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Trihydroxyphlorethols from the brown alga *Carpophyllum angustifolium*☆

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Abstract

The structural elucidation of four phloroglucinol derivatives isolated from the ethanolic extract of the brown alga *Carpophyllum angustifolium* after peracetylation is described. Three of them, trihydroxyheptaphlorethol-A octadecaacetate, trihydroxyoctaphlorethol-A eicosaacetate and trihydroxyoctaphlorethol-B eicosaacetate, are described for the first time. Characterized by three additional hydroxyl groups and two 1,2-diphenoxylated 3,4,5-triacetoxybenzene rings directly linked by an ether bond they are called trihydroxyphlorethols as a novel group of phlorotannins. The structural elucidation was carried out on the basis of spectral data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Carpophyllum angustifolium; Phaeophyceae; Sargassaceae; Phlorotannins; Trihydroxyphlorethols; Structural elucidation

1. Introduction

Carpophyllum angustifolium J. Ag. is a marine alga of New Zealand. Our specimen was collected at Panetiki Island/Cape Rodney. Brown algae very often contain a great number of phlorotannins based on phloroglucinol units (Ragan & Glombitza, 1986). Fourty-five compounds were obtained from the ethyl acetate fraction of the ethanolic extract of *C. angustifolium*. The present report will describe three phlorotannins consisting of phloroglucinol units linked exclusively by ether bonds and substituted by additional hydroxyl groups, the group was named trihydroxyphlorethols, and one fuhalol, which belongs to the fuhalol-A-series.

2. Results and discussion

After extraction of the frozen thallus with ethanol phenolic compounds were obtained from the concentrated extract by extracting with ethyl acetate. Because of the instability of the free phenols, the ethyl acetate fraction was immediately acetylated with acetic anhydride-pyridine. The high molecular mass phlorotannin acetates were separated into six partially purified fractions using a low-pressure silica gel column. Final purification of individual compounds was accomplished by HPLC. On TLC plates the compounds quenched the UV light (254 nm)-induced fluorescence. Their structures were elucidated using NMR and mass spectrometry (MS).

Three phloroglucinol derivatives were isolated in this manner, which belong to the novel group of trihydroxyphlorethols: trihydroxyheptaphlorethol-A octadecaacetate (**1**), trihydroxyoctaphlorethol-A eicosaacetate (**2**) and trihydroxyoctaphlorethol-B eicosaacetate (**3**). Additionally heptafuhalol-A octadecaacetate (**4**) was identified, the structure of which has been described previously (Sattler et al., 1977). **4** was used as a reference for the elucidation of the structural elements of compounds **1**–**3**.

Compound **1** and **2** are homologues. Compound **2** and **3** showed identical elemental compositions ($C_{88}H_{74}O_{47}$) and, therefore, gave rise to isobaric molecular ions in FAB MS ($[M+H]^+$ at m/z 1883). Judged

☆ From the dissertation of A. Schmidt, Bonn D5.

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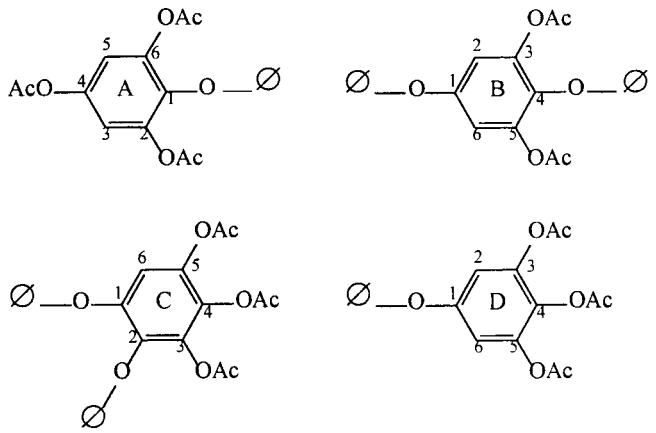


Fig. 1. Ring types A, B, C and D of peracetylated phlorotannins. ∅: aryl moiety.

by the mass difference of 208 amu between the molecular ions of compound **1** and **2** respectively **3**, compound **1** contained one 1,4-diphenoxylated 3,5-diacetoxybenzene ring (ring type B, Fig. 1) less than **2** and **3**. The FAB-mass spectrum of **1** ($C_{78}H_{66}O_{42}$) showed $(M+H)^+$ at m/z 1675.

