

POLYHYDROXYPHENYL ETHERS FROM *BIFURCARIA BIFURCATA**

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Key Word Index—*Bifurcaria bifurcata*; Phaeophyceae; Fucales; brown alga; acetylated phlorotannins; fuhalolacetates.

Abstract—In addition to the two to five-ringed fuhalol derivatives previously identified in the brown alga *Bifurcaria bifurcata*, a number of higher molecular phlorotannin acetates were isolated and characterized. These include some known and some newly identified fuhalolacetates: tetrafuhalol-B-undecacetate, desacetoxyheptafluhalol-heptadecacetate, heptafluhalol-octadecacetate, nonafluhalol-tricosacetate, undecafluhalol-octacosacetate and tridecafluhalol-tritriacontacetate.

INTRODUCTION

In previous analyses of *Bifurcaria bifurcata* Ross, phloroglucinol [2] and a number of low molecular phlorotannins [3, 4] were isolated and described in their acetyl form. An additional phlorotannin acetate fraction having higher molecular derivatives than the previously mentioned substances was obtained by column chromatographic separation (Si gel 60, CHCl_3) of acetylated algal extracts enriched following step-wise precipitation with Et_2O . This fraction was further separated by TLC and HPLC. All substances but one turned red-violet and then, later, brown on spraying with vanillin- H_2SO_4 after TLC and therefore, these are most probably derivatives of the fuhalol series.

RESULTS AND DISCUSSION

After TLC (Si gel F_{254} , CHCl_3 - Me_2CO , 22:3), eight strongly fluorescent extinguishing zones were identified in short-wave UV light. After HPLC column fractionation (Lichrosorb Si 60; gradient elution program with CHCl_3 - EtOH), the upper zone (R_f 0.61) showed peaks for three substances, 1, 2 and 3, having a relative intensity of ca 1:4:12.

Compounds 1 (R_f 45.6 cm^3) and 2 (R_f 47.4 cm^3) both showed a M^+ of m/z 992 and an eleven-times splitting of ketene (42 amu) in electron impact-induced MS. These were two isomers of tetrafluhalolacetate. The ^1H NMR spectra (CDCl_3 and $\text{Me}_2\text{CO}-d_6$) of 2 were identical with those in the literature [4, 5] for tetrafluhalolacetate. To differentiate it from other isomers, this molecule was named tetrafluhalol-A-acetate.

Resonance signals at δ 6.91 (2 H, a), 2.25 (3 H, A) and a signal at either 2.05 or 2.06 (6 H, B) ppm for a 2,4,6-triacetoxyphenoxy ring (I) were present in the ^1H NMR spectrum (CDCl_3) of 1. It is noteworthy that the frequency of the aromatic proton signals was

paramagnetically shifted ~ 3 –4 Hz and the signals of the six isochronic acetoxy protons between 2.0 and 2.1 were paramagnetically shifted ~ 7 Hz compared to the signals of the identically substituted ring I in 2 (δ 6.95 (2 H), 2.25 (3 H), 2.12 (6 H)) (Fig. 1). Moreover, the corresponding signal at δ 6.66 ppm [5] observed in 2 for ring II was missing in 1. Instead, two resonance signals δ 6.70₍₄₎ and 6.69₍₇₎ (each 2 H, b, d) were observed as in the molecular part C of other fuhalolacetates (Fig. 2). Thus, it can be concluded that the ring linked to I over an ether bridge is substituted differently in 1 than in 2.

The observed slight downfield shifting of the protons in ring I, however, could only have occurred if an aromatic ring with ether bridges in the *ortho* position (as in ring III) had been situated next to ring I, so that the protons in ring I were placed in the anisotropic range of two aromatic rings (for example, III and V of compound 1). All three acetoxy signals of the five-times substituted ring III were also shifted to a lower field. Therefore, this substance, named tetrafluhalol-B-acetate, must have structure 1 (Fig. 1) as Glombitza *et al.* [6] postulated in an earlier analysis of a mixture of both compounds but could not, at that time, positively confirm.

Compound 3 (HPLC; 50.1 cm^3) proved to be pentafluhaloltrideacetate [5, 6], whose structure was characterized by means of ^{13}C NMR spectra.

