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PHLOROTANNINS WITH DIBENZODIOXIN STRUCTURAL ELEMENTS FROM THE BROWN ALGA *EISENIA ARBOREA**

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Key Word Index—*Eisenia arborea*; Alariaceae; Phaeophyta; brown alga; phlorotannins; phenoxylated dibenzodioxins; benzofurodibenzodioxins, structure elucidation.

Abstract—Twenty-one polyhydroxyphenols, which can all be derived from phloroglucinol, were isolated from Canadian *Eisenia arborea* (Alariaceae). Most of these compounds contain dibenzo[1,4]dioxin elements and also others benzofuran moieties. The basic component is eckol, a hexahydroxyphenoxydibenzo[1,4]dioxin consisting of three phloroglucinol units. Dioxinodehydroeckol is a benzo[1′,4′]benzodioxino[1,4]benzodioxin derived from eckol. 7,7′-Bieckol, 7,9′-bieckol and 7,2″-bieckol are dimers of eckol with biaryl linkages. 8,4‴-Dieckol is a dimeric diphenyl ether. 7-Hydroxyeckol contains one and 7,7′-dihydroxy-9,9′-bieckol two additional hydroxyl groups. 3-Phloroeckol and the dehydro derivatives furodehydroeckol A, B and C are composed of four phloroglucinol rings. Halogenated compounds also occur: monobromo- and monoiodophloroglucinol, 4′-bromo- and 4′-iodoeckol as well as one bromo- and one iodophloroeckol.

INTRODUCTION

During the course of the last few years, a large number of different polyhydroxyphenols (phlorotannins) have been isolated from brown algae. These substances represent dehydropolymerizates of phloroglucinol sometimes with additional halogen or hydroxyl groups. They are divided into four classes according to their particular structural features: fucols, phlorethols, fucophlorethols and fuhalols [1].

In this paper we describe the isolation and structural determination of phenolic substances, which were extracted from the Pacific brown alga *Eisenia arborea* (Alariaceae) and are characterized by the presence of dibenzodioxin and dibenzofuran elements. Fukuyama *et al.* [2] reported recently on the isolation of similar, or the same, substances from *Ecklonia kurome*. The Otsuka Pharmaceutical Co. has patented the use of several substances from *Ecklonia* as antiplasmin inhibitors [3–5].

RESULTS AND DISCUSSION

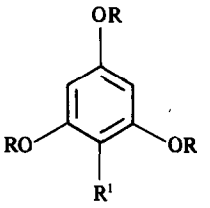
Phenols (0.1% fr. wt) soluble in ethyl acetate were obtained from an ethanolic extract of *E. arborea*. The phenolic mixture was acetylated and TLC of the acetylated total fraction displayed 17 spots which quenched UV light caused fluorescence and turned orange (**1–3**) lilac (**4**), violet (**8–10**) or red on spraying with vanillin–sulphuric acid (numbering according to descending R_f). The total mixture was pre-separated using preparative TLC and separated into 17 fractions by HPLC. It was possible to identify 21 peracetylated substances and some partially acetylated derivatives. Structures of substances **4**, **7**, **9**, **10**, **14–16** and **18** were determined by mass spectrometry, ^{1}H NMR and ^{13}C NMR spectroscopy. Of the remaining substances it was possible to measure only mass and ^{1}H NMR spectra. In these cases structures were obtained by comparing them either with the substances mentioned above or with authentic material. Phloroglucinol triacetate (**3**) and triphlorethol B hepta-acetate (**12**) have been found previously in several brown algae [6, 7].

Compound **7** is the main substance and a key compound for structure elucidation of the other substances. The empirical formula was determined by mass spectrometry to be $C_{30}H_{24}O_{15}$. A fragmentation series arises from the molecular ion at *m/z* 624 during the course of which six fragments each of 42 amu (ketene) are split off. In the region of the aromatic protons, the ^{1}H NMR spectrum (Table 1) displays an AB-system at δ 6.5 and 6.63 with a coupling constant of 2.7 Hz typical of two *meta* protons. A singlet at δ6.66 represents one proton. The AB_2-system at δ6.58 (2H) and 6.72 (1H) is typical of a 3,5-diacetoxyphenoxyl ring [7, 14]. Shift values for the protons of six acetyl groups were found between δ1.96 and 2.34. Compound **7** is, therefore, 1,3,6,8-tetra-acetoxydibenzo[1,4]dioxin with a 3,5-diacetoxylated phenoxyl ring attached to either C-2 or C-4. The bonding site can be determined with the aid of the ^{13}C NMR spectrum (Table 3) and is at C-4. The measured shift values for **7** correspond well with those calculated using increments determined by Wegner-Hambloch and Glombitza [8].

It is possible to assign the signals of the acetyl protons using two singly deacetylated derivatives of **7**. Accordingly, the signals shifted furthest upfield at δ 1.96 can be assigned to the acetyl group at C-6. At this carbon atom the methyl protons are shielded most strongly by the diamagnetic influence of the phenoxyl group. The acetyl

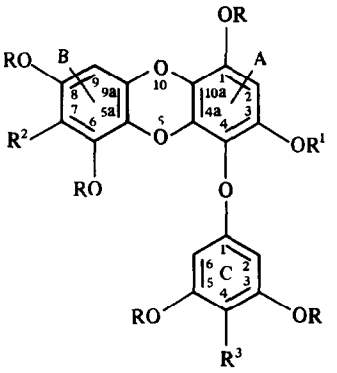
*Part 31 in the series "Antibiotics from Algae". For Part 30 see Grosse-Damhues, J. and Glombitza, K.-W. (1984) *Phytochemistry* in press.

†Part of Ph D thesis, Bonn D-5 (1984).



1 $R^1 = Br$
2 $R^1 = I$
3 $R^1 = H$

R = Ac



	R^1	R^2	R^3
5	Ac	H	Br
6	Ac	H	I
7	Ac	H	H
11	Ac	OAc	H
14	[(3—(R^1=—)]	H	H

R = Ac

group at C-1, which is *ortho* to the dioxin ether bridge and not influenced by a phenoxyl ring, is the least shielded and occurs at δ2.34. Deacetyl-**7** was isolated by Fukuyama *et al.* [2] from *Ecklonia kurome* and called eckol.