A seven-fold ketene elimination series starting with the ion at m/z 682 could be observed in the EI-mass spectra of **1** and **2** and a nine-fold ketene elimination series starting with the ion at m/z 890 in the EI-mass spectrum of **3**. The precursor ion of these series arose from fission of the ether bridge between C2 and an adjacent B type ring. Thus, generating dibenzodioxin species, as starting points for ketene eliminations, allows the assignment of an A–B1–C1–C2 ring sequence to **1** and **2** and an A–B1–B2–C1–C2 chain to **3**, respectively. Compound **2** and **3** only differ in the position of ring B2, which is situated between ring C2 and B3 and between B1 and C1, respectively.

Compound **1** and **4** contain the same number of 1,2-diphenoxylated benzene units and 1,2-diphenoxylated 3,4,5-triacetoxybenzene units. The only difference is that ring B2 of compound **1** is not situated between ring C1 and C2, but between ring C2 and B3. The ^1H NMR spectra ($CDCl_3$) of both (Table 1) showed a downfield shift of 0.01_4 ppm for the C1 ring aromatic proton in **1** compared to that of **4** (δ 6.64_8 for **1** and δ 6.63_4 for **4**, measured in $CDCl_3$). The signal for the acetoxy groups at C-3 of ring C1 is shifted upfield (δ 2.16_1 for **1** and δ 2.18_9 for **4**, respectively). There is a direct linkage between ring C1 and C2. The protons at C-2 and C-6 of ring B2 are shifted downfield (δ 6.69_6 for **1** and δ 6.66_9 for **4**, respectively), those at C-2 and C-6 of ring B3 upfield (δ 6.64_4 for **1** and δ 6.70_5 for **4**, respectively). All other chemical shifts are very similar.

A resonance signal assignment had been previously published for **4** (Sattler et al., 1997), but a correction to the original assignments must be made after examination of $^1H^1H$ ROESY NMR spectra. The signal at δ 6.69_8 should be considered to arise from the protons at C-2 and C-6 of ring D and the signal at δ 6.70_5 from the protons at C-2 and C-6 of ring B3, respectively. The chemical shifts of the acetoxy groups of ring B1 are δ 2.03_1 and of those of ring B2 δ 2.03_9 ($CDCl_3$).

By comparison of the $^1H^1H$ ROESY NMR spectra of **1** and **4** the structure proposals for compound **1** and **4** could be confirmed. In compound **1** ring B1 is linked to ring A, because there is a correlation of the protons at C-2 and C6 of ring B1 with the acetoxy groups at C-2 and C-6 of ring A. The correlation of the proton at C-6 of ring C1 with the acetoxy groups at C-3 and C-5 of ring B1 confirms the direct linkage between ring B1 and C1 resulting in a A–B1–C1 sequence. The signal correlations of the aromatic protons at C-2 and C-6 of ring D with the acetoxy groups at C-3 and C-5 of ring B3 show the direct linkage between ring B3 and D. Since **1** and **4** differ in their chromatographical behaviour they clearly present different molecular species and there remains only one

Table 1
^1H NMR spectral data of **1**, **2**, **3** and **4**. Last decimal place only shows a tendency. Assignments of signals with the same superscripts are interchangeable

