Phytochemistry, Vol. 23, No 9, pp 1951–1954, 1984
Printed in Great Britain.

0031-9422/84 $3.00 + 0.00

SESQUITERPENES FROM THE MARINE RED ALGA *LAURENCIA DISTICHOPHYLLA*

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(*Revised received* 21 *February* 1984)

Key Word Index—*Laurencia distichophylla*; Rhodophyceae; marine algae; sesquiterpenes; cuparene; isolaurenisol.

Abstract—A new cuparene-type sesquiterpene, isolaurenisol, has been isolated and identified from the New Zealand red alga *Laurencia distichophylla*. Major differences in the chemical composition of two morphologically indistinguishable samples of *L. distichophylla* are noted.

INTRODUCTION

Marine red algae from the *Laurencia* family have been studied extensively [1]. We have been examining New Zealand species of *Laurencia*, and have reported previously some of our investigations on *Laurencia thyrsifera* [2, 3]. We now report our findings for *Laurencia distichophylla*, which occurs principally on the north-east coast of New Zealand.

RESULTS AND DISCUSSION

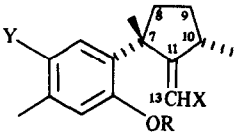
Three samples of this alga were collected from Echinoderm Reef in the Leigh Marine Reserve, New Zealand, as follows: sample 1, from the low intertidal to upper subtidal region on Oct. 25, 1980; sample 2, from the mid intertidal region on Nov. 4, 1980; and sample 3, from the upper intertidal region on Nov. 5, 1980. Each sample was immediately extracted with methanol, and then subsequently with dichloromethane. The methanol and dichloromethane extracts in each case were combined, and then analysed by TLC and GLC. These analyses revealed that the extracts from samples 2 and 3 were identical, and were thus combined and designated as extract A. The extract B, from sample 1, was quite different however.

The components of extract A were separated by a combination of column chromatography, preparative TLC and HPLC to yield debromoisolaurinterol (**1a**) (5% of extract by weight), debromoaplysin (**2a**) (3%), α-bromocuparene (**3**) (3%), a polar, non-halogenated compound (**4**) (2%), cholesterol (3%) and a new compound identified as a cuparene type (**1c**) (70%) for which the name isolaurenisol is suggested. The structures of **1a**, **2a**, **3** and **4** followed from a comparison of their spectral characteristics with those reported previously for these compounds [4–7]. The high resolution electron impact mass spectrum of **1c** showed a parent ion as a 1 : 1 doublet at *m/z* 294/296, corresponding to a molecular formula of $C_{15}H_{19}BrO$. The presence of a substituted benzene ring was shown by the UV spectrum of **1c** which had maxima at 212 nm (ε 22 200), 278 nm (ε 3400) and 284 nm (ε 3200), and the IR spectrum with absorbances at 1610 and 1500 cm^{-1}. Other IR absorptions were attributed to olefinic (1615, 950 cm^{-1}) and hydroxyl groups (3600, 1460 and 1150 cm^{-1}). The low field portion of the ^1H NMR spectrum showed resonances at δ7.2, 6.7 and 6.6, consistent with those of a trisubstituted aromatic ring as found in debromoisolaurinterol (**1a**), debromolaurinterol (**5**) and laurenisol (**6a**) [4, 5, 8]. A broad, D_2O exchangeable peak at δ5.2 could be assigned to a phenolic hydroxyl group, while a finely split one-proton doublet at δ6.0 suggested an exocyclic trisubstituted olefin, as found in laurenisol (**6a**) [8]. The remaining ^1H NMR signals of **1c**, together with the ^{13}C NMR absorptions, were consistent with the structural features of the isolaurinterol skeleton, which is isomeric with that of laurenisol (**6a**), requiring reversal of the positions of the exocyclic double bond and the tertiary methyl group.