The mass spectrum of **14** shows a molecular ion at *m/z* 832 representing a MW 208 amu more than **7**. This implies an additional acetoxylated phenoxyl ring. The ^{1}H NMR spectrum (Table 1) reveals, apart from the signals for **7**, a singlet at δ6.90 for two protons and a signal for two equivalent acetyl groups at δ2.04 and a third at δ2.27. These signals are typical of a 2,4,6-triacetoxyphenoxyl ring [7, 14]. The upfield shift of the proton at C-2 from δ6.66 in **7** to δ6.29 in **14** shows the additional ring to be linked to either C-1 or C-3 via the oxygen atom. The other shift values are practically the same as those of **7**. The ^{13}C NMR spectrum (Table 3) proves the structural proposal for **14** presented in this paper with the additional phenoxyl at C-3. This proposal is given further support by the occurrence of a mass spectral fragment at *m/z* 370, a benzodioxinodibenzodioxin which arises from the successive cleavage of phenyl ether and water. Investigations so far have shown that the appearance of such a fragment in the mass spectrum is possible only if a diphenyl ether is substituted with OR′ and OR″ at *ortho* and *ortho′* to the ether linkage. According to Fukuyama *et al.* [2], this substance would be called 2-phloroeckol octa-acetate. However, the numbering which the Japanese have adopted in this case does not conform to IUPAC rules. 3-Phloroeckol octa-acetate* is the more correct name.

Substances **15–19** are each composed of two units of substance **7**. The difference between the compounds are the binding site and the type of linkage, either biaryl or diaryl ether, with which the two units are joined. The MW of substance **15** is 1204, which corresponds to the empirical formula $C_{58}H_{44}O_{29}$. With 11 acetoxy groups it has one less than a 'dimer' of **7**. Linkage of the two components is apparently via an oxygen atom. This means that only 11 hydroxyls remain for acetylation. The ^{1}H NMR spectrum (Table 1) displays two AB-systems, one of which corresponds with that of **7** in its shift values. The other, which is shifted upfield, belongs to the protons on ring B′ joined to the phenoxyl ring carbon via a phenyl ether linkage. The diacetoxylated phenoxyl residue is the cause of the strong shielding of the protons.

Two upfield-shifted signals at δ1.93 and 2.03 for acetoxy groups *ortho* to the dioxin indicate that the linkage of the two moieties occurs via the oxygen at C-8′ or ring B′. The proposed structure is confirmed by the ^{13}C NMR spectrum (Table 3) and the substance is, therefore, referred to as 8,4‴-dieckol undeca-acetate.

The MW of 1246 and the presence of 12 acetoxyl groups show that substances **16–19** are also composed of two sub-units of **7**. In this case, however, they are joined together by a biaryl linkage. In the case of substances **16** and **18**, the linkage between the two units results in a symmetric molecule. Only a few signals can be found in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR spectra (Table 3) show the biaryl bonds to be at C-7 of rings B and B′ (7,7′-bieckol dodeca-acetate) with **16** and at C-9 of rings B and B′ (9,9′-bieckol dodeca-acetate) with **18**. Fukuyama *et al.* [2] described both non-acetylated derivatives from *Ecklonia kurome* with the incorrect names 8,8′- and 6,6′-bieckol.

The ^{1}H NMR spectrum (Table 1) of **17** shows four singlets for four aromatic protons and also two overlap-

*The names of the isolated substances in accordance with IUPAC rules can be found in the Experimental. Numbering of the formulae under Results and Discussion and also in the NMR tables does not always comply with the IUPAC rules. For the sake of clarity in this part numeration is based on that used for 7

Table 1. ^{1}H NMR data of **5–7, 11, 14–19** and **20** in deuterochloroform

	Halogenated eckols				Eckol, eckol dimers and phloroeckol					Hydroxylated eckol derivatives	
Proton	**5**	**6**	**7**	**14**	**15**	**16**	**17**	**18**	**19**	**11**	**20**
Ring A/B											
OAc-1	2.33	2.33	2.34	2.29	2.33	2.33	2.37	2.04*	2.29	2.34	2.06
C-2	6.69	6.69	6.66	6.29	6.68	6.65	6.63‡	6.69	6.59	6.68	6.64
OAc-3	2.13	2.13	2.14	—	2.12*	2.10	2.12*	2.11	1.97*	2.13	2.10
OAc-6	1.98	1.98	1.96	1.94	1.93†	1.73	1.80	1.97	1.94*	1.96	1.99
C-7	6.55	6.55	6.50	6.50	6.50	—	—	6.62	6.50	2.22* (Ac)	2.17 (Ac)
OAc-8	2.24	2.24	2.24	2.24	2.25	2.0	2.04†	2.02*	2.25	2.23*	2.01
C-9	6.67	6.67	6.63	6.63	6.64	6.70	6.84	—	6.62	6.76	—
Ring C											
C-2, C-6	6.72	6.70	6.58	6.59	6.69	6.55	6.58	6.64	—, 6.79	6.58	6.61
OAc-3, OAc-5	2.34	2.34	2.26	2.24	2.10	2.23	2.24	2.27	2.05, 2.26	2.26	2.26
C-4	—	—	6.72	6.72	—	6.70	6.73	6.73	6.96	6.74	6.72
Ring D											
OAc-2, OAc-6	—	—	—	2.04	—	—	—	—	—	—	—
C-3, C-5	—	—	—	6.90	—	—	—	—	—	—	—
OAc-4	—	—	—	2.27	—	—	—	—	—	—	—
Ring A′/B′											
OAc-1	—	—	—	—	2.28	§	2.04†	§	2.29	—	§
C-2	—	—	—	—	6.62	§	6.71	§	6.62	—	§
OAc-3	—	—	—	—	2.19*	§	2.13*	§	2.14	—	§
OAc-6	—	—	—	—	2.03†	§	1.98	§	1.89	—	§
C-7	—	—	—	—	6.31	§	6.60‡	§	—	—	§
OAc-8	—	—	—	—	—	§	2.01†	§	2.01†	—	§
C-9	—	—	—	—	6.44	§	—	§	6.52	—	§
Ring C′											
C-2, C-6	—	—	—	—	6.58	§	6.58	§	6.60	—	§
OAc-3	—	—	—	—	6.25	§	2.27	§	2.27	—	§
C-4	—	—	—	—	6.71	§	6.73	§	6.76	—	§

*,†,‡Assignments interchangeable.
§Like rings A, B and C.
AB-systems: J_{AB} = 2.7 Hz, AB_2-systems: J_{AB} = 2.0–2.1 Hz.

