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# PHLOROTANNINS FROM THE BROWN ALGA *CYSTOPHORA TORULOSA*

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**Key Word Index**—*Cystophora torulosa*; Phaeophyceae; phlorotannins; fucols; fucophlorethols; hydroxyfucophlorethols; structural elucidation.

**Abstract**—A total of 33 phlorotannins had been isolated from *Cystophora torulosa*, 20 of which have already been described. In this publication the structures of the remaining 13 compounds are described. Whereas the former substances belong to the group of fucols, branched fucophlorethols and difucol-containing fucophlorethols, it is shown here that five of the remaining 13 compounds are members of two entirely new phlorotannin subclasses, namely hydroxylated branched fucophlorethols and *bis*-fucophlorethols lacking a 1,2,3-triphenoxy-5-acetoxybenzene moiety. © 1997 Elsevier Science Ltd

## INTRODUCTION

It is well established that brown algae contain phenolic compounds with antibiotic activity [1–4]. The high-molecular members of this substance class have tannin-like properties [5, 6]. They are termed phlorotannins, since phloroglucinol is a common monomer unit. Within the algae, they are localised in specialised storage vesicles, the so-called physodes.

Phlorotannins are systematically grouped according to the bonding type between the phloroglucinol units (diphenylethers or biphenyls) as well as to the presence of additional hydroxyl groups. 33 phlorotannins had been isolated from the brown alga *Cystophora torulosa*, which is common to Australia and New Zealand. 20 of them have been characterised recently [9]. In this publication will be reported the structures of the remaining 13 compounds, which were found to belong to the class of fucols (biphenyl elements) and fucophlorethols (diphenyl ether and biphenyl elements), respectively. Among them five members of two novel phlorotannin subclasses were found. Three turned out to be branched and hydroxylated fucophlorethols, whereas another two compounds represent bisfucophlorethols lacking a 1,2,3-triphenoxy-5-acetoxybenzene moiety. Since it is difficult to isolate free phlorotannins, all substances described here were isolated and characterised by their peracetylated derivatives.

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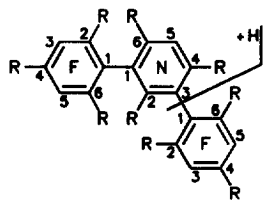
## RESULTS AND DISCUSSION

The phenols were extracted with ethanol from shredded, deep-frozen algae and pre-purified as described previously [9]. They were acetylated and purified to apparent homogeneity by HPLC on silica gel, using chloroform/ethanol mixtures as mobile phase. Critical separations were performed by the additional presence of *n*-hexane or acetonitrile [9]. The pure compounds were characterised by NMR and, occasionally, also by mass spectroscopy. The comparison of $^1$H NMR spectra was sufficient to unambiguously identify components of known structure. For novel compounds, the structural assignments were cross-checked by FAB and/or EI mass spectroscopy.

Of the 13 isolated compounds, two belonged to the group of fucols and another five to the group of branched fucophlorethols and had been isolated from other algae before: fucols: difucol hexacetate (**9**) [10]; trifucol nonaacetate (**10**) [10]; branched fucophlorethols: fucodiphlorethol-B decaacetate (**1**) [11], fucotetraphlorethol-B tetradecaacetate (**2**) [12], bisfucotriphlorethol-A pentadecaacetate (**3**) [13], bisfucotetraphlorethol-A heptadecaacetate (**4**) [12]; bisfucopentaphlorethol-A nonadecaacetate (**5**) [12].

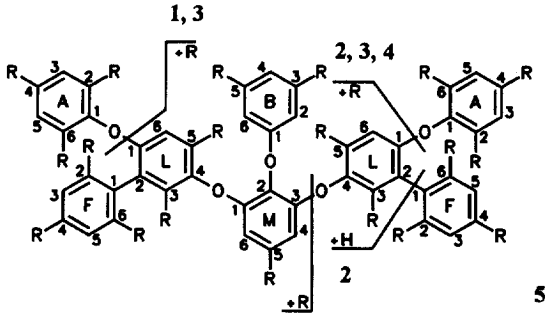
Three of the new compounds turned out to be closely related in structure to the branched hydroxyphlorethols (**1**–**5**). Instead of the 3,5-diacetoxyphenoxy unit (ring B), however, they bear a 3,4,5-triacetoxyphenoxy moiety (ring D): hydroxyfucodiphlorethol-B undecaacetate (**6**), hydroxyfucotriphlorethol-B tridecaacetate (**7**), hydroxybisfucopentaphlorethol-A eicosaacetate (**8**).



