Nonhalogenated and Halogenated Phlorotannins from the Brown Alga *Carpophyllum angustifolium*

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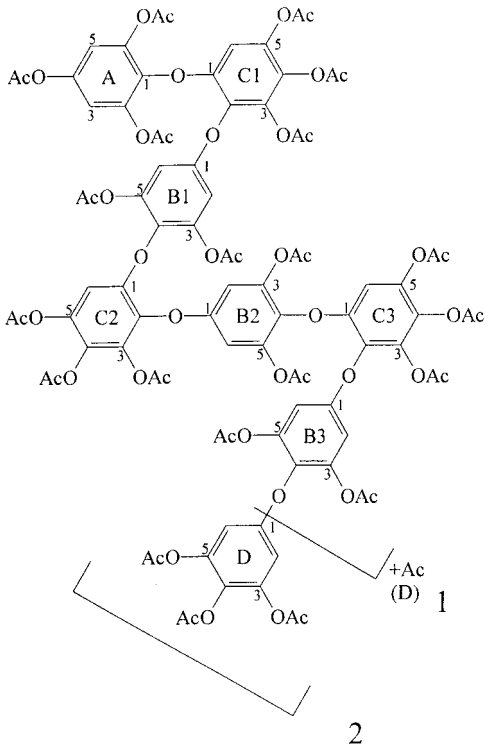
Using HPLC, forty-five phloroglucinol derivatives were obtained from an ethanolic extract of the brown alga *Carpophyllum angustifolium* after peracetylation. Compounds of the fuhalol-B series are described. The structure of hydroxyheptafuhalol-B nonadecaacetate is confirmed by the addition of ^{13}C NMR spectral data, octafuhalol-C heneicosaacetate is described for the first time. The occurrence of four halogenated phlorotannins is reported: 2-chlorophloroglucinol triacetate, 2[D′]iododiphlorethol pentaacetate, which had been isolated formerly only as a mixture, 3[A]chlorobifuhalol hexaacetate, and 3[A_4]chlorodifucol hexaacetate. All substances were characterized by means of spectral analysis.

The brown alga *Carpophyllum angustifolium* J. Ag. (Sargassaceae) is very rich in phloroglucinol derivatives, substances which have antibiotic, antifungal, antialgal and toxic activity. Forty-five compounds of this substance class were isolated. In the present report, 37 known compounds are listed and one new member of the fuhalol B series and an additional set of 2 known, and 2 new halogenated phlorotannins are described (Table 1).

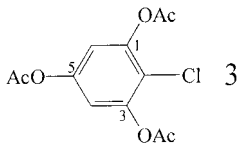
Results and Discussion

The structure of compound **2** was elucidated by comparison with the known compound **1**. The FAB-mass spectrum of the peracetylated **2** ($C_{90}H_{76}O_{49}$) showed [M + H]$^+$ at *m*/*z* 1941. Since the mass difference between the molecular ions of **1** and **2** was found to be 208 amu, it was assumed that **1** contains one less ring of type B (1,4-diphenoxylated 3,5-diacetoxybenzene) than **2**. In the EI-mass spectrum of **2** a 5-fold ketene elimination series from *m*/*z* 474 down to *m*/*z* 264 was visible, produced by a benzodioxin fragment ion consisting of ring A and C1 after the loss of six rings B_1 up to D. The chemical shifts in the ^1H NMR spectrum of compound **2** (in $CDCl_3$ and Me_2CO-d_6, respectively) were very similar to those of compound **1**. There were additional signals for the protons at C-2 and C-6 (δ 6.66 in $CDCl_3$, δ 6.79 in Me_2CO-d_6) and for the acetoxy groups at C-3 and C-5 (δ 2.07 in $CDCl_3$, δ 2.06 in Me_2CO-d_6) of an additional B type ring.



