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METABOLITES OF THE GREEN ALGAE, *CAULERPA* SPECIES

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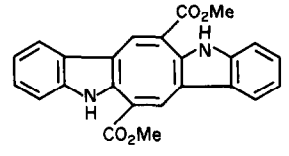
Key Word Index—*Caulerpa*; Chlorophyta; labd-7-ene; diacetoxybutadienes.

Abstract—Five *Caulerpa* species available in Western Australia have been investigated for the presence of terpenoid metabolites. A new bicyclic diterpene, containing a labd-7-ene skeleton and a 1,4-diacetoxybutadiene moiety, has been isolated from *C. trifaria*. *C. brownii*, *C. flexilis*, *C. peltata* and *C. racemosa* lacked significant quantities of terpenes and only caulerpin could be isolated from *C. peltata* and *C. racemosa*.

INTRODUCTION

The genus *Caulerpa* is represented by *ca* 60 species which are widely distributed in tropical and subtropical waters [1]. Recent chemical interest in the metabolites of the genus arose from a search for the peppery principle from those species considered edible [2–5] and from the observation that proximate herbivorous fish and invertebrates avoid consuming plants of the Caulerpaceae and Codiaceae families [6]. The metabolites isolated from different *Caulerpa* species include triterpenes and sterols [4, 7], xanthophylls [8], squalene and squalene epoxides [9, 10], the di-indolo pigment caulerpin (**1**) [5, 11] and caulerpecin, a mixture of ceramides from (2*S*, 3*R*)-sphinganine [3]. Caulerpin and caulerpecin are physiologically active and toxic to rats and mice [12]. Interestingly, *Caulerpa* species [13–17] and related green algae [6, 18] have been shown to elaborate acyclic and monocyclic sesqui- and diterpenes, some of which contain 2-substituted 1,4-diacetoxy-1,3-butadiene systems. A few of these compounds have been shown to be toxic feeding deterrents to fish [6].

We have described previously the structure of two monocyclic sesquiterpenes from *Caulerpa flexilis* var *muelleri* [17] one of which, **2**, contained a diacetoxybutadiene system.* In an extension of this work other *Caulerpa* species available in Western Australian waters have been examined for the presence of terpene metabolites and the results are documented below. Of particular interest is the isolation from *C. trifaria* of a bicyclic diterpene containing the 1,4-diacetoxy-1,3-butadiene moiety. The evidence for the structure of this new diterpene is the main topic of this report.



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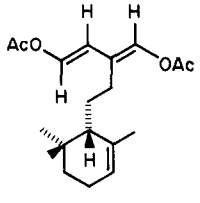

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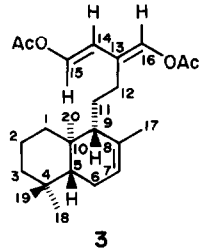

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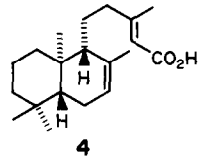

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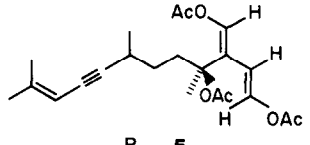

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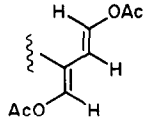

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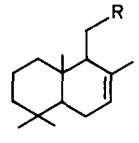

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RESULTS AND DISCUSSION

Extraction of a sample of *C. trifaria*, collected near Point Peron in Western Australia, followed by chromatography of the crude extract yielded a crystalline compound (0.4% dry wt), $C_{24}H_{36}O_4$ to which we have assigned structure **3** on the following evidence. The ^1H NMR spectrum of **3** included resonances for a 2-substituted 1,4-diacetoxy-1,3-butadiene system; a sharp singlet (δ 2.14) for two acetoxymethyl groups, two one proton doublets (δ 5.93 and 7.45, J = 12 Hz) and a one proton singlet (δ 7.19) for the olefinic protons. Comparison of the ^1H and ^{13}C NMR data (Table 1) of **3** with those of the relevant protons of **2** and of the UV (λ_{max} 251 nm, ε 17 800) and IR spectra (ν_{max} 3100, 1760 and 1625 cm^{-1}) supported this assignment. Other signals in the ^1H NMR spectrum of **3** indicated the presence of three tertiary methyl groups (δ 0.73, 3H; 0.87, 6H) and an

*In this paper the absolute configuration of **2** was assigned by comparison of the molecular rotation with that of pallescensin-1 which, because of incorrect application of the sequence role, had been assigned the (*R*)-configuration. In fact, both **2** and pallescensin-1 have the (*S*)-configuration at the lone chiral centre.

