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FUCOPHLORETHOLS FROM THE BROWN ALGAE *SARGASSUM SPINULIGERUM* AND *CYSTOPHORA TORULOSA*

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Key Word Index—*Sargassum spinuligerum*; *Cystophora torulosa*; Phaeophyceae; phlorotannins; fucophlorethols; hydroxyfucophlorethols; chlorinated fucophlorethol; structural elucidation.

Abstract—The brown alga *Sargassum spinuligerum* contains a variety of fuhalols. In addition to these compounds, a number of fucophlorethols were isolated in the form of their peracetylated derivatives and identified by means of spectral analysis. The following phlorotannins were identified: the known, fucophlorethol-B octa-acetate, fucodiphlorethol-B, -D and -F deca-acetate, hydroxyfucodiphlorethol-A undeca-acetate, bisfucotriphlorethol-A pentadeca-acetate, hydroxybisfucophlorethol-A hexadeca-acetate, bisfucotetraphlorethol-A heptadeca-acetate and the new, dihydroxyfucotriphlorethol-A and -B tetradeca-acetate, bisfucopentaphlorethol-B nonadeca-acetate, chlorobisfucopentaphlorethol-A nonadeca-acetate, difucodiphlorethol-A trideca-acetate and fucodifucotetraphlorethol-A icosa-acetate. Bisfucotriphlorethol-A pentadeca-acetate, chlorobisfucopentaphlorethol-A nonadeca-acetate and fucodifucotetraphlorethol-A icosa-acetate were also isolated from the brown alga, *Cystophora torulosa*. © 1997 Elsevier Science Ltd

INTRODUCTION

Recently we reported [1–3] on the isolation and structural elucidation of different fuhalols and phlorethols from the brown alga, *Sargassum spinuligerum*. In addition to these substances, further fucophlorethols were isolated. These phlorotannins contain a characteristic 2,4,6,2′,4′-penta-acetoxybiphenyl or a 2,4,6,2′,4′,6′-hexa-acetoxybiphenyl moiety. All of them were obtained from an ethanolic extract and peracetylated before isolation in order to prevent oxidation. Some of the described substances were also isolated from the brown alga, *Cystophora torulosa*, namely, bisfucotriphlorethol-A pentadeca-acetate, chlorobisfucopentaphlorethol-A nonadeca-acetate and fucodifucotetraphlorethol-A icosa-acetate.

RESULTS AND DISCUSSION

Separation of the crude, peracetylated mixture of phlorotannins was carried out by means of flash chromatography and HPLC [1]. Pure compounds were obtained by multiple step HPLC separation. Then, fucophlorethols were separated from fuhalols with the same number of phloroglucinol units, using a solvent system which consisted of *n*-hexane, chloroform and ethanol. Under these conditions, fucophlorethols showed a significantly shorter R_t than fuhalols with a similar M_r.

By means of ^{1}H NMR spectra and comparison with published data, fucophlorethol-B octa-acetate [4–6], fucodiphlorethol-B, -D and -F deca-acetate [4, 6–9], hydroxyfucodiphlorethol-A undeca-acetate [10], bisfucotriphlorethol-A pentadeca-acetate [6], hydroxybisfucotriphlorethol-A hexadeca-acetate [10] and bisfucotetraphlorethol-A heptadeca-acetate [6] were identified (for known fucophlorethols from *C. torulosa*, see [11]).

The FAB-mass spectrum of dihydroxyfucotriphlorethol-B tetradeca-acetate (**1**) showed $[M+H]^+$ at *m/z* 1243 ($C_{58}H_{50}O_{31}$). The EI-mass spectrum of **1** gave a signal at *m/z* 724, which is explained by the formation of a dibenzodioxin structure [9]; this type of fragmentation occurs in molecules with an 1,2-diphenoxylated benzene. The position of the 1,2-diphenoxylated element in the whole molecule was deduced from the mass of the resulting ion. **1** showed chemical shifts for the protons of rings I and II identical with dihydroxyfucotriphlorethol-A tetradeca-acetate [11]. Consequently, both substances contain the same phenoxylated fucol moiety which is characterized by proton chemical shifts at δ 6.99 and 7.10 (aromatic protons of rings I and II).