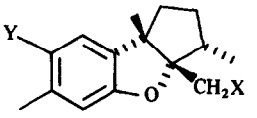
In order to confirm this structure, and to determine the stereochemistry of the tertiary methyl group, cyclisation of **1c** was attempted, as it would be expected to yield isoaplysin (**2c**), by analogy with the known cyclisation of isolaurinterol (**1b**) to aplysin (**2b**) [9]. The cyclisation was eventually achieved in trifluoroacetic acid, conditions which are somewhat stronger than required for the cyclisation of **1b** to **2b**. The cyclisation product had spectral and optical characteristics identical with those reported for isoaplysin (**2c**) [7], thus confirming the structure of **1c** and the stereochemistry at each of the chiral centres at C-7 and C-10.

It was subsequently noticed that a pure sample of **1c** had undergone cyclisation to **2c** to a small extent on standing in an ether solution over a period of several months. Further spontaneous cyclisation was prevented by converting **1c** to its acetate derivative **1d**.

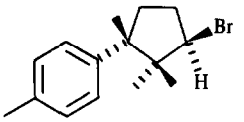
The only outstanding point of stereochemistry remaining was the geometry of the exocyclic double bond. In the original report [8] on laurenisol (**6a**) the geometry of the corresponding double bond was not assigned, but a later report [10] ascribed the (*Z*)-configuration based on the chemical shift of the C-11 proton. For this proton, a value of δ3.11 was observed, while for the same proton in the nonbrominated analogue, debromoallolaurinterol (**6b**), the C-11 proton absorbed at δ2.95. The slight downfield shift in the case of laurenisol was attributed to the proximity of the bromine, thus allowing the assignment as (*Z*). A similar effect was observed for **1c**. A complex



1a X = H, Y = H, R = H
1b X = H, Y = Br, R = H
1c X = Br, Y = H, R = H
1d X = Br, Y = H, R = Ac



2a X = H, Y = H
2b X = H, Y = Br
2c X = Br, Y = H



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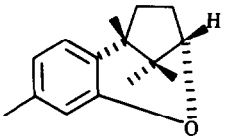

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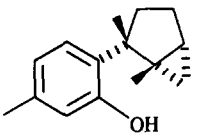

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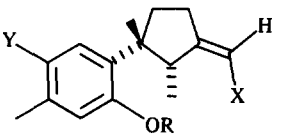

6a X = Br, Y = H, R = H
6b X = H, Y = H, R = H
6c X = H, Y = Br, R = H
6d X = Br, Y = Br, R = Ac
6e X = Br, Y = H, R = Ac

multiplet at δ3.0 was assigned to the C-10 proton, while a corresponding multiplet at δ2.8 could be assigned to the same proton in debromoisolaurinterol (**1a**). We believe that this effect in the ^{1}H NMR spectrum is too small for any conclusion to be made on the geometry of the exocyclic double bond, and would question the validity of the assignment based on this effect in the case of laurenisol (**6a**) [10].

^{13}C NMR spectroscopy has been used to good effect in assigning the stereochemistry of trisubstituted double bonds [11], and relies on the induced upfield shift in a carbon resonance brought about by the *γ-gauche* interaction when that carbon is in a *cis*-relationship with a substituent across the double bond. This method works most effectively when based on suitable models or when both isomers are available. However, assignments have been reported for acyclic systems when only one isomer was available [11]. To ascertain the validity of this approach to the present cyclopentane system, the relevant carbon chemical shifts in a model pair were examined. The model selected was the allolaurinterol (**6c**)/bromolaurenisol acetate (**6d**) or laurenisol acetate (**6e**) system. The results (Table 1) for **6c** and either **6d** or **6e** indicated that on introduction of a bromine substituent at C-13, surprisingly the resonances for both C-9 and C-11 shifted downfield by between 0.5 and 1.5 ppm. This same lack of distinction was observed for C-7 and C-10 in the pair **1c** and **1a**.

