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ISOFUHALOLS, A TYPE OF PHLOROTANNIN FROM THE BROWN ALGA *CHORDA FILUM**

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Key Word Index—*Chorda filum*, Chordaceae, brown algae, polyhydroxyphenols, phlorotannins, fuhalols, isofuhalols, structure elucidation

Abstract—From an acetylated fraction of an extract of *Chorda filum*, phloroglucinol triacetate and six 2- to 5-ringed phloroglucinol-derived polyhydroxyphenyl ethers were isolated and their structures elucidated Among these are three isofuhalols, compounds of a new structural type

INTRODUCTION

A mixture of polyhydroxyphenols soluble in ethyl acetate was extracted from *Chorda filum*, deep-frozen in a fresh state After acetylation, the higher polymers were removed from this mixture which could then be separated into their various components So far, only phloroglucinol triacetate (**1**) has been described from such a fraction [2] This paper reports on the structure elucidation of 2- to 5-ringed oligomers of peracetylated phlorotannins, some incompletely acetylated compounds as well as the examination of the polymeric portion

RESULTS AND DISCUSSION

It was possible to obtain 0 003 to 0 006 % ethyl acetate-soluble phenols (on a fr wt basis) from an evaporated ethanol extract of *C filum*, deep-frozen in a fresh state The weight of this fraction increased by between 19 and 36 % after acetylation Extraction on a semi-technical level was carried out in accordance with the method described by Glombitza [3] with the exception that a more powerful homogenizer and also a continual thin-layer evaporator were used The purest extracts were obtained when the extracts remaining in the thin-layer evaporator after the alcohol had been removed were lyophilized and the residue re-digested in ethanol (70 % v/v) The alcohol was again removed and the water phase subsequently extracted, first with petrol and then with ethyl acetate The higher polymeric portion of the phenols soluble in ethyl acetate was separated by fractional precipitation of the peracetyl derivatives with ether–petrol (1 1), as described elsewhere [4]

On TLC of the peracetylated oligomeric mixture (silica gel, chloroform–acetone, 9 1), 13 UV-quenching spots were detectable which, after spraying with vanillin–sulphuric acid, turned orange (**1**) or red and which, with the exception of two compounds, very quickly turned brown

The mixture was preseparated into 18 fractions using low-pressure column chromatography (LPLC) and HPLC on silica gel From these fractions seven peracetylated and seven incompletely acetylated phlorotannins were recovered Phloroglucinol triacetate (**1**), which has already been described [2], is found at R_f 0 71 on TLC In the R_f region between 0 66 and 0 54, the following substances already isolated from other brown algae are found diphlorethol pentaacetate (**2**), bifuhalol hexaacetate (**3**) which represents the largest yield of the substances found, and trifuhalol-A-octaacetate (**5**) (Fig 1) From the fraction containing **5**, it was also possible to enrich an isomeric compound **4** (MW 726) which has an identical R_f value (0 54) and only a slightly shorter R_t (HPLC), **6** and **7** are homologues of **4** In the mass spectrum, the intensity of the ion at m/z 126 suggests a symmetrical molecular structure with two similar terminal triacetoxyphenoxy rings At m/z 248, the last member of a ketene elimination series is found which was probably created by the cleavage of acetic anhydride and one of the two terminal rings This process involves the formation of a benzodioxin from a diphenyl ether moiety which carries acetoxy groups substituted at the *ortho* and *ortho'* position in relation to the ether bridge

In the region of the aromatic protons, the ^{1}H NMR spectrum ($CDCl_3$) of the 3-ringed substance **4** displays a singlet for four hydrogens at δ6 97 which can be assigned to two respective protons at the 3,5-position on the two terminal 2,4,6-triacetoxyphenoxy rings A singlet at δ6 20 is assigned to two hydrogens on the middle ring The acetyl protons of the middle ring give signals at δ2 24 (B) and 2 16 (C in $CDCl_3$) The structure of the middle ring can be confirmed with the aid of the ^{13}C NMR spectra of **6** and **7** (see below)