Chemical shifts found for the rings III, IV and V have been described for fuhalols [1–3, 9]. In the ^{1}H NMR spectrum, the signal of the proton at C-6 of

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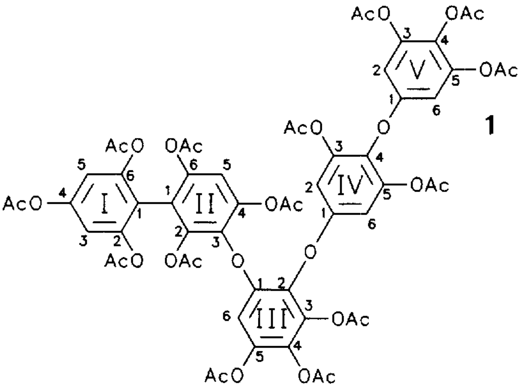

1

The numbering of carbons in **1**–**5** is not in accordance with IUPAC-rules.

ring III was found at δ 6.74(5) (acetone-d_6) instead of δ 6.65 with the fuhalol-A-analogue. This also proved the same substitution pattern of the rings but with different positions in the molecule. A difference between dihydroxyfucotriphloethol-A tetradeca-acetate and **1** is the position of the 1,2-diphenoxylated 3,4,5-triacetoxybenzene unit (ring III), having a significant influence on the neighbouring rings. The effects are similar to those reported for the fuhalol A-series with an even or an odd number of rings [1]. Characteristic for the phenoxy units attached to C-2 of ring III, are nearly identical chemical shifts for the aromatic protons of two phloroglucinol units IV and V at δ 6.69(3) and δ 6.69(5), respectively.

In the FAB-mass spectrum, bisfucopentaphlorethol-B nonadeca-acetate (**2**) gave a $[M + Na]^+$ at *m/z* 1815 ($C_{86}H_{72}O_{43}$); it contains twice the ring sequence I–II–VI. This element was isolated for the first time from the brown alga, *Cystoseira baccata*, and named, fucophlorethol-B octa-acetate [4]. Assignment of NMR shifts was carried out by comparison with this substance. Because of these two identical structure elements, the ^1H NMR spectrum of **2** showed identical shifts for the corresponding protons of both structural elements (I–II–VI and I′–II′–VI′, see Table 1). Characteristic are the signals for the aromatic protons at δ 7.0 (ring I), δ 7.11 (ring II) and δ 6.58 (ring III). These units are connected by a 1,2,3-triphenoxylated 5-acetoxybenzene unit (ring III). This element is known from fucophlorethols isolated previously [10]. Due to the high symmetry of the molecule, the signal for the two protons at C-4 and C-6 of ring III gave a singlet. In comparison to typical phlorotannin values, this signal is distinctly shifted to higher field (δ 6.27 in chloroform-*d* and δ 6.29 in acetone d_6). The single acetoxyl group at C-5 gave a signal at δ 2.17 (chloroform-*d*). C-2 of this ring is substituted by a 3,5-diacetoxyphenoxyl (IV), which is well known from phlorethols [2, 9]. The aromatic protons of this ring gave an AB_2-spin system at δ 6.58 and 6.63, with a coupling constant of 2.3 Hz (chloroform-*d*). The acetoxyl groups at C-3 and C-5 showed characteristic signals at δ 2.23.