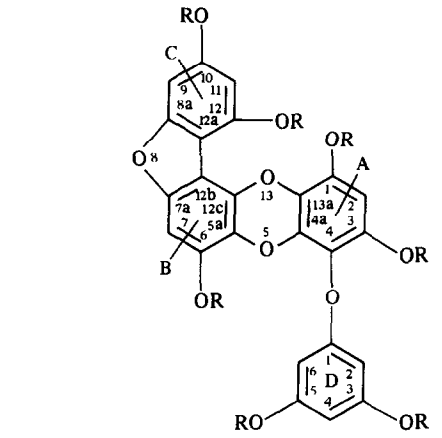
Table 2. ^{1}H NMR data of **8–10** (in deuterochloroform)

Proton	**8**		**9**		**10**
Ring A/B					
OAc-1	2.48		2.36		2.36*
C-2	6.73		6.69		6.64
OAc-3	2.14		2.13		2.10
OAc-6	1.97		2.05		—
C-7	6.98		—		—
OAc-11	—		—		2.38*
C-12	—		7.03		6.71
Ring C					
C-9	7.27	C-10	7.25	C-7	7.33
OAc-10	2.34	C-9	2.31	C-8	2.31
C-11	6.88	C-8	6.81	C-9	6.86
OAc-12	2.51	C-7	2.39	C-10	2.38*
Ring D					
C-2, C-6	6.61		6.61		6.73
OAc-3, OAc-5	2.26		2.26		2.27
C-4	6.74		6.73		6.73

*Assignments interchangeable.
AB-systems: J_{AB} = 2.0 Hz, AB_2-systems: J_{AB} = 2.0–2.1 Hz.

ping AB_2-systems. In this case also, linkage of the two subunits is via the carbon atoms of rings B and B′. The number of signals in the ^{1}H NMR spectrum and especially the 10 signals for 12 acetyl groups show that this dimeric compound is not built symmetrically. Comparison of the shift values with those of **16** and **18** suggest that a combination of both partial structures of **16** and **18** is present, i.e. the biaryl linkage is via C-7 of ring B and via C-9′ of ring B′ (7,9′-bieckol dodeca-acetate).

A further type of linkage is present in substance **19**. One **7** is attached to C-2 of the phenoxyl ring of a second one via C-7 by means of a biaryl bridge. This would explain the additional downfield shifted system at δ6.96 and 6.79 in the ^{1}H NMR spectrum. Apart from the AB-system typical of a terminal dibenzodioxin system at δ6.62 and 6.50, the ^{1}H NMR spectrum reveals three signals, each for one proton, and an AB_2-system for a terminal diacetoxyphenoxyl ring at δ6.60 (2H) and 6.76 (1H). Measured in deuterochloroform the signals tend to overlap. Recording the spectrum in acetone-d_6 separates the signals more distinctly and reveals a remarkable change in the downfield shifted AB-system: the signals coincide with one singlet at δ7.03. Compound **19** is, therefore, 7,2‴-bieckol dodeca-acetate.



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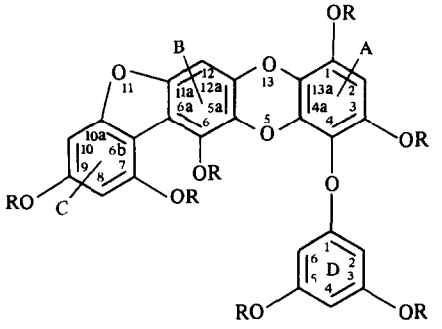

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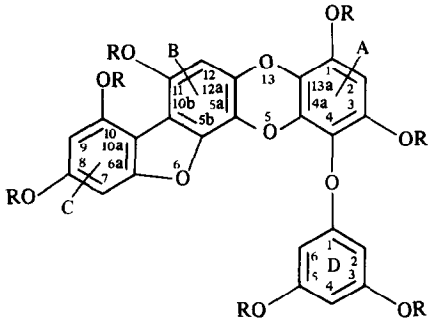


10

Substances **11** and **20** represent a new type of compound: they contain one or two acetoxy groups more than **7** or **18**, from which they are derived. Their relation to **7** and **18** is much the same as that of the fuhalols to the phlorethols. The position of the acetoxy group is indicated by the absence of an aromatic proton signal or of AB-coupling (Table 1). Compound **11** is, therefore, 7-hydroxyeckol hepta-acetate and **20** is 7,7'-dihydroxy-9,9'-bieckol tetradeca-acetate.

