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BROMINATED PHLORETHOLS AND NONHALOGENATED PHLOROTANNINS FROM THE BROWN ALGA *CYSTOPHORA CONGESTA*

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Key Word Index—*Cystophora congesta*, Phaeophyceae, brown alga, phlorotannins, phlorethols, brominated phlorethols, fucophlorethols

Abstract—The following phlorotannins were isolated from the brown alga *Cystophora congesta* and characterized as their acetates phloroglucinol triacetate, bromodiphlorethol pentaacetate, diphlorethol pentaacetate, bromotriphlorethol-A_1-heptaacetate, bromotriphlorethol-A_2-heptaacetate, tetraphlorethol-C-nonaacetate and fucodiphlorethol-D-decaacetate The substances bromodiphlorethol pentaacetate, bromotriphlorethol-A_1-heptaacetate and bromotriphlorethol-A_2-heptaacetate are the first brominated members of this series to be described Triphlorethol-A-heptaacetate was isolated previously from *C congesta*

INTRODUCTION

The phenolic substances found in various species of the genus *Cystophora* (family Cystoseiraceae) have been the subject of repeated investigations in the past In the lipophilic fractions of a *Cystophora* species not specifically identified, isoprenylated quinones and hydroquinones have been found [1] Alkenylresorcinol and alkenylphloroglucinol derivatives [2] are known from *C torulosa*, the latter also from *C congesta* Gregson and Daly [3] isolated two triphenyl diethers **1** and **3** from an aqueous extract of *C congesta* Compound **1** consists of three phloroglucinol rings which are attached to each other by two ether linkages at the *para*-position Structure elucidation was carried out mainly on peracetyl-**1** (**2**) According to the nomenclature introduced by Glombitza *et al* [4], this substance belongs to the phlorethols within the phlorotannins, which in turn are derivatives of phloroglucinol Gregson and Daly [3] obtained **1** during separation of an aqueous alcoholic extract on an XAD-2 resin column Gregson gave the Bonn laboratory a fraction containing **1** which was obtained similarly TLC of the acetylated fraction displayed a number of further spots apart from the one belonging to **2** These were separated and purified using CC, TLC and HPLC Several phlorethols and fucophlorethols were isolated in this manner, some of which were brominated derivatives The brominated two- and three-ring phlorethols are the first brominated precursors of tannins which have been isolated from plants

For a further substance **3** which was isolated with methanol on XAD-2 resin and its peracetylated derivative **4**, Gregson and Daly [3] have proposed two alternative structures whose ring components surprisingly cannot all be derived from phloroglucinol

RESULTS AND DISCUSSION

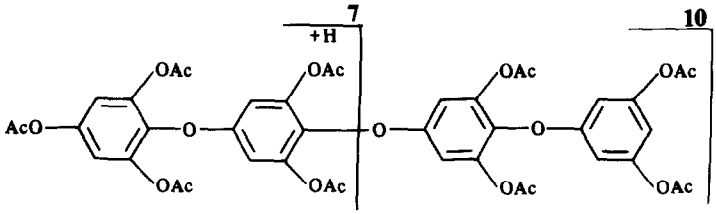
C congesta was extracted by Gregson and Daly [3] The acetylated mixture of the fraction containing **1** was then separated by CC, TLC and HPLC and the fractions obtained examined by mass and ^{1}H NMR spectroscopy

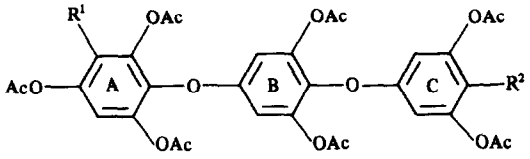
In the TLC spot at R_f 0 57 (silica gel, $CHCl_3$–Me_2CO, 19 1), phloroglucinol triacetate (**5**) was identified, which has previously been isolated several times from peracetylated phenolic fractions of various other brown algae [5]

With the aid of comparison spectra, the substance present at R_f 0 45 was identified as diphlorethol pentaacetate (**7**) This substance was first isolated from *Cystoseira tamariscifolia* by Glombitza *et al* [6] The chemical shifts of its ^{1}H NMR spectrum are listed in Table 1