Compound 4 (R_f 0.54) could be isolated by TLC and characterized in MS and ^1H NMR spectra as desacetoxyheptafluhalolheptadecacetate. This compound was identical with a substance of the same name found and isolated by Glombitza *et al.* [6] in *Halidrys siliquosa*.

Compound 5 (R_f 0.47) appeared to be the heptafluhalol-octadecacetate that Glombitza *et al.* [4] previously postulated for this seaweed based on TLC results. MS, ^1H NMR and ^{13}C NMR spectra were identical with those of the substances isolated from *Halidrys siliquosa* [6, 7], *Sargassum muticum* [5] and *Laminaria ochroleuca* [8].

Compound 6 (R_f 0.34) was a higher homologue of 5 which, according to a comparison of MS and ^1H NMR

* Part 26 in the series "Antibiotics from Algae". For Part 25, see ref. [1].

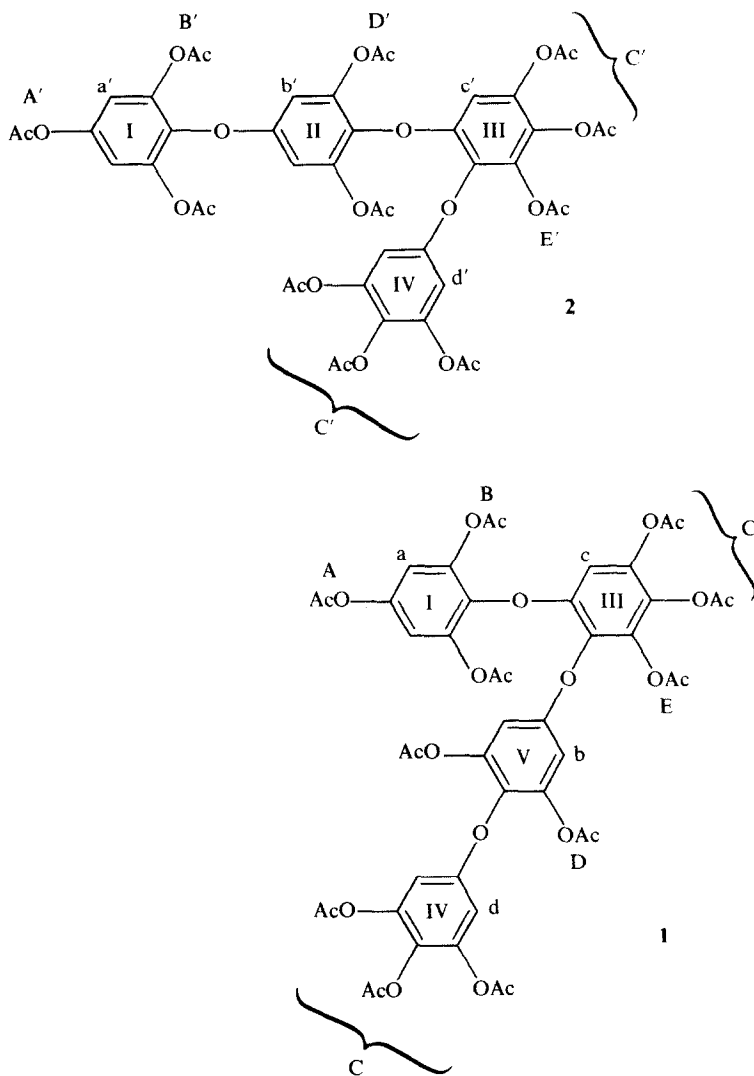


Fig. 1.

data, is identical in structure to the nonafuhaloltricosacetate previously isolated from *Sargassum muticum* [5].

Glombitza and Forster [5] were able to obtain a correlation between resonances and fuhahalolacetate protons only slightly differing from one another by means of a critical comparison of widely spread ^1H NMR spectra of fuhahalol derivatives from *Sargassum muticum*. Rules were then postulated pertaining to the influence of neighbouring substituents, even beyond ether bridges.