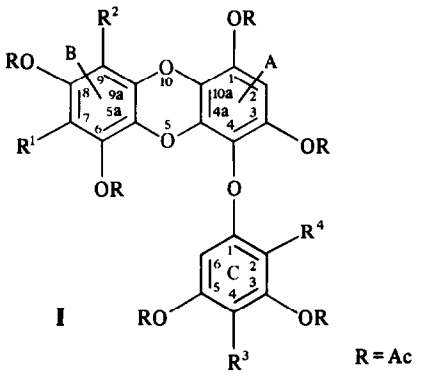
Substances **8–10** are isomeric compounds with a MW of 772. In the electron impact mass spectrum, cleavage of seven ketene units is apparent. These are compounds whose four benzene rings are linked together via a furanoid, a 1,4-dioxin structure and also a phenyl ether bridge. In the ^{1}H NMR spectrum (Table 2), the substances each reveal an AB-system for two *meta* protons which is shifted strongly downfield when compared with the values for the dibenzodioxins. The shift values are consistent with those reported by Gerstberger [9] for 1,3-diacetoxylated dibenzofurans. This shows that the benzofuran is at a terminal position and substituted with two acetoxy groups. The three compounds also have a terminal 3,5-diacetoxyphenoxyl ring in common. The characteristic AB_2-system, however, is present only in **8** and **9**. In **10**, the shift values coincide with an A_3 singlet. The ^{1}H NMR spectrum in acetone-d_6 reveals a separation of the signals to an A_2B-system. The same behaviour was found by Glombitza *et al.* [10] with the phenoxyl residue of some fucophlorethols which, for steric reasons, was strongly influenced by adjacent rings. A similar closely packed arrangement of the benzene rings can, therefore, also be assumed for **10**. Following the data obtained from the mass and ^{1}H NMR spectra, the only difference between the isomeric compounds is found in the positions of the furan and dioxin structures relative to each other. It was possible to isolate enough **9** and **10** to take ^{13}C NMR spectra (Table 4). These confirm the assumed structure of rings C and D. The value for the tertiary carbon atom of ring B of **9** is shifted far upfield to 97.9. The strong shielding can be explained only if the carbon atom is positioned between the ether bridges of both dioxin and furan. The ^{13}C NMR data indicate that in **10** the three respective ether bridges are arranged *ortho*. This arrangement also complies with the requirements arising from the ^{1}H NMR spectrum. Substitution of ring A is consistent in every case with that of **7**. If the shifts of the acetoxy groups of the relevant structure proposals are compared, there is an excellent correlation between the values obtained for the earlier described dibenzofurans [9] and those of the substances described here. The signals at *ca* δ2.39 tend to occur if the dibenzofuran system is substituted with two acetoxy groups *ortho,ortho'* to the carbon–carbon linkage. This is not true, however with **8**. The acetoxy groups at C-12 of ring C and C-1 of ring A in this case are much more influenced sterically than with **9** or **10**. This is the only explanation which can be given for the high values for the acetoxy groups at δ2.51 and 2.48.

If one assumes that these substances must have been created from phloroglucinol units, the initial compound has to be an eckol substituted by phloroglucinol via a biaryl bond at C-7 in the case of **9** and **10** and at C-9 in the case of **8**. The elimination of one molecule of water from the hydroxyl groups at C-2 of the phloroglucinol and C-8 (**8, 9**) or C-6 (**10**) of the eckol unit must then have led to the formation of the furan ring. Elimination of water leading to dibenzofurans has so far never been found *in vivo* with phlorotannins, but has been observed with electron impact mass spectrometry [13] and is a common method in organic synthesis [15]. Homologous dibenzofurans, however, can be created by a different means: starting with **7** substituted at C-6 or C-8 by phloroglucinol via an ether bridge (6-, or 8-phloroeckol, respectively), then the elimination of water between the hydroxyl on the phloroglucinol ring and the hydrogen at C-7 or C-9 of the eckol would also lead to dibenzofurans. Analogous water cleavages which take place quite readily have been observed *in vitro* in the presence of diluted aqueous alkali [16]. The aromatic hydrogens of the dibenzofuran moiety

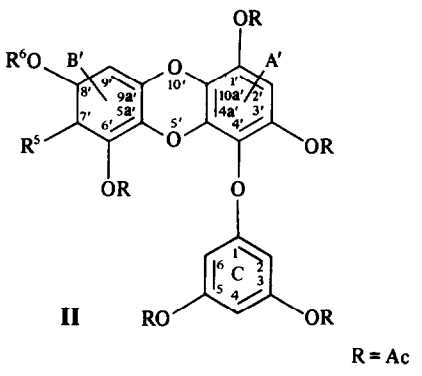
Table 3. ^{13}C NMR data of **7**, **14–16** and **18** (in deuterochloroform)

C-atom	7 Ring A/B	7 Coupling	14 Ring A/B	14 Coupling	15 Ring A/B	15 Coupling	16 Ring A/B	16 Coupling	18 Ring A/B	18 Coupling
C-1	134.8	5.5 *d*	135.0	6.0 *d*	134.9	5.6 *d*	134.8	5.1 *d*	135.3	5.6 *d*
C-2	112.7	168.0 *D*	105.7	167.0 *D*	112.5	169.0 *D*	112.8	168.0 *D*	112.8	168.0 *D*
C-3	138.7	5.0 *d*	145.9	5.4 *d*	138.9	4.8 *d*	139.3	5.9 *d*	139.3	5.5 *d*
C-4	133.1	9.0 *d*	130.4	*	133.1	8.8 *d*	132.9	8.8 *d*	132.9	9.2 *d*
C-4a	136.4	1.5 *d*	136.6	1.5 *d*	136.6	1.0 *d*	136.4	1.4 *d*	136.2	1.5 *d*
C-5a	131.2	8.5 *dd*	131.2	7.6 *dd*	131.3	7.6 *dd*	131.5	7.3 *d*	131.3	8.0 *d*
C-6	139.2	5.5 *d*	138.9	5.4, 1.5 *dd*	139.1	5.2 *d*	137.6	—	138.6	5.5 *d*
C-7	112.2	168, 6.0 *Dd*	112.0	168, 5.6 *Dd*	112.1	168, 5.6 *Dd*	114.6	6.8 *d*	112.8	168.0 *D*
C-8	145.9	5.0 *Dd*	146.0	5.3 *dd*	146.0	4.8 *dd*	144.3	4.4 *d*	144.4	5.2 *d*
C-9	107.9	168, 6.0 *Dd*	108.3	168, 6.0 *Dd*	108.0	168, 5.6 *Dd*	108.7	168.0 *D*	110.3	8.0 *d*
C-9a	141.7	5.0 *d*	141.9	5.2 *d*	141.7	4.8, 1.2 *dd*	141.3	5.2 *d*	140.2	1.5 *d*
C-10a	131.8	8.5 *d*	128.8	9.0 *d*	131.9	8.0 *d*	131.9	8.8 *d*	131.6	8.5 *d*
	Ring C		**Ring C**		**Ring C**		**Ring C**		**Ring C**	
C-1	157.9	4.5 *dd*	158.3	4.0 *dd*	153.2	4.8 *dd*	157.9	4.4 *dd*	158.0	4.8 *dd*
C-2, C-6	106.3	166, 4.5 *Ddd*	106.3	165, 5.0 *Ddd*	108.6	169, 5.6 *Dd*	106.4	165, 4.4 *Ddd*	106.5	168, 4.8 *Ddd*
C-3, C-5	151.6	6.0 *dd*	151.6	4.4 *dd*	144.5	3.5 *dd*	151.6	4.4 *dd*	151.7	5.6 *dd*
C-4	110.0	168, 4.8 *Ddd*	110.1	168, 5.0 *Ddd*	134.2	7.2 *dd*	110.2	168, 4.4 *Ddd*	110.2	169, 4.8 *Ddd*
					Ring A′/B′		**Ring A′/B′/C′**		**Ring A′/B′/C′**	
C-1	—	—	—	—	134.8	5.6 *d*	†	—	†	—
C-2	—	—	—	—	112.1	169.0 *D*	†	—	†	—
C-3	—	—	—	—	138.6	5.2 *d*	†	—	†	—
C-4	—	—	—	—	133.0	8.8 *d*	†	—	†	—
C-4a	—	—	—	—	136.4	1.4 *d*	†	—	†	—
C-5a	—	—	—	—	128.7	8.0 *dd*	†	—	†	—
C-6	—	—	—	—	139.2	5.6 *d*	†	—	†	—
C-7	—	—	—	—	106.3	*	†	—	†	—
C-8	—	—	—	—	153.9	4.8 *dd*	†	—	†	—
C-9	—	—	—	—	102.1	168, 5.5 *Dd*	†	—	†	—
C-9a	—	—	—	—	141.9	4.8 *d*	†	—	†	—
C-10a	—	—	—	—	131.8	8.0 *d*	†	—	†	—
			Ring D		**Ring C′**					
C-1	—	—	135.8	7.5 *dd*	158.0	4.0 *dd*	†	—	†	—
C-2, C-6	—	—	143.5	3.0 *dd*	106.4	168, 4.4 *Ddd*	†	—	†	—
C-3, C-5	—	—	114.9	168, 6.0 *Dd*	151.6	4.8 *dd*	†	—	†	—
C-4	—	—	146.7	5.3 *dd*	110.2	168, 4.5 *Ddd*	†	—	†	—

