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PHLOROTANNINS OF PHAEOPHYCEA *LAMINARIA OCHROLEUCA**

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Key Word Index — *Laminaria ochroleuca*; Phaeophyceae; Laminariales; acetylated phlorotannins; phlorethols; fucophlorethols.

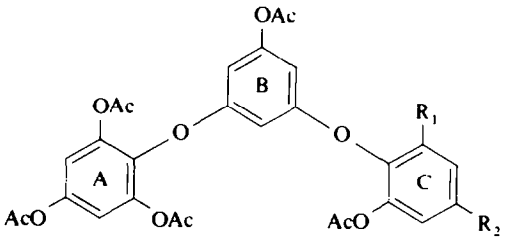
Abstract—From the mixture of acetylated phenols of *Laminaria ochroleuca* several fractions consisting of one or more components were isolated and analysed. Several substances, new or known from other seaweeds were identified: tetraphlorethol-A-nonacetate, fucophlorethol-B-octacetate, fucodiphlorethol-C-decacetate, pentafuhaloltridecacetate, and heptafuhaloloctadecacetate. A structure for an isomer of tetraphlorethol-A-nonacetate named tetraphlorethol-B-nonacetate was suggested. Additional phlorotannins were shown to be present, for which only partial structures could be proven.

INTRODUCTION

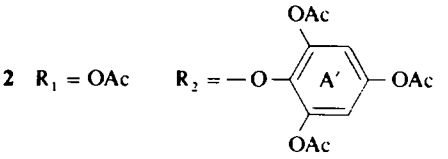
Recently we described the isolation of acetylated di- and triphlorethol C together with their monochlorinated derivatives from the Phaeophycea *Laminaria ochroleuca* [1, 2]. In the enriched acetylated extract further low molecular weight phlorotannins are present. Definite structures for some of them were determined, and others were at least partially characterized.

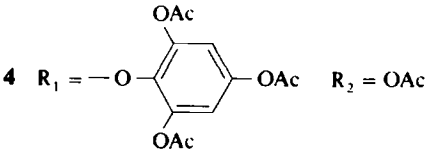
RESULTS AND DISCUSSION

A spot at R_f 0.37 on TLC (Si gel 60, $CHCl_3$–Me_2CO, 47:3) contained a mixture of compounds **2** and **3**. These compounds were eluted as a mixture from a HPLC Si gel column with various gradient programs ($CHCl_3$–EtOH). In the electron impact-induced MS, **2** had an M^+ at *m/e* 876 (corresponding to $C_{42}H_{36}O_{21}$) and lost ketene (42 mu) up to nine times to yield the free phenol $C_{24}H_{18}O_{12}$ (*m/e* 498). Compound **2** therefore seemed to be a member of the phlorethol series with four benzene rings and nine acetylated HO groups. In many respects, the ^1H NMR spectrum was very similar to that of triphlorethol-C-acetate (**1**) [1] with resonances at δ 6.95, 2.26, 2.08 and 2.06 due to two terminal 2,4,6-triacetoxylated phenoxyl groups and additional resonances for three isochronic acetoxy protons at 2.20 and an AB_2 spin system between 6.31 and 6.45 with $J_{AB} \sim 2$ Hz. Because of the low amount of isolated material, the spectra had to be taken by the CAT-technique. Therefore, the line width was large and the signals were not resolved totally. In the spectra of **2** additional resonances beyond **1** are found at 6.71 (2 aromatic H) and 2.12 (6 acetoxy-H). They must belong to a diacetoxybenzene ring linked by two *para*-situated ether bridges. Thus, compound **2** was 2,6,5′-triacetoxy-4,3′-bis(2,4,6-triacetoxyphenoxy) diphenylether, commonly called tetraphlorethol-A-nonacetate. As mentioned above, **2** was contaminated by small quantities of another substance, **3**. Up to now, the mixture could not be resolved either by TLC or by HPLC. However, the MS and the ^1H NMR resonances of **3** were identical with those of fucophlorethol-B-octacetate recently isolated from *Cystoseira baccata* [3].



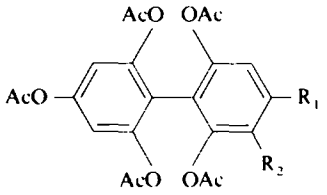
1 R_1/R_2 = OAc

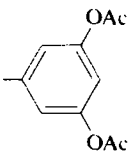




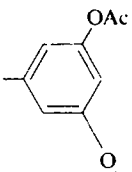
Compound **4**, combined with an approximate 10% impurity of **5**, was found at R_f 0.33. Substance **4** showed a molecular ion at *m/e* 876 which eliminated up to nine

* Part 24 in the series "Antibiotics from Algae". For Part 23 see Glombitza, K.-W., Forster, M. and Eckhardt, G. (1978) *Phytochemistry* **17**, 579. Part of the dissertation by M. Koch, 1978, Bonn D 5.





