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PHAEOPHYTINS FROM *TAPURA FISCHERI*

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Key Word Index—*Tapura fischeri*; Dichapetalaceae; phaeophytins; phaeophytin a; 17^3-ethoxyphaeophorbide a; phaeophytin a-13^2-carboxylic acid; 17^3-ethoxyphaeophorbide a-13^2-carboxylic acid; phaeophorbide a methyl ester; 17^3-ethoxyphaeophytin b.

Abstract—The ethyl acetate extract of the leaves of *Tapura fischeri* yielded four novel phaeophytins, 17^3-ethoxyphaeophorbide a, phaeophytin a-13^2-carboxylic acid, 17^3-ethoxy-phaeophorbide a-13^2-carboxylic acid and 17^3-ethoxyphaeophytin b and the known phaeophytin a. Phaeophorbide a methyl ester was isolated from the hexane extract of the leaves of *T. fischeri*. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

T. fischeri Engl. is a member of the Dichapetalaceae. The most well known member of this family is *Dichapetalum cymosum* (Hook.) Engl. or the "gifblaar". Ingestion of the leaves of this plant causes sudden death in ruminants, due to the presence of fluoroacetic acid in the leaves. Achenbach *et al.* [1] isolated dichapetalin A, a triterpenoid that shows strong cytotoxicity in a brine shrimp assay, from *Dichapetalum madagascariense*. *T. fischeri* was investigated in an attempt to find similar compounds. These were not detected but four novel phaeophytins were isolated. Plant material was collected in Northern Natal, South Africa and was identified by Mrs Anne Hutchings of the University of Zululand, who retained a voucher specimen (Hutchings 3622, Zulu). No previous work on *T. fischeri* has been reported. Structures were elucidated using ^{1}H, ^{13}C and 2D NMR spectroscopy.

RESULTS AND DISCUSSION

The dried, crushed leaves of *T. fischeri* were extracted using a soxhlet extraction apparatus with hexane and ethyl acetate. Each extract was separated into its components using column chromatography over silica gel. The hexane extract yielded phaeophorbide a methyl ester (**1**). The ethyl acetate extract yielded phaeophytin a (**2**) as well as the four novel phaeophytins, 17^3-ethoxyphaeophorbide a (**3**), phaeophytin a-13^2-carboxylic acid (**4**), 17^3-ethoxyphaeophorbide a-13^2-carboxylic acid (**5**) and 17^3-ethoxyphaeophytin b (**6**). Compounds **1** and **2** were identified by comparison of their NMR data with literature values [2, 3].

Compound **3** showed the necessary diagnostic peaks in the ^{1}H and ^{13}C NMR spectra to be identified as a phaeophytin. The 3H-7′, 3H-2′ and 3H-12′ resonances occurred as sharp singlets at δ 3.15, 3.39 and 3.65 respectively. H-5, H-10 and H-20 were found as sharp singlets at δ 9.36, 9.50 and 8.55 respectively. A vinyl group was present at C-3 as indicated by the H-3′ double doublet at δ 7.95 (J = 15.19 Hz, 11.39 Hz) and the H-3^2 (E) and the H-3^2 (Z) double doublets at δ 6.25 (J = 15.19 Hz, 2.28 Hz) and 6.15 (J = 11.39 Hz, 2.28 Hz) respectively. An ethyl group was found to occur at C-8. The 2H-8′ resonance appeared as a quartet at δ 3.66 (J = 8.35 Hz) and 3H-8^2 occurred as a triplet at δ 1.68 (J = 8.35 Hz). Similarly, as with phaeophytin a, a five membered carbocyclic ring was present at position 13. A keto group was found at C-13′, as indicated by the fully substituted carbon resonance at δ 189.6. The H-13^2 resonance occurred at δ 6.26 as with phaeophytin at and a methyl ester was also present a C-13^3. This was indicated by C-13^3 occurring as a fully substituted carbonresonance at δ 173.0 and the C-13^4 methyl carbon resonance being present at δ 53.0. The 3H-13^4

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Table 1. ^{1}H NMR data for compounds **1–6**