Compounds **6** and **7** differ from **4** in having an MW which is 208 or 2 × 208 mu greater This corresponds to one (in the case of **6**) or two (**7**) additional phloroglucinol rings, and allows calculation of the empirical formulae to be $C_{44}H_{38}O_{23}$ and $C_{56}H_{46}O_{28}$, respectively After cleavage of the diaryl ether bridges or the formation of benzodioxins, daughter ions are created which under

*Part 30 in the series "Antibiotics from Algae" For part 29 see ref [1] Dedicated to Prof Dr M Steiner on the occasion of his eightieth birthday

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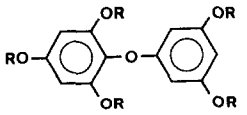
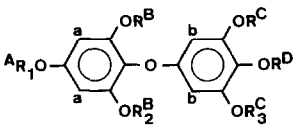


2 R = Ac

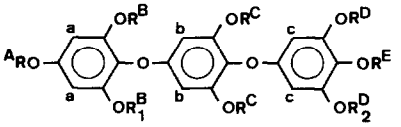


3 R_1 R_2 R_3 R = Ac

3a R_1 R_3 R = Ac R_2 = H

3b R_1 R_2 R = Ac R_3 = H

3c R_2 R_3 R = Ac R_1 = H

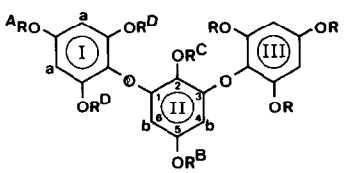


5 R_1 R_2, R = Ac

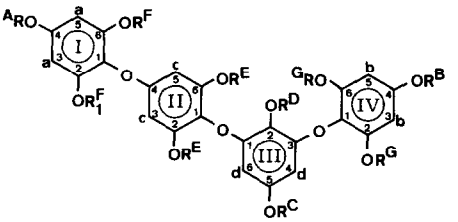
5a R_2, R = Ac R_1 = H

5b R_1, R = Ac R_2 = H

Fig 1 Structure of compounds **2**, **3** and **5** with assignment of the signals from the ^{1}H NMR spectra

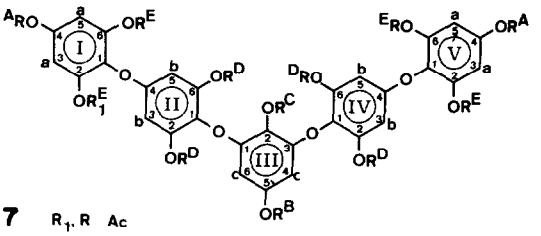


4



6 R_1 R Ac

6a R Ac R_1 H



7 R_1, R Ac

7a R Ac R_1 H

Fig 2 Structure of the isofuhalolacetates (**4**, **6**, **7**) with assignment of the signals from the ^{1}H NMR spectra

ketene elimination lead to a conspicuous series of ions with mu − 42 (748 → 580 → 496, 374 → 248)

The ^{1}H NMR spectrum ($CDCl_3$) of **6** differs from that of **7** in the region of aromatic protons by the splitting of the signal at δ6 96 into two singlets for 2 H respectively, and by the intensity of the singlets at δ6 70 Therefore, the protons of both terminal rings of **6** have in pairs a slightly different magnetic environment due to molecular asymmetry As this is evidently not the case with **7**, the 2,6-diacetoxyphenoxy moiety (two additional H at δ6 70, singlet, 4 H) which was built into the molecule must have led to the formation of a symmetrical compound

The ^{13}C NMR spectrum of **7** (Table 1) reveals fewer signals than that of **6** which has one ring less In the ^{13}C NMR spectrum of **6**, however, there are more pairs whose respective signals differ only very slightly from each other The symmetrical character of **7** results in magnetic equivalence of similar C-atoms and means that the C-atoms of rings I and V, of II and IV, and of C-4 and C-6 of ring III are indistinguishable For instance, C-4^{III} and C-6^{III} produce only one signal at 103 6 in the spectrum of **7** In the spectrum of **6**, however, a pair of signals (103 9, 103 7) is visible and they are particularly noticeable in the proton resonance coupled spectrum, in which C-3, 5^{I} and C-3, 5^{IV} give very close doublets of doublets (115 1, 115 0) and C-atoms 3, 5^{II} in turn, one doublet (109 8)