The FAB-mass spectrum of chlorobisfucopentaphlorethol-A nonadeca-acetate (**3**) gave a $[M + H]^+$ at *m/z* 1827 ($C_{86}H_{71}O_{43}Cl$). The isotope pattern of this signal showed a good correlation with the computer-simulated signal for a chlorine-containing phlorethol. The only difference from bisfucopentaphlorethol-A nonadeca-acetate [12], is C-4 of ring IV carrying a chlorine instead of a proton. This has a significant influence on the proton chemical shifts of this ring. The aromatic protons at C-2 and C-6 gave a signal at δ 6.75 (instead of δ 6.62) and the acetoxyl groups of the same ring gave signals at δ 2.09 (instead of δ 2.03, chloroform-*d*, Table 1). Due to the structural symmetry of **3**, the substituents at C-1 and C-3 of ring III showed equal chemical shifts. Rings V and V′ are known from different types of fuhalols [1–3, 9]. A signal at δ 6.93 typically indicates the aromatic protons of an 1-phenoxylated 2,4,6-triacetoxybenzene. Rings I and II are part of a fucol element, which was first described for fucodiphlorethol-F-deca-acetate [8]. Structural elucidation was carried out by comparison with the NMR data of this compound, as well as the related fucols mentioned above. Compared with **1** and **2**, ring II of **3** is phenoxylated twice. This has a significant influence on the shift of the proton at C-5 resonating at δ 6.58 (chloroform-*d*). Similar to **2**, ring III of **3** is triphenoxylated, causing an highfield shifted signal at δ 6.36 from the aromatic protons at C-4 and C-6. The acetoxyl group at C-5 gives in comparison with **2**, a nearly identical proton shift of δ 2.17.

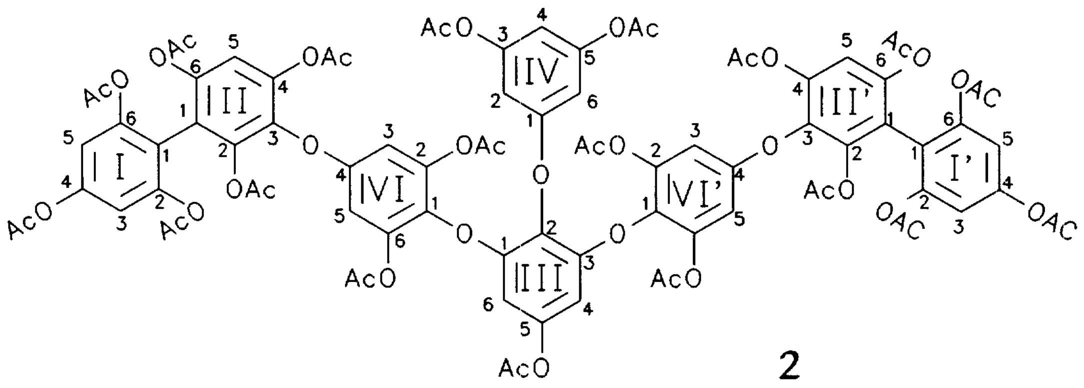

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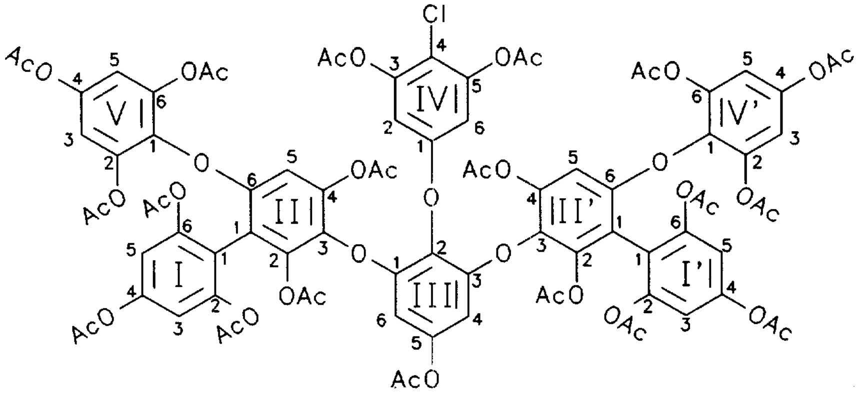
Table 1. ^1H NMR spectral data of compounds **2** to **5**

