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AN EIGHT-RING PHLOROTANNIN FROM THE BROWN ALGA *HIMANTHALIA ELONGATA**

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Key Word Index—*Himanthalia elongata*; Himanthaliaceae; Phaeophyta; Structure elucidation; field desorption mass spectrometry; phlorotannins; bisfucopentaphlorethol-A nonadecacetate.

Abstract—From an acetylated fraction of *Himanthalia elongata* extract, an eight-ring phenolacetate was isolated and its structure elucidated with the aid of field desorption mass spectrometry and other spectroscopic methods.

INTRODUCTION

In recent years a large number of polyhydroxyphenols have been isolated from numerous brown algae. In previous analyses of a mixture of acetylated phenols from *Himanthalia elongata* phloroglucinol triacetate, diphlorethol penta-acetate and difucol hexa-acetate [3] were identified.

RESULTS AND DISCUSSION

In the course of an isolation procedure from an acetylated phenol extract of this alga, in addition to oligomeric phlorotannins [12] derived from monomeric phloroglucinol, an eight-ring compound, **1**, was isolated (18 mg). This showed UV extinction and a red colouration with vanillin–sulphuric acid on TLC at R_f 0.22 [Si gel 60 F_{254}, chloroform–acetone (9:1), phloroglucinol triacetate R_f 0.67].

On electron impact-induced fragmentation by 70 eV mass spectrometry at a sample evaporation temperature of 240°, a highest mass of *m/z* 1084 could be detected, while at 250° fragments up to *m/z* 1250 were registered. For one of the other phlorotannins in *Himanthalia elongata* with R_f 0.26 (a seven-ring compound), the highest mass fragment at *m/z* 1542 could be determined. Masses above 1500 can only rarely be measured with electron impact mass spectrometry (EIMS) which sets the limit of the method.

A great advance was made in determining MWs above 1500 by using field desorption mass spectrometry (FDMS), [2, 4, 5]. With this method, the MW of the eight-ring compound **1** at mass 1792 could be determined.

By computerized calculation of the isotopic distributions of the quasimolecular ions $[M+H]^+$ and $[M+Na]^+$, the elementary composition $C_{86}H_{72}O_{43}$ was evaluated. Fig. 1 shows the mass range 1800–1850 of the FDMS of **1**. The $[M+Na]^+$ ion at *m/z* 1815 is the base peak and at *m/z* 1831, the $[M+{}^{39}K]^+$ is found when the compound was dissolved for FDMS in methanol saturated with sodium chloride.

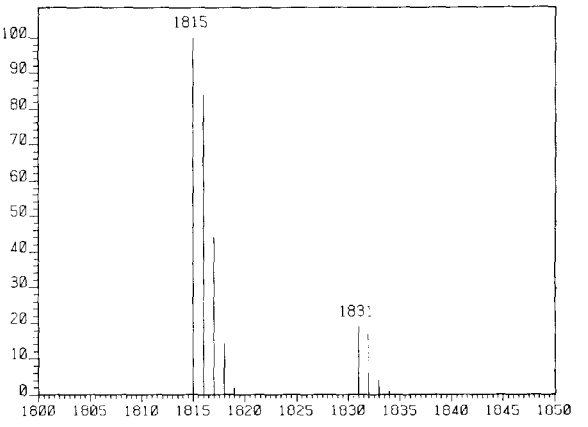

Fig. 1. Original plot of the FDMS obtained after electric registration of **1** with a Varian MAT 731 instrument and data acquisition with the SS 200 data system. The base peak equals 57 920 counts at a noise level of *ca* 50 counts.

In addition to the unambiguous assignment of the MW, the peracetylated eight-ring compound displayed a unique fragmentation pattern at slightly higher emitter heating currents, beetween 25 and 30 mA. As shown in Fig. 2, a stepwise, complete deacetylation occurs during field desorption. Starting from the $[M+Na]^+$ ion at *m/z* 1815, the loss of 42 a.m.u. is observed 19 times. This can be explained as a proton transfer from the methyl group of the protecting acetyl moiety to the phenolic oxygens of **1**. Thus, step by step the free phlorotannin derivative is generated on the field desorption emitter surface and desorbed while ketene is eliminated. In this manner the cleavage of 19 ketene fragments, as expected from the ^{1}H NMR spectrum, can be clearly confirmed.

The molecular formula corresponds to an eight-ring fucophlorethol acetate with two difucol components or one trifucol component. The latter possibility can be excluded from the ^{1}H NMR data.

* Part 29 in the series "Antibiotics from Algae". For Part 28 see ref. [1]; Part 11 in the series "Field Desorption Mass Spectrometry of Natural Products". For Part 10 see ref. [2].