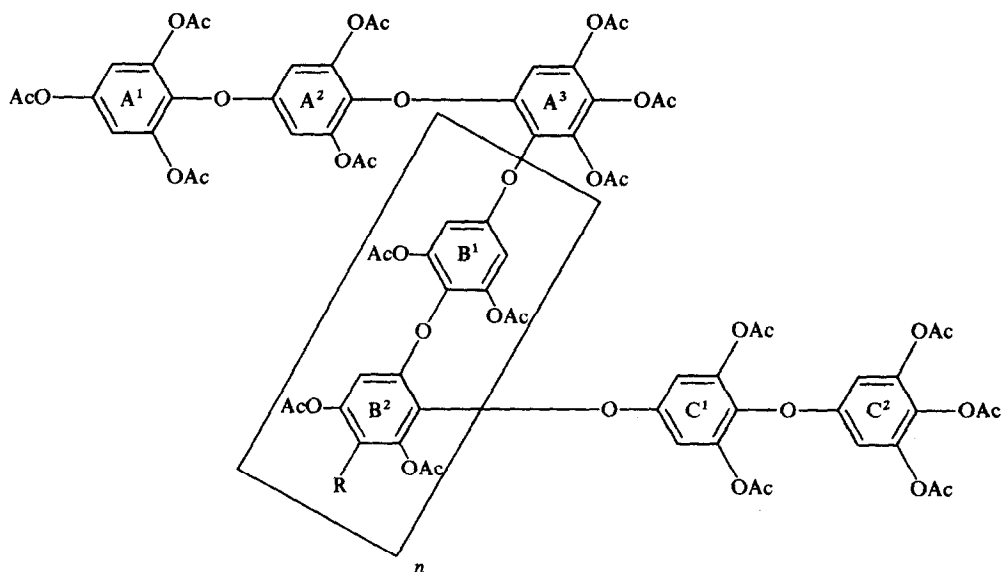
Fuhahalolacetates in discussion here contained three molecular parts (see Fig. 3): the starting part, **A**, having three rings; the terminal part, **C**, having two rings; and the middle part, **B**, having two rings, which could be present one or more times.

Part **A** caused the resonances of rings A^1 , A^2 and A^3 as shown in Fig. 3. The frequency of the aromatic protons was expressed by a shift in Hz of the signals at δ 7.04–7.06 ppm in ring A^1 (Fig. 4). Therefore, the existing deviations caused by differing conditions of measurement

could be eliminated. The methyl frequencies of the acetoxy groups were related to the most intense acetone line (Fig. 5). Part **C** had values of δ 6.83–6.82 (C^1), 6.77–6.76 (C^2), 2.25 (C^2), 2.21 (C^2) and 2.06–2.05 ppm (C^1). The middle part, **B**, consisted of one or more 3,5,3',4',5'-pentacetoxyphenyl ether units. These were substituted by additional elements of type **B** or **C** over another ether bridge in the *ortho* position to the diphenyl ether function of part **B**. The ^1H NMR frequencies were: δ 6.82–6.81 (B^1), 6.74–6.73 (B^2), 2.25 (B^2), 2.20–2.19 (B^2) and 2.07–2.06 ppm (B^1).

The fuhahalol series derivatives could be characterized by the following short forms: **3** consisted of **A** + **C** and was missing a **B** part. **5** consisted of **A** + **B** + **C** and **6** consisted of **A** + 2B + **C**.

No additional frequencies were observed in ^1H NMR spectra as the number of **B** elements increased. Instead, the signals became more intense. This intensification was most prominent in the aromatic signals of B^1 and B^2 . Compound **8** (R_f 0.24) had 2 H more in B^1 and 1 H more in B^2 than **6** to make a total of 18 H for the entire molecule. Compound **9** (R_f 0.18) had 4 H more in B^1 and 2 H more in B^2 than **6** to give the whole molecule a total of 21 H.



Compound	R	n	
3 Pentafulhaloltrideacetate	OAc	0	A + C
4 Desacetoxyheptafulhalolheptadecacetate	H	1	
5 Heptafulhaloloctadecacetate	OAc	1	A + B + C
6 Nonafulhaloltricosacetate	OAc	2	A + 2B + C
8 Undecafulhalolotacosacetate	OAc	3	A + 3B + C
9 Tridecafulhaloltritiacontacetate	OAc	4	A + 4B + C

Fig. 2.