H	1 $CDCl_3$	2 $CDCl_3$	3 $CDCl_3$	4 $CDCl_3$
Ring A				
3,5	6.938	6.936	6.943	6.93
Ac-2, Ac-6	2.114	2.110	2.117	2.11
Ac-4	2.279	2.274	2.280	2.28
Ring B1				
2,6	6.648	6.648	6.677	6.64
Ac-3, Ac-5	2.053	2.052	2.096	2.031
Ring C1				
6	6.648[a]	6.645[a]	6.623[g]	6.63
Ac-3	2.161	2.199	2.187	2.19[g]
Ac-4	2.250[b]	2.247[b]	2.249[h]	2.251[h]
Ac-5	2.218[c]	2.215[c]	2.218[i]	2.211[i]
Ring C2				
6	6.664[a]	6.632[a]	6.667[g]	6.67
Ac-3	2.196	2.192	2.190	2.19[g]
Ac-4	2.259[b]	2.255[b]	2.254[h]	2.25[h]
Ac-5	2.214[c]	2.210[c]	2.218[i]	2.211[i]
Ring B2				
2,6	6.696	6.694	6.618	6.669
Ac-3, Ac-5	2.098	2.088	2.024	2.039
Ring B3				
2,6	6.644	6.644	6.697	6.705
Ac-3, Ac-5	2.023	2.040	2.065	2.07
Ring B4				
2,6		6.699	6.667	
Ac-3, Ac-5		2.066	2.039	
Ring D				
2,6	6.709	6.694	6.705	6.698
Ac-3, C-5	2.238	2.224	2.209	2.22
Ac-4	2.245[b]	2.255[b]	2.245[h]	2.254[h]

Table 2
^{13}C NMR spectral data of **1**, **2** and **3** (in $CDCl_3$). Last decimal place only shows a tendency

C	Measured			Calculated (Wegner-Hambloch Glombitza, 1985)
	1	2	3	
Ring Type A				
1	136.59	136.59	136.64	136.8
2, 6	143.64	143.69	143.61	143.6
3, 5	115.11	115.11	115.15	113.8
4	146.76	146.77	146.71	146.2
Ring Type B				
1	153.77/153.87/154.21	153.79/153.93/154.15/154.22	153.84/153.87/154.24	153.1
2, 6	109.22/109.55	109.57/109.62	109.14/109.57/109.65	108.3
3, 5	143.69/143.76	143.75/143.94	143.69/143.75/143.76/143.94	143.9
4	134.55/134.57/134.72	134.53/134.57/134.72	134.53/134.66/134.72	134.7
Ring Type C				
1	147.86/147.89	147.87/147.90	147.84/147.89	147.5
2	134.21/134.46	134.29/134.46	134.25/134.27	134.4
3	137.94	137.93	137.94/137.98	136.6
4	131.39/131.46	131.42/131.47	131.44/131.46	131.2
5	140.16/140.20	140.15/140.17	140.20	139.2
6	109.75	109.75	109.89	109.0
Ring Type D				
1	154.91	154.67	154.67	155.2
2, 6	108.90	109.15	109.43	108.0
3, 5	144.11	144.08	144.16	144.3
4	130.38	130.30	130.31	130.2

possibility for the ring arrangements in **1** which would explain these findings.

For compound **1** the occurence of ring types A, B, C and D (Fig. 1) was additionally confirmed by ^{13}C NMR. The measured data match the calculated values very well (Table 2). Compound **1** was named trihydroxyheptaphlorethol-A octadecaacetate.

The chemical shifts of ring A, B1 and C1 of compound **2** are very similar to the homologous compound **1** (Table 1). The signal of the acetoxy group at C-3 of ring C1 is shifted downfield (δ 2.19_9 for **2** and δ 2.16_1 for **1**, respectively), the signal for the proton at C-6 of ring C2 is shifted upfield (δ 6.63_2 for **2** and δ 6.66_4 for **1**, respectively), probably because of the different three dimensional structure of compound **2** (one additional B-type ring in comparison to compound **1**).