Table 1. ^{13}C NMR resonances (δ) for selected carbons of some sesquiterpenes

	Carbon No.			
	C-7	C-9	C-10	C-11
Allolaurinterol (**6c**)*		27.9		48.4
Bromolaurenisol acetate (**6d**)*		28.7		49.4
Laurenisol acetate (**6e**)*		28.3		49.4
Debromoisolaurinterol (**1a**)†	49.9		37.8	
Isolaurinterol (**1b**)*	49.9		37.9	
Isolaurenisol (**1c**)†	52.0		39.2	

*Assignments from ref. [15].
†Assigned by comparison with ^{13}C NMR spectrum of **1b**.

Thus with no clear answer from either ^{1}H NMR or ^{13}C NMR we leave the stereochemistry of the 11,13 alkene in isolaurenisol (**1c**) unassigned.

Chromatographic isolation of the components of ex-

tract B yielded the known compounds allolaurinterol (**6c**) (62% of extract by weight), isolaurinterol (**1b**) (5%) and cholesterol (5%). The identities of the sesquiterpenes **1b** and **6c** were established by comparisons of their spectral characteristics and optical rotations with those previously reported for these compounds [7, 12].

Of some interest from these results are the possible reasons for the differences between extract B from sample 1, and extract A from samples 2 and 3. All three samples had been collected from the same region at the same time, so the regional differences found with other *Laurencia* species could be discounted. One other possibility considered was based on the differing sexual composition of the samples, since these algae may exist in three reproductive forms: the haploid male and female forms, and the diploid asexual or tetrasporangial form. At this stage of the investigation, only a small amount of sample 2 remained, and was found to consist mainly of the tetrasporangial form, with only a small amount of the female form present. Since the alga had been frozen, this may have destroyed any male plant characteristics.

Small quantities of the tetrasporangial and female forms were separated and were each extracted with dichloromethane and the extracts analysed by TLC and GLC. These analyses were identical to each other, and also to a stored sample of extract A from the combined samples 2 and 3. Since it is more likely that differences would occur between haploid and diploid forms rather than between the two haploid forms, it would seem unlikely that differences in sexual composition could have caused the observed chemical differences. This is in keeping with earlier observations on other *Laurencia* species [13].

A third possibility for the cause of the differences could be environmental factors, since it was noted that sample 1 was principally from the subtidal region, whereas samples 2 and 3 were from the intertidal region. Thus differences in sunlight distribution, exposure to air, other animals and plants in the area and perhaps other environmental factors may have engendered the chemical variation. However, culture studies by Howard *et al.* [14], in which temperature, aeration, photoperiods and seawater were varied indicated that these changes in environmental factors in nature have little or no effect on secondary metabolite chemistry in *Laurencia* species. These same workers reported cross-fertilization studies which indicated that morphologically consistent samples of *Laurencia pacifica* from different sites, but containing different metabolites, were genetically distinct so that cross-fertilization was unsuccessful. The term 'sibling species' was suggested to describe populations that are reproductively isolated, i.e. cannot be cross-fertilized, but which cannot be distinguished by obvious morphological characteristics. It is therefore possible that such genetic differences existed between sample 1 and samples 2 and 3 of *Laurencia distichophylla* examined in this study, and is the cause of the variation in secondary metabolites.

EXPERIMENTAL

Plant material. Laurencia distichophylla was collected as noted in the discussion, and voucher samples were deposited in the Herbarium, Botany Division, DSIR, Lincoln, New Zealand.

Extraction and isolation Each of the three wet samples of *L. distichophylla* was blended with MeOH, and the solid residues obtained after filtration were further extracted with CH_2Cl_2, these extracts being then combined with the appropriate MeOH extracts. The combined extract (5.1 g) from samples 2 and 3 was chromatographed on alumina (500 g) to produce several fractions (combined wt 3.4 g), the bulk of which were non polar, being eluted with CH_2Cl_2–petrol (1:19). The major portion of these fractions was a clear oil (2 g), chromatographically pure, and identified as isolaurenisol (**1c**). Other combined non polar fractions (700 mg) were separated by preparative TLC and HPLC to yield α-bromocuparene (**3**) (60 mg), debromoisolaurinterol (**1a**) (120 mg), debromoaplysin (**2a**) (24 mg), the non-halogenated compound (**4**) (10 mg) and additional **1c** (240 mg). The spectral and optical characteristics of **1a**, **2a**, **3** and **4** were identical with those previously reported [4–7].