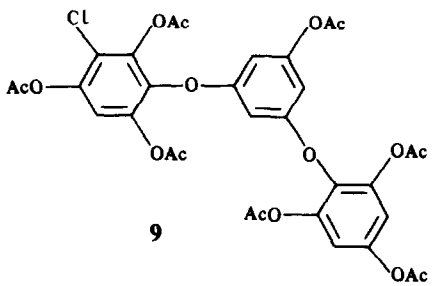
Approximately 80% of the examined fraction consisted of **2**, which had an R_f of 0 31 Below this was a substance (**10**), which displayed an $[M]^+$ at *m/z* 876 in the EI mass spectrum The $[M]^+$ loses ketene units (42 mu) as many as nine times to give an ion at *m/z* 498 Therefore, **10** appears to be a higher homologue of **7** and **2** From the ^{1}H NMR values (Table 1), it is possible to deduce that one end of **10** consists of a 2,4,6-triacetoxyphenoxy element (A) with a 3,5-diacetoxyphenoxy element (C) at the other Two signals for the two identical protons at δ6 72 and 6 69, respectively, and two further signals each for two acetylmethyl groups prove that the central part of the molecule is made up of two symmetrically substituted aromatic rings (B_1, B_2) The two rings are attached to each other and to the end components characterized above by ether linkages in the *para*-position The acetoxy frequencies belonging to the aromatic rings B_1 and B_2 have the value δ2 06 and one of the values $2\,12_{(7)}$ and $2\,12_{(0)}$, which follows from assignment of the signals of **8a** (see below) In contrast, the assignment of one of the two pairs of aromatic protons at δ6 69 and 6 72 to ring B_1 and B_2 must remain open Therefore, **10** is 4-(2,4,6-triacetoxyphenoxy)-4′-(3,5-diacetoxyphenoxy)-2,6,3′,5′-tetraacetoxydiphenyl ether This compound is referred to as tetraphlorethol-C-nonaacetate

In the TLC at a higher R_f than **7**, **6** is present, and just





2 $R^1 = H$, $R^2 = H$
8a $R^1 = Br$, $R^2 = H$
8b $R^1 = H$, $R^2 = Br$



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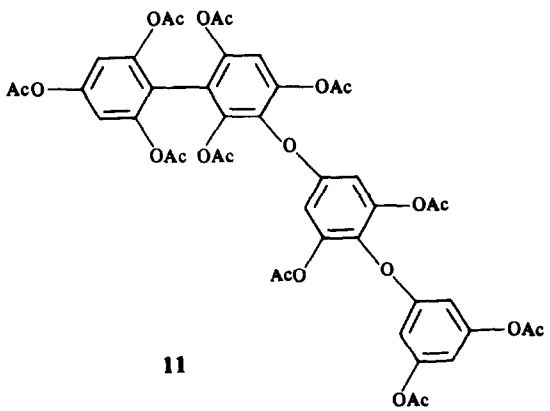

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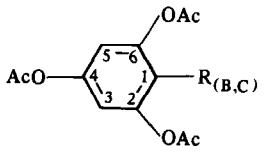
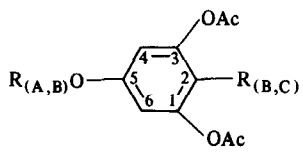
above **2** substance **8** is found In the EI mass spectra, both of these substances have a certain feature in common the $[M]^+$ ions, as well as the successive ketene cleavages down to the free phenol, display the same isotope distribution pattern as that of a monobrominated derivative In the mass spectrum of **8**, the $[M]^+$ ions are to be found at *m/z* 746 and 748 The successive cleavage of seven ketene units then takes place High resolution of the ions at *m/z* 622/620 $[M - 3 \times 42\ mu]^+$ yields 622 0317 and 620 0399 (calc 622 0146 and 620 0166), corresponding with the empirical formula $C_{26}H_{21}BrO_{13}$ The empirical formula for the $[M]^+$ is therefore $C_{32}H_{27}BrO_{16}$ However, it is indeed possible to observe more resonance signals in the ^1H NMR than would be expected judging by the mass spectrum for seven acetoxy functions and six aromatic protons Therefore, it could be a mixture of two