Proton	**1**	**2**	**3**	**4**	**5**	**6**
H-2′	3.39(*s*)	3.39(*s*)	3.39(*s*)	3.39(*s*)	3.39(*s*)	3.39(*s*)
H-3′	7.98(*dd*) (*J* = 15.19 Hz 11.39 Hz)	7.98(*dd*) (*J* = 15.19 Hz 11.39 Hz)	7.95(*dd*) (*J* = 15.19 Hz 11.39 Hz)	7.98(*dd*) (*J* = 15.19 Hz 11.39 Hz)	7.98(*dd*) (*J* = 15.19 Hz 11.39 Hz)	7.98(*dd*) (*J* = 15.19 Hz 11.39 Hz)
H-3^2(E)	6.28(*dd*) (*J* = 15.19 Hz 2.28 Hz)	6.28(*dd*) (*J* = 15.19 Hz 2.29 Hz)	6.25(*dd*) (*J* = 15.19 Hz 2.29 Hz)	6.28(*dd*) (*J* = 15.19 Hz 2.29 Hz)	6.28(*dd*) (*J* = 15.19 Hz 2.29 Hz)	6.35 (*dd*) (*J* = 15.19 Hz 2.29 Hz)
H-3^2(Z)	6.17(*dd*) (*J* = 11.39 Hz 2.28 Hz)	6.17(*dd*) (*J* = 11.39 Hz 2.28 Hz)	6.15(*dd*) (*J* = 11.39 Hz 2.28 Hz)	6.17(*dd*) (*J* = 11.39 Hz 2.28 Hz)	6.17(*dd*) (*J* = 11.39 Hz 2.28 Hz)	6.20(*dd*) (*J* = 11.39 Hz 2.28 Hz)
H-5	9.36(*s*)	9.36(*s*)	9.36(*s*)	9.36(*s*)	9.36(*s*)	10.28(*s*)
H-7′	3.21(*s*)	3.21(*s*)	3.15(*s*)	3.21(*s*)	3.21(*s*)	11.07(*s*)
H-8′	3.66(*q*) (*J* = 8.35 Hz)	3.66(*q*) (*J* = 8.35 Hz)	3.66(*q*) (*J* = 8.35 Hz)	3.66(*q*) (*J* = 8.35 Hz)	3.66(*q*) (*J* = 8.35 Hz)	3.66(*q*) (*J* = 8.35 Hz)
H-8^2	1.68(*t*) (*J* = 8.35 Hz)	1.68(*t*) (*J* = 8.35 Hz)	1.68(*t*) (*J* = 8.35 Hz)	1.68(*t*) (*J* = 8.35 Hz)	1.68(*t*) (*J* = 8.35 Hz)	1.68(*t*) (*J* = 8.35 Hz)
H-10	9.50(*s*)	9.50(*s*)	9.50(*s*)	9.50(*s*)	9.50(*s*)	9.57(*s*)
H-12′	3.68(*s*)	3.68(*s*)	3.65(*s*)	3.68(*s*)	3.68(*s*)	3.65(*s*)
H-13^2	6.26(*s*).	6.26(*s*)	6.26(*s*)	6.20(*s*)	6.20(*s*)	6.20(*s*)
H-13^4	3.88(*s*)	3.88(*s*)	3.88(*s*)			3.88(*s*)
H-17	4.20(*m*)	4.20(*m*)	4.21(*m*)	4.21(*m*)	4.21(*m*)	4.15(*m*)
H-18	4.45(*m*)	4.45(*m*)	4.46(*m*)	4.46(*m*)	4.46(*m*)	4.45(*m*)
H-18′	1.80(*d*) (*J* = 7.59 Hz)	1.80(*d*) (*J* = 7.59 Hz)	1.80(*d*) (*J* = 7.59 Hz)	1.80(*d*) (*J* = 7.59 Hz)	1.80(*d*) (*J* = 7.59 Hz)	1.80(*d*) (*J* = 7.59 Hz)
H-20	8.55(*s*)	8.55(*s*)	8.55(*s*)	8.55(*s*)	8.55(*s*)	8.55(*s*)
H-17^4	3.55(*s*)	4.35(*dd*) (*J* = 5.32 Hz, 7.69 Hz)	4.00(*m*)	4.35(*dd*) (*J* = 5.32 Hz, 7.69 Hz)	4.00(*m*)	4.00(*m*)
H-17^5		5.10(*t*) (*J* = 5.32 Hz)	1.10(*t*) (*J* = 7.59 Hz)	5.10(*t*) (*J* = 5.32 Hz)	1.08(*t*) (*J* = 7.59 Hz)	1.10(*t*) (*J* = 7.59 Hz)
Me		0.85(*d*) (*J* = 6.92 Hz)		0.85(*d*) (*J* = 6.92 Hz)		
Me		0.85(*d*) (*J* = 6.92 Hz)		0.85(*d*) (*J* = 6.92 Hz)		
Me		0.80(*s*)		0.80(*s*)		
Me		0.79(*s*)		0.79(*s*)		
Me		0.76(*s*)		0.75(*s*)		