The 2,5-diacetoxy-1,3-diphenoxy substituted ring III is the characteristic structure element of this newly-discovered phlorotannin class called isofuhalols Without the acetylated hydroxyl group at C-2^{III}, the substance would have been identical to tetraphlorethol-A-nonaacetate described by Glombitza *et al* [6] from *Laminaria ochroleuca*, and the ring would have had the same substitution pattern as the middle ring of triphlorethol-C-heptaacetate [6] for whose C-atoms the ^{13}C NMR resonances are known A shift value of 126 8 was measured for C-2^{III} of **6** and **7** and so is in accordance with the value 126 7 calculated by Wegner-Hambloch [5] Deviations between measured shifts and those calculated by Wegner-Hambloch are in the region of 0 0 and 0 1 ppm Only the value assignd to C-4, 6^{III} displays a somewhat greater difference (2 0–2 3) Altogether though, the respective values are very much in agreement with each other (Table 1)

Compounds **3a–3c**, **5a**, **5b**, **6a** and **7a** were shown to be one-fold desacetylated derivatives of substances **3**, **5**, **6** and **7** The mass spectra correspond exactly to those of the peracetylated substances It is even possible to identify the $[M]^+$ of the peracetylated substance as a consequence of a change in acetylation, either during processing or in the ion source A free hydroxyl group results in a decrease in

Table 1 Found and calculated ^{13}C NMR values of compounds 6 and 7

Found			Found		Calculated in accordance with ref [5]
6		Calculated	7		
Rings I, IV			Rings I, V		
C-1	136 6	135 7*	C-1	136 6	136 8
C-2,6	143 7	143 8	C-2,6	143 7	143 6
C-3,5	115 1	115 0	C-3,5	115 1	114 8
C-4	146 8	147 0	C-4	146 8	146 2
Ring II			Rings II, IV		
C-1	133 9*		C-1	133 8*	134 7
C-2,6	144 3		C-2,6	144 2	143 9
C-3,5	109 8		C-3,5	109 7	108 3
C-4	154 1		C-4	154 0	153 1
Ring III			Ring III		
C-1,3	150 0/149 8		C-1,3	149 9	150 0
C-2	126 8		C-2	126 7	126 7
C-4,6	103 9/103 7		C-4,6	103 6	105 9
C-5	148 1		C-5	148 0	148 9

Measured in $CDCl_3$

*In the case of the *o*-substituent being a 2,6-diacetoxyphenoxy residue, a 'steric increment' has to be included in the calculation It has not yet been examined whether other values should be used for the 2-acetoxyphenoxy residue present in this case

R_f on TLC compared to the peracetylated substance The position of the free hydroxyl groups can be determined with the aid of the ^1H NMR spectra which are characteristically changed (additional coupling possible due to ring asymmetry) As incompletely acetylated compounds are artefacts, a more detailed discussion of the spectra has been omitted Otherwise, the ^1H NMR spectra of these compounds give further support for the proposed structures of **4**, **6** and **7** and also for the assignment of the NMR-signals

The signals of the ^1H and ^{13}C NMR spectra of the high MW portion obtained by fractional precipitation are on the whole similar to those of the isolated individual substances In the ^1H NMR spectrum additional signals are visible at δ7 13, 7 00 and 6 31–6 33, which together with the signal at 133 9 in the ^{13}C NMR spectrum are usually found either with substances with a biphenyl bond (fucols), or phenoxylated biphenyls (fucophlorethols) A signal, due to a proton *meta* positioned to a biphenyl and an aryl ether bond in each case, at δ7 13 in $CDCl_3$ would be expected to appear shifted to δ7 22 in the Me_2CO-d_6 spectrum However, no such signal occurs