Table 1 Correlation of ^{1}H NMR data for the position of ring types A, B and C in **2**, **7**, **8a**, **8b** and **10** Differences of frequencies (ppm) between halogenated and nonhalogenated rings for **8a**, **8b** and **9**

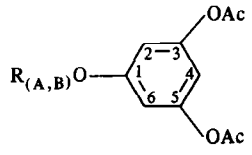
A



B



C



	OAc at C-2	C-3	OAc at C-4	C-5	OAc at C-6	OAc at C-1	OAc at C-3	C-4	C-6	C-2	OAc at C-3	C-4	OAc at C-5	C-6
7	$2.07_{(8)}$	$6.95_{(4)}$	$2.28_{(5)}$	$6.95_{(4)}$	$2.07_{(8)}$					$6.57_{(0)}$ (B)	$2.25_{(2)}$	$6.63_{(0)}$ (A)	$2.25_{(2)}$	$6.57_{(0)}$ (B)§
2	$2.13_{(5)}$	$6.95_{(6)}$	$2.28_{(1)}$	$6.95_{(6)}$	$2.13_{(5)}$	$2.06_{(5)}$	$2.06_{(5)}$	$6.70_{(5)}$	$6.70_{(5)}$	$6.55_{(4)}$ (B)	$2.24_{(7)}$	$6.65_{(0)}$ (A)	$2.24_{(7)}$	$6.55_{(4)}$ (B)§
8a	$2.25_{(0)}$	Br	$2.35_{(6)}$	$7.06_{(3)}$	$2.09_{(1)}$	$2.07_{(3)}$	$2.07_{(3)}$	$6.71_{(7)}$	$6.71_{(7)}$	$6.54_{(8)}$ (B)	$2.25_{(0)}$	$6.64_{(7)}$ (A)	$2.25_{(0)}$	$6.54_{(8)}$ (B)§
Δ(ppm) to **2**	$+0.11_{(5)}$		$+0.07_{(6)}$	$+0.10_{(7)}$	$-0.04_{(4)}$									
8b	$2.13_{(2)}$	$6.95_{(8)}$	$2.28_{(6)}$	$6.95_{(8)}$	$2.13_{(2)}$	$2.07_{(6)}$	$2.07_{(6)}$	$6.70_{(4)}$	$6.70_{(4)}$	$6.64_{(7)}$	$2.31_{(5)}$	Br	$2.31_{(5)}$	$6.64_{(7)}$
Δ(ppm) to **2**										$+0.09_{(3)}$	$+0.06_{(5)}$		$+0.06_{(5)}$	$+0.09_{(3)}$
10	$2.12_{(7)}$†	$6.97_{(3)}$	$2.28_{(0)}$	$6.97_{(3)}$	$2.12_{(7)}$†B_1	$2.12_{(0)}$†	$2.12_{(0)}$†	$6.72_{(2)}$‡	$6.72_{(2)}$‡	$6.56_{(4)}$ (B)	$2.24_{(5)}$	$6.66_{(5)}$ (A)	$2.24_{(5)}$	$6.56_{(4)}$ (B)§
					$2.12_{(7)}$†B_2	$2.06_{(5)}$	$2.06_{(5)}$	$6.69_{(5)}$‡	$6.69_{(5)}$‡					
9* A	2.16	Cl	2.32	7.03	2.03									
A′	2.06	6.94	2.24	6.94	2.06									
Δ(ppm) ring A to A′	+0.1		+0.08	+0.09	−0.03									

*Chemical shifts for ring A and A′ only, attached in C-1 and C-3 to 3-acetoxybenzene

†Positions at ring A and B may be interchanged

‡Positions at ring B_1 and B_2 may be interchanged

§J_{AB} each 1.9–2.1 Hz

isomeric substances On account of the differing signal intensities, the ratio of the two substances was computed to be 3 1 (**8a 8b**)