	2		**3**		**4**		**5**	
H	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO
				Ring I, I′				
3,5	7.00	7.04	6.98	7.05	7.01	7.04	7.01	7.04
Ac-2	2.05	2.05	2.02	2.03	2.05	*	2.05	*
Ac-4	2.30	2.28	2.28	2.27	2.28	2.28	2.28	2.27
Ac-6	2.05	2.05	1.92	1.88	2.05	*	2.05	*
				Ring I″				
3,5							6.98	7.05
Ac-2							2.01	*
Ac-4							2.28	2.27
Ac-6							1.92	1.87
				Ring II, II′				
5	7.11	7.24	6.58	6.63			6.56(5)	6.62
Ac-2	1.91	1.85	1.88	1.85			1.88	1.86
Ac-4	2.11	2.14	2.02	2.03			2.01	*
Ac-6	2.03	1.99						
				Ring II″				
Ac-2					1.67	1.58	1.66	1.58
Ac-4, Ac-6					1.82	1.83	1.79	1.78
				Ring III				
4	6.27	6.29	6.36	6.45	6.66[c]	6.61[d]	6.34[c]	6.47
6	6.27	6.29	6.36	6.45	6.77[c]	6.91[d]	6.49(5)[c]	6.47
Ac-3					2.12	2.12		
Ac-5	2.17	2.16	2.17	2.20	2.21	2.22(5)	2.15	2.19
				Ring IV				
2,6	6.58[a]	6.61[b]	6.75	6.86	6.54[e]	6.59[f]	6.58[e]	6.64
4	6.63[a]	6.72[b]			6.66[e]	6.71[f]	6.61[e]	6.64
Ac-3, Ac-5	2.23	2.23	2.09	2.09	2.24	2.22(2)	2.10	2.12
				Ring V				
3,5			6.93	7.01			6.92	7.01
Ac-2, Ac-6			2.06	*			2.06	*
Ac-4			2.28	2.27			2.28	2.27
				Ring VI, VI′				
3,5	6.68	6.79						
Ac-6, Ac-2	2.02	2.00						

*: Hidden by solvent.
[a]: AB System, $J = 2.3$ Hz.
[b]: AB System, $J = 2.0$ Hz.
[c]: AB System, $J = 2.7$ Hz.
[d]: AB System, $J = 2.6$ Hz.
[e]: AB_2 System, $J = 2.1$ Hz.
[f]: AB_2 System, $J = 2.0$ Hz.

The ^{13}C NMR spectrum showed slight differences between **3** and bisfucopentaphlorethol-A pentadeca-acetate. The chlorinated C-4 of ring IV gave a weak signal at δ 113.7; the chemical shift for the tertiary carbon at the same position of bisfucopentaphlorethol-A nonadeca-acetate is δ 109.5. The other chemical shifts of both compounds showed a good correlation.

The FAB-mass spectrum of difucodiphlorethol-A trideca-acetate (**4**) showed a $[M+H]^+$ at m/z 1169 ($C_{56}H_{48}O_{28}$), indicating the presence of five phloroglucinol units. The ^1H NMR spectrum gave a signal



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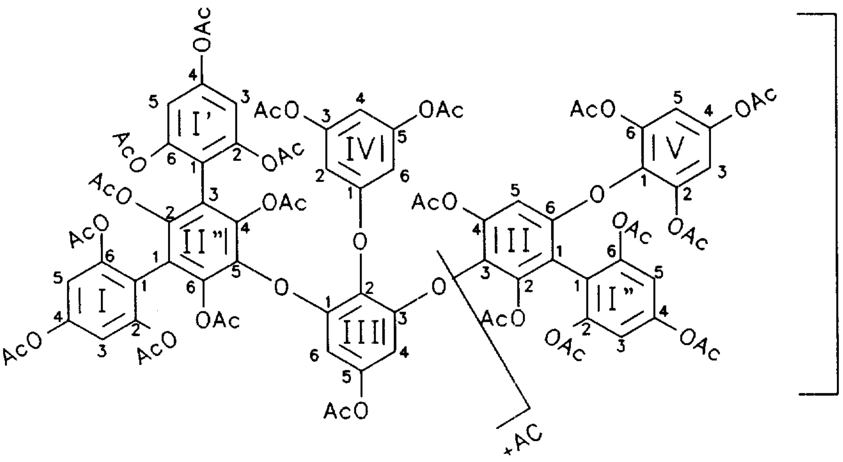