In ^{13}C NMR **2** showed the typical signals for A, B, C, and D type rings (Table 2). The identity of the rings A, C1, B1, C2, B2, and C3 of **1** and **2** was proven by the striking similarity of the signals in ^1H NMR spectrum. The additional B type ring must therefore be included in the other terminus of the molecule. The structure of compound **2** was established by a $^1H-^1H$ ROESY experiment, which showed the expected correlations and one between the C-2, C-6 ring protons of ring D and the C-3, C-5 acetoxy groups of a B type ring. Compound **2** was named octafuhalol-C heneicosaacetate.

In the EI-mass spectrum of **3** a 3-fold ketene elimination series from *m*/*z* 288/286 down to *m*/*z* 162/160 was visible. The 2 amu spacing between the peaks in conjunction with their relative intensities and the 36/34 amu difference to phloroglucinol triacetate pointed toward the monochlorinated derivative of phloroglucinol. This compound had been isolated previously from *Eisenia arborea* but only in a mixture with 2-iodophloroglucinoltriacetate.[20]



A 5-fold ketene elimination series starting with the molecular ion at *m*/*z* 586 could be observed in the EI-mass spectrum of compound **4**. The mass difference of 126 amu to diphlorethol pentaacetate ($C_{22}H_{20}O_{11}$)[10] pointed toward a substitution with iodine. Compound **4** had been isolated previously from *Cystophora congesta* but only as a mixture with 2[D′]bromodiphlorethol pentaacetate.[11] To differentiate between halogenated and nonhalogenated rings, the letter of a halogenated ring type is set in square brackets.

The FAB-mass spectrum of compound **5** showed [M + H]$^+$ at *m*/*z* 553 and 555 ($C_{24}H_{21}O_{13}Cl$) with a 6-fold ketene elimination series. The mass difference to bifuhalol hexaacetate (M$^+$ 518),[4,8] pointed toward a substitution with

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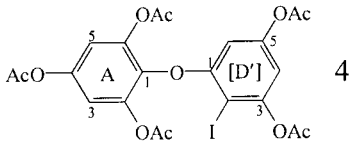
Table 1. Known Compounds Found in *C. angustifolium*

