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COMPARISON OF THE SESQUITERPENES FROM THE SEAWEED *LAURENCIA PACIFICA* AND ITS EPIPHYTE *ERYTHROCYSTIS SACCATA*

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Key Word Index—*Erythrocystis saccata*; *Laurencia pacifica*; *Rhodomelaceae*; halogenated terpenes; epiphyte–host interactions; marine natural products.

Abstract—Sesquiterpene natural products have been compared between the red seaweed epiphyte *Erythrocystis saccata* and its host *Laurencia pacifica*. Three separate collections of the host–epiphyte pair were investigated and the sesquiterpene components of the epiphyte were exactly parallel to that of the host. The major components varied as a function of collection location as follows: Stillwater Cove, aplysin and debromoaplysin; Stillwater Cove recollection, isolaurinterol and debromo isolaurinterol; Catalina Is, laurenisol and bromolaurenisol. The implications of this parallel secondary metabolite chemistry are briefly discussed.

INTRODUCTION

Seaweeds of the genus *Laurencia* (Order, Gigartinales; Family, Rhodomelaceae) produce fascinating secondary metabolites which may be useful for comparative biochemical studies. Over thirty *Laurencia* species have now been investigated, and virtually all [1, 2] contain halogenated sesquiterpenes, halogenated diterpenes and/or halogenated C_{15}-acetogenins [1–4]. With few exceptions, these particular halogenated metabolites only occur in the genus *Laurencia* [1, 2]. The remarkable biological observations involving these natural products include the following. Isomorphic populations of *Laurencia pacifica* (kylin) collected from different sites show variations in their sesquiterpenoids, presumably due to genetic differences at the species level [5]. The sea hare *Aplysia californica*, while grazing on *L. pacifica*, concentrates halogenated terpenes in its digestive gland. These compounds have been suggested to protect both the mollusc and the seaweed [6–8]. The larvae of *A. californica* must settle upon *L. pacifica* before further metamorphosis, but the involvement of seaweed metabolites in this phenomenon remains unknown [9].

Erythrocystis saccata (Order, Gigartinales; Family, Rhodomelaceae), a small parasitic red algae, grows in the coastal zone only on *Laurencia* and most often on *L. pacifica* [10]. Unlike most epiphytic algae, *Erythrocystis* is highly pigmented and is described as hemiparasitic [11]. Microscopy reveals that attachment to its host is by a large thick-walled rhizoid which does not appear to connect with adjacent host cells [12]. In culture *E. saccata* (J. Ag.) germlings apparently only develop normally when a small amount of *L. pacifica* tissue is included in the culture medium, or when *E. saccata* settles into the apical pit of *Laurencia* [12]. These latter observations and the apparent restriction of halogenated sesquiterpenes to the genus *Laurencia* stimulated us to do a tandem study of the secondary metabolite chemistry of *L. pacifica* and *E. saccata*. Described below are our results which show that similar natural products are observable for this host–epiphyte pair.

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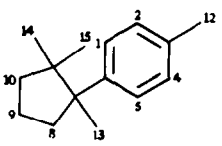
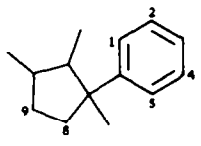
RESULTS AND DISCUSSION

We investigated three separate collections of *L. pacifica–E. saccata* pairs. High field ^{1}H NMR or GC-MS were employed to analyse their natural product mixtures. To avoid contamination the *E. saccata* was removed from the host, shortly after each seaweed collection, by a cut made well up on the epiphyte's thallus. Parallel extraction of the host and epiphyte with methanol gave crude oils which were partially purified by silica gel chromatography. The major components of *L. pacifica* were then isolated by HPLC and identified from their spectroscopic properties.