9

10

9, 10*



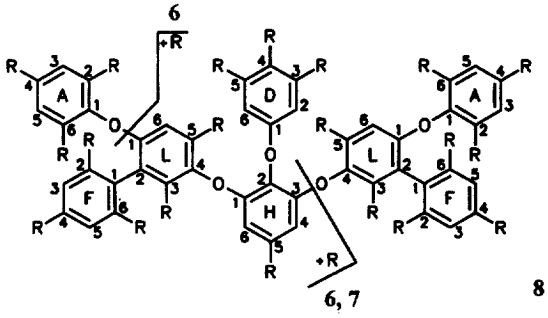
1, 3

2, 3, 4

5

1

1 - 5*



6

6, 7

8

6 - 8*

R = OAc

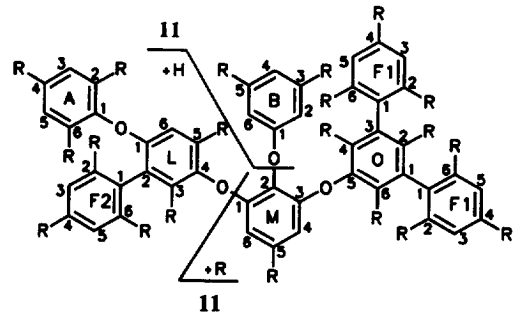
* The letters in the rings are only valid for the largest compound respectively

All of them constitute a novel and still incomplete series of homologues, of which **6** is the simplest member. It has a four-ringed, unbranched structure with a characteristic *ortho*-substitution pattern at ring H. Its five-ringed homologue **7** bears a 3,5-diacetoxy-1,4-diphenoxy-2-phenyl unit (ring L) introducing a branching site. The eight-ring homologue **8** is symmetrically branched by two L-type rings, one M-type ring and a single 5-acetoxy-1,2,3-phenoxybenzene unit. All three homologues bear an additional acetoxy group attached to C-4 of ring D (a 3,4,5-triacetoxyphenoxybenzene element), which is one more than in the closely related branched fucophlorethols.

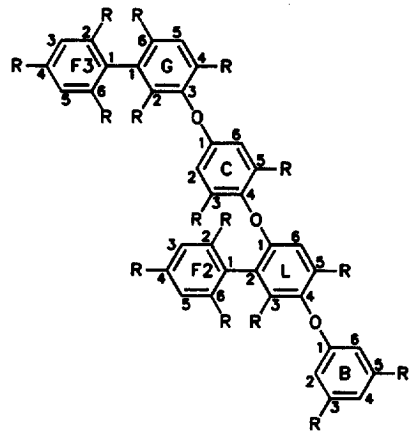
In the $FAB^+$-mass spectrum the molecular ions at $m/z$ = 977, 1185, 1851 of **6**, **7** and **8** mark the beginning of a ketene elimination series. Analogous ketene eliminations were observed in EIMS starting at $M^+(-1 \times 42)$ from $m/z$ = 934 or 1142 for **6** or **7**. It was possible to monitor 11 eliminations for **6**, and 13 for **7**. For **8**, only 11 out of the 21 possible elimination ions could be assigned.

The $^1H$ NMR spectra of **6** and **8** are highly similar to those of **1** and **5**. They only differ in an additional acetoxy group at ring B, converting it to a D type ring. Therefore, the $AB_2$-system at 6.67–6.60 ppm and 6.61–6.51 ppm typical of a B-type ring vanishes from the spectrum. Instead, a singlet of double intensity appears at 6.76–6.67 ppm. The additional acetoxy group gives rise to a singlet of triple intensity at 2.24–2.17 ppm.