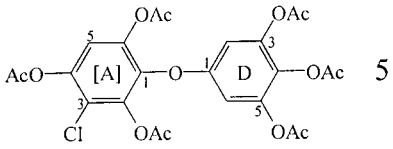
compounds of *C. angustifolium*	amount[a]	first description	ref
bifuhalol hexaacetate	++++	*Bifurcaria bifurcata*	8
trifuhalol-A octaacetate	+++	*Halidrys siliquosa*	9
hexafuhalol-A hexadecaacetate	+++	*Sargassum muticum*	3
heptafuhalol-A octadecaacetate	++++	*Halidrys siliquosa*	4
octafuhalol-A heneicosaacetate	++++	*Sargassum muticum*	3
nonafuhalol-A tricosaacetate	++++	*Sargassum muticum*	3
decafuhalol-A hexacosaacetate	+	*Sargassum spinuligerum*	5
undecafuhalol-A octadecaacetate	++	*Bifurcaria bifurcata*	6
hydroxypentafuhalol-A tetradecaacetate	++	*Carpophyllum maschalocarpum*	7
hexafuhalol-B hexadecaacetate	++	*Carpophyllum maschalocarpum*	7
hydroxyheptafuhalol-B nonadecaacetate (**1**)	++	*Sargassum spinuligerum*	2
deshydroxyoctafuhalol-C eicosaacetate	+	*Sargassum spinuligerum*	5
deshydroxyhexafuhalol-D pentadecaacetate	+	*Sargassum spinuligerum*	5
phloroglucinol triacetate	+	*Halidrys siliquosa*	4
diphlorethol pentaacetate	+	*Cystoseira tamariscifolia*	10
triphlorethol-B heptacaetate	+	*Cystoseira baccata*	12
tetraphlorethol-C nonaacetate	+	*Cystophora congesta*	11
hydroxytetraphlorethol-A decaacetate	+	*Carpophyllum maschalocarpum*	7
pseudohexafuhalol-A hexadecaacetate	++	*Sargassum spinuligerum*	13
pseudohexafuhalol-B hexadecaacetate	+	*Sargassum spinuligerum*	13
pseudoheptafuhalol-B octadecaacetate	+	*Sargassum spinuligerum*	13
pseudohexafuhalol-C hexadecaacetate	+	*Sargassum spinuligerum*	13
pseudoheptafuhalol-C octadecaacetate	++	*Sargassum spinuligerum*	13
pseudoheptafuhalol-D octadecaacetate	+	*Sargassum spinuligerum*	13
difucol hexaacetate	+++	*Fucus vesiculosus*	14
tetrafucol-A dodecaacetate	++	*Analipus japonicus*	15
fucophlorethol-B octaacetate	++	*Cystoseira baccata*	16
fucodiphlorethol-D decaacetate	++	*Cystoseira baccata*	12
fucodiphlorethol-B decaacetate	+	*Cystoseira baccata*	16
fucotriphlorethol-B dodecaacetate	+	*Cystoseira granulata*	17
bisfucotriphlorethol-A pentadecaacetate	++++ (!)	*Cystoseira baccata*	12
terfucohexaphlorethol-A tetracosaacetate	++	*Carpophyllum maschalocarpum*	7
fucodifucotetraphlorethol-A eicosaacetate	+	*Cystophora torulosa* and *Sargassum spinuligerum*	18
dihydroxyfucotriphlorethol-A tetradecaacetate	+	*Cystophora torulosa* and *Sargassum spinuligerum*	19
dihydroxyfucotriphlorethol-B tetradecaacetate	+	*Sargassum spinuligerum*	18
2-iodophloroglucinol triacetate	+	*Eisenia arborea*	20
2[D′]bromodiphlorethol pentaacetate	+	*Cystophora congesta*	11

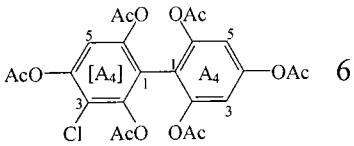
[a] Amount of isolated compounds: ++++, >30 mg (major compounds); +++, 16−29 mg; ++, 6−15 mg; +, <5 mg (minor compounds).

chlorine. The typical isotope pattern for chlorine and a 6-fold ketene elimination series starting with the ion at *m*/*z* 554/552 could be observed in the EI-mass spectrum of **5**. The only difference to bifuhalol hexaacetate[8,22] is the chlorine at C-3 of ring [A]. Due to this substitution, all resonances of ring [A] were shifted downfield significantly. The aromatic proton at C-5 appeared at δ 7.05 instead of δ 6.94. The acetoxy group at C-2 was shifted to δ 2.22, the one at C-4 to δ 2.35, and the acetoxy group at C-6 to δ 2.05. The downfield shift also extended to the proton resonances of ring D, albeit less significantly. Compound **5** was named 3[A]chlorobifuhalol hexaacetate.

The FAB-mass spectrum of compound **6** ($C_{24}H_{21}O_{12}Cl$) showed $[M + H]^+$ at *m*/*z* 537 and 539 with a 6-fold ketene elimination series. The mass difference to difucol hexaacetate (M^+ 502)[4,14] again pointed toward a chlorine substitution in one of the rings. A 6-fold ketene elimination series starting from *m*/*z* 538/536 was observed in the EI-mass spectrum. In the ^{1}H NMR spectrum the aromatic proton at C-5 of ring [A_4] was found at δ 7.11, while those at C-3 and C-5 of ring A_4 gave a signal at δ 7.02. The first one showed a significant shift downfield because of the chlorine-substitution in this ring. The signals for the acetoxy groups at C-2 and C-4 of ring [A_4] were also shifted downfield (δ 2.04 and 2.38). Even the proton resonances for C-3 and C-5 of ring A_4 (δ 7.02) were influenced by the halogen atom. Compound **6** was named 3[A_4]chlorodifucol hexaacetate.







