

STUDIES OF MARINE NATURAL PRODUCTS

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R. J. Lake

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"For the benefit of future collectors
I may mention the following localities
as good collecting grounds:
..... Kaikoura, very good."

R.M. Laing, B.Sc.
Revised List of New Zealand
Seaweeds.

Read before the Philosophical
Institute of Canterbury,
4th October 1899.

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ABSTRACT

Red algae of the *Laurencia* genus contain a wide range of unusual secondary metabolites, many of which are halogenated.

In this study the extracts of two local New Zealand species, *Laurencia thyrsoifera* and *Laurencia distichophylla* have been examined in detail resulting in the isolation of several compounds some of which were previously undiscovered.

Laurencia thyrsoifera was found to contain a range of acetylenic cyclic ethers: the isomeric diols (82a) and (82b), which were isolated as the diacetate derivatives, the mono-acetate isomeric mixture (87a-d), the isomeric diacetates (82c) and (82d), the isomeric enones (89a) and (89b), the diene (92) and finally the bromo compound (95). The structures and stereochemistries of these compounds were established by a combination of spectroscopic and chemical techniques. A g.l.c. survey of the fatty acid components of this alga was also performed.

The components of the extracts of *Laurencia distichophylla* were found to be metabolites of the cuparene-type. Two distinct chemo-types of *Laurencia distichophylla* were found to exist. The first contained the new compound (101), debromoisolaurinterol (21), debromoaplysin (7), α -bromocuparene (13) and a polar non-halogenated compound (24). The second chemo-type of *Laurencia distichophylla* contained principally allo-laurinterol (17) and isolaurinterol (11).

Cholesterol (88) was a component of both species of algae.

An extensive literature survey resulted in the collation of structural information of all the acetylenic cyclic ethers isolated from *Laurencia* species. Examination of the stereochemistry of these compounds permitted the proposal of a biogenetic sequence.

INTRODUCTION

The marine environment differs from its terrestrial counterpart in many ways and this is reflected in the character of the natural products produced by marine species. Marine natural products research has so far concentrated on identifying the compounds present in marine species but the purpose and role of such compounds still often remains unclear. The impetus for marine research is provided by pharmaceutical and/or commercial potential as well as purely scientific interest. All three factors are responsible for the considerable attention given to marine algae. From a chemist's viewpoint, the variety and novelty of the constituents of marine algae make this a rewarding field. To place this research in perspective however, it will be useful to briefly examine the nature of the marine environment and the characteristics of marine algae in general.

Seawater represents a rich and varied resource for the growth of algae. Besides water itself (which makes up 96.5% of the aquatic environment)¹ practically every single element is present in at least trace amounts. The major elements of seawater, while their actual concentration may vary, are present in almost constant ratio which permits their cumulative concentration to be expressed as "salinity" (S ‰, i.e. parts per thousand). The numerical value of salinity is related to the concentration of chloride (‰). Table 1 lists the major ions of seawater at $S = 35$ ‰.² Besides these elements, seawater also contains a wide range of minor or trace elements. Of these, it is worth mentioning iodine which has a concentration of approximately $38 \mu\text{g l}^{-1}$.²

Carbon is present in seawater in a variety of forms, the principal ones being dissolved carbon dioxide, bicarbonate and dissolved organic

Table 1: The major ions of seawater

Ion	g kg ⁻¹ at S = 35‰
Cl ⁻	19.354
SO ₄ ²⁻	2.712
Br ⁻	0.0673
F ⁻	0.0013
B	0.0045
Na ⁺	10.77
Mg ²⁺	1.290
Ca ²⁺	0.4121
K ⁺	0.399
Sr ²⁺	0.0079

material. For the algae, as photosynthetic plants, the most important source is dissolved carbon dioxide which is present at a concentration of $2 \times 10^{-3} \text{ mol l}^{-1}$ although this value is dependent on pH, salinity and temperature.³

The above represents only a very simplified view of the marine chemical constitution which is much less static than is implied here. It is worth remembering that many other factors may have an effect on the production of marine natural products. These may be chemical (pH, pollution etc.) or physical (sunlight distribution, temperature, pressure, wave and tidal effects etc.). Perhaps the most important feature of marine chemistry is the presence in significant concentration of all the halogens which undoubtedly accounts for the unusually large proportion

of halogenated marine natural products.⁴

The general characteristics of algae lie more in the domain of the biologist than the chemist; however some knowledge of the algae will help to place this research in context. The term "algae" is one that has not been rigorously defined.⁵ Algae share their obvious characteristics with other chlorophyllous plants while their unique features are more subtle. The distinguishing characteristics involve the mechanism of reproduction and do not fall within the scope of this study.

The algae, from fossil records, are probably the most ancient organisms to contain chlorophyll. The antiquity of the algae in the history of living organisms suggests that they have a primary position in the plant kingdom and this is reinforced by the relative simplicity of most algal plant bodies and biological processes (e.g. sexual reproduction).

Algae may be aquatic or subaerial (exposed to the atmosphere rather than submerged in water). Aquatic algae may grow in waters of low salinity (as low as 10 ppm) called fresh water or in marine waters where the solutes are usually 33 - 40‰.⁵ Aquatic algae may be suspended (*planktonic*) or attached and living on the bottom (*benthic*). Benthic algae grow attached to various substrates and may be classified as *epithallic* (living on stones), *epipellic* (attached to mud or sand), *epiphytic* (attached to plants), or *epizoic* (attached to animals). In addition to classification by attachment, marine algae may be categorised according to location. Some are *supralittoral* i.e. growing above the water and in the spray zone. Others are *intertidal* in that they are exposed periodically in accordance with variations in water level due

to tides. The final category is *sublittoral* i.e. constantly submerged and, depending on turbidity, these algae may grow at depths as great as 100 - 200m especially in clear tropical waters.⁵

Algae have important biological and economic roles. From the biological viewpoint algae act as oxygen producers for the water in their immediate vicinity. Algae are also highly important as primary producers of organic matter in aquatic environments due to their photosynthetic activities. At a basic level, the structural materials of algal cells and walls may be sources of food for grazing organisms. The algae also liberate substances which may be used or have an effect on other organisms associated with the algae. It has been estimated that benthic algae are responsible for the production of approximately 10% of the total dissolved organic material in the sea, through excretion or decomposition. The substances include DNA, RNA, carbohydrates, amino acids, urea, fatty acids, vitamins, proteins and enzymes. Laboratory culture studies have shown that these released substances may have stimulatory or inhibitory growth effects on other algae or organisms. It is interesting to note that there is evidence that certain algae may use dissolved thiamine and vitamin B₁₂ as growth regulating compounds.^{5,6}

Commercially the algae have been useful to mankind for centuries as foodstuffs, fertilisers and fodder for stock. More recently algal extracts have been utilized in scientific work. Agar, an extract of certain red algae, is used as an inert culture medium in microbiology. Algal extracts have also been found to have potential as pharmaceutical agents and some relevant examples of this will be given later.

The form of the algal plant body may vary from the relative simplicity of the single cell (as small as 1 x 1.5 μm) to the more

complex giant kelps and rockweeds which may grow up to 60m in length. Classification of the algae was initially related to primary pigmentation which may be brown, red, green or blue-green. More recent investigations have established that accompanying differences in pigmentation are differences in storage products and cellular organization. Thus there are now established seven divisions of algae: Chlorophyta, Euglenophyta, Chrysophyta, Phaeophyta, Pyrrophyta, Cyanophyta and Rhodophyta*. Each of these has specific characteristics but only those of the Rhodophyta (red algae) will be quoted as particularly relevant to this study and are given in Table 2.⁵

The division Rhodophyta gain their name primarily from their red coloration although there are several morphologically distinctive features as well. The red colour is usually caused by the pigment phycoerythrin (a substance which assists in photosynthesis) which often masks the green colour of chlorophyll a. This red colour is particularly noticeable in sublittoral species. In species more exposed to sunlight the phycoerythrin often partially breaks down so that the algae do not appear red but may have a range of colours including violet, purple, brown, black, yellow and green. The algae under investigation in this study were intertidal and appeared brown-green in the sea water. It was only during chromatography of the extract that the red pigment could be seen along with yellow and green coloration.

The division Rhodophyta is regarded as containing a single class: Rhodophyceae. Differences in morphology and reproductive mechanisms permit subdivision of this class into nine orders. Of these only the most well represented order, the Ceramiales, is relevant here. This

* The divisional name sometimes includes "-phyco-" to indicate the specifically algal level of cell organization e.g. Rhodophycophyta.

Table 2: Characteristics of Rhodophyta; Common name: red algae.

 Pigments and Plastid Organization in Photosynthetic Species:

Chlorophyll a, R- and C- phycocyanin, allophycocyanin, α - and β - carotene and several xanthophylls, thylakoids* single, not associated.

Storage Food: Floridean** starch (glycogen-like).

Cell Walls: Cellulose, xylans, several sulphated polysaccharides (galactans), calcification*** in some.

Flagella: absent.

Habitat: some fresh water, most brackish water or sea water.

* A vesicle, the wall of which bears photosynthetic pigments.

** A term used to specify the particular form of starch found in the Rhodophyta.

*** Deposits of CaCO_3 .

order is made up of four families: the Ceramiaceae, the Dasyaceae, the Delesseriaceae and the Rhodomelaceae. This latter family is the largest in the red algae, including more than one hundred genera. One of these is the *Laurencia* genus which is widespread in tropical and temperate seas and includes the particular seaweeds under study in this work: *Laurencia thyrsifera* and *Laurencia distichophylla*.

The *Laurencia* genus has proved to be an extremely productive source of new natural products. There are a number of reviews of this genus^{7,8,9} the most recent and comprehensive of which is contained in several chapters of "Marine Natural Products" edited by P.J. Scheuer.¹⁰

The treatment given here will be representative rather than exhaustive except for the two classes of compounds found in the algae investigated in this study. These will be covered in full.

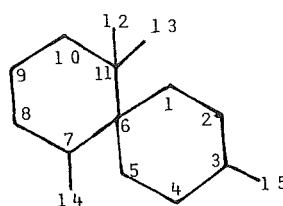
Over one hundred and fifty different natural products have presently been isolated from the approximately twenty two species of *Laurencia* investigated so far. From a chemist's viewpoint it is useful to classify the *Laurencia* natural products according to skeletal type. There are two broad classes according to biochemical origin: the terpenoids and non-terpenoids. The former contains a number of skeletal types, while the latter is mainly restricted to the acetylenic cyclic ethers which appear to be unique to the *Laurencia* genus.

Terpenoids:

Of these the majority are sesquiterpenes. Most of the sesquiterpenes may be grouped according to skeletal type of which there are three basic forms.

1. Chamigrene skeleton.

Basic skeleton*:

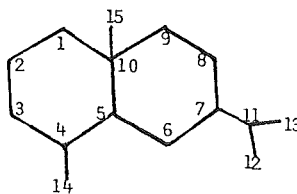


Examples of this type include 10-bromo-3,4-epoxy- α -chamigrene (1)¹¹ and elatol (2).¹² There are also a number of compounds which are similar but include a $C_1 - C_{10}$ ether link, for example structure (3).¹³

* Numbering is not always consistent in the literature

2. Selinane skeleton.

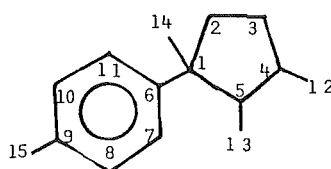
Basic skeleton:



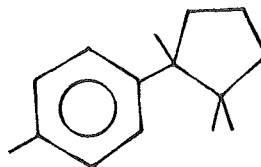
Examples of this type include 1-(*S*)-bromo-4-(*R*)-hydroxy-(-)-selin-7-ene(4)¹⁴ and heterocladol (5).¹⁵

3. Cuparene skeleton.

Basic skeleton:



or



This particular skeletal type is relevant to the study of *Laurencia distichophylla* and so all known examples will be covered.

The first compounds of this type to be isolated were aplysin (6), debromoaplysin (7) and aplysinol (8). These were originally found in the mollusc *Aplysia kurodai*¹⁶ which grazes on *Laurencia* algae. It was not until later that the suspected algal source was identified with the isolation of these three metabolites and also laurinterol (9) and debromo-laurinterol (10) from *Laurencia okamurai*.¹⁷ Isolaurinterol (11)

was later isolated from the same alga.¹⁸ Treatment of laurinterol (9) and debromolaurinterol (10) with p-toluenesulphonic acid gave aplysin (6) and debromoaplysin (7) respectively. Isolaurinterol (11) could also be converted to aplysin (6) by the same treatment. Such interrelationships are a feature of this type of molecule.

A hydrocarbon, laurene (12) has been isolated from both *Laurencia glandulifera*¹⁹ and *Laurencia nipponica*.²⁰ Two epimeric brominated hydrocarbons have also been found in *Laurencia glandulifera*.²¹ These were α -bromocuparene (13) and α -isobromocuparene (14). The stereochemistry of the epimers was decided by preparation of (13) and (14) from the corresponding known alcohols α -cuparenol (13a) and α -isocuparenol (14a). α -Isobromocuparene (14) was also isolated from *Laurencia nipponica*²¹ and, in addition, a new compound laurenisol (15).²²

Three related phenols were recently isolated from *Laurencia glandulifera*.²³ One of these was found to be laurenisol (15). The others were the bromo-analogue of laurenisol (16) and allolaurinterol (17). Three related ethers were also isolated: bromoether A (18), bromoether B (19) and the debromo-analogue of bromoether A (20). The ethers (20) and (18) were obviously related to laurenisol (15) and its bromo-analogue (16) respectively. However bromoether B (19) is somewhat unusual with its dibrominated methyl group.

Laurencia okamurai has proved to be a rich source of cuparene-type molecules^{18,24} and the constituents apparently depend on the collection site.¹⁸ Investigations of this alga have produced several new compounds: debromoisolaurinterol (21), neolaurinterol (22), isoaplysin (23), a polar nonhalogenated compound (24), isolaurene (25), dibromophenol (26) and debromoaplysinol (27).

Laurencia filiformis from Australia has produced several examples of cuparene-type molecules.²⁵ These are allolaurinterol (17), the unusual non-aromatic dihydrolaurene (28), filiformin (29) and filiforminol (30). Dihydrolaurene (28) was found to convert spontaneously to laurene (12) (also isolated from *Laurencia filiformis*) and similarly, allolaurinterol (17) cyclised to produce filiformin (29) on standing. Debromoallolaurinterol (31) has been isolated from *Laurencia subopposita*.²⁶

A species which produces unusual metabolites of this type is *Laurencia caraibica* (formerly named *Laurencia nana*). This species contains the only examples of iodinated compounds so far isolated from algae of the *Laurencia* genus:²⁷ 10-bromo-7-hydroxy-11-iodolaurene (32) and iodo-ether A (33) (the iodo-analogue of bromo-ether A (18)). Also isolated were 10-bromo-7,12-dihydroxy-^{2,3}Δ-laurene (34) and the possibly related compound caraibical (35).²⁸

These cuparene type compounds may be regarded as comprising three groups: hydrocarbons (which may be halogenated), phenols and ethers. The above review gives a slightly false view of the distribution of these compounds. Their incidence is widespread throughout the various species of *Laurencia*. Each species usually contains several examples and one compound may occur in several species. A partial listing is contained in a paper by Suzuki and Kurosawa.¹⁸ Table 3 is a more complete and up to date listing.

There are a few sesquiterpenes which do not belong to one of the three skeletal types given above. Examples include α-snyderol (36)³³ and 3β-bromo-8-epicarrapi oxide (37)³⁴ from *Laurencia obtusa*. A number of related compounds have been isolated from *Laurencia caespitosa*³⁵ of which caespitol (38) is a good example. An unusual skeletal type is

Table 3: Location and distribution of cuparene-type compounds.

-
1. *L. caraibica*: Isla Mujeres, Mexico.
 filiformin (29), allolaurinterol (17), α -bromo-
 cuparene (13), bromo-ether A (18), 10-bromo-7-hydroxy-
 11-iodolaurene (32), iodo-ether A (33), 10-bromo-
 7, 12-dihydroxy-^{2,3} Δ -laurene (34), caraibical (35).^{27,28}
 2. *L. decida*: Baja California, Mexico.
 laurinterol (9), isolaurinterol (11), aplysin (6),
 aplysinol (8).²⁹
 3. *L. filiformis* f. *heteroclada*: Port Macdonnell, South Australia.
 allolaurinterol (17), laurene (12), dihydro-laurene (28),
 filiformin (29), filiforminol (30).²⁵
 4. *L. glandulifera*: Oshoro Bay, Hokkaido, Japan.
 laurene (12),²⁰ α -bromocuparene (13), α -isobromo-
 cuparene (14),²¹ bromoether A (18), bromoether B (19),
 debromo-analogue of bromoether A (20), laurenisol (15),
 bromo-analogue of laurenisol (16), allolaurinterol (17),
 isolaurene (25).²³
 5. *L. nidifica*: Kahala Reef, Oahu Island, Hawaii.
 laurinterol (9), aplysin (6).³⁰
 6. *L. nipponica*: Moheji, Hokkaido, Japan.
 laurenisol (15),²² α -isobromocuparene (14),²¹
 laurene (12),²⁰ isolaurene (25).¹⁸
 7. *L. okamurai*: (a) Oshoro Bay, Hokkaido, Japan.

laurinterol (9), debromolaurinterol (10), isolaurinterol (11), debromoisolaurinterol (21), dibromophenol (26), aplysinol (8), debromoaplysinol (27), isoaplysin (23), polar nonhalogenated compound (24), α -bromocuparene (13), α -isobromocuparene (14), isolaurene (25).¹⁸

(b) Okino-shima, Japan.

laurinterol (9), debromolaurinterol (10), isolaurinterol (11), debromoisolaurinterol (21), neolaurinterol (22), isoaplysin (23), polar nonhalogenated compound (24), α -bromocuparene (13), α -isobromocuparene (14), isolaurene (25).¹⁸

(c) Hakata-shima, Japan.

laurinterol (9), debromolaurinterol (10), dibromophenol (26), aplysin (6), debromoaplysin (7), aplysinol (8), debromoaplysinol (27), polar nonhalogenated compound (24), α -bromocuparene (13), α -isobromocuparene (14), isolaurene (25).¹⁸

8. *L. pacifica*: (a) La Jolla, California.

laurinterol (9).³¹

(b) Ensenada, Mexico.

isolaurinterol (11).³¹

(c) Stillwater Cove, Monterey Peninsula, California.

aplysin (6), debromoaplysin (7), debromolaurinterol (10).³²

9. *L. subopposita*: La Jolla, California.

allolaurinterol (17), debromoallolaurinterol (31),

laurene (12).²⁶

displayed by aplysin (39) which again was originally isolated from a mollusc species *Aplysia angasi*³⁶ and then later from *Laurencia filiformis*.³⁷

There are only a few diterpenes so far isolated from species of the *Laurencia* genus: concinndiol (40),³⁸ and related compounds, the irieols,³⁹ exemplified by irieol A (41), and obtusadiol (42).⁴⁰ All these have quite different skeletons. The only triterpenoid isolated from the *Laurencia* genus to date is thyrseferol (43) from *Laurencia thyrsefera*.⁴¹

Non-terpenoids:

The large majority of the non-terpenoid constituents of *Laurencia* species have been found to be C₁₅ acetylenic cyclic ethers. Before covering these in detail it is worth mentioning the other non-terpenoid constituents that have been reported.

A series of four brominated indoles have been discovered in *Laurencia brongniartii*.⁴² Attention was drawn to this alga by the antimicrobial activity of its extract. The active component was found to be one of the brominated indoles (44). Another non-terpene, isolated from *Laurencia obtusa*, is obtusin (45).⁴³ This molecule has some features in common with the acetylenic cyclic ethers. The only remaining compound is poitediol (46) from *Laurencia poitei*.⁴⁴ The closely related compound dactyol (47) was also isolated along with poitediol (46). Dactyol (47) had previously been isolated from the digestive glands of the mollusc *Aplysia dactylomela*.⁴⁵

As mentioned earlier the majority of the non-terpenoid constituents of *Laurencia* species are the acetylenic cyclic ethers which constitute a distinctive feature of these algae. The first of these to be isolated

was laurencin (48) from *Laurencia glandulifera*.⁴⁶ The thorough and extensive examination of this molecule, culminating in an X-ray crystal structure determination, laid the foundation for further study of these molecules.