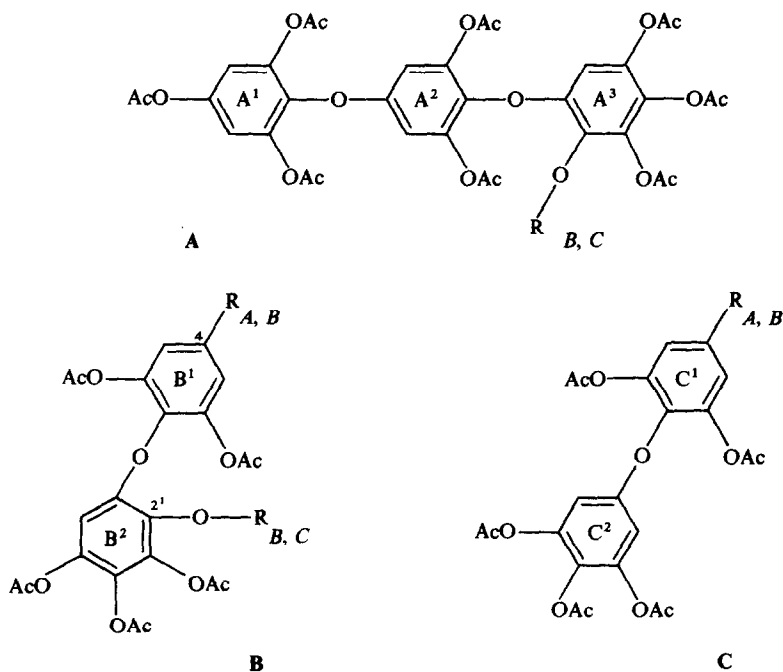


Fig. 3. Structure of elements A, B and C from fuhals.

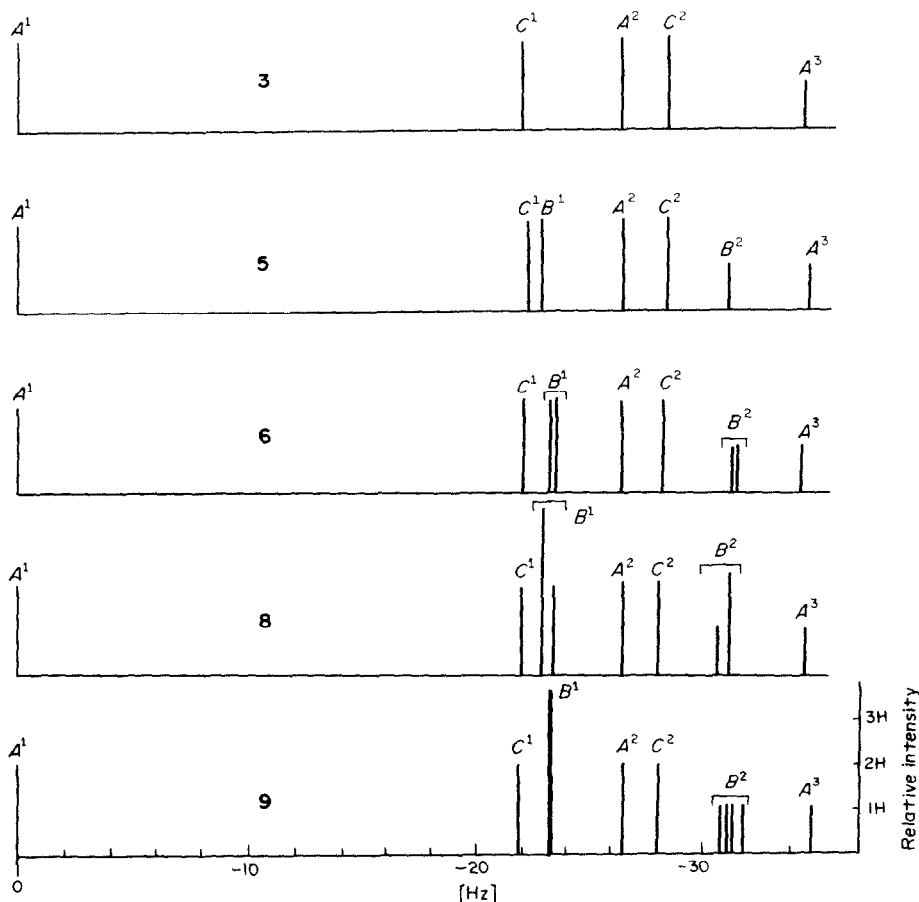


Fig. 4. Correlated aromatic ranges of the ^1H NMR spectra ($\text{Me}_2\text{CO}-d_6$, 90 MHz) of compounds **3**, **5**, **6**, **8** and **9**. The relative differences in Hz are levelled to the lowest signal (δ ca 7.06 ppm = 0 Hz).