For compound **2** the $^1H^1H$ ROESY signal correlations were very similar to compound **1** and confirmed the sequence A–B1–C1–C2. As for compound **1,** the chemical shifts for the aromatic protons of the rings C1 and C2 could not be definitely assigned.

The ring types A, B, C and D for compound **2** were confirmed by ^{13}C NMR (Table 2), it was named trihydroxyoctaphlorethol-A eicosaacetate.

Compound **3** contains the same number of phloroglucinol units as compound **2,** but the ^1H NMR showed different chemical shifts (Table 1). For the aromatic protons of ring A a chemical shift of δ 6.94_3 ($CDCl_3$) was observed. Consequently, in compound **3** there is no ring sequence A–C1 like in the fuhalol-B-series (typically δ 6.91). The proton at C-6 of ring C1 is shifted upfield in comparison to compound **2** (δ 6.62_3 for **3** and δ 6.64_5 for **2**). This additional shielding demonstrates that in **3** two rings of type B must be situated between ring A and C1.

The signal correlations in the $^1H^1H$ ROESY spectrum confirmed the structural proposal for compound **3**. There are signal correlations of the proton at C-6 of ring C1 with the acetoxy groups at C-3 and C-5 of ring B2. The protons at C-2 and C-6 of ring B2 show correlations with the acetoxy groups of ring B1. Also, the aromatic protons at C-2 and C-6 of ring B1 correlate with the acetoxy groups at C-2 and C-6 of ring A. All of these observations points towards an A–B1–B2–C1 element. The signal correlations of the aromatic protons at C-2 and C-6 of ring D with the acetoxy groups at C-3 and C-5 of ring B4 suggest a direct linkage between ring B4 and D. Also in this case, the chemical shifts for the aromatic protons of the rings C1 and C2 could not be definitely assigned.

The ^{13}C NMR data of **3** (Table 2) showed similar chemical shifts for the ring types A, B, C and D as for the compound **2**. Compound **3** obviously is an isomer of **2** and was named trihydroxyoctaphlorethol-B eicosaacetate.

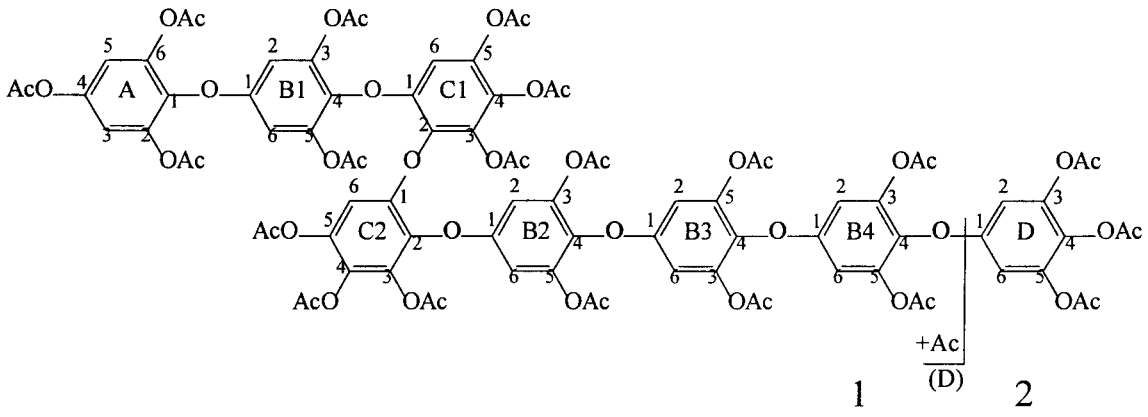
Chemical works in past have already shown, that a