Laurencia nipponica has been the most productive source of this type of compound. Laureatin (49) and isolaureatin (50) were isolated as mixtures of the $^3\Delta(E)$ and (*Z*) isomers.^{47,49} This isomerism is a common feature of these molecules. Laurefucin (51) and acetyl-laurefucin (51a) were the next molecules to be identified.⁴⁸ It is interesting to note here the co-occurrence of an alcohol, and the corresponding acetate which parallels the report that deacetyl-laurencin (48a) has also been isolated.⁴⁹

Laurencia nipponica is also the source of some interesting variations on this type of molecule. Probably significant from a biogenetic point of view are the laurediols (52a, 52b).⁴⁹ These were isolated as (*E*) and (*Z*) isomeric pairs of the C_6RC_7R and C_6SC_7S enantiomers. The corresponding diacetates (52c, 52d) were also found. From the same alga, isoprelaurefucin (53)⁵⁰ contains a seven membered ether ring instead of the more usual eight membered ring. Even more unusual is laureepoxide (54)⁵¹ which contains a five membered ether ring and also an epoxide. Finally, from *Laurencia nipponica*, laurallene (55)⁵² has also been identified. Although it does not contain an acetylenic group, the basic structure of laurallene (55) suggests that it is at least related to this type of compound.

Laurencia obtusa has provided several examples although the actual constituents of this alga appear to be quite variable according to location. Obtusenyne (56)^{53,54} is interesting in that it contains a nine membered ether ring. The other *Laurencia obtusa* compounds, laurencienyne (57),⁵⁵

laurenyne (58) and cis-isodihydrorhodophytin (59)⁵⁶ contain the more usual eight membered rings.

Chondriol (60) is an interesting example from a chemotaxonomic point of view. It was originally isolated from an alga that was thought to be *Chondria oppositoclada*.⁵⁷ However the presence of this type of molecule suggested that the alga had been erroneously classified and it was reassigned as a form of *Laurencia pacifica*.⁵⁸ Subsequent investigation of this alga revealed the presence of rhodophytin (61).^{59,60} Chondriol (60) and rhodophytin (61) were then isolated from an undescribed *Laurencia* species along with chondrin (62).⁶⁰ In the same paper epoxyrhodophytin (63) was reported from another undescribed *Laurencia* species.

The extensive study of *Laurencia subopposita* by Wratten and Faulkner²⁶ described the isolation of several known acetylenes (isoprelaurefucin (53), acetyllaurefucin (51a) and laurefucin (51)), and also a new compound, dehydrobromolaurefucin (64). Additional isolated examples of these molecules include chlorofucin (65) from *Laurencia snyderiae*,⁵⁴ poiteol (66) from *Laurencia poitei*⁵⁴ and intricenyne (67) from *Laurencia intricata*.⁶¹ *Laurencia venusta* appears to contain several non-terpenoid C₁₅ compounds, two of which, venustin A (68) and venustin B (69) have been reported.⁶²

Recently reported examples include several from *Laurencia okamurai*. Okamurallene (70)⁶³ is similar to laurallene (55) in that it does not contain an acetylenic group but appears to be a related compound. The most unusual features of okamurallene (70) are the cyclopropyl ring and the four and six membered ether rings. Also isolated from *Laurencia okamurai* are laurencenyne (71) and neolaurencenyne (72).⁶⁴ These hydro-

carbons have been proposed as earlier precursors in the biosynthetic pathway than the laurediols (52a, 52b). This will be discussed in a later section.

In late 1981, two new acetylenes, laurepinnacin (73) and isolaurepinnacin (74) were reported from *Laurencia pinnata*.⁶⁵ Laurepinnacin (73) has the same planar structure as intricenyne (67) but apparently different stereochemistry. The stereochemistry of the *Laurencia pinnata* acetylenes is of biogenetic interest as will be examined later.

All the acetylenes described above are macrocyclic ethers. A departure from this characteristic is the group of acetylenic compounds isolated from *Laurencia nidifica*.⁶⁶ These six compounds, the maneonenes and isomaneonenes, (75 a-f) contain carbocyclic as well as ether rings.

Although these acetylenic cyclic ethers are ubiquitous, there is relatively little overlap between species. However it is often important to know the collection area of the alga concerned and so Table 4 gives the distribution of these molecules in a similar way to Table 3 for the cuparenes.

Having examined the wide range of natural products found in *Laurencia* species of algae, there are several aspects worthy of comment. The first of these is chemotaxonomy. When the natural products are listed according to species of origin it is found that while many compounds occur in a range of species there is usually at least one compound that is unique to each species. While this would appear to have taxonomic potential, the situation is more complex. Despite morphological consistency, within each species the metabolic constituents may vary according to location. For this reason it is important that the area of collection is reported as well as the species. Examples of this variation

Table 4: Location and distribution of the acetylenic cyclic ethers.

-
1. *L. glandulifera*: Oshoro Bay, Hokkaido, Japan.
laurencin (48),⁴⁶ deacetyl-laurencin (48a).⁴⁹
 2. *L. intricata*: Key Largo, Florida, U.S.A.
intricenyne (67).⁶¹
 3. *L. nidifica*: (green variety) Diamond Head and Black Point Reefs,
Oahu Island, Hawaii.
maneones and isomaneones (75 a-f).⁶⁶
 4. *L. nipponica*: (a) Hakodate Bay, Japan.
laureatin (49), isolaureatin (50).⁴⁷
(b) West Shakotan, Hokkaido, Japan.
laureepoxide (54), laurencin (48), deacetyl-laurencin
(48a), laurefucin (51), acetyl-laurefucin (51a).⁵¹
(c) Oshoro Bay, Hokkaido, Japan.
laurallene (55).⁵²
(d) Not located.
laurefucin (51), acetyl-laurefucin (51a),⁴⁸ laure-
diols (52a, 52b) and diacetates (52c, 52d),⁴⁹
isoprelaurefucin (53).⁵⁰
 5. *L. obtusa*: (a) Gökceada, Aegean Sea.
obtusenyne (56),⁵³ laurenyne (58), cis-isodihydro-
rhodophytin (59).⁵⁶
(b) Positano, Italy.
obtusenyne (56).⁵⁴
(c) Castellucio, Catania, Sicily.

- laurencienyne (57).⁵⁵
6. *L. okamurai*: (a) Bikuni, Hokkaido, Japan.
okamurallene (70).⁶³
(b) Goza, Mie Prefecture, Japan.
laurencenyne (71), neolaurencenyne (72).⁶⁴
7. *L. pacifica*: Form I, Playa Estacio, Puerto Penasco, Sonora, Mexico.
chondriol (60),^{57,58} rhodophytin (61).^{59,60}
8. *L. pinnata*: Mutsuta Point, Hokkaido, Japan.
laurepinnacin (73), isolaurepinnacin (74).⁶⁴
9. *L. poitei*: Harry Harris State Park, Florida, U.S.A.
poiteol (66).⁵⁴
10. *L. snyderiae*: North Bird Rock, La Jolla, California, U.S.A.
chlorofucin (65).⁵⁴
11. *L. subopposita*: La Jolla, California, U.S.A.
isoprelaurefucin (53), acetyllaurefucin (51a),
laurefucin (51), dehydrobromolaurefucin (64).²⁶
12. *L. venusta*: Moheji, Hakodate Bay, Hokkaido, Japan.
venustin A (68), venustin B (69).⁶²
13. Undescribed *Laurencia* species: (a) Coyote Bay, Baja California, Mexico.
epoxyrhodophytin (63).⁶⁰
(b) San Carlos Bay, Guaymas, Mexico.
rhodophytin (61), chondriol (60), chondrin (62).⁶⁰

according to region may be found in Tables 3 and 4 but it is not restricted to the cuparene and acetylenic compounds. Another example concerns *Laurencia obtusa*. The major metabolite of a sample collected at Tossa de Mar in Spain was found to be α -snyderol (36).³³ However the major metabolite of another sample collected at Kimmeridge Bay, Dorset, England turned out to be the related but different compound 3 β -bromo-8-epicarrapi oxide (37).³⁴ As more samples of *Laurencia obtusa* from different regions were examined the constituents became progressively more varied and the authors of one paper concluded "that the taxonomy of this complex group should be re-evaluated".⁴⁰

Chemotaxonomy becomes an even greater problem when varieties of a single species are found at one location. Erickson and co-workers have delineated three varieties of *Laurencia nidifica* all of which were found on the Hawaiian island of Oahu.^{30,66,67} The varieties were slightly different in colour and appearance i.e. pink clumpy, pink non-clumpy and green, but all were identified as *Laurencia nidifica*. The constituents of each variety were found to be very different. There was a suggestion that the variation may have been due to physical factors since the green variety occurred where wave action was substantial.

There have been other limited attempts to systematize the chemotaxonomy,^{18,58} but much remains to be done. In general, the examination of chemical constituents has shown that morphological taxonomy is by no means definitive. A greater number of observations over longer periods of time are needed until consistent patterns of chemical constituents can be established. The taxonomic assignment of the algae according to chemical constituents may in fact not be possible until the causes of chemical variation can be firmly established. At present it is unclear whether intraspecific variations are caused by differences inherent in

the algae or by environmental factors.

The metabolites of *Laurencia* species are often unstable and decomposition under laboratory conditions may be a problem. Another effect of this instability is that the compound may undergo reactions during isolation so that the molecule identified may not be the original one present in the alga. For example allolaurinterol (17) and filiformin (29) were isolated from the original extraction of *Laurencia filiformis*.²⁵ A second extraction of stored algae contained filiformin (29) but not allolaurinterol (17). This result was explained by the observation of the spontaneous conversion of allolaurinterol (17) over some weeks to filiformin (29) despite storage at 4°. Consequently filiformin (29) may be an artefact and is not necessarily a true constituent of the algae. A second example concerns prepacifenol (76). Pacifenol (77) was originally isolated from *Laurencia pacifica*.⁶⁸ A later investigation of *Laurencia filiformis*⁶⁹ yielded the compound prepacifenol (76) which could be converted to pacifenol (77) by using p-toluenesulphonic acid in benzene. This led to uncertainty about pacifenol (77) as silica gel had been used during its isolation and this may have caused the same conversion. Accordingly, a new extraction of *Laurencia pacifica* was made using neutral material. This led to the isolation of prepacifenol (76) but no pacifenol (77). Interestingly another compound, prepacifenol epoxide (78) has been isolated from *Aplysia californica*.⁷⁰

A feature of the metabolites from *Laurencia* species is the unusually high degree of halogenation. Usually the molecules contain bromine and/or chlorine and occasionally iodine. The most common halogen is bromine and its high utilization relative to chlorine is possibly a reflection of the greater ease of oxidation of bromine.⁷ Bromonium-ion

induced cyclisations have often been postulated as contributing to the biogenesis of *Laurencia* metabolites.¹⁰ It should be noted however that although incorporating halogens into many of their metabolites, species of the *Laurencia* genus do not contain the specialised vesicular cells with high halogen content present in some members of the Rhodophyta.⁹ The *Laurencia* species do contain intracellular refractile bodies (sometimes called "gland cells") which seem to be linked to halogen metabolism although their exact role is unclear.⁹

Although the metabolites of *Laurencia* species are of interest chemically, relatively few seem to exhibit any biological activity. Exceptions are the unusual brominated indole (44) from *Laurencia brongniartii*,⁴² laurinterol (9)³⁰ and laurencienyne (57),⁵⁵ all of which exhibit some antimicrobial activity. Aplysistatin (39) has been shown to inhibit progression of leukemia cells.³⁶ This relative dearth of biological activity may not be a true reflection of pharmaceutical potential since only a few research groups seem to perform or report biological testing routinely.

Another aspect of this work that should be noted is the inter-relationship between species of *Laurencia* and the *Aplysia* mollusc (sea hares) which graze on the algae. These herbivorous molluscs consume large quantities of the algae and have been shown to concentrate the algal metabolites in their digestive glands⁹. Often the metabolites isolated from *Aplysia* species are later found to actually be *Laurencia* algal metabolites and some examples have already been given (aplysin (6), debromoaplysin (7), aplysinol (8)¹⁶ and aplysistatin (39)³⁶). If not identical, then the *Aplysia* metabolites are at least closely related to *Laurencia* metabolites. There are a few acetylenic compounds isolated from *Aplysia* species. Dactylyne (79)⁷¹ and its ³Δ isomer isodactylyne⁷²

have been isolated from *Aplysia dactylomela*. Brasilenyne (80), cis-dihydrorhodophytin (81) and cis-isodihydrorhodophytin (59) are reported from *Aplysia brasiliiana*.⁷³ With the exception of cis-isodihydrorhodophytin (59) which has been isolated from *Laurencia obtusa*,⁵⁶ the exact algal source of these compounds has not been identified although it is likely to exist. However, without knowing the algal source, it is not possible to discover how much, if any, modification has occurred between seaweed and sea hare.

The concentration of halogenated metabolites by the sea hares seems to have a role in defence. Experiments have been performed⁷³ to show that sea hares are unpalatable to a variety of fish. The metabolites appeared to be concentrated in the digestive gland and body wall as these were consistently rejected whereas other body portions were variably palatable. To confirm that the algal metabolites were responsible for this, samples of brasilenyne (80) and cis-dihydrorhodophytin (81) were coated onto small beetle larvae. When fed to swordtail fish these were rejected whereas control uncoated larvae were promptly swallowed.

The results reviewed here have been obtained by a variety of techniques and the practical aspects of algal research are worth examining briefly. Collection of samples of algae is usually straightforward either by shoreline gathering or aqualung diving. It is usually possible and always desirable to obtain manageable quantities for study without significantly disturbing the ecological balance of the collection region. Identification of the algal species and homogeneity of the sample are important requirements and the assistance of a phycologist is essential for any research team. For example the compounds laurinterol (9), debromolaurinterol (10) and isolaurinterol (11) were isolated from

a species of *Laurencia intermedia*.⁷⁴ A later examination of *Laurencia intermedia* from another location unexpectedly revealed no halogenated material and a re-examination of the first sample revealed that it actually consisted of a mixture of *Laurencia okamurai* and *Laurencia capituliformis*. Samples of all three species were obtained from the original location and the compounds were found to actually originate from *Laurencia okamurai*.¹⁸

Although classification of the *Laurencia* algae is complex, the majority of species have been categorised and only a few reports are of compounds isolated from algae for which only the genus *Laurencia* can be established. As shown by Tables 3 and 4 it is also important to report the location from which the sample is taken. Differentiation within a species between male, female or tetrasporangial reproductive forms of the algae is usually not considered although this may be important.

Extraction of the algae may be accomplished by a variety of techniques and solvents. It is usual to use methanol and some organic solvent either consecutively or in combination for the extraction. The efficiency of extraction is improved by blending the algae with the solvent rather than just standing. Soxhlet extraction also improves the yield, although as it involves the use of boiling solvent, only organic solvents with low boiling points should be used.

Following extraction, isolation of the individual components is required. For this purpose the various forms of chromatography are used. As many algal metabolites are unstable and reactive, the reagents and conditions should be as mild and neutral as possible.

Thin layer chromatography (t.l.c.) is useful as a quick routine

analytical tool, and for small scale separations may be used preparatively. The disadvantage of t.l.c. is that it gives little information as to the relative amounts of components of a mixture. Gas liquid chromatography (g.l.c.) does give relative proportions and can be useful as an analytical method. However the high temperatures required for g.l.c. analysis make decomposition a problem and for the same reason g.l.c. is of little use on a preparative scale. An exception to this involves the cuparene-type molecules. These have proved to be surprisingly stable at high temperatures. Consequently, rapid analysis of mixtures of these compounds is possible using a g.l.c. linked to a mass spectrometer (g.c.m.s.). The g.l.c. provides component separation and the mass spectrometer provides a characteristic spectrum which often permits identification of the compound. This technique requires the knowledge that cuparene-type molecules are involved and detailed information on characteristic mass spectra. However, it has been used to examine *Laurencia* samples for which the basic components of one sample had been previously established and the researchers wished to check for regional variation.⁷⁵

High pressure liquid chromatography (h.p.l.c.) is probably the most useful technique in that it is a fast analytical tool and is capable of being scaled up to preparative quantities. Resolution is usually better than can be achieved by other techniques and sample recovery is easier and usually quantitative. The solid phase materials available for h.p.l.c. are also becoming increasingly varied and specialised. In particular, the recently developed reverse phase chromatography permits separations that were previously very difficult. The use of advanced pumping machinery and microprocessor control allow a consistency and precision which makes h.p.l.c. the chromatographic technique of choice.

Having isolated the components, identification is the next priority.

The most definitive technique is X-ray crystallography but for a variety of reasons it is not always possible to obtain a suitable crystal for analysis. In these cases several alternatives have been used. In early algal work it was customary to submit the isolated sample to extensive chemical degradation in order to establish the structure. The excellent work performed to characterise laurencin (48)^{4,6} is a good example of this. Chemical reactions are still useful (notably hydrogenation) and with the increase in the range of identified metabolites, chemical conversion of an unknown to a known compound has become more common as a means of identification. However, as spectroscopic techniques become more sophisticated and reliable and the actual quantities of compound available decreases as minor components are isolated, then spectroscopy becomes more valuable. The whole range of spectroscopic techniques has been used, in particular ultraviolet and infrared absorption, and nuclear magnetic resonance (n.m.r.) spectroscopy. N.m.r. has been particularly useful as a wealth of information can be obtained from small, recoverable samples. The information available from the basic n.m.r. spectrum may be extended by the use of techniques such as homonuclear decoupling, shift reagent studies and single frequency offset resonance decoupling (s.f.o.r.d.). More recent advances in n.m.r. technique involve the use of gated broad band decoupling^{76,77} programmes and stepwise single frequency offset resonance decoupling (s.s.f.o.r.d.)⁷⁸ experiments.

The improvement in practical techniques has meant that algal research work can cope with the problems of instability and subtle variation between compounds. The wide range of background information available from identified compounds is of great assistance in further research. This is particularly true for the *Laurencia* species.

The variety of metabolites isolated from different species and also

the apparent regional variation suggested that an examination of local New Zealand species of *Laurencia* would be worthwhile. This examination was immediately rewarded with the isolation of thyrseferol (43)^{41,79} from the first alga selected for study, *Laurencia thyrsefera*. A squalene derived metabolite, thyrseferol (43) is the first example of a triterpene from this genus. An isomeric pair of acetylenic cyclic ethers was also located although the exact structures were not defined.⁷⁹ Accordingly, the identification of these compounds was chosen as a starting point for the work reported in this thesis. Further investigation identified several other acetylenic cyclic ethers from the same species. Later, attention was turned to another alga, *Laurencia distichophylla* which proved to contain a range of cuparene-type metabolites.

The large number of acetylenic cyclic ethers isolated from *Laurencia* species all have apparently similar biogenetic origins. It was felt that the amount of structural information available would be sufficient to allow the deduction of some aspects of biosynthesis. Consequently an extensive literature survey was made and the collected information, together with that obtained in this study, was examined in detail.

PART 1ANALYSIS OF LAURENCIA THYRSIFERA

Laurencia thyrsifera is a species of marine red alga widespread throughout New Zealand coastal regions.⁸⁰ It occurs in the intertidal zone as green-brown clumps attached to rocks (*epithilic*). This seaweed often occurs tangled with the bubble-like strings of another alga, *Chaetomorpha darwini*. Collections of *Laurencia thyrsifera* (J. Agardh, Voucher Number 248071, Plate 1) were made at Seal Reef, Kaikoura at low tide on three occasions: May 1978, February 1979 and February 1980. Although distinction is easily made from *Chaetomorpha darwini* only isolated untangled samples of *Laurencia thyrsifera* were collected. Male, female and tetrasporangial varieties were not differentiated.