A $\text{Me}_2\text{CO}-d_6$ spectrum of **8** showed a differentiation of acetoxy resonances in one group at δ 2.27–2.20 and another at 2.12–2.00 ppm indicative of different magnetic shielding. A group with signals for 16 acetoxyls could be found in the lower field. Signals for 19 acetoxyls were found in **9**, for which shifts from the proposed values of up to 1 Hz were observed. The frequencies between 2.00 and 2.10 ppm overlapped with the signals from the solvent, Me_2CO .

The spectra of **8** in CDCl_3 indicated the relationship between shielded and deshielded acetoxy groups to be 16 times 3 H (2.27–2.18 (48 H) ppm) to 12 times 3 H (δ 2.11, 2.07 (each 6 H), 2.04–2.02 (24 H) ppm) corresponding to the presence of 28 acetoxy groups. The relationship in **9** was 19 times 3 H (2.25–2.18 (57 H) ppm) to 14 times 3 H (δ 2.11, 2.07 (each 6 H), 2.05–2.03 (30 H) ppm), indicating the presence of 33 acetoxy substituents.

Based on the presence and the magnetic character of the 18 aromatic and the 28 acetoxy positions, it was assumed that **8** was an undecafulhalolotacosacetate with a structure homologous to **5** and **6**. In short form this can be represented by $\text{A} + 3\text{B} + \text{C}$. Compound **9** appeared to be a similarly composed tridecafulhaloltritiacontacetate, $\text{A} + 4\text{B} + \text{C}$ (Fig. 2).

The electron impact-induced MS (70 eV) for these substances indicated nothing about the calculated MW of 2622 (**8**) and 3096 (**9**). Thus, it can only be said that the fragmentation series of daughter ions (see Experimental)

supports the fact that these two compounds belong to the fuhalol derivative series.

A comparison of ^{13}C NMR spectra resonances from **6** and **8** with those of **3** and **5** showed that carbon atoms having a chemical shift which the postulated substitution model cannot account for were missing (Table 1).

Therefore, it can be concluded that the phlorotannin acetate mixture in *Bifurcaria bifurcata* is composed of a homologous series of derivatives whose structural make-up did not change until they reached very large MWs.

Compound **7** (R_f 0.29) differed from the other fuhalol derivatives in that it was a substance which turned red with vanillin- H_2SO_4 but did not turn brown rapidly thereafter. In MS, a ketene eliminating series from m/z 1084 (M^+) to the M^+ of the free phenol at m/z 622 could be observed. The position of the substance on TLC plates was in the range of 8–10 ring compounds or partly desacetylated substances with fewer rings. Because MS indicated that this substance had five rings, it must then have been a monodesacetyl derivative whose peracetyl form should have a M^+ of m/z 1126. Mass spectrally formed dibenzofuran ions from a fucol element were present, but only weakly represented. Additional ^1H NMR spectra showed typical signals for a biphenyl element (CDCl_3 , δ 6.98 (2 H), 2.27 (3 H), 2.02 (6 H) and 1.85 (3 H) ppm). However, no substituents (respectively resonances) could be assigned to the other positions of these rings.