For the proposed structure of **8a**, the bromine atom is attached to the 2,4,6-triacetoxylated terminal ring A In the case of the analogously chlorine-substituted terminal ring of chlorotriphlorethol-C-heptaacetate (**9**) from *Laminaria ochroleuca* [7], the chemical shifts for substituents in the *ortho*-position to the halogen atom are shifted downfield by 0 06–0 1 ppm compared to the non-halogenated ring (see Table 1) One signal for 1H shifted downfield occurs at δ7 06 and one for an acetyl group at 2 35, instead of one signal for two identical protons at 6 95 and for an acetyl group at 2 28 (ring A), whereas the resonance positions for the substituents and aromatic protons of the rings B and C of **8a** are only slightly or not at all shifted compared to **2**

With **8b**, both rings A and B are substituted in exactly the same manner as in **2** In comparison to the non-brominated derivative, the resonance frequencies of ring C are shifted downfield, to the same amount calculated in ppm as the frequencies of the *ortho*- or *meta*-positioned substituents to the bromine atom on ring A with **8a** Therefore, **8** is a mixture of two positional isomers 1,3-diacetoxy-2-(3,5-diacetoxyphenoxy)-5-(3-bromo-2,4,6-triacetoxyphenoxy)benzene, referred to as bromotriphlorethol-A_1-heptaacetate (**8a**) and 1,3-diacetoxy-2-(4-bromo-3,5-diacetoxyphenoxy)-5-(2,4,6-triacetoxyphenoxy)benzene, called bromotriphlorethol-A_2-heptaacetate (**8b**) With **8a** and **8b**, the first naturally occuring brominated tanning agent precursors have been identified

The signal for two identical acetoxy functions at δ2 13 is missing from the resonance frequencies in the ^{1}H NMR spectrum of **8a** This signal was present in **2** This means that because in **8a** ring A is brominated, the signal in question in **2** must be assigned to ring A The same frequency occurs twice with **10** One of these signals must be assigned to the acetoxy pair attached to ring A, and the other to ring B_1 for reasons now to be described

The dimer **7** does not possess this resonance frequency Instead, one signal shifted upfield at δ2 07 can be observed (apart from the unshielded acetoxy groups) The higher degree of shielding occurs because the acetoxy groups appear in the anisotropic range of the adjacent 3,5-diacetoxylated terminal ring For this reason, the frequency in question should be assigned to the ring adjacent to the terminal ring with **2** and **8a** to ring B_1 and to ring B_2 with **10** Comparing acetylated phlorotannins, the signals of the acetoxy-group pairs of the ring types A and B are shifted to high field (δ2 02–2 09) when (1) the second or third ring possesses ether linkages in an *ortho*-position or (2) the second ring shows ether linkages in the *meta*-position or (3) when the *para*-situated 3,5-diacetylated terminal ring can rotate freely They are more deshielded when the *para*-position is substituted by a longer chain, because the influence of the anisotropic range of the neighbourhood of the aromatic ring is less than in the above mentioned cases

With **6**, the $[M]^+$ was found at *m/z* 538 and 540 High resolution produces 538 0138 for the ^{79}Br-derivative, and 540 0098 for the ^{81}Br-derivative Deviation from the calculated values was $2\,8 \times 10^{-3}$ and $0\,8 \times 10^{-3}$ mu, respectively Consequently the empirical formula is $C_{22}H_{19}BrO_{11}$, from which ketene units are cleaved five times in succession until *m/z* 328/330 is reached This corresponds to a two-ringed derivative with an ether linkage and five acetoxy functions *M/z* 69 (18) indicates the 1,3,(5)-hydroxylation of the aromatic ring [8] Therefore **6** is a monobromodiphlorethol acetate In the mass spectrum, apart from the ketene cleavage series, one-ring cleavage products as well as debromination products can hardly be found The ^{1}H NMR-spectrum of **6** is more complex than that of **8** Compound **6** could be therefore a mixture of three different substances whose ratio can be estimated to be 3 2 1 Typical resonance frequencies are δ7 07 (1H) and 2 36 (3H) which indicate a 3-bromo-2,4,6-triacetoxyphenoxy ring Signals at δ6 97, 6 68 (each 2H), 2 31, 2 09 (each 6H) suggest a diphlorethol pentaacetate brominated at position 4' A few further signals cannot be assigned to any definite molecule All of the **6**-compounds have the same empirical formula $C_{22}H_{19}BrO_{11}$, and for this reason, it seems that a mixture of all the possible monobrominated diphlorethol acetates is present Due to the small amount of **6**, coupled systems cannot be completely identified in the ^{1}H NMR spectrum, and so it is not possible to make any definite statements as to the composition of the mixture **6**