The first collection was extracted by standing the wet algae in methanol for 12 days. The extract was then filtered, the water/methanol mixture evaporated under vacuum at a moderate temperature (40° C) to a manageable volume which was then extracted with ethyl acetate. This gave an extract that represented 2.1% based on dry weight of the extracted alga. The second collection was extracted in a similar way except that the alga was first blended with methanol which improved the yield slightly. The third collection was air dried and then ground to a coarse powder. This powder was then extracted in a Soxhlet apparatus with dichloromethane until the extracting solution became clear. This produced an extract that represented 3.3% by dry weight of algae. However, this improved yield did not in fact increase the material available for study. The initial bulk column chromatography on the first collection extract gave fractions that made up a return of 91%. Similar bulk column chromatography on the Soxhlet extract of the third collection gave a yield of 50 - 60% which reduced the amount of usable material to approximately the same as that

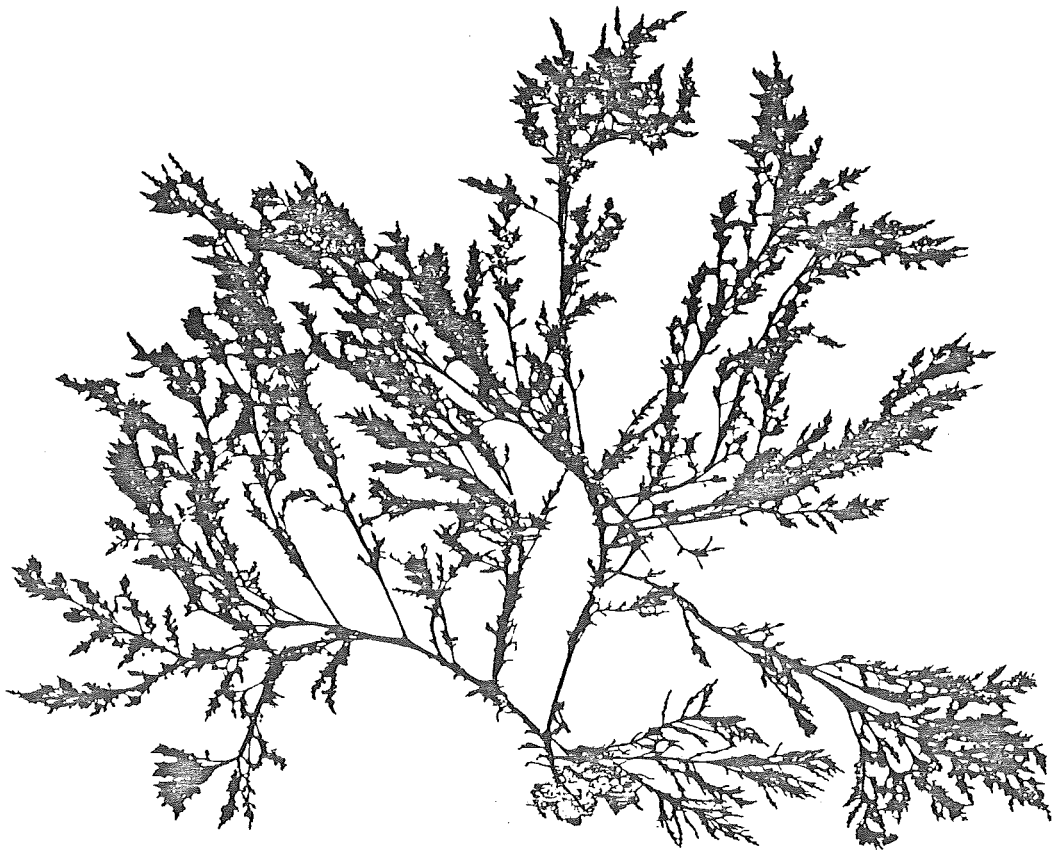


Plate 1: *Laurencia thyrsoifera*

obtained by the first extraction method. The material lost on these columns was highly coloured and appeared to be very polar as it would remain on the column even after elution with methanol. The Soxhlet extraction method was preferred however as it involved much less handling and smaller solvent volumes than the methanol extraction. The possibility that the boiling dichloromethane may have affected the extract was checked by comparison of the methanol and Soxhlet extracts. No differences were apparent by t.l.c. or in the compounds isolated from each extract. As the bulk of the results reported here were obtained from Soxhlet extracted material the percentage of extract corresponding to a particular component was calculated as a proportion of the weight of material obtained from the initial bulk column. This is believed to be a more accurate representation.

The first collection of *Laurencia thyrsifera* was tested for biological activity against *Bacillus subtilis*. No activity was observed as was expected from the results of other tests on this species.⁷⁹

Examination of the extract by t.l.c. revealed the presence of eight distinct spots: R_f 0.83, 0.73, 0.57, 0.43, 0.37, 0.27, 0.17, 0.13. A representative drawing of a t.l.c. plate is given in Figure 1. Analysis of the extract by g.l.c. (OV17) showed only six major peaks as shown in Figure 2. This discrepancy was attributed to the probable thermal instability of some compounds and that the more polar compounds would not register on g.l.c. Additionally, fatty acid ester mixtures which are homogeneous by t.l.c. are readily separated by g.l.c. and would therefore appear as more than one peak.

Fractions of the extract were obtained by column chromatography on alumina. The material could be divided into essentially three fractions.

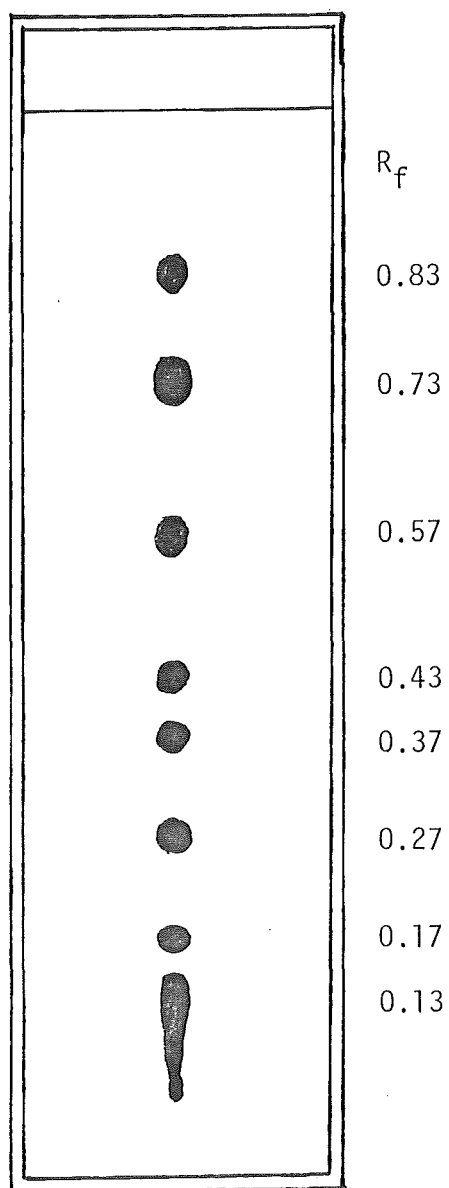


Figure 1: Representative drawing of a t.l.c. plate showing the components of *Laurencia thyrsoifera* (silica gel, developed once in 1:1 ether/pet. ether).

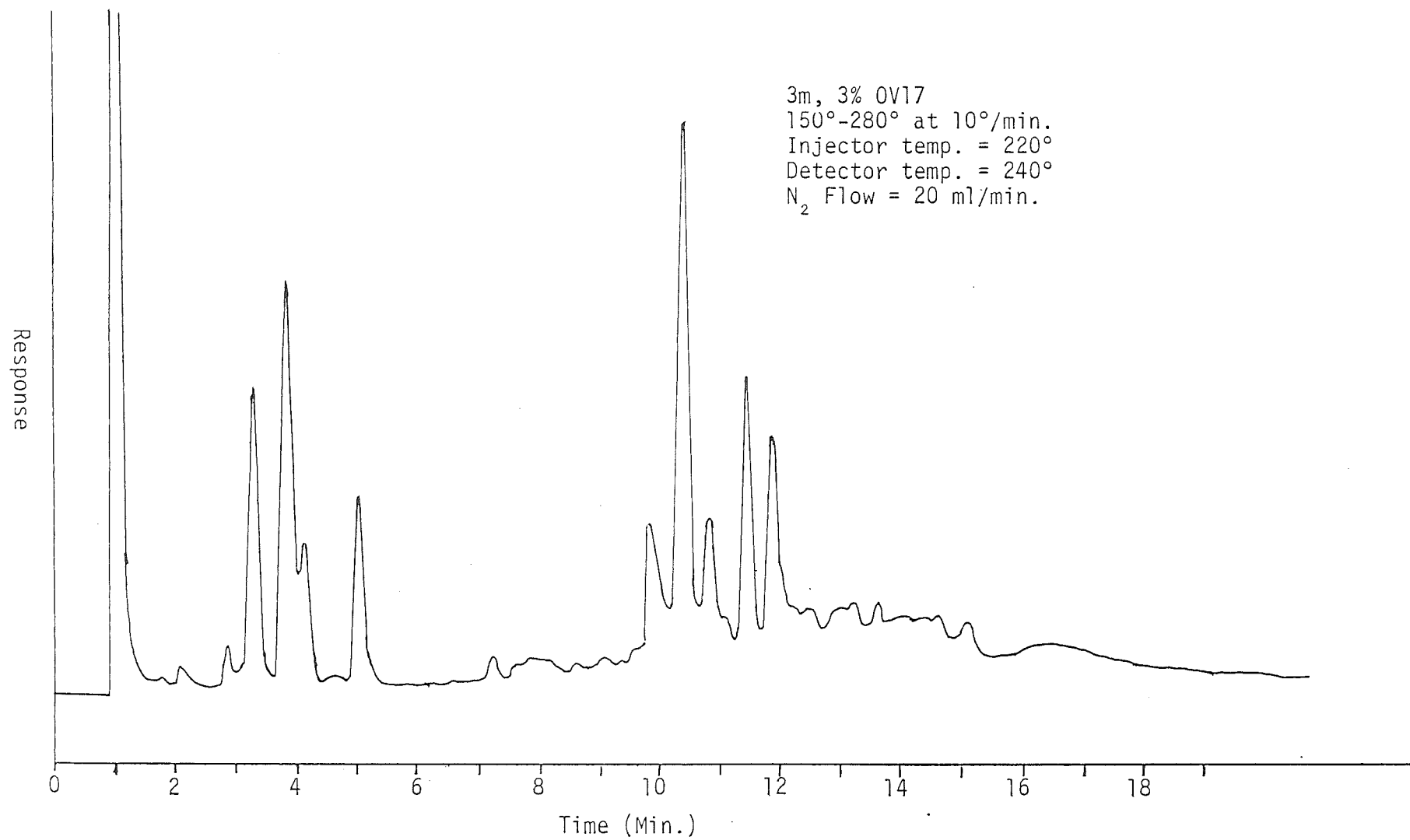


Figure 2: Gas chromatographic trace of the *Laurencia thyrsoifera* extract.

The non-polar fraction made up most of the extract, comprising 60% by weight, and included t.l.c. spots at R_f 0.83, 0.73 and 0.57. Most of the non-polar fraction could be eluted with petroleum ether. The second fraction of midrange polarity compounds contributed 18% of the weight of the extract and consisted of t.l.c. spots of R_f 0.43, 0.37 and 0.27. This was eluted with mixtures of petroleum ether and gradually increasing proportions of dichloromethane. While the first two fractions were light yellow in colour, the final polar fraction, eluted with dichloromethane and diethyl ether, was generally dark green in colour. This material corresponded to t.l.c. spots at R_f 0.17 and 0.13 and comprised 22% of the extract.

Initial investigation was directed towards the polar fraction. Attempts to isolate the components of this fraction were only partially successful due to the near chromatographic coincidence of the compounds. A small quantity of thyriferol (43) was isolated however. Accordingly the mixture was acetylated with pyridine/acetic anhydride as in the preliminary investigations.⁷⁹ This enabled the isolation of an isomeric pair of acetylenic cyclic ethers (82a) and (82b) as the diacetate derivatives (82c) and (82d) (combined 5% of the extract).

The material was non-crystalline and unstable even under nitrogen in solution at low temperature (4°C). By repeated multiple development preparative thin layer chromatography the (*Z*) and (*E*) diacetoxo-enyne isomers (82c) and (82d) were separated.

The infrared spectrum of the mixture exhibited absorptions attributable to terminal alkyne (3300, 2100 cm^{-1}), acetate (1740, 1350 cm^{-1}) and ether (1100 cm^{-1}) functionalities. The ultraviolet spectrum had maxima at 224 nm ($\epsilon = 10,000$) with inflections at 232 nm ($\epsilon = 7000$). These

absorptions are characteristic of the enyne chromophore.⁸¹ The infrared and ultraviolet spectra indicated that the molecules were examples of the acetylenic cyclic ethers common in *Laurencia* algae.

The electron impact (EI) mass spectrum of the isomeric diacetate mixture did not display any recognisable parent ions. However the low resolution chemical ionisation (CI) mass spectrum showed a parent ion (M+1) as a 3:1 doublet at m/e 369/371 suggestive of a molecular formula of $C_{19}H_{25}ClO_5$. The presence of a chlorine atom was supported by the observed loss of 36 a.m.u. (HCl) to give a daughter ion at m/e 333. Consecutive loss of two acetic acid units (60 a.m.u.) was suggested by daughter ions at m/e 309/311 and m/e 249/251. The molecular formula was supported by combustion data and implied seven double bond equivalents. This was consistent with a macrocyclic vinyl acetylenic ether with two acetate groups, a chloro group and an additional double bond.

The ^{13}C n.m.r. spectra of the individual isomers (82c) and (82d), as given in Table 5, also supported the molecular formula. Besides the usual acetate carbonyl and methyl peaks there were fifteen resonances. These included resonances for one methyl and three methylene carbons, a chlorinated methine carbon, four oxygenated methine carbons, a terminal alkyne carbon, four monoprotonated olefinic carbons, and a quaternary alkyne carbon. The different carbon types were distinguished by means of the single frequency offset resonance decoupled (s.f.o.r.d.) spectrum. Several resonances could be assigned immediately. The double bond of the enyne system consistently has absorptions at approximately δ_C 110 ppm for C_3 and δ_C 140 ppm for C_4 (carbon atom numbering is shown in structure (82a)). The quaternary alkyne proton absorbance was obvious as a singlet at δ_C 81.7 ppm for the (Z) isomer and δ_C 79.8 ppm for the (E) isomer. The absorptions at δ_C 28.2 and δ_C 10.2 ppm were assigned

Table 5: ^{13}C n.m.r. shielding data for the enyne diols and derivatives (all CDCl_3 solutions, ppm from TMS).

	82a	82b	82c	82d	84	85a	85b	86
C_1	77.0	82.6	77.0	82.6	14.0	77.0	82.6	13.8
C_2	81.7	79.8	81.7	79.8	22.4	81.7	77.0	22.3
C_3	111.8	110.8	112.0	111.0	31.4	111.8	110.9	31.0
C_4	141.7	140.8	141.1	140.2	26.3	141.5	140.8	26.2
C_5	35.5	33.9	36.5	34.0	32.9	36.0	33.1	33.1
C_6	62.8	63.4	62.4	63.1	64.7	62.7	61.9	63.9
C_7	77.0	77.3	78.2	78.7	78.7	78.6	78.6	78.5
C_8	36.4	36.2	32.5	32.4	32.7	33.5	33.5	32.1
C_9	71.8	71.8	71.3	71.3	71.4	77.6	77.6	78.0
C_{10}	72.1	72.1	72.5	72.6	72.6	79.0	79.0	79.0
C_{11}	132.2*	132.2*	127.5	127.3	127.5	129.0	129.0	128.8
C_{12}	131.1*	131.1*	133.4	133.6	133.5	130.9	130.8	130.8
C_{13}	81.1	81.1	81.4	81.4	81.3	81.7	81.7	81.5
C_{14}	28.3	28.3	28.2	28.3	28.2	28.3	28.3	21.1
C_{15}	10.3	10.3	10.2	10.2	10.2	10.2	10.2	10.0
			170.0 ⁺	170.0 ⁺	170.0 ⁺	107.6 [°]	107.6 [°]	107.3 [°]
			169.9 ⁺	169.9 ⁺	169.9 ⁺	27.0 [°]	27.0 [°]	26.9 [°]
			21.0 ⁺	21.0 ⁺	21.0 ⁺			

* Reversible

+ acetate carbonyl and methyl carbons

° acetonide quaternary and methyl carbons

as C₁₄ and C₁₅ respectively by comparison with known compounds (laurencin (48),⁴⁶ poiteol (66),⁵⁴ chlorofucin (65),⁵⁴ and obtusenyne (56)^{53,54}).

The ¹H n.m.r. spectra of each isomer were complex but similar. The only immediately obvious difference was the position of the characteristic terminal alkyne proton doublet. For (*Z*) isomers this usually occurs in the range δ_{H} 2.9 - 3.1 ppm, while for (*E*) isomers it occurs in the range δ_{H} 2.6 - 2.8 ppm.⁶⁰ Accordingly the isomer with the alkyne absorbance at δ_{H} 3.1 ppm was assigned as (*Z*), (82c), and the isomer with the absorbance at δ_{H} 2.8 ppm was assigned as (*Z*), (82d).

In order to facilitate examination of the ¹H n.m.r. spectrum in detail a sample of the (*Z*) isomer was submitted for analysis at 270 MHz. By homonuclear decoupling experiments the interrelationship of all the protons could be established as shown in the partial structure (83). From this data it was evident that the diacetates (82c) and (82d) were related to the laurencin (48)⁴⁶ type of skeleton but it was not possible to deduce the substitution pattern.

In order to eliminate the (*E*) and (*Z*) geometric isomerism and to simplify the ¹H n.m.r. spectrum a mixture of the diacetates (82c) and (82d) was hydrogenated over Pd/C. This method of hydrogenation saturated the side chain, but left the other functional groups intact. The 80 MHz ¹H n.m.r. spectrum of the hydrogenation product (84) showed the expected changes in shielding values with considerable simplification of the low field region. Here the only notable shift was that of the C₆ proton multiplet from δ_{H} 4.1 to 3.9 ppm. All other shieldings remained within \pm 0.1 ppm. Homonuclear decoupling experiments confirmed the earlier assignments of the ring protons as shown in structure (84).

Having obtained the hydrogenated product (84) it was possible to analyse the simplified ^1H n.m.r. spectrum by a technique known as step-wise single frequency off-resonance decoupling (s.s.f.o.r.d.).⁷⁸ In this technique the aim is to correlate a ^{13}C n.m.r. carbon resonance with the resonance of the proton attached to it in the ^1H n.m.r. spectrum, thus permitting further structural assignments.

The practical aspects of obtaining this correlation involve modifications of the normal s.f.o.r.d. experiment. In this process the off-resonance position of the ^1H -decoupler frequency permits a limited degree of carbon-hydrogen coupling as measured by the reduced C-H coupling constant ($^1J_{\text{R,CH}}$). In the case of a single carbon and attached hydrogen is considered, as the off-resonance decoupler frequency is moved closer to the precise ^1H -decoupling frequency then the value of $^1J_{\text{R,CH}}$ will drop. It will reach zero when the decoupling frequency is set at the same frequency as the absorbance of that particular proton.

Mathematically this is expressed in Equation (1).

$$^1J_{\text{R,CH}} = J\Delta\nu(\gamma_{\text{H}}B_2/2\pi)^{-1} \quad \text{Eqn. (1)}$$

J is the true coupling constant and $\Delta\nu$ is the decoupler offset in Hertz. B_2 represents the power of the decoupler which is usually expressed in Hertz as $\gamma_{\text{H}}B_2/2\pi$ where γ_{H} stands for the magnetogyric ratio of the proton. From Equation (1) it can be deduced that if the decoupler power B_2 is constant then the value of $^1J_{\text{R,CH}}$ depends entirely on $\Delta\nu$. When $^1J_{\text{R,CH}}$ is zero then $\Delta\nu$ must also be zero i.e. the decoupler is irradiating at the specific resonance frequency of the proton.

This is the ideal situation. In practise the decoupler frequency

is not varied continuously but set at one of a series of steps through the proton absorption range and the s.f.o.r.d. ^{13}C n.m.r. spectrum obtained at each point. By plotting the reduced C-H coupling constants against the proton irradiation frequency (ν_{H}) and applying a least squares analysis to the plot, a value of ν_{H} corresponding to $^1J_{\text{R,CH}} = 0$ may be obtained. This value of ν_{H} represents the absorption frequency of the proton attached to the carbon being analysed. The s.f.o.r.d. spectra obtained in this experiment are illustrated in Figure 3.

The ^{13}C n.m.r. spectrum of (84) is listed in Table 5. The correlations made between the carbon and proton chemical shieldings are listed in Table 6. From these correlations structure (84) was deduced. It was obvious from the carbon chemical shifts that the additional double bond lay between C_{11} and C_{12} . The C_9 and C_{10} positions were oxygenated and were assigned as the acetate positions. Of the remaining three ring carbons C_7 was also oxygenated and was assigned as one carbon of the ether linkage. Although the two remaining carbon resonances, δ_{C} 81.3 and δ_{C} 64.7 ppm, had the same s.s.f.o.r.d. frequency for $^1J_{\text{R,CH}} = 0$ this created no ambiguity as the resonances could be assigned as representing oxygenated and chlorinated carbons respectively, from their chemical shift. On the basis of comparison with known compounds of this type the oxygenated carbon was assigned to C_{13} and the chlorinated carbon to C_6 to generate a macrocyclic ether belonging to the laurencin (48)⁴⁶ family. The reverse assignment would have required an acyclic chain with an epoxide ring which would have been inconsistent with the ^1H n.m.r. data. In laureepoxide (54)⁵¹ which does contain a $\text{C}_6 - \text{C}_7$ epoxide both the C_6 and C_7 protons occur at δ_{H} 3.2 ppm.

