetrabromo-1',4-dibutyl-4'-(dibromomethylene) spiro[furan-2(5H),6'-[3]oxabicyclo-[3.2.0]heptane]-2',5-dione (6) - $C_{18}H_{18}Br_6O_4$, M 778, triclinic, space group $P\bar{1}$, a 7.510(1), b 8.134(1), c 19.608(1) Å, α 99.12(1), β 98.45(1), γ 97.38(1)° , U 1155.59 $Å^3$, D_c 2.24 g cm^{-3}, Z 2, μ (Mo Kα) 103.47 cm^{-1}. The crystal was of approximate dimensions 0.58 x 0.20 x 0.18mm; number of independent reflections measured 2679, number with $I > 3\sigma(I)$ 2091; an unique data set was collected using 2θ–ω scans to 2θ 83°; F(000) 736; g 0.0011; R 0.046; ratio of transmission factors 1.984.

(±)-trans-4,10,11,11,12,12-Hexabromo-3,9-dibutyl-1,7-dioxadispiro [4.0.4.2] dodeca-3,9-diene-2,8-dione (7) - $C_{18}H_{18}Br_6O_4$, M 778, monoclinic, space group C2/c, a 15.983(1), b 12.065(2), c 13.774(1) Å, β 115.070(1)°, U 2410.74 Å^3, D_m 2.13 g cm^{-1} (measured by flotation in ZnI_2 solution), D_c 2.14 g cm^{-1}, Z 4, μ (Cu Kα) 116.98 cm^{-1}. The crystal was of approximate dimensions 0.38 x 0.10 x 0.13mm. Number of independent reflections measured 1162, number with $I > 3\sigma(I)$ 969; a unique data set was measured to 2θ 106° with 2θ-ω scans; F(000) 1472, g 0.0038, R 0.0792; ratio of transmission factors 1.970.

(Z,Z)-5,5'-(1,2-Dibromo-1,2-ethanediylidene)bis[4-bromo-3-butyl-2(5H)-furanone] (9).- $C_{18}H_{18}Br_4O_4$, M 618, triclinic, space group $P\bar{1}$, a 7.728(1), b 10.030(1), c 14.048(2) Å, α 82.52(1), β 81.45(1), γ 86.75(1)°, U 1066.82 Å^3, D_C1.93 g cm^{-3}, Z 2, μ (Mo Kα) 74.92 cm^{-1}. The crystal was of approximate dimensions 0.16 x 0.52 x 0.09mm. Number of independent reflections measured 2795, number with $I > 3\sigma(I)$ 2276; an unique data set was collected using ω scans to 2θ 90°; F(000) 596; g 0.0013; R 0.059; ratio of transmission factors 1.827.

cis-4,11,12,13-Tetrabromo-3,10-dibutyl-1,6,8-trioxadispiro[4.1.4.2]trideca-3,10,12-triene-2,9-dione (10) - $C_{18}H_{18}O_5Br_4$, M 634, monoclinic, space group $P2_1/c$, a 16.682(2), b 16.297(1), c 8.133(1) Å, β 90.60(1)°, U 2210.97 Å^3, D_c 1.91 g cm^{-3}, Z 4, μ (Cu Kα) 85.71cm^{-1}. The crystal was of approximate dimensions 0.45 x 0.75 x 0.5mm. Number of independent reflections measured 2041, number with $I > 3\sigma(I)$ 1224; a unique data set was measured to 2θ 100° with 2θ-ω scans; F (000) 1224, g 0.0038, R 0.052; ratio of transmission factors 2.808.

(±)-cis-4,10,11,11,12,12-Hexabromo-3,9-dibutyl-1,7-dioxadispiro[4.0.4.2]dodeca-3,9-diene-2,8-dione (11) - $C_{18}H_{18}O_4Br_6$, M 778, triclinic, space group $P\bar{1}$, a 11.16(1), b 12.65(1), c 9.11(1) Å, α 109.6(1), β 87.0(1), γ 100.5(1)°, U 1191.5 Å^3, D_c 2.17g cm^{-3}, Z 2, μ (Cu Kα) 125.78cm^{-1}. The crystal was of approximate dimensions 0.25 x 0.23 x 0.05mm. Number of independent reflections measured 1307, number with $I > 3\sigma$ (I) 972; an unique data set was collected using 2θ–ω scans to 2θ 80°; F(000) 736, g 0.004; R 0.1186.

Verification of the biological origin of the compounds in D. elegans. *D. elegans* was collected on 19th March 1982 at St Kilda Rocks, Kaikoura, from a depth of fifteen metres, and placed in a black polythene bag before being brought to the surface. It was stored in seawater at 0°C until the following day when it was homogenised with dichloromethane. The green dichloromethane layer was separated from the red-grey aqueous emulsion by centrifugation, then filtered (Millipore 0.7μ) and analysed by HPLC (cyanopropyl column 4.5 x 250mm; hexane/propan-2-ol 5%; 220nm; flow program 0-2.5 mins at 0.7 ml/min, 2.5 - 5.0 mins linear increase from 0.7 to 2.5 ml/min, 5-10 mins at 2.5 ml/min). The entire process was performed in darkness to prevent the possibility of photodimerisation. No significant qualitative differences between this and other extracts were observed.

Comparison of Collections. Samples of *D. elegans* were collected on 3rd December 1980, 25th February 1981, 2nd April 1981, 14th January 1982, 19th March 1982 and April 1983, from St Kilda Rocks, Kaikoura, and on the 27th November 1981 from South Bay, Kaikoura.

In each case the samples were frozen for storage, and subsequently air dried, milled, and extracted with dichloromethane in a Soxhlet apparatus. The crude extracts were filtered through short columns of "Florisil" (20 x 30mm), in ten column volumes of ether. The oils obtained were dissolved in hexane/propan-2-ol (4%), filtered (Millipore 0.7μ) and analysed by HPLC (cyanopropyl column 4.5 x 250mm; hexane/propan-2-ol 5%; 220nm). No qualitative differences were observed between the chromatograms.

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