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DITERPENOID HYDROCARBONS IN THE SEA GRASS *AMPHIBOLIS ANTARTICA*

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Key Word Index—*Amphibolis antartica*, Potamogetonaceae, sea grass, cleistanthene, sandaracopimaradiene, isopimaradiene, *n*-hydrocarbons

Abstract—The leaves of *Amphibolis antartica* from Shark Bay, Western Australia contain a previously unreported cleistanthene hydrocarbon Other collections of the sea grass near Perth also contained sandaracopimaradiene and isopimaradiene Concentrations of the diterpenes increase with leaf age, while *n*-hydrocarbon content decreases with leaf age

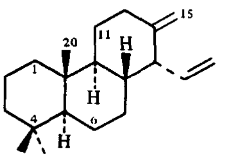
INTRODUCTION

Amphibolis antartica and *Posidonia australis* are the major sea grasses of Shark Bay, Western Australia Meadows of these grasses dominate the metahaline regions of the Bay establishing complex floral and faunal communities within monospecific stands of grass The grasses are rarely directly grazed but form a detrital energy chain via the shedding and subsequent settling of leaves

An ongoing study of the organic geochemistry of the sediments of Shark Bay required knowledge of the input of hydrocarbons from sea grass leaves *Posidonia australis* had been examined in this context [1] but no study existed for *A antartica* which provides the larger biomass of these grasses in Shark Bay A previously unreported hydrocarbon and its concentration distribution between juvenile and mature leaves of *A antartica* are documented below

RESULTS AND DISCUSSION

Examination by GC/MS of the hydrocarbons from the leaves of *A antartica* showed the presence of *n*-alkanes (C_{17}–C_{25}, odd number predominance) and a component **1** ($[M]^+$ 272, $C_{20}H_{32}$) Extraction of a large sample of leaves, followed by silicic acid and alumina chromatography of the crude extract, yielded a colourless oil, essentially homogeneous by GC (0 0002 % fr wt) to which structure **1** was assigned on the following evidence Of the five degrees of unsaturation required by its M_r, two are accounted for by the presence of five olefinic protons including an exocyclic methylene and an ethenyl group as determined by ^1H and ^{13}C NMR The compound was, therefore, a tricyclic diene Comparison of the ^1H and ^{13}C NMR spectra (Table 1) of **1** with those of the relevant protons and carbons of **2–4** assigned the skeleton as a (5α,8β,9α,10β)-4,4,10-trimethylphenanthrene * Irradiation of the doublet of doublets at δ2 81 collapsed the one-proton resonance centred at δ6 06 to a X part of an ABX system, while irradiation at δ6.06 collapsed this doubly allylic proton to a doublet and the two proton resonance at *ca* δ5 02 to an AB quartet Irradiation of the two allylic



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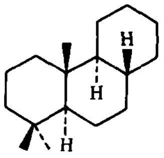

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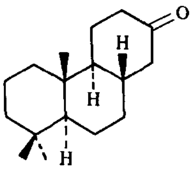

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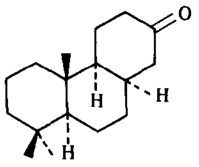

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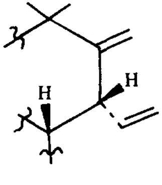

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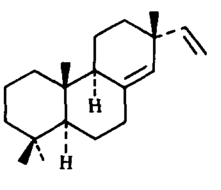

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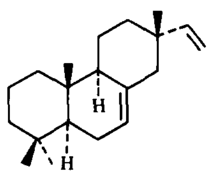

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*Relative configuration only Alternatively, **1** may be named (4aβ,4bα,8aβ,10aα)-8-ethenyl-7-methylene-tetradecahydro-1,1,4a-trimethylphenanthrene

Table 1 Comparison of the ^{13}C chemical shifts (20 1 MHz, deuterochloroform, TMS as int standard) of **1** with model compounds **2** [9], **3** and **4**