A more detailed analysis of the ^1H n.m.r. and ^{13}C n.m.r. data for (82c), (82d) and (84) supported these assignments. For example the

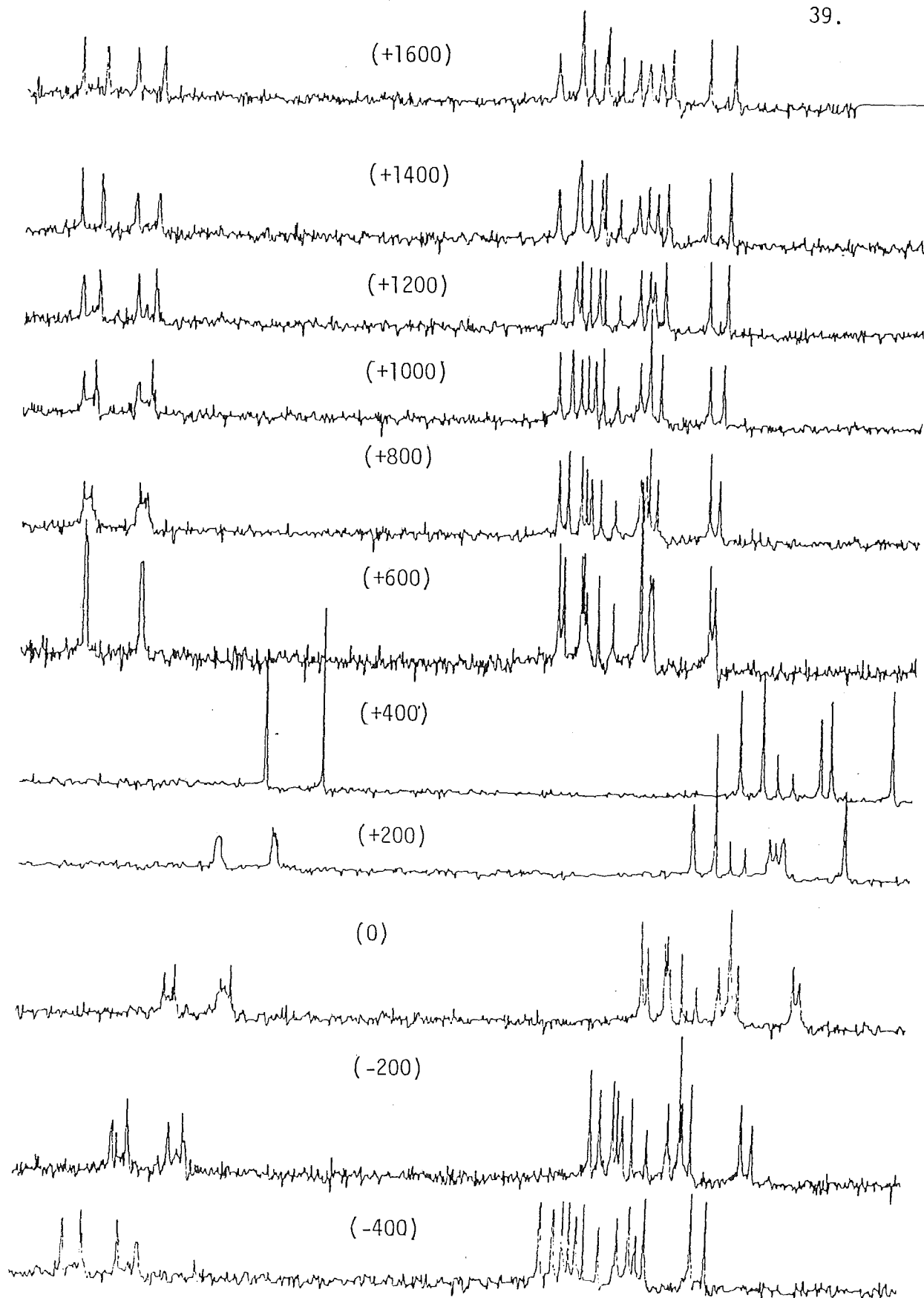


Figure 3: S.s.f.o.r.d. spectra for (84). The lower five spectra have been plotted slightly offset. Numbers in brackets refer to the decoupler offset (in Hz) relative to TMS = 0 Hz.

Table 6: S.s.f.o.r.d. results and correlations with ^{13}C n.m.r. shielding data for the hydrogenated enyne diacetate (84).

^{13}C n.m.r.* shielding	^1H n.m.r. frequency [†]	^1H n.m.r. simulation frequency [°] (and chemical shift*)
133.5	444	448 (5.6)
127.5	424	432 (5.3)
81.3	314	328 (3.9)
78.7	274	288 (3.6)
72.6	504	500 (6.3)
71.4	394	408 (5.1)
64.7	314	312 (3.9)

* In CDCl_3 ; relative to TMS

† The s.s.f.o.r.d. frequency (Hz, relative to TMS = 0) for which

$$^1J_{\text{R,CH}} = 0$$

° Hz relative to TMS = 0

resonances for the chlorinated carbons (C_6) in (82c) and (82d) are distinctive for each isomer (δ_C 63.1 and δ_C 62.3 respectively). After reduction of (82c) and (82d) to produce (84) these peaks are replaced by a single resonance at δ_C 64.7 ppm, whereas the shielding of the oxygenated carbon C_{13} alters by only 0.1 ppm. Hence the chlorine would be expected to be located at C_6 and affected by the (*E*)-(Z) isomerism of the $^3\Delta$ double bond. In addition, appropriate allylic coupling constants were obtained from 1H n.m.r. decoupling and spin-simulation experiments (See Table 7).

Homonuclear decoupling experiments on the hydrogenated diacetate (84) yielded approximate values for the proton-proton coupling constants. More precise values were obtained by computer simulation. These simulations of the 1H n.m.r. spectrum of (84) were performed on the CFT-20 spectrometer's computer/plotter system using the programme SIMEQ. With this programme the trial values of chemical shifts and coupling constants are entered and then varied manually after each simulation until a simulated spectrum is obtained which is judged to be nearest in appearance to the experimental spectrum. A result of this approach is shown in Figure 4 for part of the 1H n.m.r. spectrum of (84). The simulation shielding data is listed in Table 6 and the coupling constants in Table 7.

This left the relative and absolute stereochemistry to be assigned. In order to ascertain the relative stereochemistries of the substituents the derived coupling constants were used in Karplus-type equations to obtain values for the individual H-C-C-H torsional angles (ϕ). For sp^3 - sp^3 hybridised systems a modified Karplus equation was used which included terms to account for the important effect of electronegative substituents on the coupling constant as shown in Equation (2).⁸²

$${}^3J_{HH} = P_1 \cos^2\phi + P_2 \cos\phi + P_3 + \sum \Delta\chi_i \{P_4 + P_5 \cos^2(\xi_i \cdot \phi + P_6 |\Delta\chi_i|)\} \text{ Eqn. (2)}$$

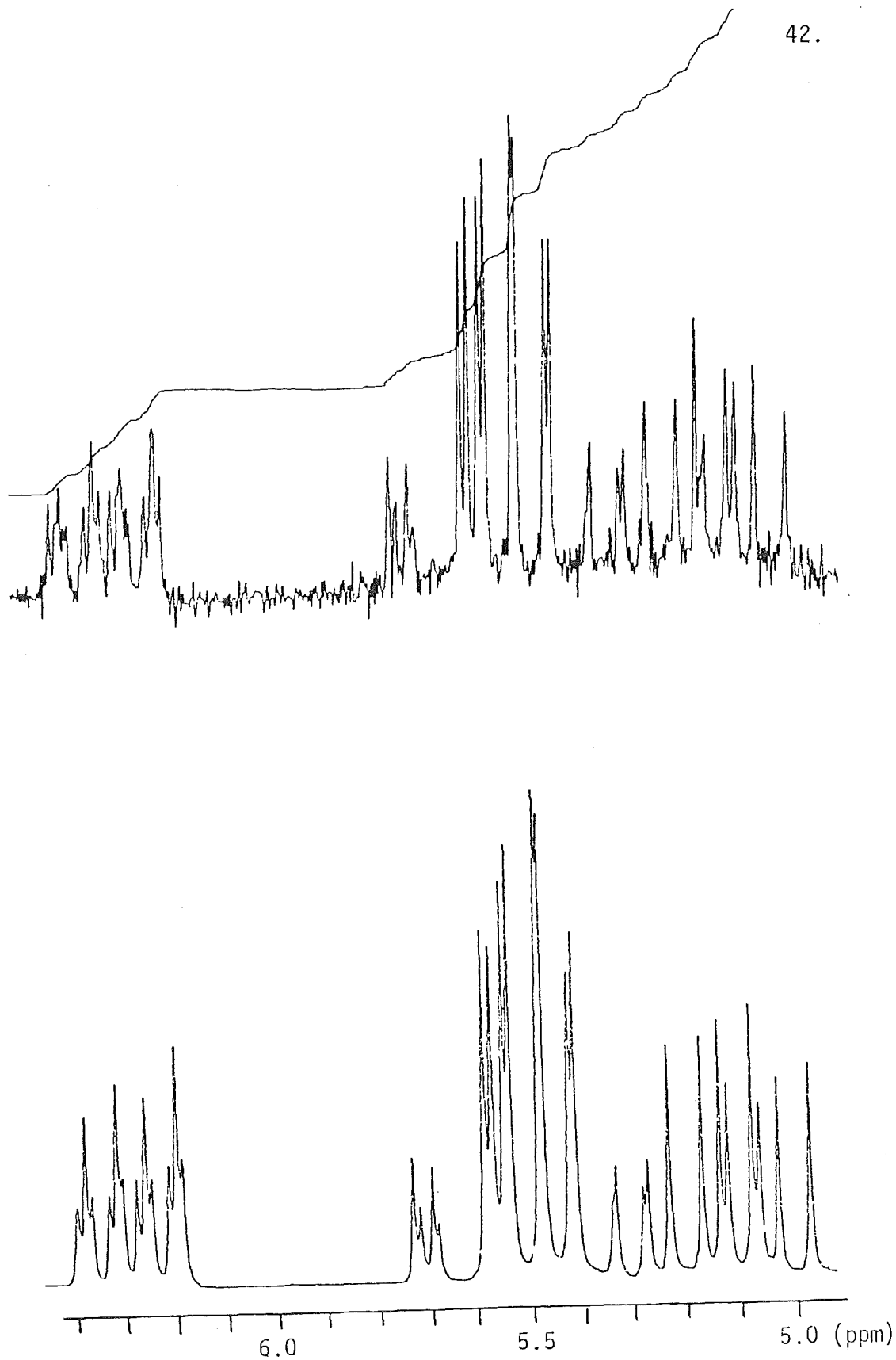


Figure 4: Experimental (above) and simulated (below) sections of the ^1H n.m.r. spectrum of (84).

Table 7: ${}^3J_{\text{HH}}$ Values for the hydrogenated diacetate (84) and hydrogenated acetonide (86) with derived torsional angles (ϕ) for (84).

	${}^3J_{\text{HH}}$		ϕ°
	84 ⁺	86 ⁺	
$J_{6,7}$	9.1		
$J_{7,8a}$	5.8		126
$J_{7,8b}$	3.1		107
$J_{8a,9}$	5.0		39
$J_{8b,9}$	7.8		137
$J_{9,10}$	9.0	8.3	180
$J_{10,11}$	5.3	4.6	160
$J_{11,12}$	11.0	11.9	
$J_{12,13}$	3.3	3.0	
$J_{13,14}$	5.0*		
$J_{14,15}$	7.0*	7.0*	
$J_{10,12}$	1.1		
$J_{11,13}$	1.1		
$J_{10,13}$	1.0		

* Not simulated

+ Simulated values accurate to within 10%

The first three terms in Equation (2) constitute the basic Karplus type equation. The final term allows for the effect of electronegative substituents where $\Delta\chi_i$ is the electronegativity difference between the substituent attached to the carbon, and hydrogen. ξ_i is a term which modifies the effect according to the relative orientation of the substituents. The constants $P_1 - P_6$ were determined empirically by fitting the equation to a large set of data for which the coupling constants and torsional angles were known. An additional term may be employed to account for the effect of electronegative β substituents. However the effect of this term is small compared to the effect of the term accounting for α substituents. A trial calculation of one of the $sp^3 - sp^3$ systems showed that the effect of the β substituent term was at the most 5° and so this term was omitted.

For the $sp^3 - sp^2$ hybridised systems Equation (3)⁸³ was used which includes the electronegative substituent effect by utilising a term involving the 1H n.m.r. chemical shift of the protons involved.

$$^3J(H_i, H_j) = 0.89 \cos^2\phi + 0.27 \sum(\delta_{Hi} + \delta_{Hj}) \quad \text{Eqn. (3).}$$

These equations were employed to obtain the various torsional angles as shown in Table 7. To determine which structure best accommodated this combination of angles, Dreiding models were constructed with differing relative chiralities for C_7 , C_9 , C_{10} and C_{13} . The absolute chirality at each of C_7 and C_{13} was assumed to be *R*. This assumption was based on the observation that in all the related compounds (dehydrobromolaurefucin (64),²⁶ laurefucin (51),⁴⁸ acetyl laurefucin (51a),⁴⁸ laurencin (48),⁴⁶ deacetyl laurencin (48a)⁴⁹ and poiteol (66)⁵⁴) the chirality at C_7 and C_{13} is consistently *R*.

The only structure which then permitted the combination of angles shown in Table 7 is that shown in (82c) and (82d) in which C₉ and C₁₀ each have the *R* configuration. This structure not only displays a good correspondence of the observed with the calculated torsional angles, but also shows a minimum of steric interactions. In arriving at this solution, it has been assumed that the ring structure is relatively immobile, and that the observed coupling constants have not arisen from rotational averaging.

As a further experiment to examine the stereochemistry of these molecules, formation of the acetonide derivative of the diols (82a) and (82b) was attempted. A mixture of thyriferol (43), (82a) and (82b) was reacted with acetone and perchloric acid, and the acetonide formation proceeded rapidly suggesting that the two alcohol groups were already coplanar or were easily able to achieve coplanarity. The acetonide derivatives were separated from the mixture to give (85a) and (85b).

The mixture of (85a) and (85b) was then hydrogenated over Pd/C to produce (86) and simplify the ¹H n.m.r. spectrum for examination. Homonuclear proton decoupling experiments at 80 MHz permitted some assignments of ring protons. At 80 MHz the clearly defined multiplets at δ_H 5.6, 5.3 and 4.9 ppm could be assigned as the absorptions of protons at C₁₁, C₁₂ and C₁₀ respectively. However the protons at C₆, C₇, C₉ and C₁₃ all had resonances in close proximity which produced a complex multiplet δ_H 3.4 - 4.1 ppm which was not amenable to analysis. Despite this difficulty decoupling experiments were performed and computer simulations made of the clearly defined multiplets. This permitted the extraction of some coupling constants for (86) and these are listed in Table 7. The marked similarity between these *J* values and those obtained for the hydrogenated diacetate (84) implied that the ring had not been substantially distorted

on forming the acetonide, thus supporting the approximately eclipsed positioning of the acetate groups as required by the angles derived from the Karplus equation study of (84).

Table 5 lists the ^{13}C n.m.r. assignments for (82a), (82b), (82c), (82d), (84), (85a), (85b) and (86). The assignments were made on the basis of s.s.f.o.r.d. results (which allowed unambiguous assignment of the ring carbons), s.f.o.r.d. results and comparisons with the shielding values of other acetylenic cyclic ethers. The elimination of the isomeric differences between (82c) and (82d) and also between (85a) and (85b) by hydrogenation clearly established that the (*E*) and (*Z*) isomerism of the $^3\Delta$ double bond was the only point of isomerism in the molecule. The isomerism was assigned on the basis of the ^1H n.m.r. chemical shifts as already described. In addition it was possible to extract the olefinic coupling constant for the $^3\Delta$ double bond from the spectra of (82c) and (82d). This was measured as 12 Hz for (82c) and 17 Hz for (82d). These values are in agreement with quoted values for other molecules with (*E*) - (*Z*) isomerism^{46,54} and confirm the assignment.

The only point of stereochemistry not established is the chirality at C_6 . The Karplus equations could not be applied as the $J_{6,7}$ coupling constant must be an average value due to the free rotation of the side chain. However in the report on laurepinnacin (73)⁶⁵ the close similarity between the splitting patterns of the C_6 proton in laurepinnacin (73) and laurencin (48) (for which the C_6R configuration had been independently determined) was used as supporting evidence for the assignment of C_6R chirality in laurepinnacin. In laurepinnacin the C_6 proton ^1H n.m.r. resonance is dt, $J = 10$ and 4 Hz and in laurencin the corresponding resonance occurs as dt, $J = 8$ and 5 Hz. In the 270 MHz spectrum of (82c) the C_6 proton absorbs at δ_{H} 4.1 ppm. The multiplet at δ_{H} 4.1 could be analysed as ddd, $J = 9, 7$ and 5 Hz. These coupling

constants are of the same order as laurepinnacin and laurencin which suggested that the chirality at C₆ in (82c) was also *R*.

The structures of (82a) and (82b) are akin to that of dehydrobromolaurefucin (64).²⁶ In an attempt to initiate a possible cyclisation of the C₉ hydroxyl group to C₆ producing dehydrobromolaurefucin (64), a sample of (82a) and (82b) was stirred in a 5% KOH/MeOH solution at room temperature. No dehydrobromolaurefucin was formed, however and this attempted cyclisation was not further pursued.

The acetylenic ether diols (82a) and (82b) occurred as a mixture with thyrseferol (43) corresponding to the t.l.c. spot at R_f 0.13. Although thyrseferol (43) was present, only small quantities could be isolated and it was estimated that the amount of thyrseferol present was only 1% of the extract. An h.p.l.c. trace of the acetylated polar fraction showed two major peaks which were isolated and identified as (82c) and (82d), and a number of minor peaks. It appeared that the *Laurencia thyrsefera* collected for this investigation contained a lesser proportion of thyrseferol than the 4.5% of extract by dry weight quoted in the initial isolation.⁴¹ As the collections for both studies were made at the same place and at approximately the same time of year it is uncertain what has caused this variation.

During attempts to isolate the lower R_f components of the polar fraction, such as the diols (82a) and (82b), the isolation of material causing the t.l.c. spot at R_f 0.17 was also achieved. This proved to be a mixture of four compounds (87a), (87b), (87c) and (87d), the four possible monoacetate isomers of the diols (82a) and (82b). This material, given the working name "Mixture A", constituted 9% of the extract but appeared to be unstable as only small quantities could be isolated.

The infrared spectrum of Mixture A contained peaks attributable to hydroxyl (3600 cm^{-1}), terminal alkyne ($3300, 2100\text{ cm}^{-1}$), acetate ($1740, 1350\text{ cm}^{-1}$) and ether (1100 cm^{-1}) functionalities. The ultraviolet spectrum contained peaks characteristic of the enyne chromophore⁸¹ i.e. maxima at 223 nm ($\epsilon = 10,600$) and inflections at 232 nm ($\epsilon = 7900$).

The EI mass spectrum of Mixture A did not display a parent ion. However in the CI mass spectrum a parent ion was shown as an (M + 1) 3:1 doublet at m/e 327/329 suggestive of a molecular formula of $\text{C}_{17}\text{H}_{23}\text{ClO}_4$. Loss of H_2O (18 a.m.u.) was observed as a 3:1 doublet at m/e 309/311 and loss of acetate (60 a.m.u.) as a 3:1 doublet at m/e 267/269.

The ^1H n.m.r. spectrum was very complex but exhibited the same general pattern as the acetylenic diacetates (82c) and (82d). The characteristic terminal alkyne proton doublets at δ_{H} 3.1 and δ_{H} 2.8 ppm could be located indicating the usual (*E*) and (*Z*) isomerism and the acetate methyl protons occurred as a singlet at δ_{H} 2.1 ppm. The ^{13}C n.m.r. spectrum of Mixture A (See Figure 5) was also similar to the spectra of (82c) and (82d). The presence of four isomers was indicated by sets of four peaks in the regions associated with C_3 , C_4 and C_6 of (82c) and (82d) i.e. at δ_{H} 110, 140 and 63 ppm respectively. The presence of hydroxyl and acetate functionalities and the (*E*) and (*Z*) isomerism indicated by the ^1H n.m.r. spectrum suggested that Mixture A was composed of the four possible monoacetate isomers of (82a) and (82b).