Isolaurenisol (**1c**). $[\alpha]_D^{25} - 42°$ ($CHCl_3$; *c* 2.5). UV λ_{max}^{EtOH} nm (log ε): 212 (4.35), 278 (3.53), 284 (3.51). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3500, 2950, 1615, 1610, 1500, 1460, 1280, 1150, 950. EIMS 70 eV, *m/z*: 294/296 $[M]^+$, 279/281, 215 (Found 294.06145, $C_{15}H_{19}BrO$ requires 294.06197). ^1H NMR (80 MHz, $CDCl_3$, TMS): δ 1.24 (3H, *d*, *J* = 7 Hz); 1.46 (3H, *s*), 2.25 (3H, *s*); 5.15 (1H, *br s*); 6.0 (1H, *d*, *J* = 2 Hz); 6.57 (1H, *br s*); 6.65 (1H, *d*, *J* = 7 Hz); 7.17 (1H, *d*, *J* = 7 Hz); a series of multiplets 1.6–3.2 (5H). ^{13}C NMR (20 MHz, $CDCl_3$, TMS): δ 160.1 (*s*, C-11), 153.3 (*s*, C-1), 138.0 (*s*, C-3), 128.7 (*s*, C-6), 128.2 (*d*, C-5), 121.3 (*d*, C-2), 118.2 (*d*, C-4), 101.3 (*d*, C-13), 52.0 (*s*, C-7), 39.2 (*d*, C-10), 39.2 (*t*, C-8), 31.0 (*t*, C-9), 26.8 (*q*, C-14), 20.6 (*q*, C-15), 19.1 (*q*, C-12).

Isolaurenisol acetate (**1d**). A sample of **1c** (152 mg) was acetylated in Ac_2O–pyridine (1:1, 1 ml) at room temp for 5 hr. Work-up in the usual manner yielded the acetate **1d** as a chromatographically pure oil (162 mg). $[\alpha]_D^{25} - 6°$ ($CHCl_3$; *c* 5.5) IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1770, 1630, 1505, 1350, 1190, 1020, 900. UV λ_{max}^{EtOH} nm (log ε): 212 (3.944), 267 (2.799), 275 (2.799). CIMS *m/z*: 337/339 $[M+1]^+$, 295/297, 257; EIMS 70 eV, *m/z*: 336.0780, $C_{17}H_{21}BrO_2$ requires 336.0725. (Found: C, 60.9; H, 6.4; $C_{17}H_{21}BrO_2$ requires C, 60.5; H, 6.3%.) ^1H NMR (80 MHz, $CDCl_3$, TMS): δ 1.1 (3H, *d*, *J* = 7 Hz), 1.4 (3H, *s*), 2.3 (6H, *s*), 6.0 (1H, *d*, *J* = 2 Hz), 6.8 (1H, *br s*), 6.9 (1H, *d*, *J* = 7 Hz), 7.2 (1H, *d*, *J* = 7 Hz), a series of multiplets 1.5–3.2 (5H). ^{13}C NMR (20 MHz, $CDCl_3$, TMS): δ 169.1, 158.0, 148.3, 137.3, 134.0, 128.6, 126.1, 124.7, 101.6, 51.9, 39.8, 39.4, 30.7, 27.1, 21.4, 20.4, 18.4.

Cyclisation of isolaurenisol (**1c**). A sample of **1c** (102 mg) was stirred with CF_3COOH (2 ml) for 30 min at room temp. After work-up, a brown oil (69 mg) was obtained, which on purification by preparative TLC gave isoaplysin (**2c**) (43 mg), identical in all respects with **2c** as reported previously [7].

Acknowledgements—Dr. M. Hawkes and Ms. D. Bonim for the collection of *Laurencia distichophylla*. The Research Committee of the New Zealand Universities Grants Committee for funds for equipment, and for a fellowship to one of us (RJL).

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