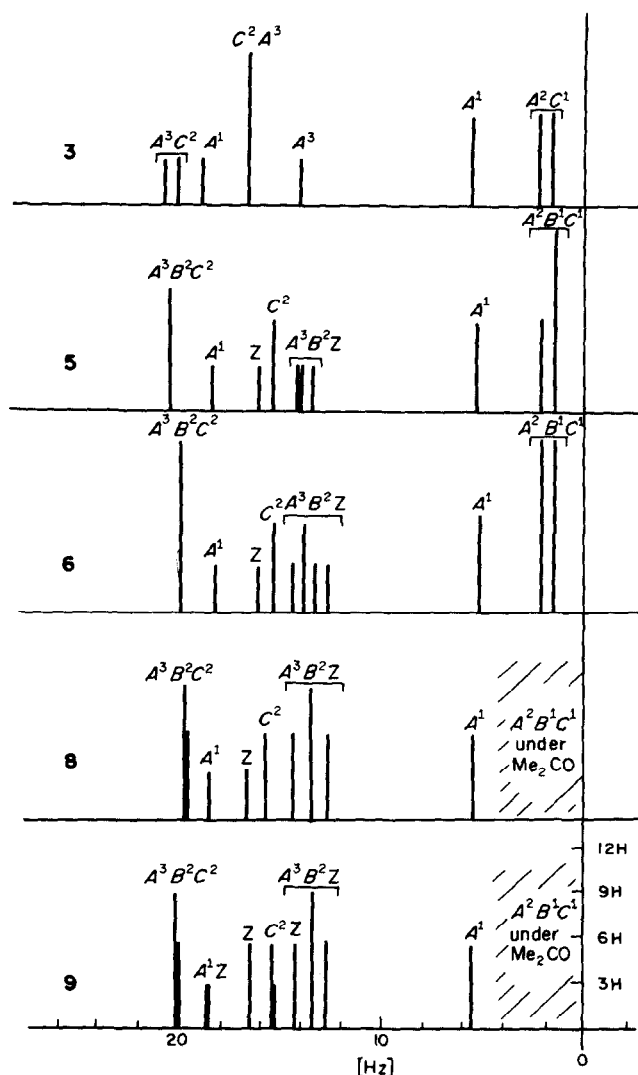


Fig. 5. Schematic correlation of ^1H NMR ($\text{Me}_2\text{CO}-d_6$, 90 MHz) resonance signals for the acetoxy groups of **3**, **5**, **6**, **8** and **9**. The relative differences in Hz are levelled to the most intense acetone signal = 0 Hz. Z indicates the unassigned signals of A^3 and/or B^2 .

The molecule also contained a 2,4,6-triacetoxyphenoxy terminal ring (δ 6.93 (2H), 2.27 (3H), 2.04 (6H) ppm). Nothing more can be said concerning substitution and bonding of the remaining aromatic rings other than the fact that a linear structure with *para*-bonded ether bridges can be eliminated from the structure possibilities due to the missing aromatic frequencies around δ 6.70–6.65 ppm.

EXPERIMENTAL

Extraction and enrichment. Freeze-dried *B. bifurcata* (4 kg), collected in Santeac/Brittanny (September 1971, April 1973), was extracted, acetylated, enriched and separated by column chromatography on Si gel with CHCl_3 as described in ref. [4]. The eluate was collected in 3 fractions. The phenol acetate mixture from the third fraction was separated by prep. TLC (Si gel F_{254} , CHCl_3 - Me_2CO , 22:3). Each zone was then detected under UV light and removed. The substances were subsequently eluted with Me_2CO and CHCl_3 - MeOH (9:1). **1**, **2**, **3** and **7** were further separated by HPLC (pumps: Knauer; columns: Lichrosorb Si 60, 5 μm ; length 25 cm, diameter 1 cm, detection at

270 nm; a gradient elution program in mixtures of CHCl_3 - EtOH). The substances were purified by precipitation of a Me_2CO soln by petrol.

Isolated substances. Tetrafulhalol-B-undecacetate (**1**), R_f 0.61, upper zone of prep. TLC; HPLC: R_f 45.6 cm^3 (0/0.9/1.05/1.15/1.45/2.4/7.0/0% EtOH); ^1H NMR (90 MHz, TMS, CDCl_3): δ 6.91 (a), 6.70, 6.69₍₇₎ (each 2H, b, d), 6.68 (1H, c), 2.25 (6H, A, C), 2.24 (3H, C), 2.22₍₈₎ (6H, C), 2.22₍₃₎ (3H, C), 2.21 (3H, E), 2.06, 2.05 (each 6H, B, D) ppm; ($\text{Me}_2\text{CO}-d_6$): δ 7.03 (2H, a), 6.79 (2H, b), 6.74 (2H, d), 6.71 (1H, c), 2.28, 2.26₍₄₎, 2.26₍₀₎ (each 3H, A, C), 2.24 (9H, C), 2.20 (3H, E) ppm; 12H *ca* 2.0 under the Me_2CO signals; equivalent to ref. [6] data; MS, ketene elimination series (70 eV, 280°) m/z : 248–416/250–418/264–474/266–476/374–(668)*/430–682/390–642/406–700/498–918/838–922/(638)*–890/514–934/530–992 of the entire molecule; UV $\lambda_{\text{max}}^{\text{MeCN}}$ nm: 271, 232 (shoulder).