Since no fucophlorethol analogues of **7** have been characterised yet, several structures can be proposed from the observed ring types A, D, F, H and L. Ring F is linked to ring L, because only these two are able




**11 - 12***




**13***

R = OAc

to form a biphenyl bond. The aromatic protons of ring A at 6.90–6.91 ppm are unusually strongly shielded. A similar phenomenon has been found for fucophlorethol-C-octaacetate [9, 12], which had also been isolated from *Cystophora torulosa*. In fucophlorethol-C-octaacetate, ring A is directly connected to C-2 of ring I. It is therefore in an *ortho*-position to ring F, which is attached to C-1 of ring I. This causes a stronger shielding of the aromatic protons of ring A. An analogous substitution pattern can be assumed for **7**: ring A connects to C-1 of ring L, which in turn is substituted with a phenyl group at the *ortho*-position (ring F). It therefore seems plausible to assume that ring H is bound to C-4 of ring L. Consequently, ring D must be attached to C-2 of ring H.

Compound **13** is a representative of still another novel structural group: bisfucotriphlorethol-B pentadecaacetate represents a bisfucophlorethol devoid of a 1,2,3-triphenoxy-5-acetoxybenzene unit (ring M in **12**). $(M+H)^+$ appeared at $m/z = 1377$ in the FAB mass spectrum and all of the 15 possible ketene eliminations were recovered in the spectrum.

Since no compounds homologous to this type have been characterised yet, the $^1H$ NMR data of **13** were compared to known compounds of similar partial structure (two F type rings and each one of type G, C, L and B). The signals of aromatic protons at 7112 ppm for 1 H and 7000 ppm for 2 H are typical of the ring-combination F–G which exists in unbranched acetylated fucophlorethols [9, 11]. Therefore, the existence of an ether bridge at C-3 of ring G has to be assumed. The second element, a F-type ring ($F_2$), gives rise to the singlet of double intensity at 6989 ppm. Such an unusual shielding had also been observed for the F-type rings of branched as well as branched and

Table 1. Correlation of $^1$H NMR-data of **6**, **7**, **8** (300 MHz, $\delta$ in ppm in $CDCl_3$ and $d_6$-$Me_2CO$)

| | Chemical shift measured | | |
|---|---|---|---|
| | **6** | **7** | **8** |
| Ring type | $CDCl_3/d_6$-$Me_2CO$ | $CDCl_3/d_6$-$Me_2CO$ | $CDCl_3/d_6$-$Me_2CO$ |
| Ring G | | | |
| C-5 | 7.090/7.211 | | |
| C-6 (Ac) | 2.049/2.077 | | |
| C-4 (Ac) | 2.009/1.969 | | |
| C-2 (Ac) | 1.810/1.797 | | |
| Ring F | | | |
| C-3,5 | 6.990/7.030 | 6.986/7.025 | 6.981/7.028 |
| C-4 (Ac) | 2.285/2.278 | 2.281/2.271 | 2.279/2.272 |
| C-2,6 (Ac) | 2.049/1.998 | 2.010/2.065 | 2.023/2.021* |
| Ring A | | | |
| C-3,5 | | 6.895/7.010 | 6.913/6.772 |
| C-4 (Ac) | | 2.269/2.270 | 2.268/2.078 |
| C-2,6 (Ac) | | 2.072/2.120 | 2.059/* |
| Ring H | | | |
| C-4 | 6.772/6.897*d* | 6.800/6.892*d* | |
| C-6 | 6.578/6.571*d* | 6.513/6.610*d* | |
| C-5 (Ac) | 2.227/2.216 | 2.235/2.232 | |
| C-3 (Ac) | 2.151/2.130 | 2.169/2.108 | |
| Ring D | | | |
| C-2,6 | 6.673/6.738 | 6.686/6.748 | 6.755/6.812 |
| C-4 (Ac) | 2.244/2.243 | 2.205/2.213 | 2.171/2.198 |
| C-3,5 (Ac) | 2.232/2.228 | 2.100/* | 2.007/2.035* |
| Ring L | | | |
| C-6 | | 6.552/6.630 | 6.82/6.635 |
| C-5 (Ac) | | 1.925/1.871 | 1.912/1.870 |
| C-3 (Ac) | | 1.910/1.862 | 1.868/1.834 |
| Ring M | | | |
| C-4,6 | | | 6.384/6.463 |
| C-5 (Ac) | | | 2.169/2.175 |
| | *d*: $J$ = 2.4/2.4 Hz | *d*, $J$ = 2.4/2.1 Hz | |

* Hidden by solvent.
The last decimal place shows only a tendency.

hydroxylated fucophlorethol acetates (**2**, **4**, **5** as well, **7**, **8**). For these subgroups, the highly shielded ring F is always connected to a L-type ring. The terminal ring $F_3$ is less shielded than ring $F_2$, which can only be the case if ring C is positioned between rings G and L.