Table 1. Comparison of the ^{13}C chemical shifts (20.1 MHz, $CDCl_3$, TMS as int. standard) of **3** with model compounds **2** and **4**

C No.	3	4	C No.	3	2*
1	39.3	39.2	11	26.0	26.1
2	19.0	18.8	12	28.1	29.1
3	42.5	42.3	13	122.4	122.4
4	33.1	33.0	14	113.3	113.3
5	50.4	50.2	15	134.3	134.3
6	24.0	23.8	16	136.1	135.9
7	122.6	122.7	OCOMe	167.4, 167.8	167.5, 167.9
8	135.1	134.4	OCOMe	20.6, 20.6	20.6, 20.6
9	55.2	54.5			
10	37.0	36.9			
17	22.0	25.3			
18	21.9	21.8			
19	33.2	33.1			
20	13.6	13.6			

*For convenience the side chain carbon atoms have been numbered as for **3**.

olefinic methyl (δ 1.81) which was shown to have coupling to an olefinic proton (δ 5.42). The two remaining degrees of unsaturation can be accommodated by considering **3** to be a bicyclic diterpene. The presence of four pendant methyl groups and their chemical shifts [19, 20] suggested that **3** contained a labd-7-ene skeleton. The mass spectrum of **3** also supported this inference showing significant ions at m/z 191, attributable to cleavage of the C-9–C-11 bond, and at m/z 204 which could be considered to arise by a cyclic rearrangement involving transfer of the hydrogen at C-9 to C-16. Corresponding ions arising from similar fragmentation processes had been observed in the mass spectrum of **2** [17].

Evidence for the structure of **3** was obtained by a comparison of the ^{13}C NMR spectrum of *ent*-labda-7,13-dien-15-oic acid (**4**) [21] which showed that the chemical shifts for the carbons associated with the bicyclic ring system were almost identical (Table 1). This comparison, in addition, serves to determine the relative configuration of **3**.

The (*E*)-configuration at C-14–C-15 follows from the value of the coupling constant (J = 12 Hz) between the vicinal protons [22]. The assignment of the stereochemistry of the double bond at C-13 is rather more difficult. The only natural diacetoxybutadiene of this type, for which evidence has been obtained for the configuration of the trisubstituted double bond, is caulerpenyne (**5**) [15]. In this case an NOE (25%) between the 1,3-protons of the butadiene system established the configuration of the double bond. For all the other naturally occurring diacetoxybutadienes the configuration shown in **6** has been assumed on the basis of the close correspondence of the spectral data with that reported for 1,4-diacetoxy-2-methylbuta-1,3-diene supposedly having the (*E,E*)-stereochemistry [23]. Subsequent revision of this assignment has shown that the model compound, in fact, had (*Z,E*)-stereochemistry [22]. The chemical shift values for the C-1, C-3 and C-4 protons of the diacetoxybutadiene systems present in trifarin, flexilin [14], didehydrotrifarin and congeners [18] are in the ranges (in deuterochloroform or carbon tetrachloride) δ 7.08–7.17, 5.82–5.88 and 7.32–7.37, which are in good agreement with those quoted for the (*E,E*)-model compound (δ 7.21, 6.04 and 7.39) but not with those for the (*Z,E*)-compound (δ 6.97, 6.42 and 7.30) [22].

In the bicyclic diterpene (**3**) the corresponding values were δ 7.19, 5.93 and 7.45, respectively, supporting an (*E,E*)-configuration. In fact, irradiation of H-16 resulted in a 21% enhancement of the signal for H-14 (reverse 13%) thus establishing an (*E*)-configuration of the double bond at C-13(16).

Lack of material and the relative instability of **3** prevented a degradative approach to establish its absolute configuration. Carman [24] has shown that for a large number of labdane diterpenes, in which as asymmetric ring structure is separated by two methylene groups, the molecular rotations of the two portions are approximately additive, provided no interaction occurs between the two segments. The contribution to the molecular rotation value of ring system A to a molecule has been calculated to be $-1°$ but these calculations were based on only two model compounds (only a few labdane diterpenes containing the Δ^7-olefin are known).

Since Carman's work [24] other examples of compounds incorporating ring system A have become available and similar calculations suggest that the contribution of this ring system to the molecular rotation is greater, ranging from $+17°$ to $+73°$ (three examples in the labd-7-ene series) [25, 26] and from $-45°$ to $-82°$ (three examples in the *ent*-labd-7-ene series) [19, 20]. In so far as the molecular rotation of **3** ($-31°$) in negative and within the order of magnitude predicted it appears that **3** has the *ent*-labdane configuration.