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at δ 7.01 (chloroform-*d*, Table 1) representing four protons. This signal was explained by a complex fucol moiety, which is made up by rings I, I′ and II″. Ring II″ was characterized by two signals at δ 1.67 (acetoxyl at C-2) and δ 1.82 (acetoxyl at C-4 and C-6). This structural element was described for tetrafucol-B deca-acetate, first isolated from the brown alga, *Fucus vesiculosus* [13], and also was noted for difucophlorethol-A undeca-acetate [14]. Ring III is a characteristic moiety of dehydroxyfuhalols [1, 2] and typically showed an AB-spin system at δ 6.66 and 6.77 (protons at C-4 and C-6). Ring IV is well known as an element of phlorethols [9]. The aromatic protons at C-2, C-4, and C-6 formed an AB_2-spin system (δ 6.54 and 6.66).

Fucodifucotetraphlorethol-A icosa-acetate (5) differs from 4 in C-3 of ring III carrying an additional fucol moiety, which is also described for 3 (rings I″, II and V). As an effect of this substitution, the aromatic protons at C-4 and C-6 of ring III of 5 are shifted to higher field giving signals at δ 6.34 and 6.49(5), respectively (Table 1). In comparison with 4, a high-field shift was observed for the protons of ring IV. As an effect of complex steric hinderance, the protons of the acetoxyl groups at C-3 and C-5 gave resonances at δ 2.10, instead of the more usual δ 2.24. The molecular formula of 5 of $C_{88}H_{74}O_{44}$ was confirmed by a FAB-mass spectrum showing a $[M+Na]^+$ at *m/z* 1857.

EXPERIMENTAL

EI-MS operation. 70 eV, 200–300°; positive ion FAB-MS: Xe gun, 3-nitrobenzylalcohol as matrix. ^{1}H NMR spectra (90 and 300 MHz) and ^{13}C NMR (75 MHz) were recorded using solvents as int. standards.

Extraction and isolation. Extraction and sepn are described in ref. [1].

Isolated compounds. Yields are given for *S. spinuligerum* if not mentioned otherwise. Fucophlorethol-B octa-acetate. 6 mg, ^{1}H NMR data identical with refs [4–6].

Fucodiphlorethol-B deca-acetate. 3 mg, ^{1}H NMR data identical with refs [4, 6].

Fucodiphlorethol-D deca-acetate. 5 mg, ^{1}H NMR data identical with ref. [7].

Fucodiphlorethol-F deca-acetate. 3 mg, H NMR data identical with refs [8, 9]. Hydroxyfucodiphlorethol-A undeca-acetate. 1 mg, H NMR data identical with ref. [10].

Bisfucotriphlorethol-A pentadeca-acetate. 11 mg, (*C. torulosa*: 3 mg), ^{1}H NMR data identical with ref. [6].

Table 2. ^{13}C NMR spectral data of compound **3** in comparison with bisfucopentaphlorethol-A nonadeca-acetate (in chloroform-*d*).

C	3 [ppm]	* [ppm]
	Ring I, I′	
1	115.8	115.8
2,6	149.0	149.1
3,5	113.9	114.0
4	150.2	150.5
	Ring II, II′	
1	114.4	114.6
2	146.0	146.2
3	136.2	136.3
4	142.5	143.6
5	109.8	109.3
6	151.6	152.8
	Ring III	
1,3	150.4	150.4
2	130.8†	130.3
4,6	103.3	103.2
5	148.2†	147.7
	Ring IV	
1	156.8†	158.7
2,6	107.3	106.3
3,5	151.4	151.4
4	113.7†	109.5
	Ring V, V′	
1	133.8	133.8
2,6	143.5	143.7
3,5	114.8	114.8
4	146.9	147.0

*: Bisfucopentaphlorethol-A nonadeca-acetate [12].
†: Weak signal.

Hydroxybisfucotriphlorethol-A hexadeca-acetate. 1 mg, ^{1}H NMR data identical with ref. [10].

Dihydroxyfucotriphlorethol-A tetradeca-acetate. 3 mg, (*C. torulosa*: 3 mg). NMR and MS data given in ref. [11]. Bisfucotetraphlorethol-A heptadeca-acetate. 2 mg, ^{1}H NMR data identical with ref. [6].