C No *	1	2	3	4
1	39 6	39 1	39 4	40 5
2	18 9	19 0	18 7	18 3
3	42 2	42 2	42 1	42 1
4	32 1	33 3	33 1	33 3
5	55 2	55 5	55 2	54 7
6	21 5	21 8	21 6	18 1
7	31 4	35 9	37 9	31 7
8	40 7	36 8	35 7	34 4
9	54 8	56 3	54 5	46 6
10	39 6	36 9	37 0	38 5
11	27 0†	25 1	25 6	27 7
12	29 6†	27 1	41 3	38 9
13	152 8	26 4	216	214
14	49 4	35 5	49 1	45 0
15	106 3	—	—	—
16	138 1	—	—	—
17	115 7	—	—	—
18	33 6	33 6	33 7	33 5
19	22 1	22 0	21 9	21 8
20	14 0	14 3	14 2	17 4

*For convenience, the rings have been numbered as for **1**

†These assignments may be interchanged

Table 2 Leaf hydrocarbons from a single head of *Amphibolis antartica*

Leaf No *	Dry leaf wt (mg)†	Hydrocarbon (ηg) (μg/g)		
		1	n-C_{19}	n-C_{21}
1	68	2632 (39)	ND	ND
2	40	1378 (34)	186 (5)	Trace
3	59	1390 (23)	181 (3)	Trace
4	49	1339 (27)	267 (5)	218 (4)
5	34	978 (29)	448 (13)	268 (8)
6	26	535 (21)	1071 (41)	475 (18)
7	15	190 (13)	1391 (93)	672 (45)
8	8	39 (5)	1614 (201)	723 (90)
9	4	Trace	1833 (458)	817 (204)
10	2	ND	1416 (708)	777 (388)
E‡	141	ND	Trace	Trace

*Leaf No 1, Most mature leaf, leaf No 10, juvenile leaf

†Most of the epiphyte was scraped off leaves Nos 1–6 Leaves Nos 7–10 were essentially epiphyte free

‡E, Epiphyte from leaves Nos 1–6 Contains n-heptadecane 3834 ηg (27 μg/g)

ND, Not detected

protons at δ2 16 collapsed the finely coupled multiplet at δ4 57 to a doublet These results demand the partial structure **5** Finally, irradiation at δ1 5 collapsed the doublet of doublets at δ2 81 to a doublet The coupling constant between these two protons, H-8 and H-14, was measured as 3 5 Hz and supports the 8-ethenyl substituent as axial [2]

Lack of material and the relative instability of **1** prevented any definitive chemical degradation Conversion of **1** to its tetrol with osmium tetroxide, periodate oxidation and oxidation with Jones reagent gave a complex mixture Analysis of this mixture by GC/MS showed one component to be consistent, by its mass spectrum, with that of **3** No further material was available to confirm the formation of the expected β-keto acid and its subsequent decarboxylation to **3**

Collections of *A antartica* from near Perth contained not only **1** but also the known isomers sandaracopimaradiene (**6**) and isopimaradiene (**7**), identified, for **6**, by its mass spectrum [3] and for **7** by GC/MS comparison of an authentic sample

Analysis of individual heads of leaves of *A antartica* from Shark Bay showed (a) that n-alkanes are concentrated in juvenile leaves and diminish as the leaf ages, and (b) that **1**, while not detected in juvenile leaves increases with leaf age and persists in leaves which have become detritus In samples from near Perth, the same distribution of n-alkanes and all three diterpenes is observed Epiphytes on the leaves contribute a significant proportion of n-heptadecane The results for the hydrocarbon analysis of a single *A antartica* head of leaves are given in Table 2

This is the first report of diterpenoid hydrocarbons in sea grass Furthermore, **1** contains the cleistanthane skeleton, of which there have been only a few reports [4–6] It is difficult to gauge the importance of **1** as a geochemical marker because, first, it has not been previously reported and, secondly, while it is stable in leaf detritus and fragments of buried leaf (unpublished results) the isolated oil, in the laboratory, is unstable Sandaracopimaradiene and isopimaradiene, isolated from collections near Perth, are more stable and have been found in recent sediments [7]