The substance occurring at R_f 0 20 received the number **11** In the EI mass spectrum it displays an $[M]^+$ of *m/z* 918, as well as a successive cleavage of ten ketene units to *m/z* 498, which represents the ion of the free phenol Furthermore, an ion at *m/z* 480 ($[498 - H_2O]^+$, 66% of *m/z* 498) can be identified which suggests a fucol moiety (*o,o'*-tetrahydroxylated biphenyl) in a four-ringed derivative The ^{1}H NMR spectrum gives resonance frequencies for three acetoxy group pairs [δ2 25 (6H), 2 06 (12H)] and four single acetoxy groups [δ2 29, 2 11, 2 03, 1 93] as well as two times a pair and one single aromatic H [δ7 13 (1H), 7 02, 6 72 (each 2H)] and also for an AB_2 system [ν_A = 6 65 (1H), ν_B = 6 55 (2H), J_{AB} = 2 0 Hz] The values are identical to those published by Glombitza *et al* [9] for fucodiphlorethol-D-decaacetate from *Cystoseira baccata* Therefore, fucophlorethols have been proved to occur in *Cystophora congesta*

Indications were also obtained that several more, partly brominated, phlorotannin acetates are present The angular trimer **3**, not derivable form phloroglucinol, and its peracetyl derivative **4**, both described by Gregson and Daly, were not present in this fraction

EXPERIMENTAL

C congesta Womersley and Nizamuddin was collected at Whangaparoa Peninsula (North Island, New Zealand) and immediately frozen An aq extract of the alga was chromatographed on Amberlite XAD-2, then on silica gel for isolating **1**, all prepared and described in ref [3] A portion (400 mg) of this fraction was acetylated with Ac_2O–pyridine (1 1, 20 ml, room temp, 24 hr) to yield a mixture of acetates (590 mg) The mixture was preseparated by CC on silica gel Merck 60 (0 040–0 062 mm diam) with gradient elution with $CHCl_3$ and $CHCl_3$–Me_2CO (4 1) Final separation was carried out on silica gel Merck 60 F_{254} precoated TLC plates in $CHCl_3$–Me_2CO (19 1) and $CHCl_3$–Me_2CO (93 7) and by HPLC on LiChrosorb Si 60 (7 μm) using various gradient elution programmes with *n*-hexane–$CHCl_3$ (stabilized with 0 3% MeOH) or $CHCl_3$–MeOH for high MW compounds ^{1}H NMR spectra were recorded at 90 MHz, δ-values were measured in $CDCl_3$ with TMS as standard R_f values for the following substances are based on TLC development in $CHCl_3$–Me_2CO (19 1), spray reagent 1% vanillin in conc H_2SO_4, heating to 100–105° for 5–10 min

Percentages and wts are given in relation to the fraction of 590 mg phlorotannin acetates

Phloroglucinol triacetate (**5**) 0 5 mg (0 08 %), R_f 0 57, orange, ^{1}H NMR values identical with values given in ref [5]

Bromodiphlorethol pentaacetate (**6**) 1 5 mg (0 25 %), R_f 0 49, bright red, ^{1}H NMR δ7 07, 7 05, 6 99, 6 97$_{(5)}$, 6 70, 6 62, 6 59, 2 36, 2 31, 2 28, 2 25, 2 21, 2 19, 2 11, 2 09, 2 03 MS *m/z* 540/538 ([M]$^+$, 3/3), 498/496 (21/20), 456/454 (40/40), 414/412 (65/65), 372/370 (45/44), 330/328 (28/29), (460), 418 (1), 376 (1), 334 (3), 292 (6), 250 (2), 248 (12), 231 (9), 69 (18), 43 (100), HRMS 540 0098 found (calc 540 0090), 538 0138 found (calc 538 0110)