Dihydroxyfucotriphlorethol-B tetradeca-acetate: 2,4,6,2′,4′,6′-hexa-acetoxy-3-{3,4,5-triacetoxy-2-[3,5-diacetoxy-4-(3,4,5-triacetoxyphenoxy)phenoxy] phenoxy} biphenyl (**1**). 1 mg. EI-MS ketene elimination series: *m/z* 1158 → 654, 1014 → 930, 892 → 514, 700 → 406, 684 → 390, 682 → 388, 624 → 372, 622 → 370, 500 → 374, 416 → 248, 376 → 250, 316 → 232, 266 → 142. FAB-MS ketene elimination series: 1281 [M+K]$^+$, 1265 [M+Na]$^+$ → 1223, 1243 [M+H]$^+$ → 823. ^{1}H NMR (CDCl$_3$/Me$_2$CO-d_6) δ: Ring I: 6.99/7.03 (2H, *s*, C-3,5), 2.07/hidden by solvent (6H, *s*, Ac-2,6), 2.29/2.28 (3H, *s*, Ac-4), ring II: 7.10/7.22 (1H, *s*, C-5), 1.85/1.84 (3H, *s*, Ac-2), 2.08/2.11 (3H, *s*, Ac-4), 2.00/1.97 (3H, *s*, Ac-6), ring III: 6.72/6.74(5) (1H, *s*, C-6), 2.20/2.19(5) (3H, *s*, Ac-3), 2.25/2.24(5) (3H, *s*, Ac-4), 2.22/2.22(5) (3H, *s*, Ac-5), ring IV: 6.69(3)/6.73(5) (2H, *s*, C-2,6), 2.04/2.00 (6H, *s*, Ac-3,5), ring V: 6.69(5)/6.79 (2H, *s*, C-2,6), 2.22/2.21 (6H, *s*, Ac-3,5), 2.26/2.27(5) (3H, *s*, Ac-4).

Bisfucopentaphlorethol-B nonadeca-acetate: 4,3′,5′-triacetoxy-2,6-*bis*{2,6-diacetoxy-4-[2,4,6-triacetoxy-3-(2,4,6-triacetoxyphenyl) phenoxy]phenoxy}diphenylether (**2**). 2 mg. FAB-MS ketene elimination series: 1831 [M+K]$^+$, 1815 [M+Na]$^+$ → 1689, 1793 [M+H]$^+$ → 1499. ^{1}H NMR: Table 1.

Chlorobisfucophlorethol-A nonadeca-acetate: 4,3′,5′-triacetoxy-2,6-*bis*[2,6-diacetoxy-3-(2,4,6-triacetoxy phenyl) - 4 - (2,4,6 - triacetoxy - phenoxy)phenoxy] - 4′-chlorodiphenylether (**3**). 1 mg, (*C. torulosa*: 5 mg). EI-MS *m/z* 1827 [M+H]$^+$ → 1617. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2.

Difucodiphlorethol-A trideca-acetate: 2,4,6-triacetoxy-1-[3,5-diacetoxy-2-(3,5-diacetoxyphenoxy) phenoxy]-3,5-*bis*(2,4,6-triacetoxyphenyl)benzene (**4**). 1 mg. FAB-MS ketene elimination series: 1191 [M+Na]$^+$, 1169 [M+H]$^+$ → 705. ^{1}H NMR: Table 1.

Fucodifucotetraphlorethol-A-icosa-acetate: 4,3′,5′-triacetoxy-2-[2,4,6-triacetoxy-3,5-*bis*(2,4,6-triacetoxyphenyl)phenoxy]-6-[2,6-diacetoxy-3-(2,4,6-triacetoxyphenyl)-4-(2,4,6-triacetoxyphenoxy) phenoxy] diphenylether (**5**). 2 mg, (*C. torulosa*: 4 mg). FAB-MS ketene elimination series: 1873 [M+K]$^+$ → 1831, 1857 [M+Na]$^+$ → 1731, 1835 [M+H]$^+$ → 1709. ^{1}H NMR: Table 1.

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