On account of the intensity ratio of the ^1H NMR signals, phenoxylated bifuhalol acetate is to be regarded as the main structural element Signals at δ6 18–6 20 and in the ^{13}C NMR spectrum at 126 8 indicate the presence of isofuhalol elements

EXPERIMENTAL

Extraction and isolation Pulverized thalli from *C filum* (L) Stackhouse (130 kg) deep-frozen while still fresh (Le Caro/Brest, Brittany, France, August 1979) were divided into portions of 8–10 kg Each portion was suspended in 22 l 70% EtOH using an Ultraturrax X TP 65 for 90 min in an N_2 atmosphere with a controlled temp programme (−1 5 to 43°) and at slightly acidic pH (pH 5 8, addition of $K_2S_2O_5$) After settling, insoluble material was removed by filtration through glass wool The pellet was pressed hydraulically and the fluid obtained from this process was combined with the supernatant The solvent was removed under red pres in a thin-layer evaporator at 10–12 l/hr The evapn process was adjusted so that not more than 1 1–1 5 l aq concentrate remained (variation 1) With variation 2, a large excess of Me_2CO (6 l) was added to the aq concentrate with careful stirring and the ppt passed through a suction filter After repeating precipitation once, the ppt was made Me_2CO-free With variation 3, the aq concentrate (1 2 l) was lyophilized (yield 180 g from a 9 kg portion) and digested for 15 min in 600 ml EtOH (60%) The suction-filtered substance which did not dissolve was re-extracted with 400 ml 70% EtOH and the EtOH filtrate evapd The aq concentrate obtained (250 ml) was treated in the following manner shaking with petrol as well as 5–6 times with EtOAc, the combined EtOAc phases were dried, filtered, reduced to dryness under red pres and acetylated Yield 0 003–0 006% free phenols and 0 004–0 009% acetylated phenols, altogether 9 2 g acetyl derivatives

Enrichment of the oligomeric phlorotannins was carried out in three steps Separation of the higher MW portion by fractional precipitation with Et_2O–petrol (1 1) [4], LPLC, glass column 280 × 15 mm packed with silica gel 60, 0 040–0 063 mm/230–400 mesh ASTM, absorbance monitor, mobile phase $CHCl_3/CHCl_3$–Me_2CO (9 1)/$CHCl_3$–Me_2CO (7 5 2 5), flow rate 640 ml/hr, gradient elution, amount of sample per column 100 mg HPLC preseparation 2 HPLC pumps with gradient former on LiChrosorb Si 60, 10 μm, 9 6 × 250 mm, with $CHCl_3$–EtOH gradients (up to 10% EtOH), detection at 270 nm, flow rate 8 ml/min, amount of sample per run 20 mg Of the 18 fractions obtained from preseparation, 11 were chosen for further HPLC separation which in each case was carried out with 1 5–3 5 mg sample

Isolated substances Yield in reference to 130 kg fresh alga, R_f on silica gel $CHCl_3$–Me_2CO (9 1), detection 1% vanillin in conc H_2SO_4, 5–10 min heating at 105–110° Phloroglucinol triacetate (**1**), 2 0 mg, R_f 0 71, UV, MS, ^1H NMR identical to authentic material Diphlorethol pentaacetate (**2**), 3 0 mg, R_f 0 66, UV, MS, ^1H NMR values as in ref [7] Bifuhalol hexaacetate (**3**), 42 0 mg, R_f 0 60, UV, MS, ^1H NMR values as in ref [7] Trifuhalol-A-octaacetate (**5**), 13 mg, R_f 0 54, UV, MS, ^1H NMR values as in ref [8] Trisofuhalol octaacetate, 1,3-*bis*(2,4,6-triacetoxyphenoxy)-2,5-diacetoxybenzene (**4**), 1 8 mg (mainly consisting of **4**), R_f 0 54, UV λ_{max}^{MeCN} nm 212, 271, 228 (shoulder), MS (230°, 70 eV, main ketene elimination series) *m/z* 726 ([M]$^+$) → 390, 668 → 374, 624 → 372, 476 → 266, 376 → 250, 374 → 248, 184 → 142, 168 → 126, ^1H NMR ($CDCl_3$) δ6 97 (4H, a), 6 20 (2H, b), 2 28[a] (6H, A), 2 24[a] (3H, B), 2 16 (3H, C), 2 13 (12H, D, [a] = can be derived only from intensity differences because together with **5**), (Me_2CO-d_6) (same sequence as above) δ7 08, 6 27, 2 28[a], 2 25[a], 2 14, 2 13