The apparent instability of Mixture A and the small amounts of material available precluded any attempts at isolation of the individual isomers. Accordingly, to confirm that Mixture A was composed of monoacetate isomers (87a - d), a sample was acetylated with pyridine/acetic anhydride. This produced a sample with spectral characteristics identical

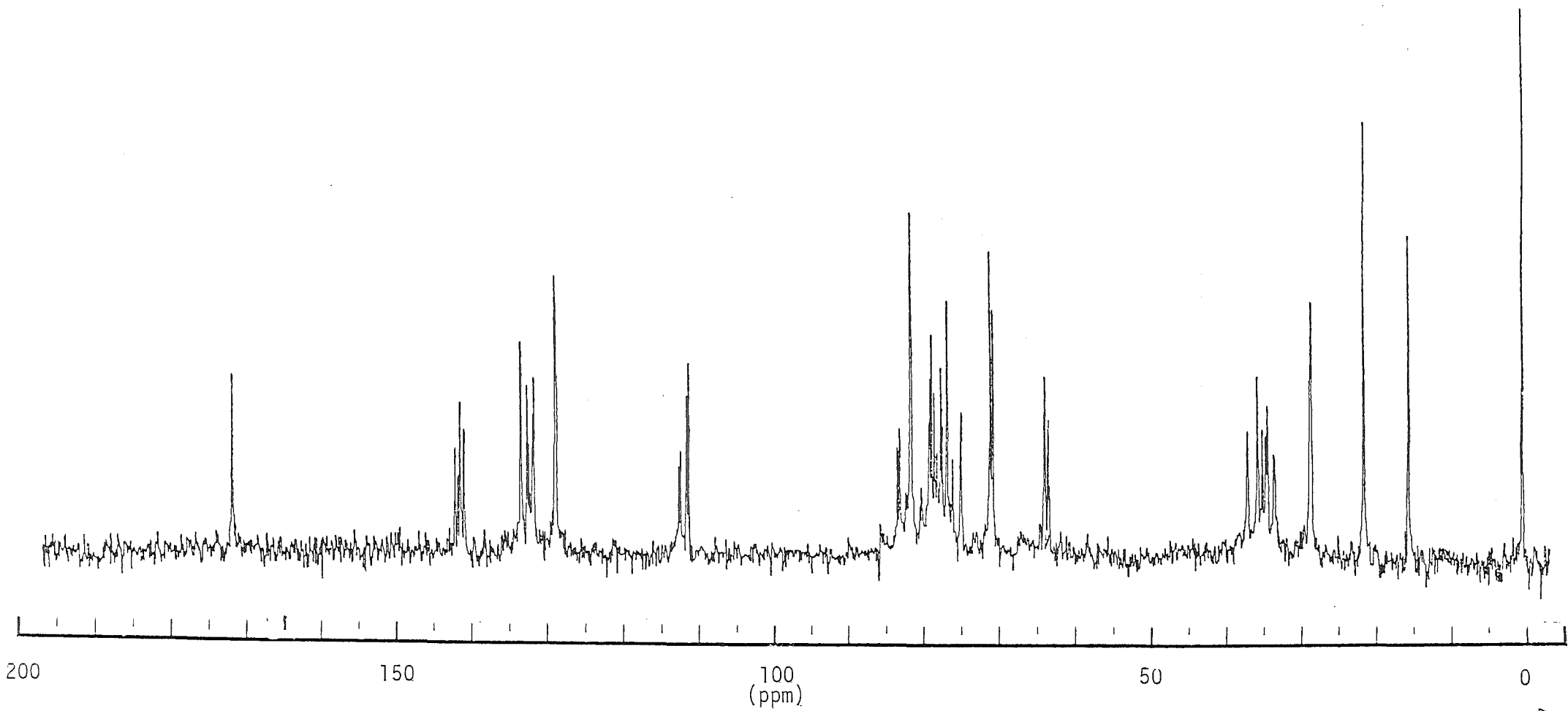


Figure 5: ^{13}C n.m.r. spectrum of the mixture of (87a), (87b), (87c) and (87d) (ppm, TMS = 0).

to those of the mixture of (82c) and (82d) and confirmed the structures of (87a - d).

The remainder of the polar fraction appeared to be highly coloured polar constituents with R_f of approximately zero. These components were not investigated further.

Attention was then turned to the fraction of midrange polarity. The t.l.c. spots at R_f 0.43 and 0.37 were investigated and were found to arise from the acetylenic ether diacetates (82c) and (82d) respectively (combined 7% of extract by weight). Thus it appeared that the diols (82a) and (82b), the monoacetate isomers (87a - d) and the diacetates (82c) and (82d) all occurred in the same alga.

There are precedents for this co-occurrence of alcohols and their corresponding acetates in this class of compound from *Laurencia* algae. Laurefucin (51) and acetyl laurefucin (51a) have both been isolated from *Laurencia subopposita*^{2,6} and *Laurencia nipponica*.^{4,8} Laurencin (48) was isolated from *Laurencia glandulifera*^{4,6} and the corresponding alcohol deacetyl laurencin (48a) has also been reported.^{4,9} The laurediols (52a) and (52b) from *Laurencia nipponica* also occurred as the corresponding diacetate mixture.^{4,9} Hence the co-occurrence of the diol, monoacetate and diacetate isomers of (82a) and (82b) in *Laurencia thyrsifera* is not remarkable.

The possibility that some of the molecules were artefacts could not be overlooked however. The extraction and isolation procedures did not utilise any reagents that could have caused acetylation. The inverse possibility, that hydrolysis of the diacetates (82c) and (82d) had occurred to produce the other molecules could not be completely excluded. In an attempt to check this possibility an experiment was set up to

simulate the extraction conditions. A sample of *Laurencia thyrsifera* extract was refluxed with dichloromethane and a few drops of water for several days and this extract was compared by t.l.c. with the original extract after each day. The t.l.c. spots for all the isomers had been identified and if hydrolysis had been occurring then the spots for the diacetate and monoacetate isomers could be expected to diminish or disappear. No changes were apparent after refluxing for five days. As the original extraction procedure usually took only two days it is a reasonable assumption that hydrolysis during extraction had not occurred.

The other major component of the fraction of midrange polarity appeared as a t.l.c. spot at R_f 0.27. This was found to be a mixture of cholesterol (88) (5% of extract by weight) and two more isomeric acetylenic cyclic ethers (89a) and (89b) in an approximately 1:1 ratio (combined 4% of extract by weight).

The chromatographic coincidence of these three compounds caused some difficulty in isolation. While cholesterol (88) could be crystallised from the mixture it was usually necessary to resort to h.p.l.c. to purify the mixture of (89a) and (89b). Cholesterol was identified by comparison of its melting point, ^1H n.m.r.⁸⁴ and ^{13}C n.m.r.⁸⁵ spectra with published data.

The infrared spectrum of the mixture of (89a) and (89b) showed the expected absorptions attributable to alkyne ($3300, 2100\text{ cm}^{-1}$), ether (1100 cm^{-1}) and olefinic (960 cm^{-1}) functionalities. There was also a carbonyl absorption at 1690 cm^{-1} . The ultraviolet spectrum of the mixture had maxima at 223 nm ($\epsilon = 15,500$) with inflections at 233 nm ($\epsilon = 11,000$) and 215 nm ($\epsilon = 13,400$). The first two absorptions were as expected for the enyne chromophore.⁸¹ The inflection at 215 nm together

with the infrared carbonyl absorption at 1690 cm^{-1} suggested that the molecules contained an α,β unsaturated ketone. The normal ultraviolet absorption for an unsubstituted α,β unsaturated ketone is a maximum at 215 nm^{81} and this was assumed to be partially obscured by the more intense enyne absorption.

No obvious parent ions were visible in a high resolution EI mass spectrum of the mixture. However, a low resolution CI mass spectrum displayed a parent ion ($M + 1$) 3:1 doublet at m/e 267/269 showing the presence of a chlorine atom and suggesting a molecular formula of $C_{15}H_{19}ClO_2$. A daughter ion singlet at m/e 231 showed the loss of HCl (36 a.m.u.).

Careful chromatography enabled the isolation of the individual isomers (89a) and (89b). The ^{13}C n.m.r. spectra of these (See Table 8) displayed resonances attributable to a carbonyl carbon, four olefinic methine carbons, two oxygenated methine carbons, terminal and quaternary alkyne carbons, a chlorinated methine carbon, four methylene and one methyl carbon. The general pattern of the ^{13}C n.m.r. spectra was very similar to those for the diols (82a) and (82b) and a similar structure was deduced i.e. an eight membered ring with a chlorine attached to the side chain.

The 1H n.m.r. spectra of the isomers (89a) and (89b) were again complex although the usual terminal alkyne proton-derived doublets at δ_H 3.1 and 2.9 ppm were obvious and indicated (*E*) and (*Z*) isomerism in the enyne group. In order to simplify the spectra an hydrogenation over Pd/C was attempted as before. Unfortunately this produced a compound which gave 1H n.m.r. and ^{13}C n.m.r. (See Table 8) spectra which contained no olefinic resonances. The infrared spectrum still exhibited

Table 8: ^{13}C n.m.r. shielding data for (89a), (89b) and (90) (all CDCl_3 solutions, ppm from TMS).

	(89a)	(89b)	(90)
C_1	82.8	77.1	14.0
C_2	79.8	81.7	22.5
C_3	111.1	112.1	31.2
C_4	140.2	141.0	26.3
C_5	33.9	36.3	32.6
C_6	62.5	61.8	64.5
C_7	83.3	83.1	82.0 ⁺
C_8	28.6	28.4	31.0 [°]
C_9	40.1	40.3	39.2 [†]
C_{10}	183.4	183.4	*
C_{11}	128.6	128.9	37.5 [†]
C_{12}	136.3	135.8	29.6 [°]
C_{13}	81.8	81.8	80.9 ⁺
C_{14}	28.9	28.9	26.4 [°]
C_{15}	9.9	10.0	10.8

* Not observed

+ Reversible

° Reversible

† Reversible

a carbonyl peak but this had shifted to 1700 cm^{-1} . From this it was deduced that the hydrogenation had completely saturated the isomeric mixture of (89a) and (89b) to produce (90).

Consequently a sample of the (*E*) isomer (89b) was submitted for 270 MHz ^1H n.m.r. spectral analysis. Homonuclear decoupling experiments on this spectrum provided assignments as shown in the partial structure (91). This effectively located the carbonyl at C_{10} and the second double bond at $^1\Delta$ establishing the α,β unsaturated ketone structure.

The 270 MHz ^1H n.m.r. spectrum also permitted the extraction of the majority of the coupling constants for this molecule. These values were then refined by computer simulation as before. The $^3J_{\text{HH}}$ values are listed in Table 9. From the $J_{3,4}$ value of 15.9 Hz the assignment of the $^3\Delta$ double bond as (*E*) in (89b) was confirmed.

The chirality at C_7 and C_{13} was assumed to be *R* in (89a) and (89b) for the reasons described previously (p.44) for (82c) and (82d) i.e. that this chirality is common to all the related compounds. This assumption is supported by the similar $J_{12,13}$ value in (89b) (2.2 Hz) and (84) (3.3 Hz). A Karplus analysis was made for the $\phi_{12,13}$, $\phi_{7,8a}$ and $\phi_{7,8b}$ H-C-C-H torsional angles using the same Karplus type equations as before. This gave a best fit for $\text{C}_7\text{RC}_{13}\text{R}$ but the correlation between the derived ϕ values and those observed in Dreiding model studies was not as good as for (84). This is perhaps to be expected as the eight membered ether ring in (89a) and (89b) has fewer substituents and includes a carbonyl group and is therefore likely to be more mobile leading to averaging of the coupling constants.

Table 9: ${}^3J_{\text{HH}}$ Values for (89b) (Hz).

$J_{1,3}$	2.2	$J_{11,12}$	12.2
$J_{3,4}$	15.9	$J_{12,13}$	2.2
$J_{4,5a}$	7.2	$J_{14,15}$	7.5
$J_{4,5b}$	7.2		
$J_{5a,5b}$	15.0	$J_{3,5a}$	1.4
$J_{5a,6}$	9.2	$J_{3,5b}$	1.4
$J_{5b,6}$	3.2	$J_{11,13}$	2.2
$J_{6,7}$	5.8		
$J_{7,8a}$	8.2		
$J_{7,8b}$	3.3		
$J_{8a,8b}$	13.5		
$J_{8a,9a}$	6.6		
$J_{8a,9b}$	7.9		
$J_{8b,9a}$	5.7		
$J_{8b,9b}$	6.2		

The compounds in the nonpolar fraction of the extract appeared as t.l.c. spots at R_f 0.57, 0.73 and 0.83. The material corresponding to the spot at R_f 0.83 (15% of extract) was found to consist of fatty acid esters. These were possibly artefacts arising from trans-esterification of acetyl-CoA esters by the methanol used in extraction. The composition of the mixture was analysed by the "equivalent chain length" (ECL) method. This method utilises a plot of the logarithm of the relative retention time ($\log r$) against chain length. A reference line is obtained from the retention times of a mixture of known saturated fatty acids. The ECL of an unknown fatty acid ester may then be determined from its $\log r$ value. For unsaturated fatty acids the ECL is usually a decimal value (e.g. 16.5 = 16:1 ω 7*). The identity of the fatty acid may be tentatively assigned by reference to tables listing ECL values.⁸⁶ Table 10 lists the ECL values and tentative identifications for the fatty acid esters of *Laurencia thyrsifera*. The g.l.c. analysis was performed using a 2 m, 3% DEGS column operated isothermally at 180°C.

T.l.c. analysis in various solvents of the material corresponding to the spot at R_f 0.57 (5% of extract) revealed that it contained several compounds. Attempts were made to isolate these and the ¹³C n.m.r. and ¹H n.m.r. spectra of the partially purified components displayed absorbance characteristics similar to those of the other acetylenic cyclic ethers. However, the chromatographic homogeneity of the material and the small quantities involved prevented complete purification.

* The shorthand notation for fatty acids is based on the notation n: x ω y, where n is the number of carbon atoms in the fatty acid chain, x is the number of double bonds and ω y denotes the number of carbon atoms from the centre of the double bond farthest removed from the carboxyl group to, and including, the terminal methyl group.

Table 10: ECL values and tentative identifications for fatty acid esters from *Laurencia thyrsoifera*.

	ECL	Structure
1.	12	12:0
2.	14	14:0
3.	16*	16:0
4.	17.5	16:2 ω 7
5.	18	18:0
6.	18.5*	18:1 ω 9
7.	19.25	18:2 ω 6
8.	19.6	18:2 ω 5
9.	20	20:0
10.	20.35	
11.	20.5	
12.	21.0	
13.	21.35	
14.	22.0	22:0
15.	22.7	20:4 ω 6
16.	23.2	20:5 ω 3
17.	23.95	22:3 ω 6
18.	25.85	
19.	26.35	

* Major constituents

Investigation of the material with a t.l.c. spot at R_f 0.73 showed that it was composed of an approximately 1:1 mixture of two more acetylenic cyclic ethers (20% of extract each). These were extremely difficult to separate from each other and from the small quantities of fatty acids also present. In fact it was initially thought that the two molecules were $^3\Delta$ (*E*) and (*Z*) isomers. Partial purification could be achieved by multiple development preparative t.l.c., but it was necessary to use h.p.l.c. in the reverse phase mode to complete the purification.

The first of these cyclic ethers to be investigated was found to have structure (92). The ether (92) occurred only as the (*Z*) isomer; no evidence for the existence of an (*E*) isomer was found. It had been thought that the silica gel used in the isolation of the *Laurencia thyrsoifera* acetylenes may have caused the (*E*) - (*Z*) isomerism but the occurrence of (92) as only the (*Z*) isomer indicates that the isomerism for some of the acetylenic ethers does occur in the alga.

The infrared spectrum of (92) displayed the appropriate resonances for a terminal alkyne ($3300, 2100 \text{ cm}^{-1}$), an ether link (1100 cm^{-1}) and carbon-carbon double bonds ($1620, 900 \text{ cm}^{-1}$). The ultraviolet spectrum had a maximum at 223 nm ($\epsilon = 9600$) with inflections at 214 nm ($\epsilon = 7400$) and 233 nm ($\epsilon = 7400$). The absorptions at 223 and 233 nm were characteristic of the enyne chromophore.⁸¹ The expected ultraviolet absorption appropriate to the diene chromophore was not observed. This was due to the fact that although homoannular dienes in six membered rings absorb at approximately 253 nm^{81} this absorption is markedly dependent on ring size. The wavelength of the absorption maximum decreases as ring size expands from six to nine and then increases as the ring expands further.⁸⁷ The absorption maximum for 1,3-cyclooctadiene is 226 nm.⁸⁸ To this value of 226 nm must be added 5 nm for the chlorine substituent according to the

rules of diene absorption⁸¹ which gives a maximum of 231 nm. As the extinction coefficients for homoannular dienes ($\epsilon \sim 7000$) are lower than those for the enyne chromophore the diene absorption would be obscured. However, the diene absorptions are rather broad⁸⁸ and the inflection at 214 nm may be part of the obscured peak.

The low resolution CI mass spectrum of (92) displayed parent ion ($M + 1$) peaks at m/e 285/287/289 in the appropriate ratio to indicate the presence of two chlorine atoms and in keeping with a molecular formula of $C_{15}H_{18}Cl_2O$. Loss of HCl (36 a.m.u.) was visible as a 3:1 doublet at m/e 249/251 and loss of a further chlorine atom (35 a.m.u.) resulted in a peak at m/e 214. The EI high resolution mass spectrum did not display a recognisable parent ion. The molecular formula $C_{15}H_{18}Cl_2O$ represents six double bond equivalents which is consistent with a monocyclic vinyl acetylenic ether with a chloro group and two additional double bonds.

The presence of two additional double bonds was confirmed by the ^{13}C n.m.r. spectrum of (92). There were six resonances in the olefinic region at δ_C 141.0 (d), 135.3 (d), 131.9 (s), 127.1 (d), 126.9 (d) and 110.9 (d) ppm. The peaks at δ_C 141.0 and 110.9 were assigned as C_4 and C_3 of the enyne group respectively. This left three methine and one quaternary carbon representing two additional double bonds, one obviously disubstituted and the other trisubstituted. The midrange portion of the spectrum contained the expected quaternary and methine absorptions for the terminal alkyne at δ_C 79.9 (s) and 82.6 (d) ppm respectively. These were assigned on the basis of comparison with (82a) and (82c) since it was expected that the double bond of the enyne group was of (*Z*) configuration (vide infra). In addition there were peaks due to two oxygenated methine carbons at δ_C 77.6 and δ_C 76.4 and a chlorinated methine carbon absorbance at δ_C 64.3 ppm. The high field portion of the spectrum displayed

three methylene resonances at δ_C 35.0, 33.2 and 29.8 ppm and a methyl peak at δ_C 9.9 ppm. The proton count from the ^{13}C n.m.r. spectrum is in keeping with a molecular formula of $C_{15}H_{18}Cl_2O$.

The 1H n.m.r. spectrum of (92) displayed a similar pattern to the other acetylenic cyclic ethers from *Laurencia thyrsoifera*. It contained an alkyne proton doublet resonance at δ_H 3.1 ppm indicating that the double bond of the enyne system had (Z) configuration. As the 1H n.m.r. spectrum was complex, hydrogenation of (92) was attempted to give a product with a simplified spectrum. Unfortunately despite several attempts it was found that it was impossible to saturate the side chain without partially or completely saturating the rest of the molecule as well. The 1H n.m.r. spectrum of the products of the hydrogenation was obtained and if, as was usual, olefinic region peaks were absent, the products were not pursued. One hydrogenation attempt gave a product that displayed a residual triplet at δ_H 5.7 ppm and a small quantity of the product (93) was isolated by h.p.l.c. The infra red spectrum of (93) displayed peaks attributable to an ether linkage (1060 cm^{-1}) and carbon-carbon double bonds ($1650, 920\text{ cm}^{-1}$). The ^{13}C n.m.r. spectrum (See Table 11) contained only one olefinic peak at δ_C 124.2 and it was therefore assumed that the remaining double bond was trisubstituted with the quaternary carbon resonance not being observed. The rest of the ^{13}C n.m.r. spectrum was compatible with this assessment as was the 1H n.m.r. spectrum which contained the triplet at δ_H 5.7 ($1H, J = 8\text{ Hz}$) and two methyl triplets just distinguishable at δ_H 0.87 and 0.88. The structure (93) was deduced, but the 1H n.m.r. spectrum of (93) was not amenable to analysis at 80 MHz.

Accordingly, despite its complexity, homonuclear decoupling experiments were performed on the 1H n.m.r. spectrum of the original ether (92). The appropriate portion of the spectrum, excluding the C_{15} methyl triplet, is

Table 11: ^{13}C n.m.r. shielding data for (92), (93), (95), (98) and (100).

	(92)	(93)	(95)	(98)	(100)
C ₁	82.6	14.0	82.6	14.0	14.0
C ₂	79.9	22.5	79.9	22.5	22.5
C ₃	110.9	31.3	110.8	31.3	31.3
C ₄	141.0	26.6	141.2	26.6	26.6
C ₅	35.0*	33.3	34.3*	33.3	33.2
C ₆	64.3	65.6	63.5	65.3	65.2
C ₇	76.4	84.3*	83.7	86.2*	84.8*
C ₈	33.2*	31.6°	32.3	33.0°	32.3
C ₉	127.1	124.2	129.4°	25.3°	129.8°
C ₁₀	131.9	**	128.9°	25.2°	128.7°
C ₁₁	126.9	33.7	25.6	28.1°	25.8
C ₁₂	135.3	31.3	56.0	58.5	56.5
C ₁₃	77.6	81.2*	84.6	85.3*	84.2*
C ₁₄	29.8	29.2	29.7	28.7°	29.7
C ₁₅	9.9	10.3	9.3	9.9	9.6

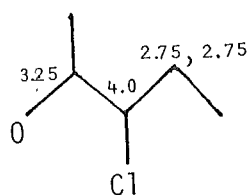
* Reversible

° Reversible

** Not observed

reproduced in Figure 6.