*Due to signal overlapping not determinable.
†Like rings A, B and C.



I R = Ac



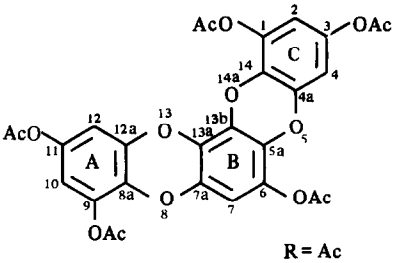
II R = Ac

	R^1	R^2	R^3	R^4	R^5	R^6
15	H	H	[II-(OC-8')]	H	H	
16	[II-(C-7')]	H	H	H		Ac
17	[II-(C-9')]	H	H	H	H	Ac
18	H	[II-(C-9')]	H	H	H	Ac
19	H	H	H	[II-(C-7')]		Ac
20	OAc	[II-(C-9')]	H	H	OAc	Ac

Table 4. ^{13}C NMR data of substances **9** and **10**

C-atom	**9** Ring A/B	**10** Ring A/B	Coupling (Hz)
C-1	134.9	134.7	5.6 *d*
C-2	112.7	113.0	170.0 *D*
C-3	139.2	138.9	5.6 *d*
C-4	133.0	133.1	8.4 *d*
C-4a	136.6	136.4	1.6 *d*
C-5a	132.9	125.3	6.4 *d*
C-6	130.4	C-5b 144.5	—
C-6a	113.0	C-10b 113.9	*
C-11a	152.6	C-11 139.7	5.6 *d*
C-12	97.9	106.7	167.0 *D*
C-12a	141.5	140.1	5.2 *d*
C-13a	131.6	132.0	8.0 *d*
	Ring C	Ring C	
C-6b	114.3	C-10a 114.2	*
C-7	144.4	C-10 144.5	5.0, 1.2 *dd*
C-8	111.7	C-9 112.1	167.0, 4.8 *Dd*
C-9	149.9	C-8 150.1	5.2 *dd*
C-10	103.6	C-7 104.1	169.0, 4.8 *Dd*
C-10a	157.4	C-6a 157.7	4.4 *d*
	Ring D	Ring D	
C-1	158.2	158.4	4.8, 1.6 ddd
C-2, C-6	107.3	107.5	168.0, 5.2 *Ddd*
C-3, C-5	151.7	151.8	4.8 *dd*
C-4	110.3	110.6	169.0, 4.8 *Ddd*

*Coupling cannot be determined due to signal overlapping.



R = Ac

4

would not be at C-2 and C-4, but at C-1 and C-3 instead. The chemical shifts of the protons would hardly differ in both these cases. The coupling constant with C-2 and C-4 protons is J_{AB} = 1.8–2.0 Hz and *ca* 2.2 with C-1, C-3 protons [9]. As J_{AB} = 2.0 Hz was found for **8–10**, this indicates that the assumed structure proposals are correct. Moreover, the sum of the deviations between calculated and measured ^{13}C NMR shifts is smaller with the proposed structures for **8–10** than they would be for the respective isomers.

Compound **4** differs from all the other compounds by its very poor solubility. It is more or less insoluble in polar solvents such as ethanol as well as apolar ones (cyclohexane and petrol). Its MW is 580. Elimination of five ketene fragments can be observed with electron impact mass spectrometry. This corresponds to a pentaacetoxylated benzodioxino-dibenzodioxin. In the aromatic region, the ^{1}H NMR spectrum (Table 5) displays a

Table 5. ^{1}H NMR data of **4**, deacetylpermethyl-**4** (**4a**), deacetyl-**4** (**4b**) and deacetylperfluorbenzoyl-**4** (**4c**)

Proton	**4** ($CDCl_3$)	**4a** ($CDCl_3$)	**4b** (CD_3OD)	**4c** ($CDCl_3$)
OAc-1	2.31*	MeO 3.81*		
C-2	6.60‡	6.19‡	5.96§	6.89§
OAc-3	2.27†	MeO 3.73†		
C-4	6.66‡	6.28‡	6.02§	6.73§
OAc-6	2.38	MeO 3.89*		
C-7	6.33	6.30	6.18	6.43
OAc-9	2.32*	MeO 3.86*		
C-10	6.59	6.22	5.97‡	6.80‡
OAc-11	2.28†	MeO 3.75†		
C-12	6.59	6.22	6.01‡	6.31‡