Experimental Section

General Experimental Procedures. EI-MS operation, 70 eV, ion source 200−300°; positive ion FAB-MS, Xe gun, 3-nitrobenzyl alcohol as matrix. ^{1}H NMR spectra (300 MHz), ^{1}H−^{1}H ROESY NMR spectra (500 MHz) and ^{13}C NMR (125 MHz) were recorded using solvents as internal standards.

Table 2. ^{13}C NMR Spectral Data of **1** and **2** (in $CDCl_3$)

	measd		
carbon	**1**	**2**	calcd[21]
ring type A			
1	138.0	136.2	136.8
2, 6	143.2	143.2	143.6
3, 5	115.0	115.0	113.8
4	146.6	146.6	146.2
ring type B			
1	154.2/154.2	154.2/154.3/154.3	153.1
2, 6	109.4	109.2/109.4/109.7	108.3
3, 5	143.6/143.7	143.7/143.8	143.9
4	134.7/134.7	134.7/134.7	134.7
ring type C			
1	147.6/147.9	147.6/147.9/147.9	147.5
2	134.2/134.3/134.6	134.3/134.5	134.4
3	137.9	138.1/137.9	136.6
4	131.4/131.6	131.4/131.6	131.2
5	140.2/140.2	140.2	139.2
6	109.2/109.3/109.7	109.5	109.0
ring type D			
1	155.0	154.7	155.2
2, 6	109.0	109.1	108.0
3, 5	143.7	143.9	144.3
4	130.4	130.3	130.2

Plant Material. The marine alga *C. angustifolium* J. Ag. was collected in October 1987 at Panetiki Island/Cape Rodney/New Zealand and transported frozen to Germany. It was identified by Dr. Dromgoole (University of Auckland) and a voucher specimen (No. 8714) deposited at the herbarium of the University of Auckland.

Extraction and Isolation. An alcoholic extract of 20 kg of frozen alga after concentration was shaken successively with petrol, chloroform, and ethyl acetate. After evaporation of the solvent, the ethyl acetate fraction (yield 68.1 g) was immediately acetylated with acetic anhydride/pyridine (yield 88.5 g). High molecular weight polymers were removed by precipitation using a mixture of ether and petrol; yield of low-M_r oligomers: 9.4 g. After a crude separation of 500 mg portions by flash chromatography on silica gel, the six fractions obtained were separately purified in several HPLC steps on silica gel columns by different gradients of $CHCl_3$ and *n*-hexane, MeOH, EtOH, and MeCN. Extraction and separation are described in detail by Glombitza and Schmidt.[1]

Hydroxyheptafuhalol-B nonadecaacetate (1): 8 mg; ^1H NMR data identical with ref 2, ^{13}C NMR see Table 2.