resonance was found as a sharp singlet at δ 3.88. Phaeophytin a is substituted with a phytyl ester at C-17^3. No evidence for such an ester was found for compound **3**. However, the ^{13}C NMR spectrum showed an additional methylene carbon resonance at δ 61.0 and an additional methyl group carbon resonance at δ 14.0. The HETCOR spectrum indicated a correlation between the peak at δ 61.0 and a two proton multiplet at δ 4.00 as well as a correlation between the methyl group carbon resonance at δ 14.0 and a three proton triplet at δ 1.10 (J = 7.59 Hz). The COSY spectrum showed coupling between these two proton resonances. As such it was concluded that an ethyl ester was present at C-17^3. The stereochemistry was assigned by spectral correlations with phaeophytin a [2]. Thus compound **3** was identified as 17^3-ethoxyphaeophorbide a. The stereochemistry at C-13^2 was further confirmed by an NOE experiment. Irradiation of H-17 gave a positive NOE for 3H-18′ and for H-13^2.

The ^{1}H and ^{13}C NMR spectra of compound **4** were very similar to those found for phaeophytin a. The only difference occurred at C-13^3. The H-13^2 resonance occurred at δ 6.20, as expected and the C-13^2 resonance was present in the usual position at δ 64.7. However, no evidence was found for a methyl ester at C-13^3. The fully substituted carbonyl resonance at δ 173, typical of a methyl ester at C-13^3 was absent and an additional fully substituted carbon resonance was present at δ 169.5. The 3H-13^4 singlet, usually appearing at δ 3.88, was also absent. It was thus suspected that a carboxylic acid group was present at C-13^2. The IR spectrum indicated the possibility of a carboxylic acid, a broad band could be seen at 3388 cm^{-1}, but this was not conclusive as the NH stretch also occurs in this region.

Compound **5** was isolated in very small quantities and only a ^{1}H NMR spectrum and an IR spectrum were obtained. Comparisons with the ^{1}H NMR spectra and the IR spectra of compound **3** and compound **4** led to the suggested structure, 17^3-ethoxyphaeophorbide a-13^2-carboxylic acid. As with compound **3**, the ester at C-17^3 was an ethyl ester. This was indicated by the 2H-17^4 resonance occurring at δ 4.00 and the 3H-17^5 resonance occurring at δ 1.10. The fact that a carboxylic acid group was present at C-13^2, as with compound **4**, was indicated by the absence of a fourth sharp three proton singlet at δ 3.88, assigned to 3H-13^4. The H-13^2 resonance, as with compound **4**, appeared at δ 6.20 as a sharp singlet. The IR spectrum of compound **5** also contained a broad band at 3400 cm^{-1}, but as with compound **4**, this could also have been due to the N–H stretch.