*,†Assignments interchangeable.
‡,§AB-spin-system.

singlet for two, a singlet for one and a very poorly resolved AB-system for two further aromatic protons. Using a 400 MHz apparatus, resolution of the signals is much better and an unambiguous identification and determination of the coupling constants of the AB-system (2.7 Hz) can be achieved. Four of the five acetoxy groups are found in pairs at δ2.32 and 2.31 as well as at δ2.27 and 2.26. Only the signal at δ2.38 is isolated. Accordingly, **4** is a C-1, C-3, C-6, C-9, C-11 acetoxylated benzodioxinodibenzodioxin. A slight asymmetrical arrangement is caused by the angular linkage of the benzene rings which is reflected in the ^{1}H NMR spectrum. However, one would expect a separation of the signals into an AB-system, not only for the protons at ring C, but also for those at ring A. Probably the acetyl group at C-1 causes a stronger shielding of the proton at C-12 so that its shift value coincides with that of the proton at C-10. The prerequisite for a proton coupling, therefore, does not exist. This presumption is confirmed by the ^{1}H NMR spectrum of deacetyl-**4** (Table 5). The influence of the acetyl group on a proton at C-12 is missing in this case and the signals of both proton pairs are split into AB-systems. The same coupling pattern is shown also by the *p*-fluorobenzoyl derivative of **4**. The proton at C-12 is strongly influenced by the benzoyl residue at C-1. However, the methyl derivative of **4**, on the other hand, displays only a singlet for the two protons on ring A (Table 5). It was not possible to take a satisfactory ^{13}C NMR spectrum of **4** due to its poor solubility, but a number of 'signal pairs' were detected for two at a time very similar carbon atoms (see Experimental) thus confirming the proposed structure of **4** which should be referred to as dioxinodehydroeckol penta-acetate.

It was not possible to separate the fraction with the highest R_f value any further using HPLC. The mass and ^{1}H NMR spectra showed it to be a mixture of a monobrominated (**1**) and a monoiodated (**2**) triacetoxybenzene. The nonacetylated fraction was silylated and separated by GC. GC/MS confirmed the presence of monohalogenated compounds. Di- or trihalogenated derivatives could not be detected, in contrast to reports by Blackman and Matthews [11] for a red alga. It was possible to assign the signals of the ^{1}H NMR spectrum to the two substances by assessing the integrals: each of the substances shows a singlet for two protons in the aromatic region at δ6.96 (**1**) and 6.94 (**2**), and in the acetyl region a singlet for one acetyl group at δ2.26 (C-5), and for two acetyl groups (C-1, C-3) at 2.34 (**1**) and 2.36 (**2**). Halogenation causes a shift of the *ortho* acetoxy groups by *ca* $\Delta\delta$ 0.1 downfield. The aromatic *meta* protons are shifted downfield by *ca* $\Delta\delta$0.08 and 0.1. Observations by Koch and Gregson [12] on halogenated phlorethols also confirm this. An unambiguous assignment of the signals was possible by comparison with synthetic 2-bromophloroglucinol triacetate. Compound **1** was found to be 2-bromophloroglucinol triacetate and **2** was 2-iodophloroglucinol triacetate.

Separation of **5** and **6**, which was not possible by means of TLC, was also not successful using HPLC. Electron impact mass spectrometry showed the fraction to be a mixture of a brominated and an iodinated **7** with molecular ions at *m/z* 704/702 and 750. Interpretation of the ^{1}H NMR spectrum was, therefore, again possible only by comparing the integration of the signals (Table 1). The AB-system for C-7, C-9 is hardly changed compared to **7**, which is also true for the singlet for the aromatic proton at C-2. An AB_2-system is missing. Instead, a singlet for two protons appear at δ6.70 which, referring back to observations with **1** and **2**, is now assigned to **6** and the singlet at δ6.72 to **5**. Shift values of the acetoxy groups correspond with those of **7**, except for the signal of the two magnetically equivalent acetoxy groups of the phenoxyl ring. This is shifted downfield by *ca* $\Delta\delta$0.08. This means that the 3,5-diacetoxyphenoxyl residue is brominated at C-4 (**5**, 4′-bromoeckol hexa-acetate) and, in the case of **6**, iodinated (4′-iodoeckol hexa-acetate).

Two additional halogenated substances could only be found in the fraction by electron impact mass spectrometry and consisted mainly of **12**. The molecular ions at *m/z* 912/920 (**13a**) and 958 (**13b**) correspond to a brominated and an iodinated derivative of **14** or an isomeric compound of **14**. The occurrence of a fragment at *m/z* 370 indicates that the basic structure is the same as **14**. As there are no ^{1}H NMR data available, it is not possible to determine the position of halogenation. So, **13a** can be described as a bromophloroeckol octa-acetate and **13b** as an analogous iodo compound.

Higher MW polyhydroxyphenols than those described here were not found, in contrast to results from other brown algae. Only deacetylated derivatives of compounds mentioned above were found in the lower R_f region by TLC.

EXPERIMENTAL

Extraction and isolation. Freeze-dried material (500 g) or deep-frozen thallus (1 kg) of *E. arborea* Areschoug (Bamfield, Canada, September 1982) were extracted in accordance with the method described in [13] and, subsequently, processed. A total of 25 kg fresh alga material was used. Dark brownish oxidized substances were removed from the acetylated phlorotannin extract by silica gel CC (3 × 10 cm) with $CHCl_3$–Me_2CO (9:1). The enriched fraction was pre-fractionated on prep. silica gel layers [14] with $CHCl_3$–Me_2CO (9:1). Isolation of the substances was carried out by HPLC on various silica gel columns: LiChrosorb SI 60, 10 μm, 7.5 mm i.d. 25 cm, or Partisil 10 μm, 5 mm i.d. 50 cm isocratic with $CHCl_3$–EtOH as solvent (0.3–2% EtOH). Detection was by UV (275 nm).