Diphlorethol pentaacetate (**7**) 4, 5 mg (0 75 %), R_f 0 45, bright red, ^{1}H NMR data ($CDCl_3$) comparable to data of ref [6]

Bromotriphlorethol-A_1-heptaacetate (**8a**) 1,3-Diacetoxy-2-(3,5-diacetoxyphenoxy)- 5-(3-bromo -2,4,6- triacetoxyphenoxy)-benzene, R_f 0 37, bright red, ^{1}H NMR δ7 06$_{(3)}$ (1H), 6 71$_{(7)}$ (2H), ν_A = 6 64$_{(7)}$ (1H), ν_B = 6 54$_{(8)}$ (2H, AB_2 system, J_{AB} = 2 1 Hz) 2 35$_{(6)}$ (3H), 2 25$_{(0)}$ (9H), 2 09$_{(1)}$ (3H), 2 07$_{(3)}$ (6H), MS *m/z* 748/746 ([M]$^+$, 3/3), 706/704 (15/15), 664/662 (33/31), 622/620 (23/22), 580/578 (19/20), 538/536 (21/19), 496/494 (6/7), 454/452 (5/5), 418 (2), 376 (5), 334 (13), 292 (9), 250 (5), 248 (3), 69 (17), 57 (35), 43 (100), together with **8b** (both 2 5 mg, 0 43 %)

Bromotriphlorethol-A_2-heptaacetate (**8b**) 1,3-Diacetoxy-2-(4-bromo-3,5-diacetoxyphenoxy)-5-(2,4,6-triacetoxyphenoxy)-benzene, R_f, colour and MS identical to **8a** ^{1}H NMR δ6 95$_{(8)}$, 6 70$_{(4)}$, 6 64$_{(7)}$ (each 2H), 2 31$_{(5)}$ (6H), 2 28$_{(6)}$ (3H), 2 13$_{(2)}$, 2 07$_{(6)}$ (each 6H)

Triphlorethol-A-heptaacetate (**2**) 490 mg (83 %), R_f 0 31, bright red, ^{1}H NMR δ6 95$_{(6)}$, 6 69$_{(7)}$ (each 2H), ν_A = 6 65 (1H), ν_B = 6 55$_{(4)}$ (2H, AB_2 system, J_{AB} = 2 0 Hz), 2 28$_{(1)}$ (3H), 2 24$_{(7)}$, 2 13$_{(5)}$, 2 06$_{(5)}$ (each 6H), MS identical with ref [3]

Tetraphlorethol-C-nonaacetate (**10**) 5 mg (0 85 %), 4-(2,4,6-triacetoxyphenoxy)-4′-(3,5-diacetoxyphenoxy)-2,6,3′,5′-tetraacetoxydiphenylether, R_f 0 25, bright red, ^{1}H NMR δ6 97$_{(3)}$, 6 72$_{(2)}$, 6 69$_{(5)}$ (each 2H), ν_A = 6 66$_{(5)}$ (1H), ν_B = 6 56$_{(4)}$ (2H, AB_2 system, J_{AB} = 2 0 Hz), 2 28 (3H), 2 24$_{(5)}$, 2 12$_{(7)}$, 2 12$_{(0)}$, 2 06$_{(5)}$ (each 6H) MS *m/z* 876 ([M]$^+$, 1 5), 834 (11), 792 (40), 750 (60), 708 (72), 666 (55), 624 (41), 582 (27), 540 (17), 498 (26), 684, 642, 600, 558, 516, 474, 432, 390 (each 5 %), 626 (16), 584 (17), 542 (18), 500 (15), 458 (8), 416 (6), 374 (5), 414, 372, 356, 334, 292, 250 (8), 248 (8), 126 (8), 69 (9), 57 (12), 43 (100)

Fucodiphlorethol-D-decaacetate (**11**) 3 mg (0 5 %), R_f 0 20, deep red, ^{1}H NMR and MS identical with ref [9]

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