The sesquiterpene carbon skeletons from *L. pacifica* range from cuparanes (**A**), lauranes (**B**), cyclolauranes (**C**), to chamagranes (**D**), [1, 2, 13, 14]. We have previously described [15] the five major constituents isolated from *L. pacifica* collected intertidally at Stillwater Cove, Monterey, CA, in February 1978. These include aplysin (**1**) debromoaplysin (**2**), debromolaurinterol (**3a**), pacifenol (**4**) and pacifidiene (**5**). Collectively, these are all examples of type **B**, **C**, or **D** frameworks. GC-MS analysis of the semi-pure oil from the Stillwater Cove *L. pacifica* showed the presence of all the above compounds except **4** which decomposed on the GC column yielding **5** and other unidentified compounds (compare Fig. 1A and 1B). To circumvent this latter problem, compound **4** was quantitatively converted to its TMS ether **6** by treatment with *N*-trimethylsilylimidazole. Subsequent GC-MS analysis of the silylated *L. pacifica* crude oil revealed all five components, with **3a** and **4** as their respective TMS ethers **7** and **6**. *Laurencia pacifica* infected with *E. saccata* was then collected from Stillwater Cove in August 1978. Analysis of the semi-pure oil obtained from the separated



A



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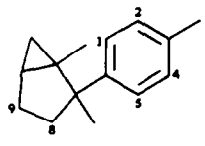

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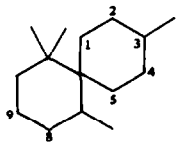

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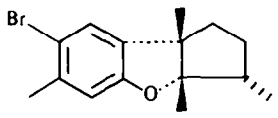
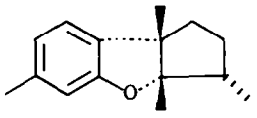


1



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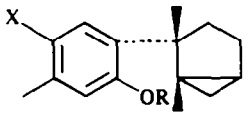
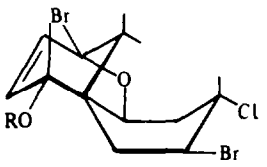
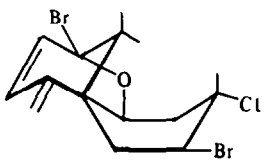


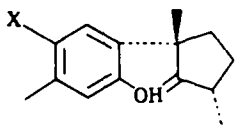
3a R = H, X = H
3b R = H, X = Br
7 R = TMS, X = H



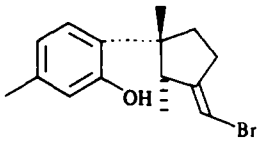


4 R = H
6 R = TMS

5



8 X = Br
9 X = H



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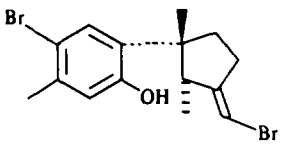
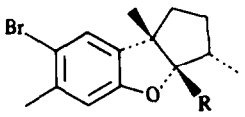


11



12 R = CH_2OH
13 R = CHO

L. pacifica revealed **1** and **2** as major components by GC-MS (Fig. 2A). Similar analysis of the *E. saccata* oil by GC-MS again revealed **1** and **2** as the major sesquiterpene components (Fig. 2A).

A new collection was made subtidally at Stillwater Cove, in 1980. In contrast to what was observed previously, GC-MS analysis of the oils from either *L. pacifica* or *E. saccata* showed major components **1** and **2** were absent. The new major components have been previously described and were isolaurinterol (**8**) [16] and debromoisolaurinterol (**9**) [16]. Definitive evidence that only these sesquiterpenes were present, in about 1:1 relative amounts, in both the *Laurencia* and *Erythrocystis* crude oils was provided by ^{1}H NMR (360 MHz, benzene-d_6) spectra (Fig. 3). The ^{1}H NMR spectrum of the *L. pacifica* crude oil clearly shows characteristic signals for compounds **8** and **9** including methyl (δ1.05, 1.2, 2.2), exomethylene (δ4.95) and aromatic ring protons (δ6.5–7.6), and these compounds are estimated as being about 16% and 12%, respectively, of the extract. By comparison, the *E. saccata* crude oil contains much less than 1% of these compounds, but in the same relative percentages (Fig. 3b).