GC/MS analysis. Non-acetylated crude phenolic extract (1 g) was chromatographed on a silica gel column (2.5 × 25 cm) with $CHCl_3$–EtOH (9:1) 120 ml (fractions I–II) and $CHCl_3$–EtOH (4:1) 400 ml (fractions II–VI). Fraction I was silylated with

MSTFA [9]. The TMSi derivatives were analysed by GC/MS using a 30 m, 0.25 mm i.d. fused silica SE-30 column, carrier gas H_2, injector temp. 250°, temp. program 160–260° at 10°/min.

Isolated substances. Yield in reference to 10 kg fresh alga; R_f on silica gel $CHCl_3$–Me_2CO (9:1); detection by 1% vanillin in conc. H_2SO_4, 3 min at 120°.

2-Bromophloroglucinol triacetate (**1**) *and 2-iodophloroglucinol triacetate* (**2**). R_f 0.79, together 10 mg; UV λ_{max}^{MeCN} nm: 200, 225 shoulder; MS (180°, 70eV, main ketene elimination series): *m/z* **1** 332/330 → 206/204; **2**: 378 → 252 → 126.

Phloroglucinol triacetate (**3**). R_f 0.70, 15 mg; identical to authentic material.

Dioxinodehydroeckol penta-acetate (**4**) [1,3,6,9,11-*pentaacetoxybenzo*[5',6'][1,4]*dioxino*[2',3':5,6]*dibenzo*[b,e][1,4]*dioxin*]. R_f 0.69, 30 mg; UV λ_{max}^{MeCN} nm: 223, 248 (sh), 288; MS (150°, 70 eV, ketene elimination series) *m/z*: 580 → 370. ^{13}C NMR ($CDCl_3$): 105.5 (C-7), 107.9, 108.0 (C-4, C-12), 112.1, 112.2 (C-2, C-10), 125.5 (C-13a), 128.9, 129.0 (C-8a, C-14a), 137.0, 138.0, 138.5 (C-1, C-7a, C-9), 142.1, 142.2 (C-4a, C-12a), 145.7, 146.0 (C-11, C-3), chemical shifts for C-13b, C-5a and C-6 could not be determined exactly.

4'-Bromoeckol hexa-acetate (**5**) [4-(4-*bromo*-3,5-*diacetoxyphenoxy*)-1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dioxin*] *together with 4'-iodoeckol hexa-acetate* (**6**) [4-(4-*iodo*-3,5-*diacetoxyphenoxy*)-1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dioxin*]. R_f 0.64, together 8 mg; UV λ_{max}^{MeCN} nm: 205, 233, 280 (sh); MS (220°, 70 eV, main ketene elimination series) *m/z*: **5**: 704/702 → 452/450; **6**: 750 → 498 → 372. ^{1}H NMR (Me_2CO-d_6): **5**: δ6.93 (2H), 6.89 (1H), 6.78, 6.67 (AB-system), 2.33 (3H), 2.30 (6H), 2.23 (3H), 2.12 (3H), one signal under Me_2CO-d_6; **6**: δ6.90 (2H), 6.89 (1H), 6.78, 6.67 (AB-system), 2.33 (3H), 2.30 (6H), 2.23 (3H), 2.12 (3H).

Eckol hexa-acetate (**7**) [4-(3,5-*diacetoxyphenoxy*)-1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dioxin*]. R_f 0.63, 210 mg; UV λ_{max}^{MeCN} nm: 233, 289; MS (180°, 70 eV, ketene elimination series) *m/z*: 624 → 372, 264, 232, 126.

Fucofuroeckol A hepta-acetate (**8**) [1,3,7,10,12-*penta-acetoxy*-9-(3,5-*diacetoxyphenoxy*)*benzo*[4',5']*furo*[2',3':5,6]*dibenzo*[b,e][1,4]*dioxin*] R_f 0.59, 17 mg; UV λ_{max}^{MeCN} nm: 224, 260 (sh), 286; MS (230°, 70 eV, ketene elimination series) *m/z*: 772 → 478, 370, 354, 248, 232, 126.

Fucofuroeckol B hepta-acetate (**9**) [1,3,8,10,13-*penta-acetoxy*-11-(3,5-*diacetoxyphenoxy*)*benzo*[4',5']*furo*[2',3':4,5]*dibenzo*[b,e][1,4]*dioxin*]. R_f 0.57, 28 mg; UV λ_{max}^{MeCN} nm: 230, 254, 289 (sh), 298, 320; MS (230°, 70 eV, ketene elimination series) *m/z*: 772 → 478, 370, 354, 248, 232, 126.

Fucofuroeckol C hepta-acetate (**10**) [2,4,5,8,10-*penta-acetoxy*-11-(3,5-*diacetoxyphenoxy*)*benzo*[4',5']*furo*[3',2':5,6]*dibenzo*[b,e][1,4]*dioxin*]. R_f 0.55, 42 mg; UV λ_{max}^{MeCN} nm: 225, 248 (sh), 254, 275 (sh), 285, 325 (sh); MS (230°, 70 eV, ketene elimination series) *m/z*: 772 → 478, 412 → 370, 354, 248, 232, 126.

7-Hydroxyeckol hepta-acetate (**11**) [4-(3,5-*diacetoxyphenoxy*)-1,3,6,7,8-*penta-acetoxydibenzo*[b,e][1,4]*dioxin*]. R_f 0.51, 10 mg; UV λ_{max}^{MeCN} nm: 230, 287 (sh); MS (230°, 70 eV main ketene elimination series) *m/z*: 683 → 388.

Triphlorethol B hepta-acetate (**12**). R_f 0.50, together with **13a** and **13b** 7 mg, ^{1}H NMR values as in Ref. [7].

Bromophloroeckol octa-acetate (**13a**) *together with iodophloroeckol octa-acetate* (**13b**) *together with* **12**. 7 mg; MS (230°, 70 eV, main ketene elimination series) *m/z* **13a**: 912/910 → 702/700; **13b**: 958 → 622 → 496, 696 → 528, 694 → 526, 660 → 492, 412 → 370, 290 → 248, 276 → 234.