The multiplet at $\delta_{\text{H}}4.0$ (1H) was in the same position as the resonance of the proton of the chlorinated C_6 carbon of the other acetylenes from this alga. As a starting point for the decoupling experiments it was assumed that the $\delta_{\text{H}}4.0$ multiplet was the absorption of a comparable H - C - Cl fragment in (92). Irradiation at $\delta_{\text{H}}4.0$ did not affect the olefinic region but simplified the multiplet at $\delta_{\text{H}}3.25$ (1H,ddd, $J = 1.5, 3, 9$ Hz) to a doublet of doublets ($J = 1.5, 9$ Hz). From this, and also on the basis of its chemical shift, the multiplet at $\delta_{\text{H}}3.25$ was assigned to the proton at C_7 of the ether link adjacent to the H - C - Cl fragment at C_6 . This left the residual couplings of 1.5 and 9 Hz which were assigned as couplings to the methylene protons at C_8 and gave $J_{6,7} = 3$ Hz. Irradiation at $\delta_{\text{H}}4.0$ also affected the complex multiplet at $\delta_{\text{H}}2.75$ (2H). This multiplet had the same chemical shift as the C_5 methylene protons in (82c) and (89a) and so was assigned as the resonance of the C_5 methylene protons in (92). This irradiation suggested the partial structure below.



The frequency of irradiation was then changed to $\delta_{\text{H}}3.25$. This reduced the multiplet at $\delta_{\text{H}}4.0$ to a doublet of doublets ($J = 6, 5$ Hz) which was attributed to the residual couplings to the two protons on C_5 . The irradiation at $\delta_{\text{H}}3.25$ caused slight changes in the olefinic region but this was attributed to the effect of irradiating close to the alkyne proton multiplet at $\delta_{\text{H}}3.1$. Considerably more affected were the two multiplets at

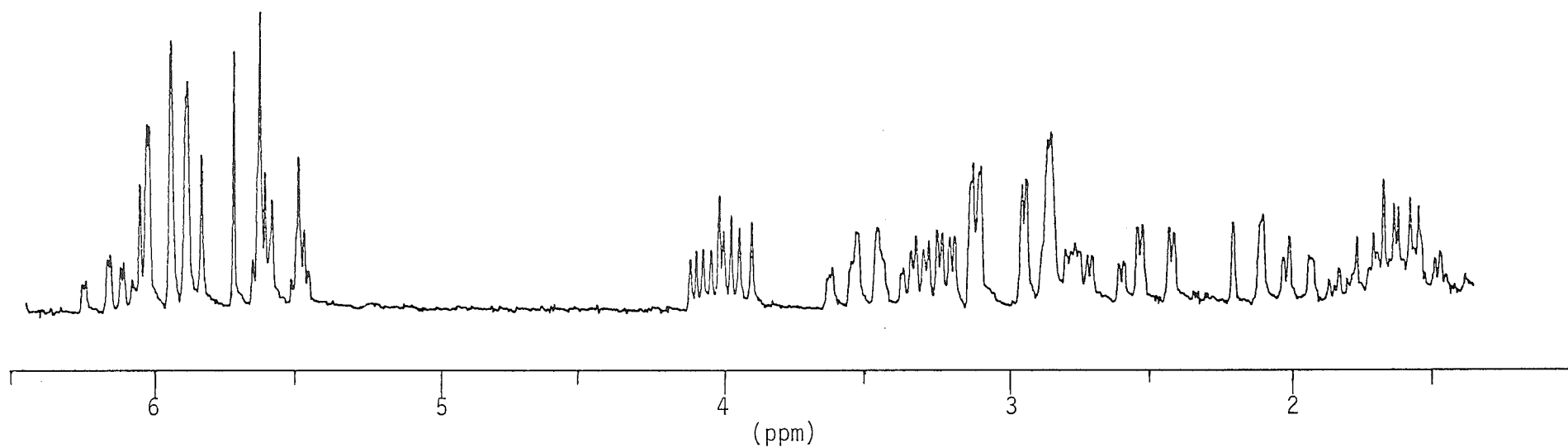
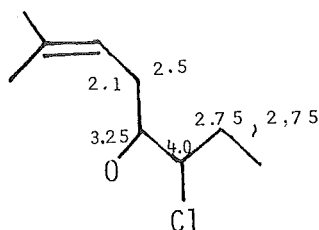


Figure 6: ¹H n.m.r. spectrum of (92) (excluding triplet at $\delta_{\text{H}}0.95$)(ppm, TMS = 0).

$\delta_{\text{H}}2.1$ and 2.5 (both 1H). The multiplet at $\delta_{\text{H}}2.1$ did not have any obvious pattern but the other at $\delta_{\text{H}}2.5$ could be recognised as (1H,ddd, $J = 1.5, 9, 14$ Hz). The irradiation at $\delta_{\text{H}}3.25$ simplified both these multiplets to $\delta_{\text{H}}2.1$ (dd, $J = 8, 14$ Hz) and $\delta_{\text{H}}2.5$ (dd, $J = 9, 14$ Hz). These two multiplets were assigned to the two C_8 protons, $\text{H}_{8\text{a}}$ and $\text{H}_{8\text{b}}$ respectively, from these decouplings and the fact that their chemical shieldings were comparable to the corresponding $\text{H}_{8\text{a}}$, $\text{H}_{8\text{b}}$ multiplets of (82c) and (89a). The coupling constant value of 14 Hz was assigned as the geminal coupling of $\text{H}_{8\text{a}}$ and $\text{H}_{8\text{b}}$ while the remaining coupling appeared to be to a single proton on C_9 . This was assigned as the C_9 proton of a $^9\Delta$ double bond. Furthermore the absence of allylic $J_{8\text{a},10}$ or $J_{8\text{b},10}$ couplings indicated that C_{10} was quaternary and thus substituted with the second chlorine atom. This established the fragment below.



Further decouplings supported this partial structure. Irradiation at $\delta_{\text{H}}2.5$ simplified the multiplet at $\delta_{\text{H}}3.25$ to (dd, $J = 3, 7$ Hz). Assigning $J_{6,7} = 3$ Hz as before, this left $J_{7,8\text{a}} = 7$ Hz whereas the original multiplet at $\delta_{\text{H}}3.25$ suggested $J_{7,8\text{a}} = 9$ Hz. This slight reduction in $J_{7,8\text{a}}$ was attributed to the effect of irradiating close to $\delta_{\text{H}}2.1$ ($\text{H}_{8\text{a}}$). In addition the irradiation at $\delta_{\text{H}}2.5$ caused changes in the olefinic region

at approximately $\delta_{\text{H}}5.9$ so this was assigned as the approximate shift of the C_9 proton absorbance. Irradiation at $\delta_{\text{H}}2.1$ ($\text{H}_{8\text{a}}$) caused the expected effects at $\delta_{\text{H}}3.25$ and in the olefinic region at $\sim \delta_{\text{H}}5.9$.

These irradiations accounted for most of the high field multiplets except for the broad doublet of doublets at $\delta_{\text{H}}3.45$ and the complex multiplet at $\delta_{\text{H}}1.6$. This latter absorbance was assigned to the C_{14} methylene protons on the basis of changes caused by irradiation of the triplet at $\delta_{\text{H}}0.9$ ppm. Irradiation at $\delta_{\text{H}}1.6$ changed the multiplet at $\delta_{\text{H}}3.45$ to a broad doublet ($J = 4$ Hz) indicating that it was the resonance of the other proton of the ether link i.e. H_{13} . This $\delta_{\text{H}}3.45$ multiplet had not been affected by any of the other decouplings. Irradiation at $\delta_{\text{H}}3.45$ caused substantial changes in the olefinic region at $\delta_{\text{H}}5.5$ and slight changes at approximately $\delta_{\text{H}}5.9$. This was taken to indicate that a $^{11}\Delta$ double bond existed in (92) with protons at C_{11} and C_{12} absorbing at $\delta_{\text{H}}5.9$ and $\delta_{\text{H}}5.5$ respectively. Irradiation at $\delta_{\text{H}}3.45$ would eliminate vicinal coupling to H_{12} ($\delta_{\text{H}}5.5$) and small long range couplings to H_{11} ($\delta_{\text{H}}5.9$).

Irradiations in the olefinic region were only of limited value due to the overlap of multiplets. However some useful information was obtained. Irradiation at $\delta_{\text{H}}5.5$ caused the terminal alkyne proton resonance at $\delta_{\text{H}}3.1$ (dd, $J = 1, 2$ Hz) to collapse to a broad singlet indicating the removal of coupling to the C_3 proton. Conversely irradiation of $\delta_{\text{H}}5.9$ collapsed the $\delta_{\text{H}}3.1$ multiplet to a doublet ($J = 2$ Hz) indicating the removal of long range coupling to the C_4 proton.

These decoupling experiments were difficult to interpret. The separation of the multiplets at 80 MHz was not sufficient to permit precise irradiation of a single resonance only, especially in the extremely complex olefinic region. However the observed effects permitted the assignments

shown in structure (94) and the extraction of the approximate coupling constants listed in Table 12.

Following the homonuclear decoupling experiments, s.s.f.o.r.d. experiments were performed to correlate the proton absorbances with peaks in the ^{13}C n.m.r. spectrum. The results are given in Table 13. The derived ^1H n.m.r. chemical shifts from the s.s.f.o.r.d. experiments are given along with those from the decoupling experiments.

The s.s.f.o.r.d. experiments permitted the assignment of most of the ^{13}C n.m.r. resonances as listed in Table 11. The carbon peaks at δ_{C} 127.0, 126.9 and 140.9 all had similar s.s.f.o.r.d. derived ^1H n.m.r. chemical shifts for the attached protons, as did the carbon resonances at δ_{C} 135.2 and 110.9. However this presented no difficulty as the peaks at δ_{C} 140.9 and 110.9 could be assigned as C_4 and C_3 respectively by comparison with other compounds of this type. The resonance at δ_{C} 9.9 was assigned to the methyl carbon at C_{15} . The three methylene resonances at δ_{C} 35.0, 33.2 and 29.8 could not be assigned unequivocally although the C_{14} methylene was tentatively assigned to δ_{C} 29.8 by comparison with known compounds.

The stereochemistry has again been assumed to be $\text{C}_7\text{R}\text{C}_{13}\text{R}$ based on the reasoning described previously for (82c) and (82d). In support of a common relative stereochemistry it is noted that the $J_{12,13}$ coupling constant (~ 4 Hz) is close to those of the diacetates (82c) and (82d) (3.3 Hz) and the enones (89a) and (89b) (2.2 Hz).

The second major component of the material with a t.l.c. spot at R_f 0.73 was found to have the structure (95). The infra red spectrum displayed the appropriate peaks indicating terminal acetylene ($3300, 2100\text{ cm}^{-1}$), ether ($1090, 1060\text{ cm}^{-1}$) and double bond (950 cm^{-1}) functionalities. The ultraviolet absorptions were a maximum at 222 nm ($\epsilon = 15,500$) and an

Table 12: ${}^3J_{HH}$ values obtained for (92)(Hz).

$J_{1,3} = 2$	$J_{7,8b} = 1.5$
$J_{1,4} = 1$	$J_{8a,8b} = 14$
$J_{5a,6} = 6$	$J_{8a,9} = 8$
$J_{5b,6} = 5$	$J_{8b,9} = 9$
$J_{6,7} = 3$	$J_{12,13} = 4$
$J_{7,8a} = 9$	$J_{14,15} = 7$

inflection at 231 nm ($\epsilon = 11,400$), appropriate for the enyne chromophore.⁸¹ No parent ions were visible in the EI mass spectrum, but the low resolution CI mass spectrum displayed a parent ion multiplet ($M + 1$) at m/e 331/333/335. The peak ratios indicated the presence of chlorine and bromine atoms and suggested a molecular formula of $C_{15}H_{20}BrClO$. Loss of HCl (36 a.m.u.) was visible as a daughter ion 1:1 doublet at m/e 295/297,

Table 13: S.s.f.o.r.d. results and correlations with ^{13}C n.m.r. shielding data for (92).

^{13}C n.m.r.* shielding	^1H n.m.r.* shielding	^1H n.m.r. shielding* from decoupling experiments
127.0	6.1	5.9
126.9	6.1	5.9
140.9	5.9	5.9
135.2	5.6	5.5
110.9	5.6	5.5
64.3	4.1	4.0
77.5	3.6	3.5
76.4	3.2	3.3
82.8	3.0	3.1

* In CDCl_3 ; relative to TMS

† The s.s.f.o.r.d. ^1H n.m.r. shielding value for which $^1J_{\text{R,CH}} = 0$.

and loss of HBr (80 a.m.u.) was indicated by a 3:1 doublet at m/e 251/253.

The ^1H n.m.r. spectrum contained a terminal acetylenic proton doublet at $\delta_{\text{H}}3.1$ indicating that the double bond in the enyne group had (*Z*) configuration. Although there was some overlap of multiplets at 80 MHz, decoupling experiments allowed the location of proton multiplets beginning with irradiation of the C_{15} methyl triplet at $\delta_{\text{H}}0.95$ and tracing through the skeleton to the enyne group. This established an eight membered ether ring with a $^9\Delta$ double bond and substituents at C_6 and C_{12} . The chlorine was placed at C_6 and the bromine at C_{12} on the basis of comparison with the other acetylenic ethers of *Laurencia thyrseifera*. This established the planar skeleton (96) (*vide infra*).

The ^{13}C n.m.r. spectrum (See Table 11) contained peaks consistent with such a skeleton i.e. four olefinic methine carbons, two oxygenated methine carbons, acetylenic methine and quaternary carbons, chlorinated and brominated methine carbons, three methylene and one methyl carbon.

Having established the planar skeleton (96) the assignment of stereochemistry was required. Although the planar skeleton was identical to that of intricenyne (67),⁶¹ the stereochemistry of intricenyne was unfortunately not determined. An additional problem was that another compound has been reported with the same basic skeleton but with an (*E*) double bond in the enyne group and $\text{C}_{12}^R\text{C}_{13}^R$ stereochemistry: laurepinnacin (73).⁶⁵ The ^{13}C n.m.r. and ^1H n.m.r. spectra of (95) were different from those quoted for laurepinnacin (73). However, it was uncertain whether these differences were due to the (*Z*) double bond of the enyne group of (95) i.e. was (95) the $^3\Delta(\text{Z})$ isomer of laurepinnacin, or were the differences due to alternative stereochemistry?

The 80 MHz ^1H n.m.r. spectrum of (95) did not resolve the multiplets sufficiently to permit extraction of coupling constants and so a sample was submitted for 270 MHz ^1H n.m.r. analysis. Homonuclear decoupling experiments on the 270 MHz ^1H n.m.r. spectrum (See Figure 7) confirmed the derived shifts displayed in structure (96) and permitted the extraction of most of the coupling constants as listed in Table 14. Comparison of this chemical shift data and coupling constants with the quoted values for laurepinnacin (73) and intricenyne (67) gave a much better correlation with intricenyne, especially for the protons attached to C_{12} , C_{13} , C_{14} and C_{15} where the (*E*)-(Z) isomerism of the $^3\Delta$ double bond would be expected to have little effect. The only difference between the quoted ^1H n.m.r. data for intricenyne and that found for (95) could be explained by a different assignment for one of the C_8 and C_{11} protons. Intricenyne (67) is reported as $\delta_{\text{H}}2.26$, $\text{H}_{11\text{a}}$ and $\delta_{\text{H}}2.45$, $\text{H}_{8\text{a}}$ whereas $\delta_{\text{H}}2.31$, $\text{H}_{8\text{a}}$ and $\delta_{\text{H}}2.50$, $\text{H}_{11\text{a}}$ were found for (95). It is therefore highly probable that the compound (95) from *Laurencia thyrseifera* is the same as intricenyne (67) from *Laurencia intricata*.

A method of deducing the stereochemistry of (95) was suggested by the experiments performed by Fukuzawa and Masamune to establish the stereochemistry of laurepinnacin (73).⁶⁵ In their work laurepinnacin was hydrogenated to produce octahydro laurepinnacin (97), which had C_{12}R chirality. The C_{12}S epimer (98) was also generated by a series of reactions on deacetyl laurencin (48a). Deacetyl laurencin was hydrogenated to produce deacetyloctahydro laurencin (99) which was then treated with thionyl chloride to produce the corresponding chloro derivative (98). It seemed likely that hydrogenation of (95) to obtain the octahydro-derivative would produce either (97) or (98) which would enable the stereochemistry to be deduced.

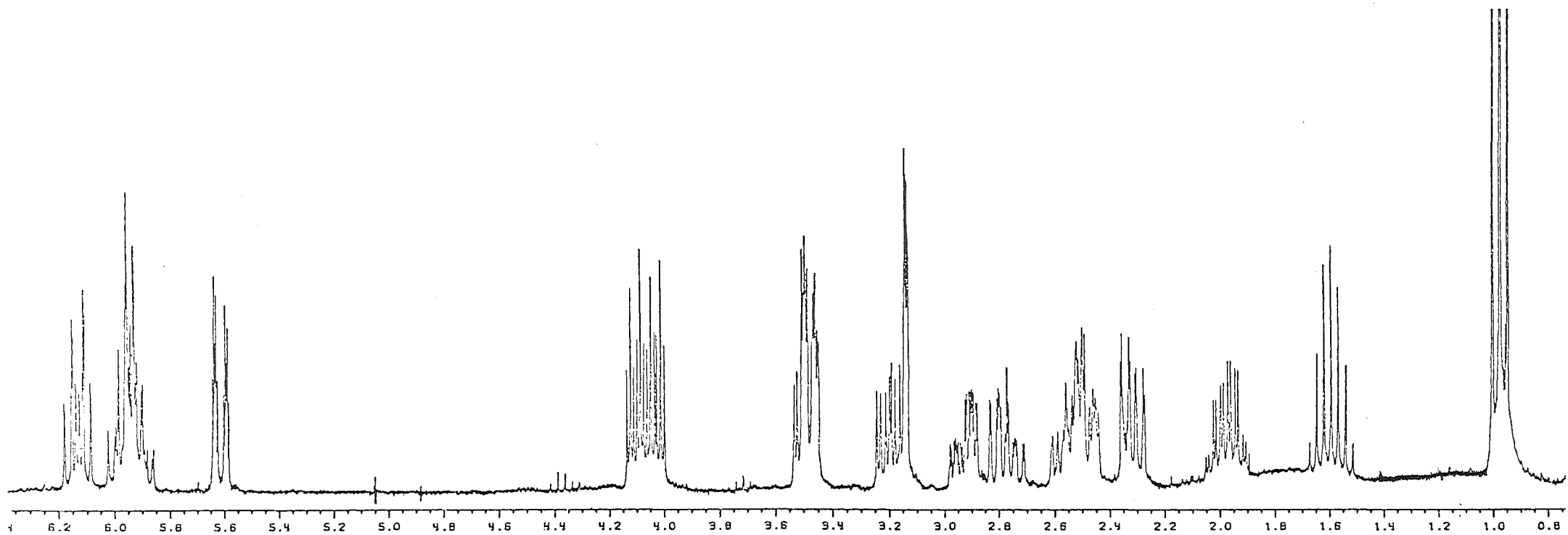


Figure 7: ^1H n.m.r. spectrum of (95) (ppm, TMS = 0).

Table 14: ${}^3J_{\text{HH}}$ values obtained for (95)(Hz).

$$J_{1,3} = 2.3$$

$$J_{11b,12} = 3.5$$

$$J_{1,4} = 1.0$$

$$J_{11a,11b} = 14.0$$

$$J_{3,4} = 10.8$$

$$J_{12,13} = 9.9$$

$$J_{3,5a} = 1.5$$

$$J_{14,15} = 8.0$$

$$J_{3,5b} = 1.5$$

$$J_{4,5a} = 7.1$$

$$J_{4,5b} = 7.1$$

$$J_{5a,5b} = 14.2$$

$$J_{5a,6} = 9.7$$

$$J_{5b,6} = 3.8$$

$$J_{6,7} = 3.8$$

$$J_{8a,8b} = 14.0$$

$$J_{8a,10} = 0$$

$$J_{9,10} = 10.0$$

$$J_{9,11b} = 0$$

$$J_{10,11b} = 8.4$$

$$J_{11a,12} = 3.4$$

^1H n.m.r., ^{13}C n.m.r. and infra red data on (97) and (98) were kindly provided by Professors Masamune and Fukuzawa through private correspondence. A sample of (95) was then hydrogenated over Pd/C in ethyl acetate. The difficulty of isolation of (95) and the small quantities involved called for some caution with this reaction and the hydrogenation was stopped after a short time (15 min.) to check the procedure. The product of the reaction at this stage appeared to be (100). The ^1H n.m.r. spectrum of (100) contained an olefinic multiplet at $\delta_{\text{H}} 5.9$ (2H) and the ^{13}C n.m.r. spectrum (See Table 11) exhibited two olefinic peaks at $\delta_{\text{C}} 129.8$ and 128.7 , suggesting that the sidechain had been saturated and that the $^9\Delta$ double bond in the ring was still intact. This product was not purified and the sample was rehydrogenated to completely saturate the molecule. Further reaction was successful and the resulting product exhibited ^1H n.m.r., ^{13}C n.m.r. and infra red spectral characteristics identical with (98). This established the relative stereochemistry as $\text{C}_6\text{RC}_7\text{RC}_{12}\text{SC}_{13}\text{R}$ or $\text{C}_6\text{SC}_7\text{SC}_{12}\text{RC}_{13}\text{S}$. The former absolute stereochemistry was preferred on the basis of the reasons given previously for the assumption of $\text{C}_7\text{RC}_{13}\text{R}$ chirality in the other acetylenic cyclic ethers from *Laurencia thyrsefera*. The question of absolute stereochemistry can be resolved by comparison of $[\alpha]_{\text{D}}$ values. The $[\alpha]_{\text{D}}$ for the octahydro-laurencin derivative (98) was not quoted in the publication on laurepinnacin.⁶⁵ The $[\alpha]_{\text{D}}$ value has been requested from Professor Masamune and Fukuzawa but at the time of writing no reply had been received.