Another collection of *L. pacifica* bearing *E. saccata* was made subtidally at Avalon Harbor, Catalina Is., CA in 1978. Parallel GC-MS analysis of their respective crude extracts (Fig. 2B) shows both contain two known major

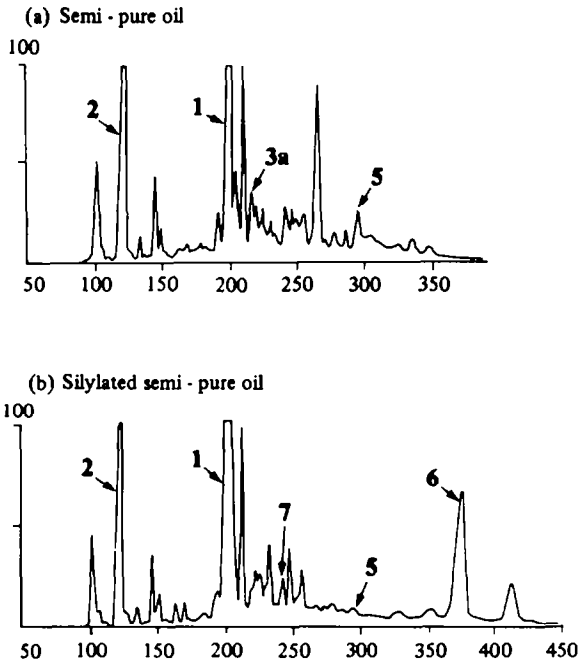


Fig. 1. GC of *L. pacifica* (Stillwater Cove, Feb. 1978).

components, laurenisol (**10**) [17] and bromolaurenisol (**11**) [17]. Pure samples of **10** and **11** were obtained by preparative HPLC work-up of the crude *L. pacifica* oil.

Several control experiments were next initiated to insure that the sesquiterpenes observed from *Erythrocystis* were not artifacts or contaminants from *Laurencia*. If, during transportation of the host–epiphyte pair back to the laboratory, the *Laurencia* began to decompose, then rupture of cells [18] might release sesquiterpenes which could adhere to the epiphyte. This possibility was ruled out because a portion of the 1980 Stillwater Cove subtidal collection was kept submerged while the epiphytes were removed. GC-MS of the resultant *Erythrocystis* crude extract again showed **8** and **9** as the two major terpene components.

The only other possibility to consider was the *Erythrocystis* tissue extracted could be contaminated with *Laurencia* tissue. Electron microscopy [11, 12, 19] shows that *E. saccata* grows principally in the apical pit at the end of the *L. pacifica* branches. Moreover, when in these pits, the *E. saccata* germling extends tendrils into the tissue of the host plant and not vice versa (see Fig. 4). Thus, *Laurencia* does not extend into the *Erythrocystis* tissue.

It is unmistakable that the sesquiterpene natural products of *E. saccata* exactly parallels that of its host, but at relative percentages which are much lower. If somehow *Erythrocystis* recognizes *Laurencia* on the basis of the natural products described herein, then it would appear that no single *L. pacifica* metabolite is responsible. Similarly, no one metabolite can be involved if *L. pacifica* is responsible for the development of *Erythrocystis*.

The question as to the origin of *Erythrocystis* sesquiterpenes is complex and can not be directly answered at this time. The most obvious explanations of the *E. saccata* chemistry we observed are (a) *Laurencia* and *Erythrocystis* are taxonomically similar and produce analogous natural products by a parallel biogenetic pathway, (b) The *Laurencia* natural products are internally translocated to *Erythrocystis*, or (c) *Erythrocystis* is able to 'concentrate' metabolites dispersed in seawater which are produced by *Laurencia*. The low yield of sesquiterpenes from *Erythrocystis* might argue against its ability to biosynthesize these metabolites. Several separate studies [20] have demonstrated that exchange of photosynthetically fixed ^{14}C occurs from an algal host to its parasite and this represents a strong precedent for possibility (b). Minimal circumstantial evidence can be found as a precedent for possibility (c). This comes from studies on contiguous red algae which have recently been summarized [8]. Also of relevence are results from two prior studies where genera other than *Laurencia* have been observed as sources of metabolites typical of this genus.