At the same time a compound of another new group of phlorotannins was isolated from *Xiphophora chondrophylla* [14]: fucophlorethol with a difucol unit. The isolated difucophlorethol-A-undecaacetate is identical to **11**. Compound **12** is a novel, eight-ringed homologue of this series. In the FAB mass spectrum, **12** gives rise to a $(M+H)^+$ at $m/z$ = 1835. 16 of the 20 possible ketene eliminations starting from this ion were identified. The $^1$H NMR spectrum of **12** shows signal groups characteristic of one each of ring types A, B, $F_2$, L and M, respectively. Therefore, **12** is a close relative of the branched fucophlorethols (**1**–**5**). A different substituent must however be located at C-3 (or C-1) of ring M. An asymmetrical structural pattern results from this proposition, which would explain, the protons of ring M appearing as an AB system. Analogous to **11**, the remaining $^1$H NMR signals are assigned to one O-type and two $F_1$-rings.

## EXPERIMENTAL

*Plant material. Cystophora torulosa* (R. Br.) I. Ag. collected at Whangaparoa, New Zealand was transported frozen to Germany. Voucher specimen: Herbarium of the University of Auckland.

*EIMS.* MS 50 from Kratos, 200–300°C, 70 eV; pos. FABMS: Concept 1 H from Kratos, Xe gun, 3-nitrobenzyl alcohol as matrix.

*$^1$H NMR.* 300 MHz XL-300 from Varian. The $^1$H NMR spectra were recorded in both $CDCl_3$ and $d_6$-$Me_2CO$ because signals differ in each solvent. The chemical shifts have been estimated to the third decimal place, in order to distinguish between closely

Table 2. Correlation of $^1$H NMR-data of **12**, **13** (300 MHz, $\delta$ in ppm in $CDCl_3$ an $d_6$-$Me_2CO$)

| Ring type | Chemical Shift measured **12** $CDCl_3/d_6$-$Me_2CO$ | **13** $CDCl_3/d_6$-$Me_2CO$ |
|---|---|---|
| Ring G | | |
| C-5 | | 7.112/7.240 |
| C-6 (Ac) | | 2.118/* |
| C-4 (Ac) | | 2.025/* |
| C-2 (Ac) | | 1.911/* |
| Ring $F_1$ | | |
| C-3,5 | 7.007/7.036 | |
| C-4 (Ac) | 2.280/2.274 | |
| C-2,6 (Ac) | 2.045/* | |
| Ring $F_2$ | | |
| C-3,5 | 6.979/7.049 | 6.989/7.021 |
| C-4 (Ac) | 2.280/2.274 | 2.285/2.274 |
| C-2,6 (Ac) | 2.007/* | 2.048/* |
| Ring $F_3$ | | |
| C-3,5 | | 7.000/7.041 |
| C-4 (Ac) | | 2.292/2.280 |
| C-2,6 (Ac) | | 2.055/* |
| Ring A | | |
| C-3,5 | 6.923/7.010 | |
| C-4 (Ac) | 2.278/2.268 | |
| C-2,6 (Ac) | 2.054/* | |
| Ring C | | |
| C-2,6 | | 6.688/6.800 |
| C-5 (Ac) | | 2.055/* |
| C-3 (Ac) | | 2.050/* |
| Ring B | | |
| C-4 | 6.613/6.640*t*, $J$ = 1.95/$A_3$ | 6.673/6.721*t* |
| C-2,6 | 6.583/6.640*d* | 6.575/6.624*d* |
| C-3,5 (Ac) | 2.102/2.126 | 2.240/2.232 |
| Ring L | | |
| C-6 | 6.563/6.620 | 6.629/6.740 |
| C-5 (Ac) | 1.915/1.872 | 1.941/* |
| C-3 (Ac) | 1.882/1.860 | 1.911/* |
| Ring M | | |
| C-6 | 6.498/6.468*d*, $J$ = 2.7/2.6 Hz | |
| C-4 | 6.343/6.457*d* | |
| C-5 (Ac) | 2.146/2.193 | |
| Ring O | | |
| C-4,6 (Ac) | 1.786/1.783 | |
| C-2 (Ac) | 1.665/1.581 | |

* Hidden by solvent.
The last decimal place shows only a tendency.

neighboured signals, which could clearly be differentiated by visual inspection of the spectra.