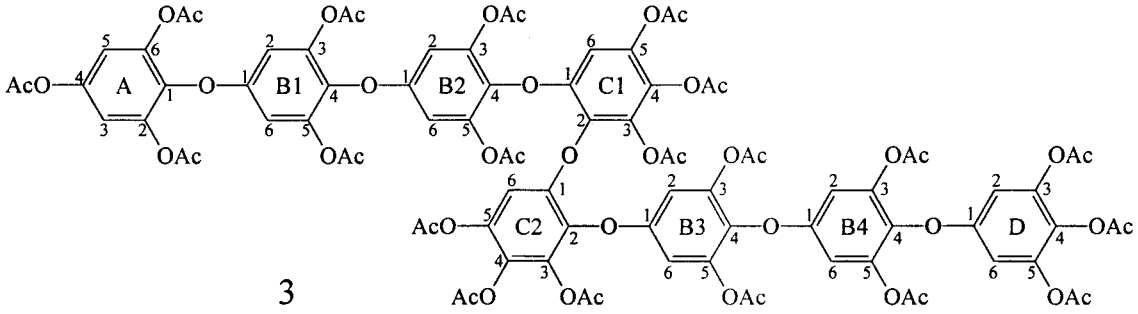
chemotaxonomic delimitation of Sargassaceae and Cystoseiraceae is very difficult. Fuhalols or fucophlorethols as main components have been isolated in both families (Glombitza, Wiedenfeld, & Eckhardt, 1978). Isolated from *Carpophyllum angustifolium*, Sargassaceae, trihydroxyphlorethols as a novel group of phlorotannins are described for the first time.

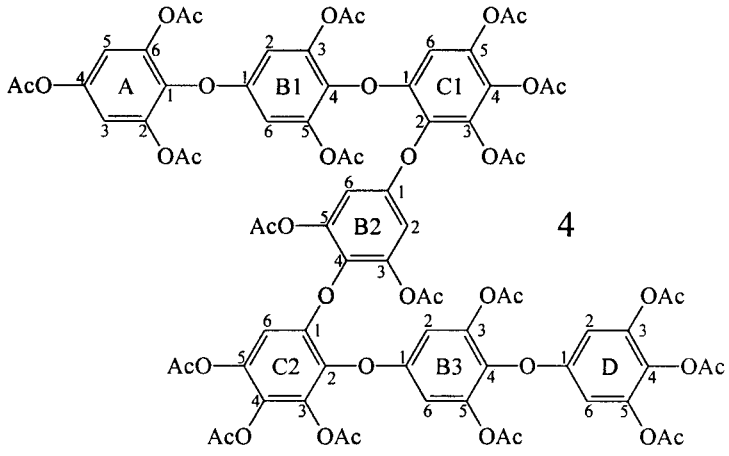
3. Experimental

3.1. EI–MS operation

MS 50 (Kratos), ion source 200–300°C, 70 eV, Finnigan MAT 95SQ, source-temperature 260°C, electron-energy 70 eV. Positive ion FAB–MS. Concept 1H







(Kratos) Xe gun, 3-nitrobenzylalcohol as matrix. ^{1}H NMR spectra (300 MHz) XL-300 (Varian), ^{11}H ROESY NMR spectra (500 MHz) and ^{13}C NMR (125 MHz) AMX500 (Bruker) were recorded using solvents as int. standards.

3.2. Plant material

Thalli of *Carpophyllum angustifolium* were collected in October at Panetiki Island/Cape Rodney/New Zealand at low water level (voucher specimen: Herbarium of the University of Auc1kland).