* Elimination series whose beginning or end signal was weak.

Tetrafuhalol-A-undecacetate (**2**), TLC zone and HPLC program the same as **1**, R_f 47.4 cm³; ¹H NMR spectrum equivalent to refs. [5, 6].

Pentafluhaloltrideacetate (**3**), TLC zone and HPLC program the same as **1**, R_f 50.1 cm³; ¹H NMR spectrum equivalent to refs. [4, 6].

Desacetoxyeptafluhalolheptadecacetate (**4**), R_f 0.54, second zone of TLC; MS, ¹H NMR spectrum equivalent to ref. [6].

Heptafluhalolotadecacetate (**5**), R_f 0.47, 3rd zone of TLC; MS, ¹H NMR (CDCl₃, Me₂CO-*d*₆), ¹³C NMR spectra (CDCl₃, proton-decoupled, TMS), equivalent to refs. [6, 7].

Nonafuhaloltricosacetate (**6**), R_f 0.34, 5th zone of TLC; MS, ketene elimination series (70 eV, 300°) m/z : 248–416/250–334/264–474/266–434/370–622/372–624/374–500/386–680/388–682/390–684/404–740/406–700/496–874/498–(708)*/510–930/512–932/514–934/526–988/528–990/530–992/(754)*–1174/636–1140/638–1100/654–1158/836–(1130)*/778–1156/(918)*–1044/(886)*–(1954)*/(902)*–1070/916–1042/1124–1166/1182–1124 of the entire molecule; ¹H NMR (CDCl₃, Me₂CO-*d*₆) and UV spectra equivalent to ref. [5]; ¹³C NMR spectrum (CDCl₃, proton-decoupled, TMS), see Table 1.

Compound **7**, R_f 0.29, 6th zone of TLC; R_f 60.9 cm³ (0/1/1.1/1.5/1.8/2.5/7.0/0% EtOH); MS ketene elimination series (70 eV, 300°) m/z : 232–316/248–458/250–334/264–474/266–434/(354)*–606/(314)*–608/(370)*–706/372–708/374–(668)*/390–684/488–740/490–700/496–832/498–(834)*/596–848/514–808/572–908/(690)*–(942)*/(692)*–1028/(730)*–1066/(578)*–(956)*

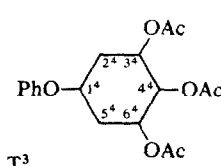
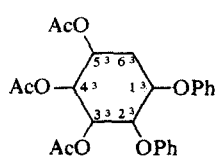
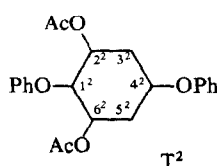
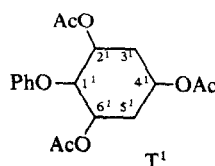
702–(954)*/762–1056/622–1084 of the entire molecule; ¹H NMR (CDCl₃): δ 6.98, 6.93, 6.61, 6.58, 6.38, 2.27, 2.17, 2.04–2.02, 1.91, 1.86 ppm; (Me₂CO-*d*₆): δ 7.07, 7.04, 6.68, 6.66, 6.47, 2.27, 2.20, 1.90, 1.86 ppm; range 2.1–2.0 covered by the solvent signals.

Undecafluhalolotacosacetate (**8**), R_f 0.24, 7th zone of TLC; MS ketene elimination series (70 eV, 300°) m/z : 248–416/250–334/264–474/266–434/370–622/372–624/374–500/386–680/388–682/390–684/404–740/406–700/496–(832)*/498–834/(510)*–888/512–890/514–892/(526)*–946/528–948/530–950/(636)*–(1098)*/638–(1058)*/654–1116/802–1180/762–1140/776–1154/778–1156/1128–1170 of the entire molecule; ¹H NMR (CDCl₃): δ 6.94, 6.72, 6.71_(s), 6.70 (each 2 H), 6.68 (5 H), 6.67, 6.66 (each 2 H), 6.65 (1 H), 2.27 (3 H), 2.24–2.18 (45 H), 2.11, 2.07 (each 2 H), 2.03, 2.02 (each 12 H) ppm; (Me₂CO-*d*₆): δ 7.06, 6.81 (each 2 H), 2.80 (6 H), 6.76, 6.74 (each 2 H), 6.71 (3 H), 6.67 (1 H), 2.27 (15 H), 2.26 (3 H), 2.22–2.23 (9 H), 2.20 (21 H), 2.12 (6 H) ppm; range 2.08–2.00 covered by the solvent signals; ¹³C NMR spectrum (CDCl₃, proton-decoupled), see Table 1; UV $\lambda_{\max}^{\text{MeCN}}$ nm (ϵ): 231 (shoulder, 109 250), 272 (24 470).