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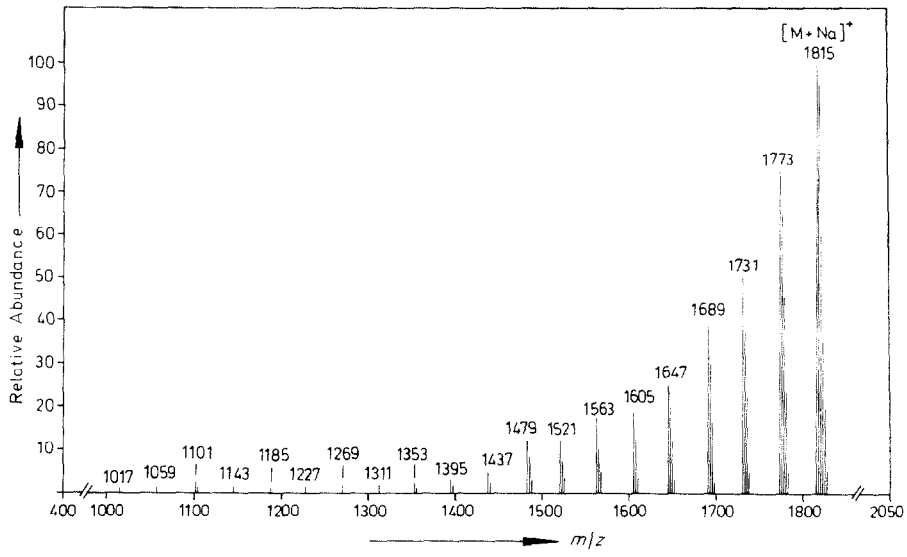

Fig. 2. FDMS of **1** averaged between 25 and 30 mA emitter heating current. Six spectra were accumulated giving 386 700 counts for the base peak. Weaker series of an overall loss of 42 a.m.u. starting from $[M + H]^+$ at m/z 1793 and $[M + {}^{39}K]^+$ at m/z 1831 were omitted for the sake of clarity. An accelerating voltage of 6 kV was used with the normal 5 mm gap of the magnet.

The ^{1}H NMR spectrum (90 MHz, $CDCl_3$) shows a very simple grouping of the aromatic proton resonance signals in the form of five singlets at δ6.98, 6.93, 6.62, 6.59 and 6.37 for a total of 15 H in the integration ratio of 4:4:3:2:2. The ^{1}H NMR spectrum (90 MHz, Me_2CO–d_6) shows three singlets at δ7.06, 7.02 and 6.46 in the ratio 4:4:2 and also at 6.65 the f_4-signal of an A_2B system (3H) superimposed by a singlet for 2H. The transitions of the A part of the A_2B system are reduced to three signals (f_7, f_8 and $f_{5/6}$) at δ6.70, 6.69 and 6.67; those of the B part show three signals (f_3–f_1) at 6.63, 6.62 and 6.61. The shift values found for the protons of the A_2B-systems, are $\nu_A = \delta 6.68$ and $\nu_B = \delta 6.63$. The coupling constant J_{AB} is 2.0 Hz (typically for *meta* H). The methyl region of the ^{1}H NMR spectra shows seven signals for 19 acetyls ($CDCl_3$) or seven separate signals for 15 acetyls and two signals at δ2.00 and 2.03 (Me_2CO–d_6) which are partially hidden and whose areas cannot be calculated. The small number of signals for 19 acetyls indicates a high molecular symmetry. The groupings of the signals according to lit. data [3, 6–8] and according to some of our own comparison data [12] are reproduced in Fig. 3.

This shows that **1** possesses a 5-acetoxy-1,2,3-triphenoxy-substituted middle ring, linked via ether bridges in position 2 with a 3,5-diacetoxysubstituted ring and in positions 1 and 3 with two difucol units, which themselves carry a 2,4,6-triacetoxyphenoxy substituent. Thus, **1** has a six-ring bisfucotriphlorethol-A element* [8], whose difucol units also each possess a 2,4,6-triacetoxyphenoxy substituent [signals at δ6.93 or 7.02 (in $CDCl_3$ and Me_2CO–d_6, respectively) for four aromatic hydrogens and at 2.03 and 2.04 ppm for four acetyls, and 2.26_5 and 2.28_3 for two acetyls] which in principle can be bound in the C-2, C-4 or C-6 positions of rings 2 and 6.