3.3. Extraction and isolation

Frozen thalli of *Carpophyllum angustifolium* (20 kg) were extracted with two portions of 20 l EtOH (96%) each. After concn to 3 l the aq. soln was extracted with petrol, $CHCl_3$ and EtOAc, respectively. The EtOAc layer was dried over Na_2SO_4 and evapd under red. press., yield 68.13 g. The extract was subsequently acetylated with Ac_2O-pyridine. The crude acetylated phlorotannins (88.46 g) were dissolved in $CHCl_3$ and treated with a mixt. of Et_2O and petrol (1:1). Small amounts of polymeric phlorotannins were pptd and removed by filtration. The soln contained the low-M_r oligomers. Portions of 500 mg (total 9.4 g) were separated by flash chromatography using a silica gel column (20 × 430 mm) with a step gradient: $CHCl_3$–*n*-hexane (1:1), $CHCl_3$, $CHCl_3$–MeOH (99:1), $CHCl_3$–MeOH (91:9), $CHCl_3$–MeOH (80:20). This separation was monitored by an UV detector (Serva chromatocord) and TLC (silica gel F_{254}, $CHCl_3$–MeOH, 9:1). Six frs were obtained, which were further sepd by HPLC (2 Knauer HPLC pumps, FR 30 or 64, gradient former or programmer 50) on Lichrosorb Si 60 (5 μm, 250 × 8 mm) with a $CHCl_3$–EtOH gradient and were detected at 254 nm, 270–275nm and 250–255 nm, respectively. For the sepn of related compounds solvent systems consisting of $CHCl_3$–*n*-hexane (1:1) or $CHCl_3$ and $CHCl_3$–MeCN (6:4) were used. Higher oligomers were separated on Lichrospher Si 60 (5 μm, 250 × 8 mm).

3.4. Isolated compounds

3.4.1. Trihydroxyheptaphlorethol-A octadecaacetate: 2,3,4,3′5′ -pentaacetoxy-6-(2,3,4-triacetoxy-6-(2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy)phenoxy) phenoxy)-4′-(3,5-diacetoxy-4-(3,4,5-triacetoxyphenoxy)phenoxy)-diphenylether (**1**)

7 mg. FAB–MS ketene elimination series: *m*/*z* 1713 $(M+K)^+$, 1697 $(M+Na)^+ \rightarrow$ 613, 1675 $(M+H)^+ \rightarrow$ 919. EI–MS ketene elimination series: 950 → 530, 948 → 528, 742 → 448, 648 → 390, 682 → 388, 476 → 264, 474 → 306, 226 → 142. ^{1}H NMR: $CDCl_3$: Table 1, d_6-Me_2CO: 7.044 (A, 2H, C-3,5), 2.100 (A, 6H, Ac-2, 6), 2.269 (A, 3H, Ac-4), 6.753 (B1, 2H, C-2,6), 2.068 (B1, 6H, Ac-3, 5), 6.653 (C1, 1H, C-6[a]), 2.154 (C1, 3H, Ac-3), 2.260 (C1, 3H, Ac-4[b]), 2.221 (C1, 3H, Ac-5[c]), 6.657 (C2, 1H, C-6[a]), 2.194 (C2, 3H, Ac-3[b]), 2.260 (C2, 3H, Ac-4), 2.217 (C2, 3H, Ac-5[c]), 6.760 (B2, 2H, C-2,6), 2.087 (B2, 6H, Ac-3, 5), 6.748 (B3, 2H, C-2,6), signal overlapped by signals of acetone (B3, 6H, Ac-3, 5), 6.805 (D, 2H, C-2,6), 2.233 (D, 6H, Ac-3, 5), 2.245 (D, 3H, Ac-4[b]), ^{13}C NMR: Table 2. [a–c] Assignments of signals with the same superscripts are interchangeable.