Tridecafluhaloltritriacontacetate (probably) (**9**), R_f 0.18, 8th zone of TLC; MS ketene elimination series (70 eV, 300°) m/z : 248–416/(250)*–334/264–474/266–434/370–622/372–624/374–458/386–680/388–682/390–684/404–740/406–700/494–(704)*/496–(874)*/498–(750)*/510–888/512–890/514–892/526–904/528–990/530–992/636–972/1004–1046/(886)*–1096/(960)*–1086/916–958/998/1068/(1070)*/1072/1080 of the entire molecule; ¹H NMR (CDCl₃): δ 6.94 (2 H), 6.72, 6.71_(s), 6.68,

Table 1. ¹³C NMR shift values (ppm) of compounds **3**, **5**, **6** and **8** in the fuhalol series with assignments to carbon atoms of ring type (T^x)

3 [1]	5 [1]	6	8	
Numbers of T ^x				
1T ¹ , 2T ² , 1T ³ , 1T ⁴	1T ¹ , 3T ² , 2T ³ , 1T ⁴	1T ¹ , 4T ² , 3T ³ , 1T ⁴	1T ¹ , 5T ² , 4T ³ , 1T ⁴	Assignment
168–166	168–166	168–166	168–166	Carbonyls
154.7	154.7	154.7	154.7	1 ⁴
154.2, 153.8	154.3, 153.8	154.4, 154.3, 154.2, 153.8	154.4, 154.3, 153.8	4 ²
147.9	148.0, 147.9	147.9, 147.8 _(s)	147.9, 147.8 _(s)	1 ³
146.8	146.8	146.8	146.8	4 ¹
144.0	144.0	144.0	144.0	3 ⁴ , 5 ⁴
143.8	143.8	143.8	143.8	2 ¹ , 6 ¹ , 2 ² , 6 ²
140.2	140.3	140.3	140.3	5 ³
138.0	138.0	138.0	138.0	3 ³
136.7	136.6	136.6	136.6	1 ¹
134.8	134.8	134.8	134.7	2 ³
134.6, 134.5	134.6, 134.5, 134.3	134.6, 134.5, 134.3	134.6, 134.4, 134.3	1 ²
131.6	131.5	131.5	131.5, 130.9	4 ³
130.4	130.4	130.3	130.4	4 ⁴
115.1	115.1	115.1	115.1	3 ¹ , 5 ¹
109.8, 109.6	109.7 ₍₃₎ , 109.6 ₍₆₎ , 109.5	109.7, 109.5	109.6 ₍₆₎ , 109.5	3 ² , 5 ²
109.1, 108.8	109.1	109.1	109.1	6 ³ , 2 ⁴ , 6 ⁴
21–20	21–20	22–20	22–20	Methyls



6.66, 6.65₍₅₎, 6.64₍₈₎ (together 20 H), 2.25, 2.22, 2.20, 2.18 (together 57 H), 2.11, 2.07 (each 6 H), 2.05, 2.03 (30 H) ppm; (Me₂CO-*d*₆): δ 7.06, 6.81 (each 2 H), 6.80 (8 H), 6.76, 6.74 (each 2 H), 6.70 (4 H), 6.67 (1 H), 2.27 (15 H), 2.25₍₅₎ (6 H), 2.23, 2.22₍₅₎ (15 H), 2.20 (21 H), 2.11 (6 H), 2.10–2.00 (covered by solvent) ppm; UV $\lambda_{\text{max}}^{\text{MeCN}}$ nm (ϵ): 231 (shoulder, 151 955), 272 (31 630).

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