3 R_1 = OAc; R_2 = —O—



5 R_1 = [OPh(OAc)$_2$]$_2$OAc R_2 = —H

6 R_1 = OAc R_2 = —O—

ketene units. It consisted of four ether-linked benzene rings substituted by nine acetoxy groups, therefore **4** appeared to be an isomer of **2**. Compound **4** showed the same ^{1}H NMR resonances for the rings A, B, respectively A', as **1** or **2**. The AB system of ring C (ν_A = 6.68, ν_B = 6.71 ppm, J_{AB} = 2 Hz) was overlapped by some signals of **5**. As the Me signals around 2 ppm cannot be assigned exactly, it is impossible to absolutely prove the existence of a 2,3-diphenoxy-1,4-diacetoxybenzene structure element for ring C. Such an element would be in agreement with the theory of biosynthesis. It should be pointed out that Glombitza *et al.* [4] demonstrated the presence of the same substitution pattern in a desacetoxyheptafuhalol with different ^{1}H NMR values (ν_A = 6.51, ν_B = 6.72 ppm). Attempts to synthesize a model substance with a similar substitution pattern have failed up to now. Tentatively, **4** is thought to have the structure of a tetraphlorethol-B-nonacetate isomeric to **2**.

A signal at *m/e* 912 was attributed to the molecular ion of **5**. The elimination of H_2O from partially deacetylated fragments containing three or four rings leading to ions with a dibenzofuran structure [5], together with the ^{1}H NMR spectrum, gives evidence that **5** is a fucodiphloretholdecacetate. The resonances at δ 6.99, 6.70 (each 2 aromatic H) and 2.04, 1.97 (each 6 isochronic acetoxy protons) were typical signals of a 4'-phenoxy substituted 2,4,6,2',6'-pentacetoxybiphenyl moiety. Partial structures like this have been described for some of the phlorotannins found in *Fucus vesiculosus* [5]. As yet, nothing can be said about the two other rings of **5**.

From a spot at R_f 0.27, a MS was taken with a molecular ion at *m/e* 918 ($C_{44}H_{38}O_{22}$, **6**). A free phenol (*m/e* 498, $C_{24}H_{18}O_{12}$) was left after a ten-fold ketene elimination. Some of the partially or totally deacetylated ions lost H_2O. This behaviour is typical of the above-mentioned fucophlorethols. Resonances at δ 7.09 (1 H), 7.0 (2 H), 2.27 (3 H), 2.06 (9 H), 2.03 (3 H) and 1.86 (3 H) gave evidence that **6** contained a 2,4,6,2',4',6'-hexacetoxybiphenyl-3-phenoxy element like **3**. Thus, **6** seemed to be an isomer of **5**. Between δ 6.29 and 6.46, **6** showed a poorly resolved three-spin coupling system similar to **1**. A distinct signal at 6.95 and further resonances in the acetoxy-range showed the presence of a symmetrically substituted 2,4,6-triacetoxyphenoxy group. According to the spectral data, **6** had the structure of a 2,4,6,2',4',6'-hexacetoxy-3-[5-(2,4,6-triacetoxyphenoxy)-3-acetoxyphenoxy] biphenyl (fucodiphlorethol-C-decacetate).

The spot at R_f 0.26 was an inseparable phlorotannin mixture. One component, **7**, gave a molecular ion at *m/e* 1126 ($C_{54}H_{46}O_{27}$) which fragmented by successive loss of ketene to an ion at *m/e* 748. Compound **7** seemed to be the next higher homologue of either **6** or **5**. The ^{1}H NMR spectrum did not allow further conclusions as to the structure of **7**.

Laminaria ochroleuca specimens from two different locations, Roscoff/Le Loup and Ménéham, were investigated. The Ménéham material contained two more derivatives (**8** and **9**) of the fuhalolacetate type. Compound **8** was detected on the TLC at R_f 0.28 and 0.8 mg was isolated. The MS and ^{1}H NMR spectrum were taken. Compound **8** proved to be identical with a pentafuhaloltridecacetate previously isolated from *Halidrys siliquosa* [4] and *Bifurcaria bifurcata* [6], the structure of which was confirmed by ^{13}C NMR spectroscopy. Substance **9** was isolated from a spot at R_f 0.15. The MS showed fragmentation patterns that varied distinctly according to the operation conditions. Electron impact energy of 70 eV and temperatures up to 240° resulted in fragments with 2–5 rings and of a low degree of hydroxylation in agreement with previous results [4, 7]. When the temperature was increased to 280° and the ionization to 100 eV, ions of the ketene elimination series, starting from the molecular ion, and daughter ions consisting of 3 or 5 phloroglucinol units with three additional HO groups became visible. These 3 and 5 rings originated from the central part of the molecule. Further temperature increase resulted in catalytic dehydrogenation reactions, which led to series differing from the main series by −2 H or −4 H. Possibly derivatives of *o*-quinones, dibenzodioxines, or both are formed. A sophisticated analysis of the fragments seemed establish the structure proposal published recently [4, 7] for heptafuhaloloctadecacetate.