Tetraisofuhalol decaacetate, 2,6,2',5'-tetraacetoxy-4-(2,4,6-triacetoxyphenoxy)-3'-(2,4,6-triacetoxyphenoxy)diphenyl ether, (**6**), 16 mg, R_f 0 48, UV λ_{max}^{MeCN} nm 216, 271, 228 (shoulder), MS (230°, 70 eV, main ketene elimination series) *m/z* 934 ([M]$^+$) → 514, 876 → 498, 748 → 500, 684 → 390, 668 → 374, 434 → 266, 418 → 250, 374 → 248, 142, 168 → 126, ^1H NMR ($CDCl_3$) δ6 96$_3$ (2H, a), 6 96 (2H, b), 6 70 (2H, c), 6 18 (2H, d), 2 28 (9H, A, B, C), 2 15 (3H, D), 2 14, 2 12, 2 11 (each 6H, E, G, F), (Me_2CO-d_6) δ7 08 (4H, a, b), 6 81 (2H, c), 6 25 (2H, d), 2 28 (6H, A, B), 2 26 (3H, C), 2 15 (3H, D), 2 14, 2 13$_3$, 2 13 (each 6H, E, F, G)

Pentaisofuhalol dodecaacetate, 1,3-*bis*[2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy)phenoxy]-2,5-diacetoxybenzene, (**7**), 19 mg, R_f 0 43, UV λ_{max}^{MeCN} nm 216, 272, 228 (shoulder), MS (250°, 70 eV, main ketene elimination series) *m/z* 1142 [M]$^+$ → 848,

1084 → 622, 892 → 514, 876 → 498, 790 → 496, 642 → 390, 668 → 374, 540 → 372, 476 → 266, 376 → 250, 332 → 248, 184 → 142, 168 → 126, ^{1}H NMR ($CDCl_3$) δ6 96 (4H, a), 6 70 (4H, b), 6 17 (2H, c), 2 28$_2$ (6H, A), 2 28 (3H, B), 2 14 (3H, C), 2 12, 2 09 (each 12 H, D, E), (Me_2CO-d_6) (same sequence as above) δ7 07, 6 80, 6 23, 2 28, 2 26, 2 15, 2 13, 2 11

Partially acetylated derivatives Bifuhalol pentaacetate, 3,4,5,2',4'-pentaacetoxy-6'-hydroxydiphenylether, (**3a**), R_f 0 34, ^{1}H NMR ($CDCl_3$) δ6 75 (1H, C-3'), 6 61 (1H, C-5', AB-system), 6 73 (2H, b), 2 29 (3H, A), 2 26 (3H, D), 2 25 (6H, C), 2 01 (3H, B), (Me_2CO-d_6) δ (same sequence as above) 6 75, 6 60, 6 68, 2 29, 2 26, 2 25, 2 03

Bifuhalol pentaacetate, 3,4,2',4',6'-pentaacetoxy-5-hydroxydiphenyl ether (**3b**), R_f 0 29, ^{1}H NMR ($CDCl_3$) δ6 94 (2H, a), 6 44 (1H, C-2), 6 40 (1H, C-6, AB-system), 2 29 (3H, A), 2 27 (3H, D), 2 25 (3H, C), 2 09 (6H, B), (Me_2CO-d_6) δ7 02, 6 34, 6 34 (a very compact AB-system), 2 27, 2 26, 2 24, 2 08