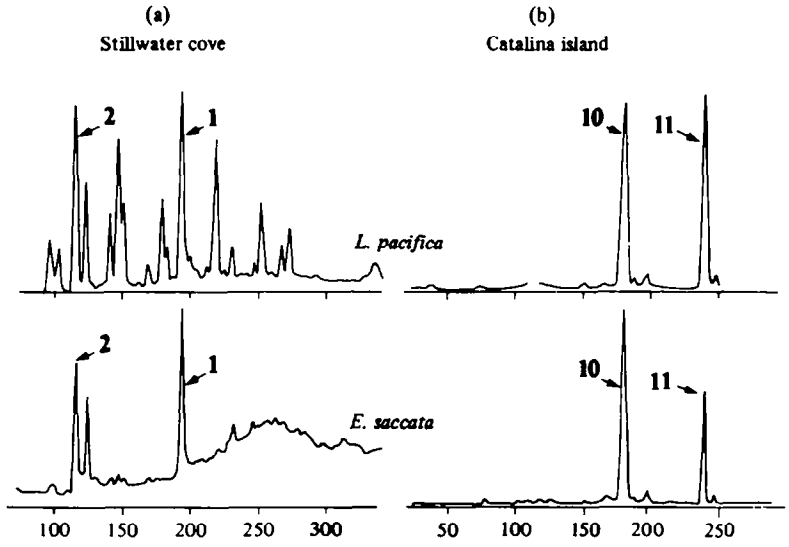


Fig. 2. Major lauranes from *L. pacifica* and *E. saccata*. A, Stillwater Cove, Aug. 1978; B, Catalina Is., Sept. 1978.