3.4.2. Trihydroxyoctaphlorethol-A eicosaacetate: 2,3,4,3′5′-pentaacetoxy-6-(2,3,4-triacetoxy-6-(2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy)phenoxy) phenoxy)-4′-(3,5-diacetoxy-4-(3,5-diacetoxy-4-(3,5-diacetoxy-4-(3,4,5-triacetoxyphenoxy)phenoxy)-phenoxy)phenoxy)diphenylether (**2**)

11 mg. FAB–MS ketene elimination series: *m*/*z* 1921 $(M+K)^+$, 1905 $(M+Na)^+ \rightarrow$ 1821, 1883 $(M+H)^+ \rightarrow$ 1253. EI–MS ketene elimination series: 950 → 740, 948 → 738, 684 → 390, 682 → 388, 476 → 264, 474 → 306. ^{1}H NMR: $CDCl_3$: Table 1, d_6-Me_2CO: 7.044 (A, 2H, C-3,5), 2.099 (A, 6H, Ac-2, 6), 2.268 (A, 3H, Ac-4), 6.756 (B1, 2H, C-2,6), 2.061 (B1, 6H, Ac-3, 5), 6.659 (C1, 1H, C-6[a]), 2.195 (C1, 3H, Ac-3), 2.223 (C1, 3H, Ac-4[b]), 2.218 (C1, 3H, Ac-5[c]), 6.648 (C2, 1H, C-6[a]), 2.195 (C2, 3H, Ac-3), 2.247 (C2, 3H, Ac-4[b]), 2.218 (C2, 3H, Ac-5[c]), 6.756 (B2, 2H, C-2,6), 2.088 (B2, 6H, Ac-3, 5), 6.729 (B3, 2H, C-2,6), signal overlapped by signals of acetone (B3, 6H, Ac-3, 5), 6.808 (B4, 2H, C-2,6), 2.074 (B4, 6H, Ac-3, 5), 6.801 (D, 2H, C-2,6), 2.218 (D, 6H, Ac-3, 5), 2.258 (D, 3H, Ac-4[b]),^{13}C NMR: Table 2.

3.4.3. Trihydroxyoctaphlorethol-B eicosaacetate: 2,3,4,3′,5′-pentaacetoxy-6-(2,3,4-triacetoxy-6-(2,6-diacetoxy-4-(2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy) phenoxy)phenoxy)phenoxy)-4′-(3,5-diacetoxy-4-(3,4,5-triacetoxyphenoxy)phenoxy)diphenylether (**3**)

9 mg. FAB–MS ketene elimination series: *m*/*z* 1921 $(M+K)^+$, 1905 $(M+Na)^+ \rightarrow$ 1821, 1883 $(M+H)^+ \rightarrow$ 1337. EI–MS ketene elimination series: 950 → 530, 948 → 780, 890 → 512, 684 → 390, 476 → 266, 474 → 264. ^{1}H NMR: $CDCl_3$: Table 1, d_6-Me_2CO: 7.052 (A, 2H, C-3,5), 2.108 (A, 6H, Ac-2, 6), 2.264 (A, 3H, Ac-4), 6.773 (B1, 2H, C-2,6), 2.089 (B1, 6H, Ac-3, 5), 6.727 (B2, 2H, C-2,6), signal overlapped by signals of acetone (B2, 6H, Ac-3, 5), 6.652 (C1, 1H, C-6[a]), 2.190 (C1, 3H, Ac-3), 2.264 (C1, 3H, Ac-4[b]), 2.199 (C1, 3H, Ac-5[c]), 6.696 (C2, 1H, C-6[a]), 2.199 (C2, 3H, Ac-3), 2.264 (C2, 3H, Ac-4[b]), 2.220 (C2, 3H, Ac-5[c]), 6.784 (B3, 2H, C-2,6), 2.067 (B3, 6H, Ac-3, 5), 6.730 (B4, 2H, C-2,6), signal overlapped by signals of acetone (B4, 6H, Ac-3, 5), 6.796 (D, 2H, C-2,6), 2.213 (D, 6H, Ac-3, 5), 2.246 (D, 3H, Ac-4[b]), ^{13}C NMR: Table 2.

*3.4.3. Heptafuhalol-A octadecaacetate: (**4**) (Sattler et al., 1997)*

30 mg. ^{1}H NMR: Table 1.

$^{1}H^{1}H$ ROESY NMR spectra and tables can be requested from the author of correspondence.

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