Bifuhalol pentaacetate, 3,4,5,2',6'-pentaacetoxy-4'-hydroxydiphenyl ether (**3c**), R_f 0 13, ^{1}H NMR ($CDCl_3$) δ6 58 (2H, a), 6 69 (2H, b), 2 25 (3H, D), 2 24 (6H, C), 2 09 (6H, B), (Me_2CO-d_6) δ6 66, 6 70, 2 25, 2 23, 2 09

Trifuhalol-A-heptaacetate, 2,6-diacetoxy-1-(3,4,5-triacetoxyphenoxy)-4-(2,4-diacetoxy-6-hydroxyphenoxy)benzene (**5a**), R_f 0 29, ^{1}H NMR ($CDCl_3$) δ6 77 (1H, a = C-3), 6 62 (1H, b = C-5, AB-system), 6 71 (2H, b), 6 70 (2H, c), 2 29 (3H, A), 2 26 (3H, E), 2 24 (6H, D), 2 09 (6H, C), 2 04 (3H, B), (Me_2CO-d_6) δ6 76, 6 59 (AB-system), 6 71, 6 70, 2 26, 2 25, 2 25, 2 08, 2 05

Trifuhalol-A-heptaacetate, 2,6-diacetoxy-1-(3,4-diacetoxy-5-hydroxyphenoxy)-4-(2,4,6-triacetoxyphenoxy)benzene (**5b**), R_f 0 24, ^{1}H NMR ($CDCl_3$) δ6 95 (2H, a), 6 69 (2H, b), 6 38 (2H, c), 2 29 (3H, A), 2 27 (3H, E), 2 25 (3H, D), 2 12 (6H, C), 2 07 (6H, B), (Me_2CO-d_6) δ7 06, 6 76, 6 35, 6 34 (each 1H, c, AB-system) 2 28, 2 24, 2 13, 2 08

Tetraisofuhalol nonaacetate, 2,6,2',5'-tetraacetoxy-4-(2-hydroxy-4,6-diacetoxyphenoxy)-3'-(2,4,6-triacetoxyphenoxy)diphenyl ether, (**6a**), R_f 0 24, ^{1}H NMR ($CDCl_3$) δ6 76 (1H, on C-5^I), 6 61 (1H, on C-3^I, AB-system), 6 71 (2H, c), 6 17 (2H, d), 6 96 (2H, b), 2 28 (9H, A, B, C), 2 13 (3H, D), 2 11 (12H, E, G), 2 03 (3H, F), (Me_2CO-d_6) δ6 77, 6 61 (AB-system), 6 74, 6 24 (1H on C-6^{III}), 6 20 (1H on C-4^{III}, AB-system), 7 07, 2 27 (3H, B), 2 25 (6H, A, C), 2 14 (3H, D), 2 11, 2 12 (each 6H, E, G), 2 07 (3H, F)

Pentaisofuhalol undecaacetate, 1-[2,6-diacetoxy-4-(2-hydroxy-4,6-diacetoxyphenoxy)phenoxy]-3-[2,6-diacetoxy-(2,4,6-triacetoxyphenoxy)phenoxy]-2,5-diacetoxybenzene, (**7a**), R_f 0 17, ^{1}H NMR ($CDCl_3$) δ6 76 (1H on C-5^I), 6 61 (1H on C-3^I, AB-system), 6 71, 6 70 (each 2H, b), 6 16 (2H, c), 6 96 (2H, a^V), 2 27 (9H, A, B, A), 2 13 (3H, C), 2 12 (6H, b^{IV}), 2 10 (6H, b^{II}), 2 09 (6H, E^V), 2 04 (3H, E^I), (Me_2CO-d_6) δ6 76, 6 61 (AB-system), 6 73, 6 20, 6 21 (AB-system), 6 79, 7 06, 2 28, (3H, A^V), 2 25 (6H, A^I, B), 2 14 (3H, C), 2 12 (6H, D^{IV}) (signals for E^I and D^{II} under Me_2CO)

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