*Isolated compounds.* Yields correspond to 18 kg of frozen alga.

*Difucol hexaacetate* (**9**). Yield 6 mg, identical with substance in ref. [10].

*Trifucol nonaacetate* (**10**). Yield 4 mg, identical with substance in ref. [10].

*Fucodiphlorethol-B decaacetate* (**1**). Yield 4 mg, identical with substance in ref. [11].

*Fucotetraphlorethol-B tetradecaacetate* (**2**). Yield 5 mg, identical with substance in ref. [12].

*Bisfucotriphlorethol-A pentadecaacetate* (**3**). Yield 3 mg, identical with substance in ref. [13].

*Bisfucotetraphlorethol-A heptadecaacetate* (**4**). Yield 3 mg, identical with substance in ref. [12].

*Bisfucopentaphlorethol-A nonadecaacetate* (**5**). Yield 6 mg, identical with substance in ref. [12].

*Hydroxyfucodiphlorethol-B undecaacetate*, 2,4,6,2′, 4′,6′-*hexaacetoxy*-3-[3,5-*diacetoxy*-2-(3,4,5-*triacetoxyphenoxy*)*phenoxy*]*biphenyl* (**6**). Yield 3 mg, EIMS ketene elimination series: $m/z$ 934–514, FABMS: $m/z$ $[M+K]^+$ 1015, $[M+Na]^+$ 999, ketene elimination series: $m/z$ 977–557.

*Hydroxyfucotriphlorethol-B tridecaacetate*, 2,6,3′,5′-*tetraacetoxy*-3-(2,4,6-*triacetoxyphenyl*)-4-(2,4,6-*triacetoxyphenoxy*)-2′-(3,4,5,-*triacetoxy*)*biphenylether* (**7**). Yield 3 mg, EIMS ketene elimination series: *m/z* 1142–638, FABMS: *m/z* $[M+K]^+$ 1223, $[M+Na]^+$ 1207, ketene elimination series: *m/z* 1185–933.

*Hydroxybisfucopentaphlorethol-A eicosaacetate*, 5-*acetoxy*-2-(3,4,5-*triacetoxyphenoxy*)-1,3-*bis*[3-(2,4,6-*triacetoxyphenyl*)-4-(2,4,6-*triacetoxyphenoxy*)-2,6-*diacetoxyphenoxy*]*benzene* (**8**). Yield 2 mg, FABMS: *m/z* $[M+K]^+$ 1889, $[M+Na]^+$ 1873, ketene elimination series: 1851–1389.

*Bisfucotriphlorethol-B pentadecaacetate*, 3,5,2′,6′-*tetraacetoxy*-4-(3,5-*diacetoxyphenoxy*)-2-(2,4,6-*triacetoxyphenyl*)-4′-(2,4,6,2′,4′,6′-*hexaacetoxybiphenyl*-3-*oxy*)*diphenylether* (**13**). Yield 3 mg, EIMS ketene elimination series: *m/z* 1292–746, 1168–622, 1142–638, 710–374, 726–390, 502–250, 518–266, FABMS: *m/z* $[M+K]^+$ 1415, $[M+Na]^+$ 1399, ketene elimination series: *m/z* 1377–915.

*Difucophlorethol-A undecaacetate* (**11**). Yield 3 mg identical with substance in ref. [14].

*Fucodifucotetraphlorethol-A eicosaacetate*, 5,2′,6′-*triacetoxy*-2-(3,5-*diacetoxyphenoxy*)-3′-(2,4,6-*triacetoxyphenyl*)-4′-(2,4,6-*triacetoxyphenoxy*)-3-[2,4,6-*triacetoxy*-3,5-bis(2,4,6-*triacetoxyphenyl*)*phenoxy*] *diphenylether* (**12**). Yield 4 mg, FABMS: *m/z* $[M+K]^+$1873, $[M+Na]^+$ 1857, ketene elimination series: *m/z* 1835–1163.

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