No further detailed analysis of additional fractions consisting of mixtures of 4- to 7-ring compounds was possible. However, partial structures for some could be derived from the spectral data. They contained only members of the phlorethol (ether-linked phloroglucinols) or fucophlorethol series (phenoxy substituted biphenyls with phloroglucinol pattern). Some of these compounds are chlorinated.

EXPERIMENTAL

Extraction and *chromatography*. 15 kg lyophilized, pulverized thalli from Roscoff/Brittany (April 1974) and 10 kg from Ménéham/Brittany (May 1973) were extracted as described in ref. [1]. The phenolic fraction was enriched and prefractionated. Separation was carried out by combination of TLC and HPLC.

For PLC, Si gel plates (Merck 60, F_{254} Fertigplatten, 0.25 mm) were used with different solvent systems (mainly $CHCl_3$–Me_2CO, 47:3; CCl_4–$CHCl_3$, 4:1) and repeated development. Gradient elution systems with various $CHCl_3$–EtOH mixtures (0.2–5% EtOH) were used with a Si gel HPLC column (Partisil 10, 25 cm, 9 mm ϕ). Detection by UV absorption at 275 nm. All given R_f values are in reference to Si gel plates (Merck 60, F_{254}, 0.25 mm) with the solvent system $CHCl_3$-Me_2CO (47:3).

Tetraphlorethol-A-nonacetate (**2**). 5th zone of TLC, R_f 0.37, 2,6,5′-triacetoxy-4,3′-bis (2,4,6-triacetoxyphenoxy) diphenylether, 6.5 mg/15 kg algae; ^{1}H NMR ($CDCl_3$, 100 MHz, CAT): δ 6.95 (4 H), 6.71 (2 H), 6.45–6.31 (3 spin coupling system, $J_{AB} \sim 2$ Hz), 2.26 (6 H), 2.20 (3 H), 2.12, 2.08, 2.06 (each 6 H).

Fucophlorethol-A-nonacetate (**3**). 2,4,6,2′,4′,6′-Hexacetoxy-3-(3,5-diacetoxyphenoxy)biphenyl, 5th zone of TLC, R_f 0.37; in a ratio of 1:7 together with **2**; corresponding to 1 mg/15 kg algae; ^{1}H NMR and MS values were identical with the described values in [3].

Tetraphlorethol-B-nonacetate (**4**). Tentatively identified as 3,1′,5′-triacetoxy-1,3′-bis(2,4,6-triacetoxyphenoxy)diphenylether; 7th zone of the TLC, R_f 0.33; 8 mg/15 kg algae; ^{1}H NMR ($CDCl_3$, 100 MHz, CAT): δ 6.94 (4 H), AB system (2 H, $\nu_A = 6.46$, $\nu_B = 6.33$, $J_{AB} = 2.1$ Hz), 2.26, 2.20, 2.07, 2.06 (in a ratio of 2.5:1.5:4:0.8).

Fucodiphloretholdecacetate (**5**). 7th zone of the TLC, R_f 0.33; in a ratio of 1:10 together with **4**, corresponding to 1 mg/15 kg algae.

Fucodiphlorethol-C-decacetate (**6**). 2,4,6,2′,4′,6′-hexacetoxy-3-[5-(2,4,6-triacetoxyphenoxy)-3-acetoxyphenoxy]biphenyl; 10th zone of the TLC, R_f 0.27; 2.7 mg/15 kg algae; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.09 (1 H), 7.0, 6.95 (each 2 H), 6.45–6.29 (3 spin coupling system, $J_{AB} \sim 2$ Hz), 2.27 (6 H), 2.20 (3 H), 2.10 (6 H), 2.06 (9 H), 2.03, 1.86 (each 3 H).

Fucotriphloretholdodecacetate (**7**). 11th zone of the TLC, R_f 0.26.

Pentafuhaloltridecacetate (**8**). 9th zone of the TLC, R_f 0.28; 0.8 mg/10 kg algae, ^{1}H NMR and MS values are identical with those in refs. [4] and [6].

Heptafuhaloloctadecacetate (**9**). R_f 0.15; 43 mg/10 kg algae; IR, MS (70 eV), UV, ^{1}H NMR ($CDCl_3$ and Me_2CO-d_6) are identical with the description in refs. [4] and [7]. MS 100 eV, temp. >280°): ketene elimination starting and ending with: *m/e* 430–262, 432–264, 532–280, 530–278, 680–386, 682–388, 698–404, 696–402, 756–420, 712–628, 1090–712, 948–738, 1030–736, 1130–752, 1070–944, 1212–960.

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