Risch *et al.* [4] published the ^{13}C NMR data for phaeophytin b and the ^{13}C NMR data for compound **6** compared favorably except for the absence of the phytyl ester at C-17^3. Instead, as with compounds **3** and **5**, evidence was found for the presence of an ethyl ester at C-17^3. The 2H-17^4 resonance was present at δ 4.00(*m*) and 3H-17^5 was present at δ 1.10. The C-17^4 methylene carbon resonance appeared at δ 61.0 and C-17^5 as a methyl group carbon resonance at δ 14.0. Thus compound **6** was identified as 17^3-ethylphaeophytin b. The stereochemistry was assigned by spectral correlations with phaeophytin b [4].

EXPERIMENTAL

The dried leaves (300 g) of *Tapura fischeri* were crushed and extracted successively with hexane and EtOAc using a soxhlet apparatus for 24 h. Repeated CC over silica gel (Merk Art. 9385, solvent: hexane–EtOAc) of the hexane extract (5.81 g) yielded phaeophorbide a methyl ester **1** [2] and likewise repeated CC of the EtOAc extract yielded phaeophytins **2–6**. The ^1H NMR data for compounds **1–6** are given in Table 1 and the ^{13}C NMR data for compounds **1–4** and compound **6** are given in Table 2. The NMR spectra were run in $CDCl_3$, relative to TMS, at room temp on a Gemini 300 MHz spectrometer. All IR spectra were recorded as liquid films using NaCl discs. The HRMS were obtained by Dr Boshoff at the Cape Technikon, South Africa.

17^3-*Ethoxyphaeophorbide a* (**3**)

Amorphous (60 mg), HR-MS *m/z* 620.3008 $[M^+]$ ($C_{37}H_{40}N_4O_5$ requires 620.2998). EIMS *m/z* 620 $[M^+]$, 588, 562; IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 2927, 2854, 1749, 1705, 1624, 1550, 1459, 1347, 1216, 1163; $[\alpha]_D = +7.81°$ ($c = 1.600$ g/100 cm^3, solvent $CHCl_3$).

Phaeophytin a-13^2-*carboxylic acid* (**4**)

Amorphous (30 mg). IR ν_{max} ($CHCl_3$) cm^{-1}: 3388, 2927, 2854, 1736, 1703, 1617, 1459, 1400, 1163, 1038.

17^3-*Ethoxyphaeophorbide a*-13^2-*carboxylic acid* (**5**)

Amorphous (25 mg). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 2920, 2861, 1729, 1700, 1610, 1459, 1400, 1097, 1037.

17^3-*Ethoxyphaeophytin b* (**6**)

Amorphous (30 mg). IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 2914, 2848, 1736, 1703, 1640, 1459, 1374, 1235, 1163, 1038° $[\alpha]_D = +366.67°$ ($c = 0.300$ g/100 cm^{-1}, solvent $CHCl_3$).