PART 2ANALYSIS OF LAURENCIA DISTICHOPHYLLA

Laurencia distichophylla is a species of red alga which occurs principally on the north-east coast of the North Island of New Zealand.⁸⁰ In the water it has a red-brown colour. Three samples of this alga were received, all of which had been collected from the "Echinoderm Reef" of the Leigh Marine Reserve, North Island, New Zealand. The details of each collection were:

1. Collected 25/10/80 from the low intertidal to upper subtidal region. (Voucher No. CHR 368013, See Plate 2).
2. Collected 5/11/80 from the upper intertidal region.
3. Collected 4/11/80 from the mid-intertidal region.

The three samples were kept separate during extraction. The wet alga was blended with methanol and left standing for 18 hours. Filtration produced the methanol extract. The solid residue was then extracted in a Soxhlet apparatus with dichloromethane to produce a second extract. Examination by t.l.c. and g.l.c. revealed no apparent differences between the methanol and dichloromethane extracts and so they were combined giving one extract for each sample.

The extracts of Samples 2 and 3 appeared identical by t.l.c. and g.l.c. Both had one major component and several other minor peaks. The extract of Sample 1 however proved to be different. The major component of Sample 1 had both a lower R_f value by t.l.c. and a longer retention time by g.l.c. than the major component of both 2 and 3. The g.l.c. trace of Sample 1 is reproduced in Figure 8 and the g.l.c. trace of Samples 2 and 3 in Figure 9. The extract of Sample 1 was therefore kept separate during analysis.

Samples 2 and 3 were investigated first. The extract (3.6% by dry

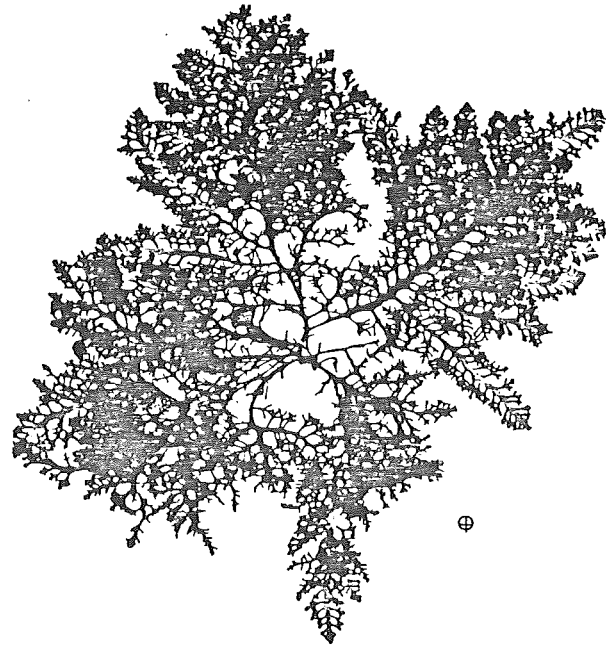
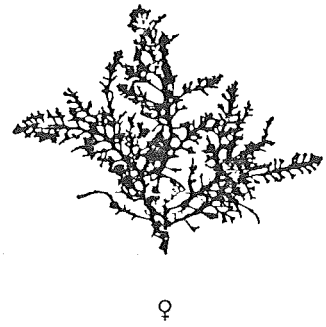
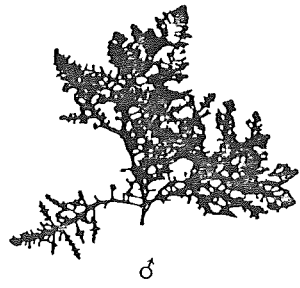


Plate 2: *Laurencia distichophylla* Sample 1.

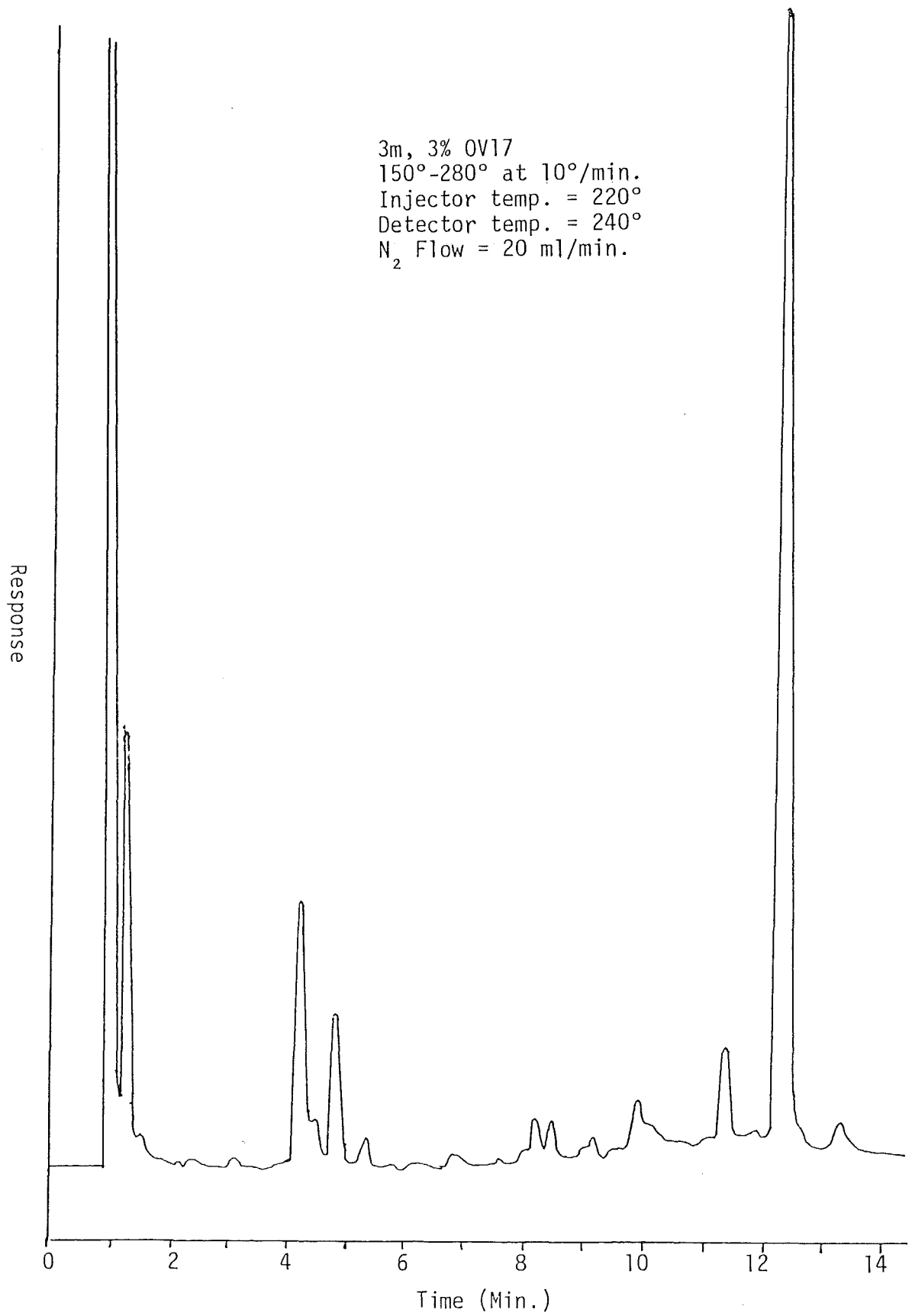


Figure 8: Gas chromatographic trace of *Laurencia distichophylla* Sample 1.

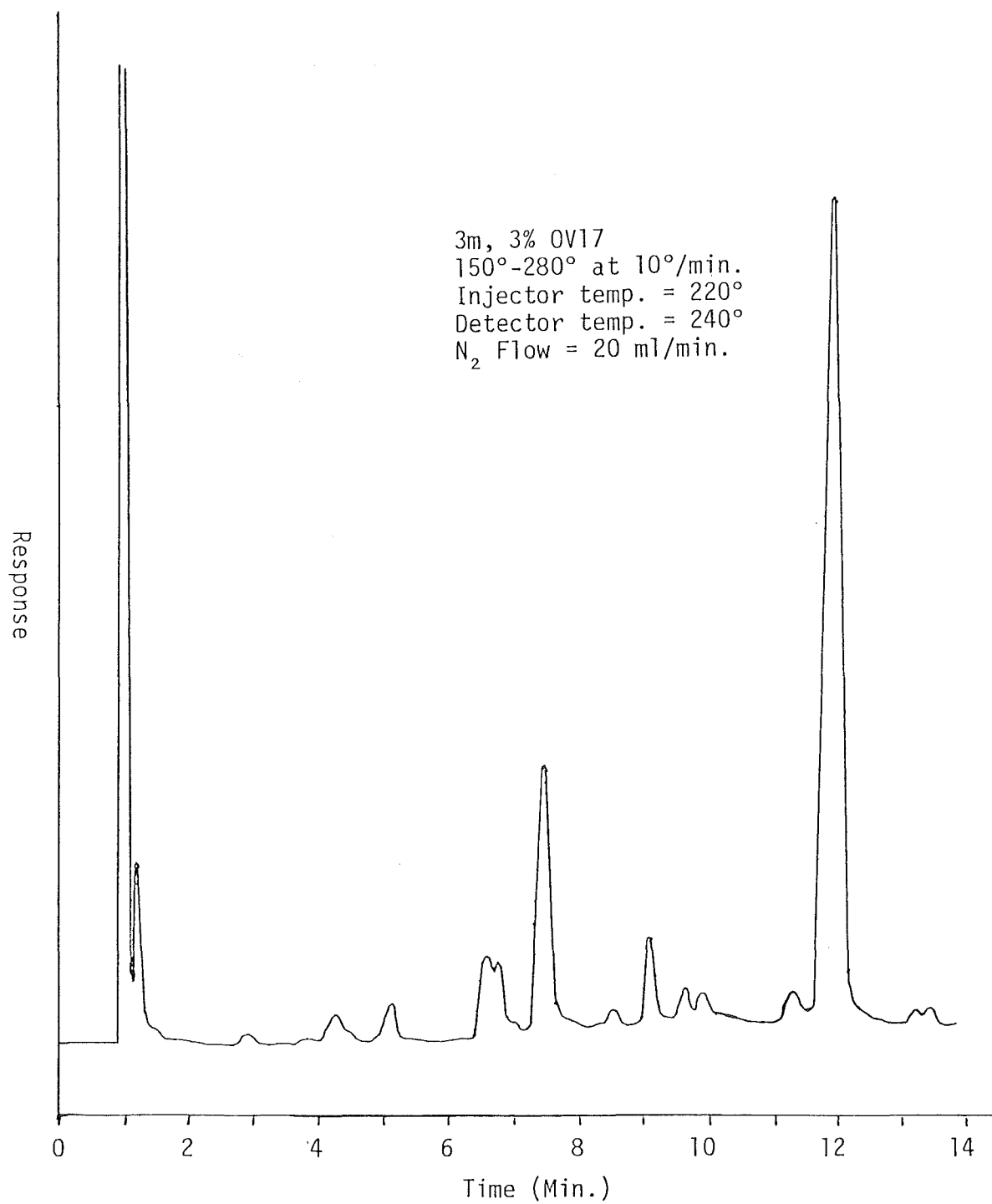


Figure 9: Gas chromatographic trace of *Laurencia distichophylla* Samples 2 and 3.

weight of alga) was chromatographed on alumina. A high proportion of the sample was eluted with petroleum ether in the early fractions and consisted of three spots by t.l.c. (R_f 0.73, 0.65 and 0.57). Of these the spot at R_f 0.57 corresponded to the major component (70% by weight of the extract). This was isolated and proved to be a new compound of the cuparene type (101).

The ultraviolet spectrum of (101) had maxima at 212 ($\epsilon = 22,200$), 278 ($\epsilon = 3400$) and 284 nm ($\epsilon = 3200$) which indicated the presence of a substituted benzene ring.⁸⁹ This was supported by absorbances in the infra red spectrum of (101) at 1610 and 1500 cm^{-1} . Other absorbances in the infra red spectrum were peaks attributable to olefinic (1615, 950 cm^{-1}) and hydroxyl groups (3600, 1460 and 1150 cm^{-1}).

The high resolution EI mass spectrum of (101) showed a parent ion (M^+) 1:1 doublet at m/e 294/296 which indicated the presence of a bromine atom and a molecular formula of $C_{15}H_{19}OBr$. Loss of bromine was observed as a daughter ion at m/e 215.

The ^1H n.m.r. spectrum of (101) contained peaks which were characteristic of the cuparene-type skeleton, many examples of which have been found in *Laurencia* species. The low field portion of the spectrum displayed resonances at δ_H 7.2 (d, $J = 7$ Hz), 6.7 (bd, $J = 7$ Hz), 6.6 (bs), 6.0 (d, $J = 2$ Hz) and 5.2 (bs) ppm (all 1 H). The absorptions at δ_H 7.2, 6.7 and 6.6 ppm were consistent with those of a trisubstituted aromatic ring as found in debromoisolaurinterol (21),¹⁸ debromolaurinterol (10)¹⁷ and laurenisol (15).²² The broad peak at δ_H 5.2 ppm represented an hydroxyl proton as it could be exchanged with D_2O and was assigned as phenolic by comparison with other compounds of this type. The finely split doublet at δ_H 6.0 suggested the presence of an exocyclic trisubstituted double bond since an identical

peak occurs in the ^1H n.m.r. spectrum of laurenisol (15).²²

The high field portion of the ^1H n.m.r. spectrum displayed resonances at $\delta_{\text{H}}1.2$ (3 H, d, $J = 7$ Hz), 1.5 (s, 3 H), 2.3 (s, 3 H) and a series of multiplets between $\delta_{\text{H}}1.5 - 3.2$ (5 H). The singlets at $\delta_{\text{H}}1.5$ and 2.3 were consistent with those of the quaternary methyl group (C_{14}) and aromatic methyl group (C_{15}) respectively which always occur in compounds of this type. The remaining doublet was assigned as a methyl group attached at a tertiary site and the literature provided evidence as to its position. All the molecules of this type so far isolated have at least one methyl group attached to the five membered ring always at C_4 or C_5 . If attached at C_5 the appropriate methyl doublet occurs between $\delta_{\text{H}}0.6 - 0.8$ ppm in the ^1H n.m.r. spectrum. If attached at C_4 the doublet occurs between $\delta_{\text{H}}1.0 - 1.2$ ppm.^{17,18,25} As the doublet for (101) occurred at $\delta_{\text{H}}1.2$ the methyl group was deduced to be attached at C_4 .

Comparison of the spectral data with that for laurenisol (15)²² indicated that the new compound (101) was identical except for the reversal of the positions of the exocyclic double bond and the tertiary methyl group.

The ^{13}C n.m.r. spectrum of (101) contained fifteen resonances of which eight occurred in the low field olefinic region. These were $\delta_{\text{C}}160.1$ (s), 153.3 (s), 138.0 (s), 128.7 (s), 128.2 (d), 121.3 (d), 118.2 (d) and 101.3 (d). Allowing six resonances for the carbons in an aromatic ring this left two peaks appropriate for a double bond. These two peaks were assigned to be those at $\delta_{\text{C}}160.1$ and 101.3 on the basis of comparison with the exocyclic disubstituted double bond peaks in the spectra of allolaurinterol (17),²³ 10-bromo-7-hydroxy-11-iodolaurene (32),²⁷ and laurene (12).¹⁹

The high field portion of the ^{13}C n.m.r. spectrum contained three

methyl resonances (δ_c 19.1, 20.6, 26.8), two methylene peaks (δ_c 31.0, 39.2) and a methine and quaternary carbon absorbances (δ_c 39.2 and 52.0 respectively). The coincident methylene and methine peaks at δ_c 39.2 were distinguished by use of the gated broad band decoupling technique^{76,77} which utilises a pulse sequence to produce a normal decoupled ^{13}C n.m.r. spectrum but with the methylene and quaternary peaks inverted. Two peaks, one positive and one negative at δ_c 39.2 revealed the presence of coincident methylene and methine peaks.

In order to confirm the structure of (101) and also to establish stereochemistry, cyclisation of (101) was attempted. If the structure was correct then cyclisation in the same manner as the conversion of isolaurinterol (11) to aplysin (6),⁹⁰ should convert (101) to isoaplysin (23),²⁴ as illustrated in Figure 10.

Interestingly the cyclisation of (101) was relatively difficult to achieve quickly. The normal reagent used to effect this type of cyclisation is p-toluene-sulphonic acid in chloroform with a trace of acetic acid but after stirring a sample of (101) in this reagent for 7 days only a very small amount of the cyclised product was formed. Increasingly stronger acid conditions were tried until a good yield was achieved after stirring a sample of (101) in 100% trifluoroacetic acid for 30 minutes. However, the cyclisation reaction does occur spontaneously, but it is slow. After several months it was found that purified samples of (101), stored in solution at 4°C, had cyclised.

After purification the cyclised product displayed spectral characteristics identical with those quoted for isoaplysin (23)²⁴ and consequently the structure (101) was confirmed for the original molecule.

The only outstanding point of stereochemistry remained the geometry

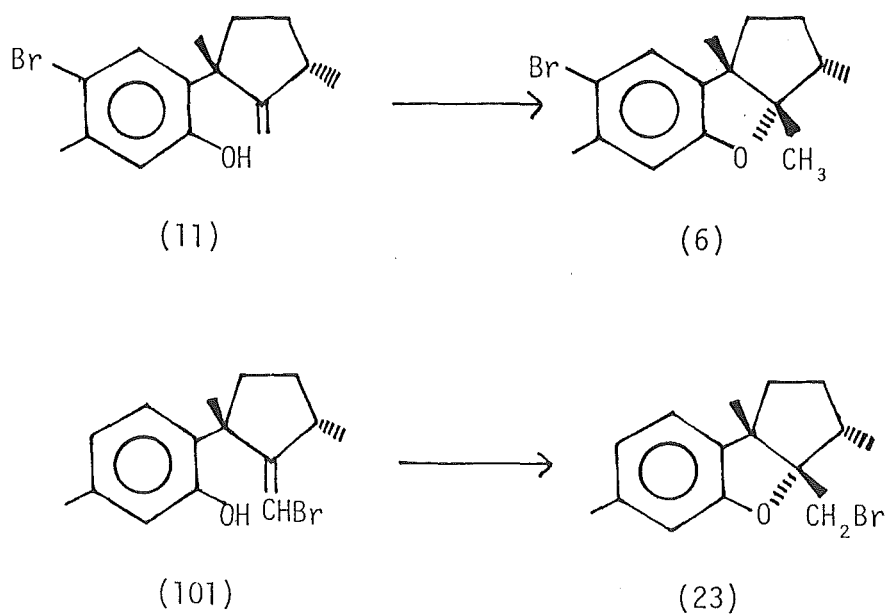


Figure 10: Cyclisation of isolaurinterol (11) to aplysin (6)(above) and (101) to isoaplysin (23)(below).

of the exocyclic double bond. In the original report on laurenisol (15)²² the geometry of the corresponding double bond was left open but in a later paper^{23b} it was ascribed (*Z*) configuration on the basis of the chemical shift of the C₅ proton. The shift of that proton in laurenisol

was $\delta_{\text{H}}3.11$ whereas in the nonbrominated analogue, debromoalloylaurinterol (31) the C_5 proton absorbed at $\delta_{\text{H}}2.95$. The slight downfield shift in the case of laurenisol was attributed to the proximity of the bromine and (Z) configuration was deduced. A similar effect was observed for (101). A complex multiplet at $\delta_{\text{H}}3.0$ was observed in the ^1H n.m.r. spectrum of (101) and assigned to the C_4 proton. A corresponding multiplet at $\delta_{\text{H}}2.8$ was observed in the spectrum of the nonbrominated analogue of (101), debromo-isolaurinterol (21) which was also isolated from *Laurencia distichophylla* (vide infra). However it was felt that the effect on the shift of the C_4 proton would occur whether the exocyclic double bond was (E) or (Z) and so the geometry was left unassigned.

In order to preserve some of the original (101) a sample was acetylated in the usual manner to produce (101a) and prevent cyclisation.