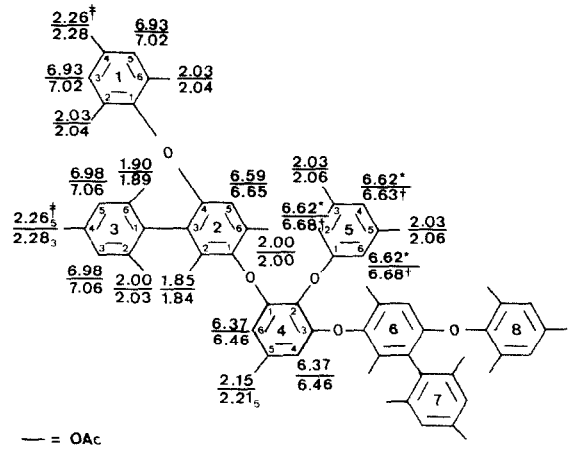

Fig. 3. Assignment of ^{1}H NMR signals of **1**. Underlined, $CDCl_3$; not underlined, Me_2CO–d_6; *$A_2B \cong A_3$ system; †A_2B system (J_{AB} = 2.0 Hz); ‡interchangeable.

The symmetrical structure of the whole compound requires that both triacetoxyphenoxy substituents are bound to each difucol moiety in the same position. C-2 substitution is excluded on the basis of ^{13}C NMR data (see below). For C-6 substitution, a shift of δ6.80 ($CDCl_3$) for aromatic hydrogen at C-5 should be obtained. For C-4 substitution, a value of δ6.54, which is very near to the measured value of 6.59, is found. In the acetyl region, on C-6 substitution, only one signal at higher field (below δ1.98 in $CDCl_3$ or Me_2CO–d_6) for acetyl at C-$2^{2,6}$ is to be expected. This is a typical signal for acetyl groups in sterically hindered positions in fucophlorethol-B structure elements [9]. C-4 substitution would give two high field acetyl signals, one at δ1.80–1.86 for acetyls at C-$2^{2,6}$

*For systematic reasons this unit previously [8] called bisfucodiphlorethol should be named bisfucotriphlorethol A.

and the other one at 1.90–1.98 for acetyls at C-6[3,7]; the latter is comparable to similar positions in fucophlorethol-C types [12].

Two signals at δ1.85 and 1.84 and at 1.90 and 1.89 (in $CDCl_3$ and Me_2CO-d_6, respectively) were measured each for two acetyl groups on two identical structure elements with phenoxyls at C-1 and C-4. This requirement is only fulfilled for C-4 substitution. The fucophlorethol acetates B and C, as well as the bisfucotriphlorethol-A acetate [12], co-occur non acetylated in *Himanthalia elongata*.

The ^{13}C NMR spectrum shows 22 signals from ring carbon atoms. The shift values measured (Table 1) give an excellent correlation with the values calculated by Forster [10]. The groupings can be seen in Table 1.

Table 1. Measured and calculated ^{13}C NMR shift values of **1**

^{13}C NMR shift values measured [δ-values (ppm)]	^{13}C NMR shift values calculated according to Forster [10] [δ-values (ppm)]	Assignment of carbon atom in ring* [δ-values (ppm)]
158.7	157.5	1^5
151.8	152.1	$4^{2,6}$
151.4	151.7	$3, 5^5$
150.5	150.6	$4^{3,7}$
150.4	150.4	$1, 3^4$
149.1	149.4	$2, 6^{3,7}$
147.7	146.9	5^4
147.0	147.7	$4^{1,8}$
146.2	144.1	$2^{2,6}$
143.7	143.4	$2, 6^{1,8}$
143.6	142.1	$6^{2,6}$
136.3	135.8	$1^{2,6}$
133.8	134.1	$1^{1,8}$
130.3	131.3	2^4
115.8	115.8	$1^{3,7}$
114.8	114.0	$3, 5^{1,8}$
114.6	112.0	$3^{2,6}$
114.0	113.8	$3, 5^{3,7}$
109.5	110.8	4^5
109.3	110.0	$5^{2,6}$
106.3	107.7	$2, 6^5$
103.2	106.2	$4, 6^4$

The signals at δ151.8, 147.0, 146.2, 143.7, 143.6, 133.8, 114.8 and 109.3 in the ^{13}C NMR spectrum of **1** are absent in the spectrum of aforementioned bisfucodiphlorethol A acetate [12]. They are due to the new structural units: from the two 2,4,6-triacetoxyphenoxy rings (147.0, 2C, 143.7, 4C, 133.8, 2C, 114.8, 4C) and the penta-substituted rings of the difucol units (151.8, 2C, 146.2, 2C, 143.6, 2C, 109.3, 2C). In order to decide in which position the triacetoxyphenoxy rings are bound at rings 2 and 6, respectively, the theoretical shift values for all three possible binding positions were calculated (see Table 2) and compared with the measured values. The comparison indicates substitution in the C-4 position.