The isolation of the bicyclic diterpene, **3**, from *C. trifaria* was surprising since previous work on a Tasmanian collection of *C. trifaria* has resulted [14] in the isolation of trifarin, an acyclic diterpene containing the diacetoxybutadiene moiety. In view of the difference in metabolites between two taxonomically similar species we decided to investigate the metabolites of other *Caulerpa* species available in Western Australian waters.

Samples of *C. racemosa*, *C. peltata*, *C. brownii* and *C. flexilis*, collected at different points along the Western Australian coast, were examined for the presence of terpenoid metabolites. No significant amount of terpenes was detected and, from *C. racemosa* and *C. peltata*, only

caulerpin (**1**) could be isolated in low yield (0.003%). The presence of caulerpin in *C. racemosa* collected in Sri Lanka and Western Australia [11] and in the var. *clavifera* from the Phillippines [5] has been noted previously. However, the absence of isolatable quantities of terpenoids from *C. brownii* and *C. flexilis* was surprising as samples of these species collected in Tasmania contained significant amounts of the diterpene caulerpol [13] and the sesquiterpene flexilin [14], respectively.

EXPERIMENTAL

General details have been described previously [27].

Extraction of C. trifaria. A sample of *C. trifaria* (102 g dry wt), collected off Point Peron, near Perth, Western Australia, was coarsely homogenized and extracted with CH_2Cl_2–MeOH (1:1). The CH_2Cl_2–soluble extract (4.2 g, 4% dry wt) was fractionated by rapid elution (light petrol to CH_2Cl_2 to EtOAc gradient elution) through silicic acid to yield a fraction (402 mg), eluted with light petrol–CH_2Cl_2 (1:3), which crystallized from light petrol–CH_2Cl_2 as white rods of the bicyclic diterpene **3**, mp 62.5–63.0°, $[\alpha]_D - 8°$ ($CHCl_3$; *c* 6.6). (Found: C, 74.53; H, 9.74. $C_{24}H_{36}O_4$ requires C, 74.19; H, 9.34%.) IR ν_{max}^{film} cm^{-1}: 3100, 1760, 1625; UV λ_{max}^{EtOH} 251 nm (ε 17800); ^1H NMR (90 MHz; $CDCl_3$): δ 0.73 (3H) and 0.87 (6H) (*br s*, H_3-18, H-19, H-20), 1.81 (*m*, H_3-17), 2.14 (*s*, 2 × –OCOMe), 5.42 (*br m*, H-7), 5.93 (*d*, *J* = 12.0 Hz, H-14), 7.19 (*br s*, H-16), 7.45 (*d*, *J* = 12.0 Hz, H-15); ^{13}C NMR (see Table 1). MS(EI) *m/z* (rel. int.): 388 $[M]^+$ (1), 328 (9), 286 (8), 204 (91), 191 (34), 189 (19), 161 (25), 149 (27), 133 (19), 109 (71), 43 (100); MS (CI, isobutane) *m/z*: 389 $[M+1]^+$, (6), 329 (36), 287 (100).

Extraction of C. peltata *and* C. racemosa. A sample of *C. peltata* was collected in a tidal lagoon on Big Nook Island in the Southern Abrolhos Group in Western Australia (individual plants ranged from 30–50 cm in length). Extraction of the freshly thawed sample (825 g, wet wt), as described above, afforded a CH_2Cl_2-soluble extract (1 g) which was fractionated through silicic acid. A fraction eluted with CH_2Cl_2 afforded an orange-red powder (26 mg, 0.003%) which recrystallized from Et_2O as pale red crystals of caulerpin (**1**), mp 311–314.5° (lit. [5, 11] mp 317°). ^1H NMR, IR, UV and MS characteristics were identical with those reported [11].

Samples of *C. racemosa* were collected from Augusta to Lancelin along the Western Australian coastline. Screening of the CH_2Cl_2-soluble extracts of these samples by TLC (Si gel, CH_2Cl_2) showed them to contain caulerpin (**1**).

Examination of C. brownii *and* C. flexilis. A number of samples of these two species were collected from Augusta to Lancelin along the Western Australian coast. Extraction of the samples as described above gave CH_2Cl_2-soluble fractions which were screened by ^1H NMR. Although the ^1H NMR spectra in some cases showed the presence of traces of other compounds besides fat, these could not be isolated by prep. TLC.

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