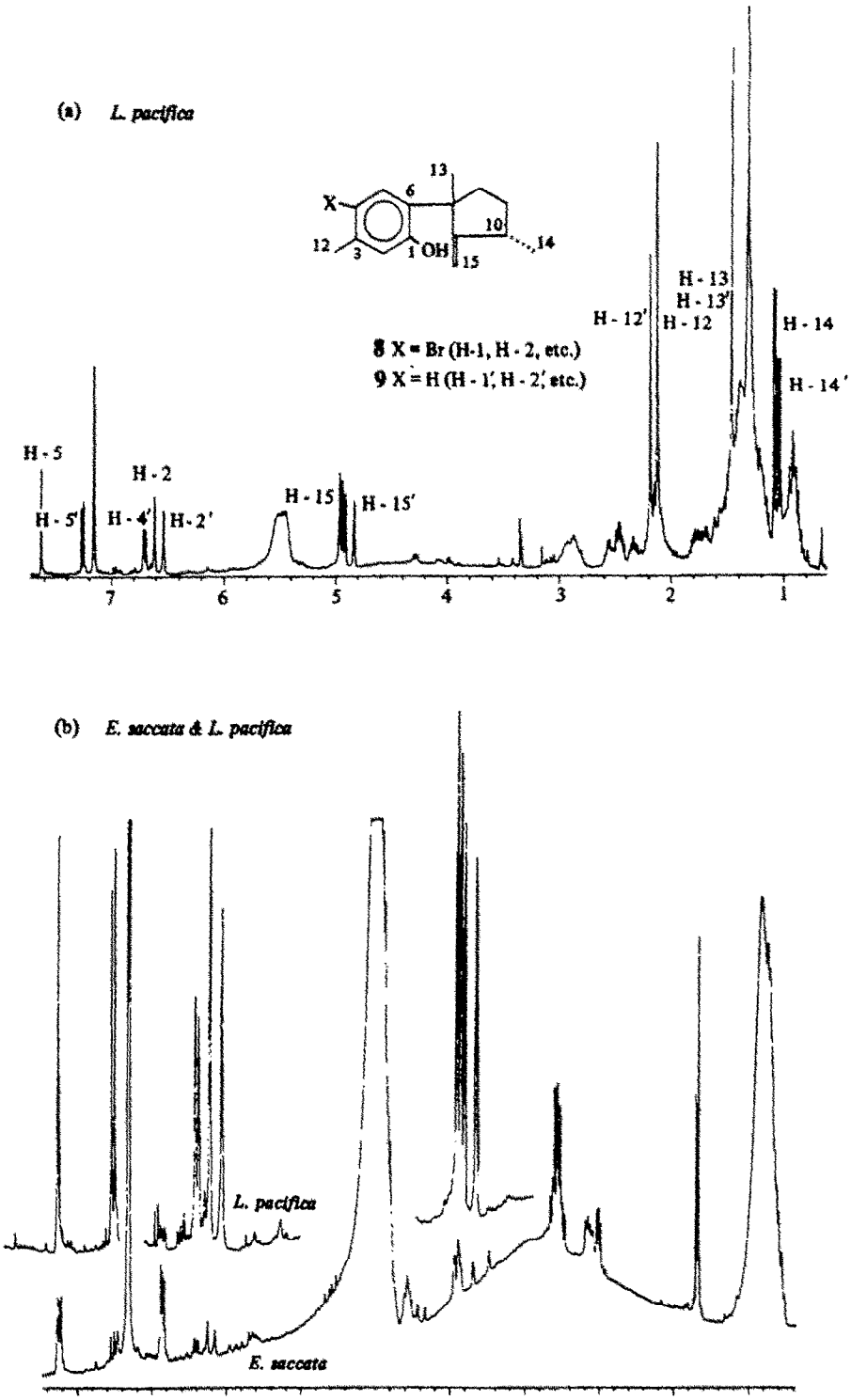


Fig. 3. ^{1}H NMR of crude oils at 360 MHz in C_6D_6 (Stillwater Cove, Aug. 1980).

Three coralline algae from Japan including (Order, Cryptonemiales; Family, Corallinaceae) *Marginiosporum aberanns*, *Amphiroa zonata* and *Corrallina pilulifera* were reported to contain, in minute yields, laurinterol (**3b**), isolaurinterol (**8**), aplysin (**1**), aplysinol (**12**) and aplysinal (**13**) [21]. GC-MS was used to survey compounds from organisms in the Sea of Cortez, and halogenated sesquiterpenes common to *Laurencia* were observed from a variety of other seaweeds including *Plocamium cartilagineum* and in other organisms including a sponge, brittle star and a bryozoan [22]. We suspect that the halogenated sesquiterpenes observed in these two studies are due to

Fig. 4. Transmission electron micrograph through a portion of a rhizoidal cell of a mature specimen of *E. saccata* showing: plastids (P), mitochondria (M), dictyosome (D), cell wall (CW) and primary vacuole (1°V).

contamination from *Laurencia* species which are present in the collection locales of all of these organisms.

EXPERIMENTAL

Our general analytical, chemical and chromatographic methods have been described previously [15]. ^{1}H NMR spectra were recorded at UCSC on a JEOL FX-100 PFT spectrometer operating at 99.55 MHz, or at the Stanford Magnetic Resonance Lab at 360 MHz. GC-MS data were obtained on a Finnigan 4000 instrument equipped with an 1/8 in. × 6 ft. glass column packed with 3% OV-17 on chromosorb Q and temperature programmed from 130 to 225° at either 5 or 10°/min.