EXPERIMENTAL

Extraction and chromatography. Lyophilized, pulverized thalli (3 kg) from *Himanthalia elongata* (Roscoff/Brittany, March 1979) were extracted as described in ref. [11]. The phenolic fraction was enriched and prefractionated. Separation was carried out by HPLC, controlled by TLC. Gradient elution systems with various $CHCl_3$–EtOH mixtures (5–15% EtOH) were used with different Si gel HPLC columns (LiChrosorb SI 60, 7 μm, 25 cm, 16 mm ϕ; 5 μm, 25 cm, 7, 5 mm ϕ; Partisil 10 μm, 25 cm, 9 mm ϕ; 50 cm, 6 mm ϕ). Detection by UV absorption at 270 nm. R_fs are from Si gel plates (Merck 60, F_{254}, 0.25 mm) run in $CHCl_3$–Me_2CO (9:1).

Bisfucopentaphlorethol-A nonadecacetate, **1** {5-*acetoxy*-2-[3,5-*diacetoxyphenoxy*)-1,3-bis-[3-(2,4,6-*triacetoxyphenyl*)-4-(2,4,6-*triacetoxyphenoxy*)-2,6-*diacetoxyphenoxy*]-*benzene*}. R_f 0.22, 18 mg/3 kg alga; UV λ_{max}^{MeCN} nm: 208, 235 (shoulder), 275 (shoulder); EIMS (70 eV) (highest mass found at 240° *m/z* 1084, at 250° *m/z* 1252); ketene elimination series ($-x \cdot 42$) of fragments generated by ether splitting: 1120–1036, 1206–870, 1250–788, 1100–680, 1126–622, 1040–620, 984–606, 982–646, 892–514, 876–498. 874–496, 776–482, 858–480, 684–432, 668–374, 456–372, 610–358, 440–356, 424–340, 548–338, 434–266, 418–250, 332–248, 276–234, 358–232, 226–142, 210–126.

Field desorption mass spectrometry. The FDMS were produced on a Finnigan MAT 731 double-focusing spectrometer equipped with a combined electron impact field ionization–field desorption ion source. All spectra were recorded electrically with scan times between 4 and 8 sec/decade and at a mass resolution of better than 3000 (10% valley definition). Data acquisition and processing were performed using the Varian Spectra-System 200. For mass calibration, the EIMS of a mixture of perfluoro-

Table 2. ^{13}C NMR shift values δ(ppm) at the difucol element due to the three theoretically possible binding positions of the two 2,4,6-triacetoxyphenoxy terminal rings, compared with measured values (according to Forster [10])

Carbon positions on the five-fold substituted difucol rings (rings 2 and 6) of **1**	Calculated shift values with a 2,4,6-triacetoxyphenoxy ring in position						^{13}C NMR shift values found
	C-2	Δppm	C-4	Δppm	C-6	Δppm	
1	133	−3.6	135.8	−0.5	133.1	−3.2	136.3
2	148.6	+2.4	144.1	−2.1	144.1	−2.1	146.2
3	112	−2.6	112	−2.6	114.7	+0.1	114.6
4	145.9	−5.9	152.1	+0.3	145.6	−6.2	151.8
5	114.0	+4.7	110.0	+0.7	110.0	+0.7	109.3
6	142.4	−1.2	142.1	−1.5	148.6	+5.0	143.6
		Σ num. 20.3		Σ num. 7.7		Σ num. 17.5	

Δppm, Deviation from measured value.

kerosene and *tris*-(perfluorononyl)-*s*-triazine (PCR Research Chemicals, Gainesville, Florida) were taken. The field desorption emitters were prepared by high temp. activation [13] of 10 μm diameter W wires in a multiple, home built activation chamber. Emitters with an average length of 30 μm for the grown carbon microneedles were used and their efficiency and adjustment were determined by means of the signal at m/z 58, the molecular ion of Me_2CO, in the field ionization mode. All spectra were produced at ion source potentials of 6 kV for the emitter and -4 kV for the slotted cathode plate, an ion source pres. of *ca* 10^{-7} Torr and an ion source temp. between 50 and 70°. The samples were desorbed by indirect heating using an Ar ion laser [14] type Coherent Innova 90 (max. power 5 W available at all lines) and by controlling the emission of the field desorption ions roughly by the total ion monitor.

MeOH was used as solvent and an estimated amount of *ca* 1 μg was transferred to the emitter by the syringe technique. The analysis time required for the field desorption investigations of one compound was *ca* 30 min, including sample preparation, field desorption measurements, data processing and output through the Statos 33 plotter (Varian, Palo Alto, California).

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