3-Phloroeckol octa-acetate (**14**), [4-(3,5-*diacetoxyphenoxy*)-3-(2,4,6-*triacetoxyphenoxy*)-1,6,8-*triacetoxydibenzo*[b,e][1,4]*dioxin*]. R_f 0.48, 42 mg; UV λ_{max}^{MeCN} nm: 210 (sh), 233, 270 (sh), 285 (sh); MS (230°, 70 eV, main ketene elimination series) *m/z*: 832 → 496, 626 → 416, 540 → 372, 454 → 370, 440 → 356; ^{1}H NMR (Me_2CO-d_6): δ7.02 (2H), 6.74 (1H), 6.70 (2H) AB_2-system, 6.77, 6.65 (AB-system), 6.36 (1H), 2.29 (3H), 2.27 (3H), 2.22 (9H), 2.01 (6H), 2.0 (3H).

8,4'''-Dieckol undeca-acetate (**15**) [1,3,6-*triacetoxy*-4-(3,5-*diacetoxyphenoxy*)-8-[2,6-*diacetoxy*-4-(1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dioxin*-4-*yloxy*)*phenoxy*]*dibenzo*[b,e][1,4]*dioxin*]. R_f 0.43, 110 mg; UV λ_{max}^{MeCN} nm: 233, 278, 290 (sh); MS (280°, 70 eV, ketene elimination series) *m/z*: 1204 → 742, 806 → 512, 790 → 496, 648 → 354, 646 → 478, 582 → 372, 564 → 354, 348 → 264, 374 → 248, 316 → 232; ^{1}H NMR (Me_2CO-d_6): δ6.90 (1H), 6.89 (2H), 6.87 (1H), 6.78, 6.68 (AB-system), 6.76 (1H), 6.69 (2H, AB_2-system), 6.54, 6.44 (AB-system), 2.34 (3H), 2.28 (3H), 2.24 (3H), 2.23 (6H), 2.17 (3H), 2.12 (3H), 2.08 (6H), 1.98 (3H), one signal under Me_2CO-d_6.

7,7'-Bieckol dodeca-acetate (**16**) [1,1',3,3',6,6',8,8'-*octa-acetoxy*-9,9'-*bis*(3,5-*diacetoxyphenoxy*)*bis*(*dibenzo*[b,e][1,4]*dioxin*-2,2'-*yl*)]. R_f 0.36, 72 mg; UV λ_{max}^{MeCN} nm: 237, 295 (sh); MS (270°, 70 eV, main ketene elimination series) *m/z*: 1246 → 742, 912 → 618, 848 → 512, 748 → 496, 704 → 494, 688 → 478, 412 → 370, 316 → 232; ^{1}H NMR (Me_2CO-d_6): δ6.88 (2H), 6.87 (2H), 6.74 (2H), 6.68 (4H, AB_2-system) 2.33 (6H), 2.21 (6H), 2.11 (6H), 1.97 (6H), 1.76 (6H).

7,9'-Bieckol dodeca-acetate (**17**) [1,2',3,4',6,7',8,9'-*octa-acetoxy*-6',9-*bis*(3,5-*diacetoxyphenoxy*)*bis*(*dibenzo*[b,e][1,4]*dioxin*-1',2-*yl*)]. R_f 0.28, 3 mg; UV λ_{max}^{MeCN} nm: 230, 290 (sh); MS (270°, 70 eV, main ketene elimination series) *m/z*: 1246 → 742, 806 → 638, 748 → 370, 688 → 478, 540 → 372, 396 → 354, 392 → 266, 274 → 232, 248.

9,9'-Bieckol dodeca-acetate (**18**) [2,2',4,4',7,7',9,9'-*octa-acetoxy*-6,6'-*bis*(3,5-*diacetoxyphenoxy*)*bis*(*dibenzo*[b,e][1,4]*dioxinyl*)]. R_f 0.26, 55 mg; UV λ_{max}^{MeCN} nm: 235, 290 (sh); MS (280°, 70 eV, main ketene elimination series) *m/z*: 1246 → 742, 934 → 724, 792 → 372, 744 → 618, 688 → 478, 414 → 372, 412 → 370, 396 → 354, 392 → 266, 306 → 264, 274 → 232, 248, 126, 43; ^{1}H NMR (Me_2CO-d_6): δ6.87 (2H), 6.81 (2H), 6.78 (2H), 6.782 (4H, AB_2-system), 2.24 (12H), 2.12 (6H), 2.0 (6H), 1.96 (6H), one signal under Me_2CO-d_6.

7,2'''-Bieckol dodeca-acetate (**19**) [2-2,4-*diacetoxy*-6-(1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dioxin*-4-*yloxy*)*phenyl*-9-(3,5-*diacetoxyphenoxy*)-1,3,6,8-*tetra-acetoxydibenzo*[b,e][1,4]*dixoin*]. R_f 0.19, 8 mg; UV λ_{max}^{MeCN} nm: 223, 285 (sh); MS (270°, 70 eV main ketene elimination series) *m/z*: 1246 → 742, 1084 → 832 → 496 → 370, 934 → 514, 876 → 624 → 372; ^{1}H NMR (Me_2CO-d_6): δ7.03 (2H), 6.84 (1H), 6.82 (1H), 6.73 (2H, AB_2-system), 6.74, 6.64 (AB-system), 6.62 (1H), 6.60 (1H), 2.31 (3H), 2.28 (3H), 2.24 (6H), 2.23 (3H), 2.22 (3H), 2.14 (3H), 2.01 (3H), 1.95 (6H), 1 93 (3H), 1 78 (3H).

7,7'-Dihydroxy-9,9'-bieckol tetradeca-acetate (**20**) [2,2',3,3',4,4',7,7',9,9'-*deca-acetoxy*-6,6'-*bis*(3,5-*diacetoxyphenoxy*)-*bis*(*dibenzo*[b,e][1,4]*dioxinyl*)]. R_f 0.14, 11 mg; UV λ_{max}^{MeCN} nm: 230, 290 (sh); MS (300°, 70 eV main ketene elimination series) *m/z*: (1362) → 942, 1290 → 828, 846 → 510, 832 → 664, 830 → 536, 434 → 288, 376 → 292.

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