Collections and extractions. Laurencia pacifica bearing *E. saccata* was collected at Stillwater Cove, Monterey, CA (intertidally: February 1978; August 1978 and subtidally; September 1980) and at Avalon Harbor, Catalina Is, CA (subtidally: September 1978). The samples collected in 1978 were returned to the laboratory in ice and the epiphytes were removed as described in the text. For the 1980 collection, a portion of the plants were kept submerged after collection and the epiphytes were immediately removed and immersed in MeOH.

Once separated the plants and epiphytes (I, *L. pacifica*, Stillwater Cove, Feb. 1978, 1200 g dry weight; II, *L. pacifica*, Stillwater Cove, Aug. 1978, 100 g; III, *E. saccata*, Stillwater Cove, Aug. 1978, 99 g; IV, *L. pacifica*, Catalina Is., 72 g; V, *E. saccata*, Catalina Is., 1.6 g; VI, *L. pacifica*, Stillwater Cove, Sept. 1980, 28.8 g; VII, *E. saccata*, Stillwater Cove, Sept. 1980, 12.9 g) were extracted in refluxing MeOH for 1 hr. The solvent was removed under vacuum and the residue was dissolved in hexane (2 × 50 ml), dried over $MgSO_4$ and concd to give crude oils (I, 1.5 g, 0.12%; II, 0.45 g, 0.45%; III, 0.3 g, 0.3%; IV, 0.52 g, 0.72%; V, 0.02 g, 1.2%; VI, 1.19 g, 4.1%; VII, 0.38 g, 2.9%). Chromatography (activated silica gel, 60–200 mesh) of the above crude oils gave semi-pure oils which were analysed by GC-MS or ^{1}H NMR (I, 0.67 g, 0.05%; II, 0.12 g, 0.12%; III, 0.1 g, 0.1%; IV, 0.2 g, 0.27%; V, 0.10 g, 0.62%; VI, 0.87 g, 3.0%; VII, 0.18 g, 1.4%).

Isolation and identification of sesquiterpenes. Our previously described procedure [15] was used to isolate the various metabolites from the *L. pacifica* Feb. 1978 semi-pure oil: aplysin (**1**), 130 mg, 0.1%; debromoaplysin (**2**), 30 mg, 0.0025%; debromolaurinterol (**3a**), 30 mg, 0.0025%; pacifenol (**4**), 50 mg, 0.004%; and pacifidiene (**5**), 12 mg, 0.001%.

The *L. pacifica* Sept. 1980 crude extract was analysed by ^{1}H NMR (360 MHz, C_6D_6) and displayed signals (Fig. 3A) identical to that in the lit. [16] for: isolaurinterol (**8**), 16% and debromoisolaurinterol (**9**), 12% of the crude oil (% by ^{1}H NMR integration).

The *L. pacifica* Aug. 1978 semi-pure oil was further purified by HPLC (hexane–C_6H_6) to give laurenisol (**10**), 10 mg, 0.1%, HPLC fraction #6, ^{1}H NMR spectrum identical to that in the lit. [17]; and bromolaurenisol (**11**), 5 mg (0.005%), HPLC fraction #9, ^{1}H NMR spectrum identical to that in the lit. [17].

Conversion of pacifenol (**4**) *to pacifenol trimethylsilyl ether* (**6**). *N*-6-Trimethylsilyimidazole (10 μl) was added to **4** (0.5 mg) dissolved in 10 μl of dry pyridine. This mixture was warmed to 70° for 2 hr, followed by addition of 1% NaOH (1 ml), extraction with $CHCl_3$ (2 × 10 ml), drying over $MgSO_4$ and concn under vacuum. Analysis by GC-MS (Fig. 4, p. 12, ref. [19]) showed **6**, mass spectrum, *m/z*, 498, 500, 502 (3:7:5) $[M]^+$; 483, 485, 487, 489 (3:7:5:1) $[M-Me]^+$; 419, 421, 423 (3:4:1) $[M-Br]^+$; 391, 393, 395 (2:3:1); 329, 331, 333 (3:4:1); 293, 295 (1:1); 249, 251 (1:1); 221, 223 (1:1); 170; 73 (base peak).

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