Octafuhalol-C heneicosaacetate (2): 10 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.90 (2H, s, ring A, H-3, 5), 6.71 (2H, s, ring B2, H-2, 6), 6.70 (2H, s, ring D, H-2, 6), 6.68 (2H, s, ring B1, H-2, 6), 6.67 (1H, s, ring C3, H-6), 6.66 (4 H, s, ring C1, C2, H-6; ring B3, H-2, 6), 2.26 (3H, s, ring A, Ac-4), 2.25 (6H, s, ring C1, C2; 3H, s, ring C3; 3H, s, ring D, Ac-4, respectively), 2.22 (3H, s, ring C2, Ac-3; 9H, s, ring C1, Ac-5, ring D, Ac-3, 5), 2.20 (9H, s, ring C1, Ac-3, ring C2, C3, Ac-5), 2.17 (3H, s, ring C3, Ac-3), 2.07 (6H, s, ring B3, Ac-3, 5), 2.04 (6H, s, ring B2, Ac-3, 5), 2.03 (6H, s, ring A, Ac-2, 6), 2.02 (6H, s, ring B1, Ac-3, 5); (Me_2CO-d_6) δ 7.02 (2H, s, ring A, H-3,5), 6.80 (2H, s, ring B3, H-2, 6), 6.79 (2H, s, ring D, H-2, 6), 6.77 (2H, s, ring B2, H-2, 6), 6.73 (2H, s, ring B1, H-2, 6), 6.70 (1H, s, C3, H-6), 6.69 (2H, 2s each 1H, ring C1, C2 H-6), 2.27 (3H, s, ring A, Ac-4), 2.26 (6H, s, ring C3, D Ac-4), 2.25 (6H, 2s each 3H, ring C1, C2, Ac-4), 2.22 (3H, s, ring C1, Ac-5), 2.21 (6H, s, ring D, Ac-3, 5; 3H, s, ring C1, Ac-3), 2.20 (3H, s, C2, Ac-3), 2.19 (6H, 2s each 3H, ring C 2, C3, Ac-5), 2.18 (3H, s, ring C3, Ac-3), 2.07 (6H, s, ring A, Ac-2, 6), 2.06 (6H, ring B3, Ac-3, 5), 2.05 (12H, 2s each 6H, ring B1, B2, Ac-3, 5); ^{13}C NMR see Table 2. FAB-MS ketene elimination series: *m/z* 1979 $[M + K]^+$, 1963 $[M + Na]^+ \rightarrow$ 1921, 1941 $[M + H]^+ \rightarrow$ 1353. EI-MS ketene elimination series: 1216 → 1090, 1214 → 1088, 992 → 530, 990 → 528, 934 → 514, 742 → 406, 740 → 404, 726 → 390, 724 → 388, 476 → 266, 474 → 264, 226 → 142.

2-Chlorophloroglucinol triacetate (3): 0.7 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (2H, C-4,6), 2.35 (6H, s, Ac C-1, 3), 2.28 (3H, s, Ac C-5). EI-MS ketene elimination series: 288/286 → 162/160.

2[D′]Iododiphlorethol pentaacetate (4): 1 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ ring A, 6.98 (2H, C-3, 5), 2.11 (6H, Ac C-2, 6), 2.29 (3H, Ac C-4); ring [D′], 6.74 (1H, C-4), 6.49 (1H, C-6) AB, J = 2.5 Hz), 2.36 (3H, Ac C-3), 2.22 (3H, Ac C-4). EI-MS ketene elimination series: 586 → 376.

3[A]Chlorobifuhalol hexaacetate (5): 2 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ ring [A], 7.05 (1H, C-5), 2.22 (3H, Ac C-2), 2.35 (3H, Ac C-4), 2.05 (3H, Ac C-6); ring D, 6.73 (2H, C-2,6), 2.26 (6H, Ac C-3,5), 2.27 (3H, Ac C-4). FAB-MS ketene elimination series: *m/z* 591 $[M + K]^+$, 575 $[M + Na]^+$, 553 $[M + H]^+ \rightarrow$ 301. EI-MS ketene elimination series: 554/552 → 302/300.

3[A_4]Chlorodifucol hexaacetate (6): 2 mg; ^1H NMR ($CDCl_3$, 300 MHz) δ ring [A_4], 7.11 (1H, C-5), 2.04 (3H, Ac C-2), 2.38 (3H, Ac C-4), 2.02 (3H, Ac C-6); ring A_4, 7.02 (2H, C-3,5), 2.07 (6H, Ac C-2,6), 2.30 (3H, Ac C-4). FAB-MS ketene elimination series: 559 $[M + Na]^+$, 537 $[M + H]^+ \rightarrow$ 285. EI-MS ketene elimination series: 538/536 → 286/284.

By means of ^1H NMR spectroscopy ($CDCl_3$ and Me_2CO-d_6, 300 MHz) and comparison with published data, thirty-seven known compounds were identified (Table 1).

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