Table 2. ^{13}C NMR data for compounds **1–6**

C	**1**	**2**	**3**	**4**	**6**
1	142.2(*s*)	142.9(*s*)	142.0(*s*)	142.0(*s*)	143.5(*s*)
2	131.8(*s*)	131.8(*s*)	131.8(*s*)	131.8(*s*)	132.3(*s*)
2′	12.1(*q*)	12.1(*q*)	12.1(*q*)	12.1(*q*)	12.1(*q*)
3	136.5(*s*)	136.5(*s*)	136.5(*s*)	136.5(*s*)	137.5(*s*)
3′	129.0(*d*)	129.0(*d*)	129.0(*d*)	129.0(*d*)	128.6(*d*)
3^2	122.8(*t*)	122.8(*t*)	122.8(*t*)	122.8(*t*)	123.3(*t*)
4	136.2(*s*)	136.2(*s*)	136.2(*s*)	136.2(*s*)	136.9(*s*)
5	97.5(*d*)	97.3(*d*)	97.5(*d*)	97.5(*d*)	101.5(*d*)
6	155.5(*s*)	155.5(*s*)	155.5(*s*)	155.5(*s*)	151.0(*s*)
7	136.1(*s*)	136.1(*s*)	136.1(*s*)	136.1(*s*)	132.5(*s*)
7′	11.2(*q*)	11.2(*q*)	11.2(*q*)	11.2(*q*)	187.1(*s*)
8	145.2(*s*)	145.2(*s*)	145.2(*s*)	145.2(*s*)	158.8(*s*)
8′	19.7(*t*)	19.7(*t*)	19.7(*t*)	19.7(*t*)	18.5(*t*)
8^2	16.5(*q*)	16.3(*q*)	17.3(*q*)	16.3(*q*)	19.5(*q*)
9	151.0(*s*)	151.0(*s*)	151.0(*s*)	153.5(*s*)	146.8(*s*)
10	104.0(*d*)	104.4(*d*)	104.4(*d*)	104.0(*d*)	106.5(*d*)
11	138.0(*s*)	137.9(*s*)	137.9(*s*)	137.9(*s*)	137.9(*s*)
12	129.1(*s*)	129.1(*s*)	129.1(*s*)	129.0(*s*)	132.2(*s*)
12′	12.2(*q*)	12.2(*q*)	12.2(*q*)	12.2(*q*)	11.9(*q*)
13	129.0(*s*)	129.0(*s*)	129.0(*s*)	129.0(*s*)	129.7(*s*)
13′	189.6(*s*)	189.6(*s*)	189.6(*s*)	189.6(*s*)	189.6(*s*)
13^2	64.7(*d*)	64.7(*d*)	64.7(*d*)	64.7(*d*)	64.7(*d*)
13^3	173.0(*s*)	173.0(*s*)	173.0(*s*)	169.5(*s*)	169.5(*s*)
13^4	53.0(*q*)	53.0(*q*)	53.0(*q*)		53.0(*q*)
14	150.0(*s*)	150.0(*s*)	150.0(*s*)	150.0(*s*)	150.8(*s*)
15	105.2(*s*)	105.2(*s*)	105.2(*s*)	105.2(*s*)	105.1(*s*)
16	161.3(*s*)	161.3(*s*)	161.3(*s*)	161.3(*s*)	164.0(*s*)
17	51.1(*d*)	51.1(*d*)	51.1(*d*)	51.1(*d*)	51.5(*d*)
17′	29.8(*t*)	29.8(*t*)	29.8(*t*)	29.8(*t*)	31.3(*d*)
17^2	31.2(*t*)	31.2(*t*)	31.2(*t*)	31.2(*t*)	29.8(*t*)
17^3	172.0(*s*)	172.0(*s*)	172.0(*s*)	173.0(*s*)	174.0(*s*)
18	50.0(*d*)	50.1(*d*)	50.1(*d*)	50.1(*d*)	50.0(*d*)
18′	22.7(*q*)	22.7(*q*)	22.7(*q*)	22.7(*q*)	23.1(*q*)
19	170.0(*s*)	170.0(*s*)	170.0(*s*)	172.2(*s*)	173.0(*s*)
20	93.1(*d*)	93.1(*d*)	93.1(*d*)	93.7(*d*)	93.1(*d*)
17^4	52.0(*q*)	61.0(*t*)	61.0(*t*)	61.0(*t*)	61.0(*t*)
17^5		118.0(*d*)	14.0(*q*)	118.0(*d*)	14.0(*t*)
17^6		142.0(*s*)		142.5(*s*)	
17^7		62.0(*t*)		62.0(*t*)	
CH_2		39.5(*t*)		39.5(*t*)	
		37.0(*t*)		37.0(*t*)	
		37.0(*t*)		37.0(*t*)	
		36.5(*t*)		36.5(*t*)	
		25.0(*t*)		25.0(*t*)	
		24.8(*t*)		24.8(*t*)	
		24.2(*t*)		24.2(*t*)	
		40.0(*t*)		40.0(*t*)	
CH		28.0(*d*)		28.0(*d*)	
		32.5(*d*)		32.5(*d*)	
		32.5(*d*)		32.5(*d*)	
Me		22.7(*q*)		22.7(*q*)	
		22.6(*q*)		22.6(*q*)	
		19.6(*q*)		19.6(*q*)	
		19.4(*q*)		19.4(*q*)	
		16.2(*q*)		16.2(*q*)	

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