The differences observed in different collections of this sea grass have not been correlated with spatial or temporal variations The concentrations of **1**, **6** and **7** in maturing leaves appear to reach a limit and may reflect the point at which the leaf stops growing The possibility that these diterpenes are produced in response to epiphytic growth, which may be considerable, was negated by analysis of the leaf hydrocarbons of a laboratory cultured seedling containing no epiphytes, and which showed the same diterpene pattern as field collections It is possible that these diterpene hydrocarbons are a shunt pathway incorporated into the biosynthesis of kaurene and gibberellins [8] which are no longer needed as leaf growth diminishes resulting in a build-up of the observed diterpenes Knowledge of the absolute stereochemistry of **1** is required to further support this postulate

The leaves of *A antartica* are rarely grazed but contribute to the energy budget via a detrital food chain Mature leaves fall to the sediment while juvenile leaves continue to mature on the stem Therefore, whereas n-hydrocarbons and diterpene hydrocarbons are found in the leaves of this sea grass, it is the diterpenes which significantly contribute to the sediment hydrocarbon profile It is possible during high physical energy conditions, such as cyclones, that whole *Amphibolis* beds may be disturbed and, subsequently, buried thereby contributing both n-hydrocarbons and diterpenes to the sediment hydrocarbon profile

EXPERIMENTAL

Extraction Fresh frozen *A antartica* leaves (8 8 kg) collected in Shark Bay, Western Australia, were removed from their stems and blended, in batches, with MeOH (~ 8 l) The leaves were further extracted with CH_2Cl_2 and petrol to yield a combined extract of 24 g The extract was fractionated by rapid elution with petrol through silicic acid to yield a colourless oil (400 mg) CC of this fraction on neutral alumina (III) with *n*-pentane gave, as a colourless oil, **1** homogeneous by GC (16 mg, 0 0002 % fr wt), $[\alpha]_D$ +42° ($CHCl_3$, *c* 8 0), ^{1}H NMR (80 MHz, $CDCl_3$) δ0 81, 0 83 and 0 84 (H_3-18, H_3-19, H_3-20), 2 16 (*m*, H_2-12), 2 81 (*dd*, *J* = 9 0, 3 5 Hz, H-14), 4 57 (*m*, H-15), 4 65 (*d*, *J* = 2 3 Hz, H-15), 5 01 (*dd*, *J* = 2 4, 10 4 Hz, H-17), 5 03 (*dd*, *J* = 2 4, 16 4 Hz, H-17), 6 06 (*ddd*, *J* = 16 4, 10 4, 9 0 Hz, H-16), ^{13}C NMR (see Table 1), EIMS *m/z* (rel int) 272 $[M]^+$ (31), 257 (64), 230 (10), 187 (14), 177 (25), 175 (20), 163 (21), 161 (21), 149 (20), 148 (34), 147 (24), 135 (32), 134 (49), 133 (36), 125 (28), 123 (38), 121 (32), 119 (42), 109 (43), 107 (44), 105 (45), 95 (55), 93 (50), 91 (47), 81 (60), 79 (68), 69 (100), 67 (46), 55 (37)

Analysis of hydrocarbons Leaves, collected in Shark Bay, Pt Peron and Cottesloe near Perth and Rottnest Island, Western Australia, were extracted with CH_2Cl_2–MeOH (1 1) for 2–3 hr The CH_2Cl_2 layer was concd and the pentane solubles eluted from a column of neutral alumina (I) The total pentane eluant was analysed by GC Samples were analysed by FID on a capillary instrument in the splitless mode Either a 12 5 m or 50 m × 0 2 mm crosslinked Me silicone capillary column was used for analyses The column was temp programmed from 70° to 130° at 30°/min and then to 270° at 3°/min Under these conditions (on the 50 m column), the retention indices were determined sandaracopimaradiene, 1956, cleistanthene, 1958, isopimaradiene, 1985 Selected alkene fractions were analysed by capillary GC/MS using a quadrupole mass spectrometer

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