The remaining nonpolar fraction of the extract was found to be a mixture of several metabolites, all previously isolated from other species of *Laurencia*. The components were debromoisolaurinterol (21)¹⁸ (5% of extract by weight), debromoaplysin (7),¹⁷ α -bromocuparene (13)²¹ (each 3% of extract by weight) and a polar nonhalogenated compound (24)²⁴ (2% of extract by weight).

In their paper reporting the isolation of α -bromocuparene (13) and α -isobromocuparene (14)²¹ Suzuki et. al. differentiated the two epimers on the basis of fine ^1H n.m.r. chemical shift differences, in particular the shifts of the C_{14} methyl group and the C_4 proton. Unfortunately the ^1H n.m.r. solvent was not reported. The spectrum of the compound isolated from *Laurencia distichophylla*, using CDCl_3 as solvent, did not correlate with the quoted shifts for either α -bromocuparene or α -isobromocuparene. As the ^1H n.m.r. solvent preferred by Suzuki et. al. in other reports was

CCl_4 the spectrum was obtained using that solvent. In this spectrum the C_{14} methyl group and C_4 proton shifts corresponded exactly with those for α -bromocuparene and it was on this basis that the structure was assigned.

The samples of debromoisolaurinterol (21) were observed to cyclise slowly to debromoaplysin (7) in storage over several weeks. Thus it is uncertain whether debromoaplysin is a true metabolite of *Laurencia distichophylla*. Interestingly however no evidence was found for the presence of isoaplysin (23) in the nonpolar fraction.

Of the remaining extract the major component (3% by weight) was cholesterol (88) which was isolated as white crystals. In addition there were two minor midrange components (R_f 0.35 and 0.31) which were not present in sufficient quantities to be isolated. The remainder of the extract occurred at the origin on t.l.c. and was highly coloured. This material was not investigated.

Attention was then turned to Sample 1 of the *Laurencia distichophylla*. The major components of this extract were found to be allolaurinterol (17) (62% of extract by weight), isolaurinterol (11) and cholesterol (88) (each 5% of extract by weight). The allolaurinterol was found to cyclise to filiformin (29) on storage, as experienced by other workers.²⁵

All the components of both types of *Laurencia distichophylla*, except for (101), were compounds that had been previously isolated. The components were identified by comparison of their ^1H n.m.r., ^{13}C n.m.r., infra red, ultraviolet and mass spectra with quoted values. This established the planar structure and relative stereochemistries. In order to establish absolute stereochemistry the $[\alpha]_D$ values of filiformin (29) and isoaplysin (23) isolated from *Laurencia distichophylla* were determined. These were found to have the same sign and comparable values to those quoted.^{24,25} This was taken to indicate that the absolute stereochemistry at C_1 of

all the compounds were the same as the quoted stereochemistries. This optical data is consistent with the observation that the chirality at C₁ is the same for all the compounds of this type reported to date (April 1982).

Sample 1 also contained other minor components, two of which were partially purified and characterised. The first of these, from the non-polar fraction, appeared to be a cuparene type compound as the ¹H n.m.r. spectrum contained the characteristic methyl absorptions at δ_{H} 1.05 (d, J = 7 Hz), 1.45 (s) and 2.30 (s). The olefinic region contained two peaks at δ_{H} 7.1 and 7.2 (which could be interpreted as two single proton singlets or a 2H doublet, J = 8 Hz) and a broad singlet at δ_{H} 6.6. In addition there was a broad doublet at δ_{H} 3.75 (2 H, bd, J = 5 Hz). The ultraviolet spectrum contained the expected phenyl absorptions at 206, 232, 292 and 298 nm, as did the infrared spectrum with peaks at 715 and 910 cm⁻¹. Also in the infra red spectrum were peaks at 1720 cm⁻¹ (indicating a carbonyl) and 1210 and 1260 cm⁻¹, indicating an ether link as there was no hydroxyl absorption. The EI mass spectrum displayed two possible parent ions (M⁺) as 1:1 doublets at m/e 308/310 and 310/312. As the sample was not completely pure it could not be determined which parent ion was correct but obviously the compound contained bromine. The ¹³C n.m.r. spectrum contained peaks at δ_{C} 126.4, 110.8, 64.0, 42.4, 31.7, 29.7, 23.1, 22.9, 13.8 and small, presumably singlet peaks at δ_{C} 137.1, 100.3 and 54.6.

This spectral data suggested a cuparene type compound with an ether link, a C₄-methyl group and a tetrasubstituted phenyl ring, similar to aplysin (6) but the rest of the structure could not be deduced.

The other partially characterised compound from Sample 1 was inter-

esting in that it appeared to be a saturated version of the enyne compounds. The infra red spectrum displayed peaks attributable to hydroxyl (3650 , 1215 cm^{-1}), acetylene (3350 , 2100 cm^{-1}), olefinic (990 cm^{-1}) and halogen (650 , 620 cm^{-1}) functionalities. However the ultraviolet spectrum did not display the characteristic enyne absorptions, but instead had maxima at 238 and 258 nm . The ^1H n.m.r. spectrum contained a broad doublet at $\delta_{\text{H}}3.2$ which suggested a terminal alkyne. The ^{13}C n.m.r. spectrum exhibited fourteen peaks suggesting a C_{15} compound with the quaternary alkyne carbon not being observed. The fourteen peaks comprised six olefinic resonances, a terminal alkyne carbon resonance, an oxygenated carbon resonance, five alkyl methylene resonances and a methyl carbon resonance at $\delta_{\text{C}}14.0$. This data suggested a linear carbon chain with a methyl group at one end, an acetylene at the other and three double bonds and an hydroxyl substituent inbetween.

The occurrence of these two potentially interesting compounds suggests that a re-examination of this alga would be worthwhile. However the question remained, why was Sample 1 different from Samples 2 and 3? All three samples had been collected from the same region at approximately the same time, so the regional differences found with other *Laurencia* species could be discounted. Another possibility was that the difference was due to the sexual composition of the collections. These algae exist in three reproductive forms: male, female and tetrasporangial. The latter is an asexual reproductive form. Unfortunately all of Samples 1 and 3 had been used for extraction but a portion of Sample 2 was available. This appeared to be mainly composed of tetrasporangial plants with small amounts of female as well. No male plants were found but this may have been due to the method of storage. The algae had been frozen and this may have destroyed the male plant characteristics.

A small quantity of tetrasporangial and female specimens were positively identified. These were extracted with dichloromethane and the extracts analysed by t.l.c. and g.l.c. Both were identical to each other and also to a stored sample of the extract of Sample 2. It is more likely that differences would occur between haploid (male or female) and diploid (tetrasporangial) forms of the alga, than between the two haploid forms. Consequently it seemed unlikely that a difference in sexual composition could have caused the chemical variation.

It is possible that differing environmental conditions may have caused the variation. Sample 1 had been collected from the low intertidal to upper subtidal region whereas Samples 2 and 3 were collected from the mid- and upper intertidal regions. Differences in sunlight distribution, exposure to air, other animals and plants in the area etc. may have engendered the chemical variation. However culture studies by Howard, Nonomura and Fenical⁹¹ in which temperature, aeration, photoperiods and seawater were varied indicated that changes in environmental factors in nature have little or no effect on secondary metabolite chemistry in *Laurencia* species. In the same paper cross-fertilization culture studies were reported which indicated that despite morphological consistency, samples of *Laurencia pacifica* from different sites, which contained differing 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 differentiated by obvious morphological characteristics. It is possible that such genetic differences exist between the samples of *Laurencia distichophylla* examined in this study which may have caused the variation in secondary metabolites.

BIOGENESIS OF THE ACETYLENIC CYCLIC ETHERS

The biogenetic pathway for the formation of the acetylenic cyclic ethers of the *Laurencia* genus has not yet been established. However it is possible to draw some conclusions from consistent features apparent in the structures which have so far been established.

The first point to be considered is that in each of the acetylenic cyclic ethers the basic skeleton consists of an unbranched chain of carbon atoms. This has led to the assumption that the biogenetic precursors of these compounds are derived from straight chain primary metabolites such as the fatty acid esters.⁹ A polyketide origin is possible, but arguments in favour of such a source are less persuasive.

The biogenesis of fatty acids begins with acetyl-CoA, a complex thiol ester which is produced in the mitochondria by the metabolism of lipids, carbohydrates and proteins.⁹² The overall process of fatty acid synthesis is catalysed by a cluster of seven proteins, known as the fatty acid synthetase complex.⁹³ This complex is basically similar for animals and plants, but it has been found that it is possible to resolve the components of the plant synthetase complex without loss of activity whereas this is not possible for the animal synthetase complex. The other major difference between plant and animal fatty acid biosynthesis is that the major product in animal systems is saturated palmitate (C_{16}) with smaller amounts of myristate (C_{14}) and stearate (C_{18}), while the plant synthetases yield mainly C_{18} acids.⁹²

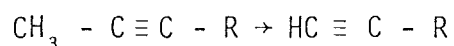
During the fatty acid biogenesis the acyl intermediates in the process of chain lengthening are thiol esters, not of CoA, but of a low molecular weight protein called acyl carrier protein (ACP). This protein can form a complex with the six other enzyme proteins in the synthetase

complex. A crucial first step in the biosynthesis is the conversion of acetyl-CoA to malonyl-CoA by the enzyme system acetyl-CoA carboxylase.⁹³ It is this reaction which explains the requirement of CO₂ for fatty acid biosynthesis. While a single molecule of acetyl-CoA serves as the primer in the process the C₂ units which are added subsequently are taken from malonyl-CoA. The reaction steps are summarized in Figure 11.⁹² As this process involves the addition of C₂ units the resulting fatty acids usually contain an even number of carbon atoms.

A second feature of the acetylenic cyclic ethers is the terminal acetylene functionality and other unsaturated positions along the carbon chain. Unsaturation is a common feature of fatty acids in nature and is produced by either an aerobic or anaerobic pathway. The latter proceeds *via* dehydration of a hydroxy acyl-ACP derivative and occurs in certain bacteria. In plants, animals and algae the aerobic mechanism involves the desaturation of the acyl derivative of the corresponding fully formed fatty acid although the details may vary between species. The process is aerobic since molecular oxygen is required as an electron acceptor. The electron donor is usually NADPH. The basic reaction is given in Figure 12.⁹³ Further desaturation to produce polyenoic fatty acids is believed to occur in the same manner.

Naturally occurring acetylenic compounds are widespread in nature. Labelling studies have shown that these are derived from fatty acids by further desaturation of unsaturated compounds. Terminal alkynes are relatively uncommon however and the production of these seems to involve one of two further steps:⁹⁴

1. Removal of a terminal methyl group i.e.



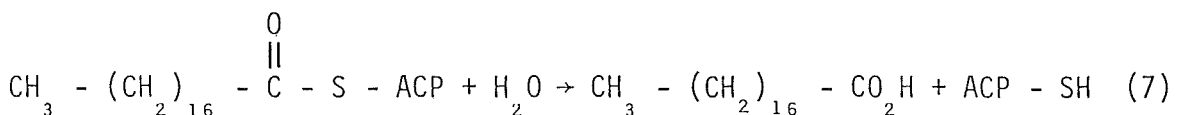
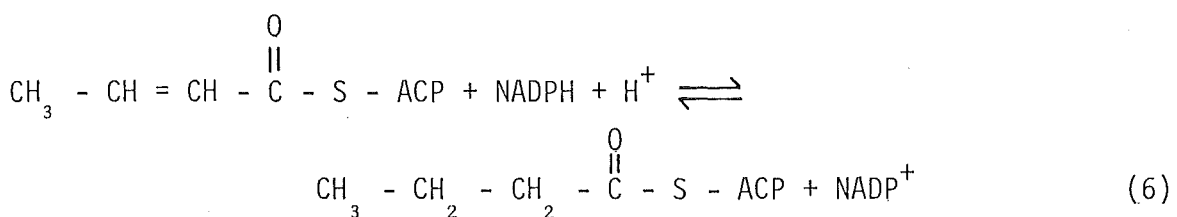
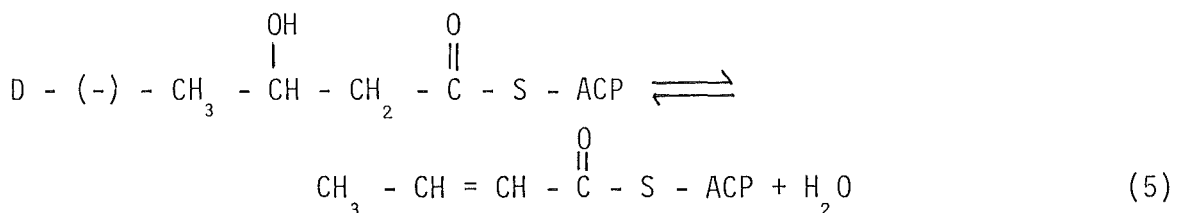
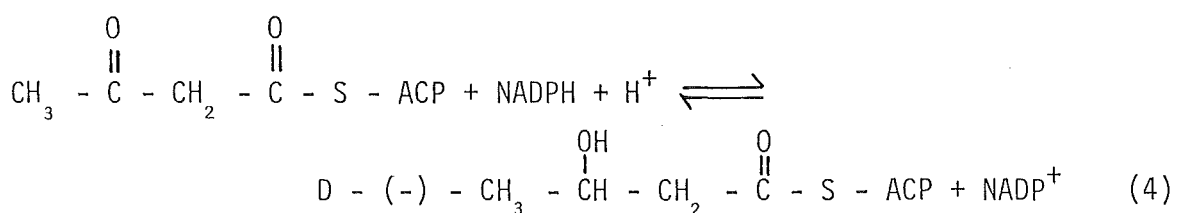
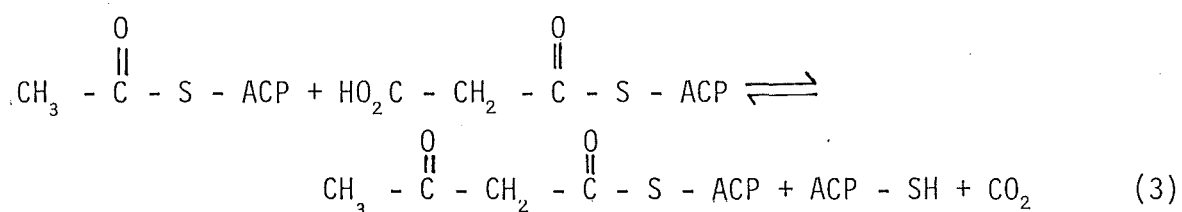
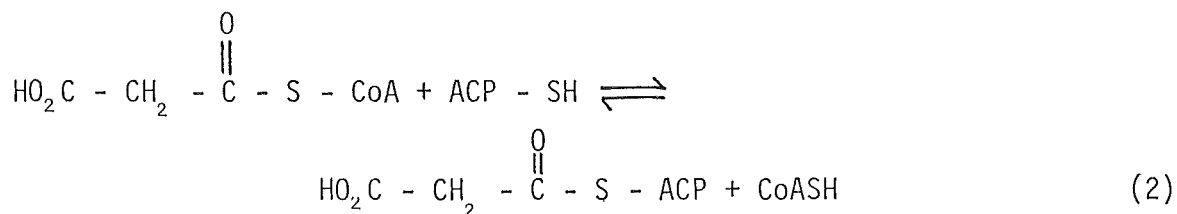
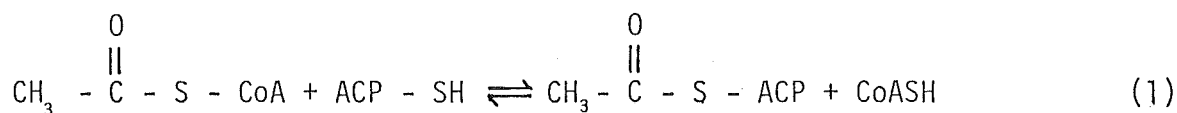


Figure 11: Mechanism of saturated fatty acid biosynthesis.

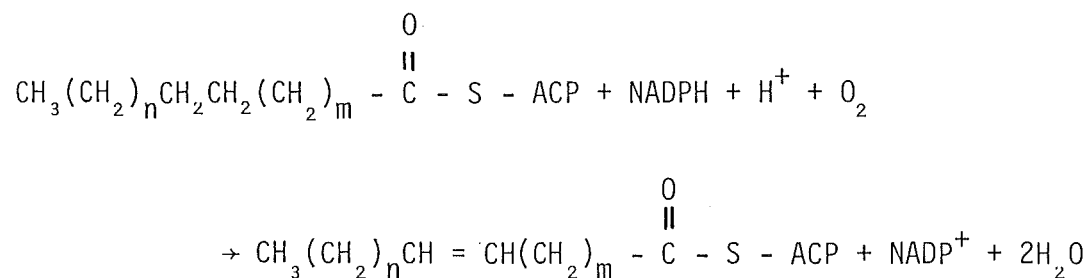
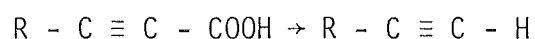


Figure 12: Fatty acid desaturation.

2. Decarboxylation i.e.



Both these steps have been observed in nature. The absence of substitution at the non-acetylenic end of the carbon chain of the *Laurencia* acetylenes suggests that decarboxylation may be the process involved in their production. In addition the creation of a terminal alkyne by this method allows the production of a C₁₅ chain from a C₁₆ precursor. As mentioned earlier it is usual for fatty acids to contain even numbers of carbon atoms and all the acetylenic compounds so far isolated from *Laurencia* species contain fifteen carbons.

For the case of okamuraallene (70)⁶³ which contains a cyclopropane

ring it is interesting to note that although cyclopropane acids are rare elsewhere they do occur in relatively high concentration in some bacteria. Cyclopropane biogenesis involves the addition of a C_1 unit across a carbon-carbon double bond. This reaction has been studied in *E. coli* and the C_1 unit was found to be derived from the methyl group of S-adenosyl methionine.⁹²

Another feature of these acetylenic cyclic ethers is the presence of oxygen as a common substituent and of course as a component of the ether ring. Introduction of oxygen may occur either by direct oxidation of C-H bonds or by epoxidation of a carbon-carbon double bond and subsequent opening of the epoxide. These reactions are performed by enzymes known as mono oxygenases.^{93,94}

After the discovery of the first of the acetylenic cyclic ethers, laurencin (48), it was suggested⁹⁵ that it was biogenetically derived from hexadeca-4,7,10,13-tetraenoic acid (102) (which has been isolated from an alga of the *Scenedesmus* genus) or a closely related compound. This suggestion has been supported by the isolation of the mixture of laurediols (52a), (52b), and their diacetates (52c) and (52d) from *Laurencia nipponica* and more recently the identification of laurencenyne (71) and neolaurencenyne (72)⁶⁴ from *Laurencia okamurai*. All these compounds have been suggested as precursors of the acetylenic cyclic ethers, with laurencenyne and neolaurencenyne postulated as earlier precursors than the laurediols.

Accepting this as a reasonable postulate, it is of interest to consider the details of how the hydroxyl functionalities may be introduced into laurencenyne or neolaurencenyne to produce the laurediols (ignoring for the moment the fact that laurencenyne has a $^{12}\Delta(Z)$ double bond while

the laurediols have a $^{12}\Delta(E)$ double bond). A likely biogenetic route would be the epoxidisation of the $^6\Delta$ double bond followed by the opening of the epoxide by water. Figure 13 illustrates the stereochemistries resulting from the normal trans opening of an epoxide derived from a (*E*) or (*Z*) double bond. The laurediols (and diacetates) occurred in the alga as mixtures of the $C_6R C_7R$ and $C_6S C_7S$ enantiomers. From Figure 13 it is obvious that these resulting stereochemistries are entirely consistent with this method of producing the laurediols from the $^6\Delta(Z)$ double bond of laurencenyne and neolaurencenyne.

It is now pertinent to consider the C_6C_7 stereochemistries of the acetylenic cyclic ethers. These are listed in Table 15*. From this it can be seen that the stereochemistries are consistently $C_6R C_7R$ or $C_6S C_7S$ except for chondrin (62) and this exception may be rationalised if it is assumed that chondrin results from an intramolecular cyclisation of chondriol (60) with resulting inversion of stereochemistry at C_7 . This stereospecificity strongly suggests that the C_6C_7 substituents are introduced into the molecule *via* epoxidation of a $^6\Delta(Z)$ double bond.

The substituents at C_6 and C_7 are observed to be either two oxygens or a chlorine and oxygen. This suggests that an alternative nucleophile for the epoxide opening is chloride.

The high degree of stereospecificity at C_6 and C_7 lends support to the proposed epoxide intermediate, and in addition allows the introduction of two alternative nucleophiles (OH^- or Cl^-). This intermediate would

* This discussion will consider only the "conventional" acetylenic cyclic ethers. The allenic compounds laurallene (55) and okamurallene (70) have been excluded as have the maneonenes and isomaneonenes (75a-f) which contain carbocyclic rings. The *Aplysia* acetylenes have also been omitted as they may have undergone chemical modification from the algal source.

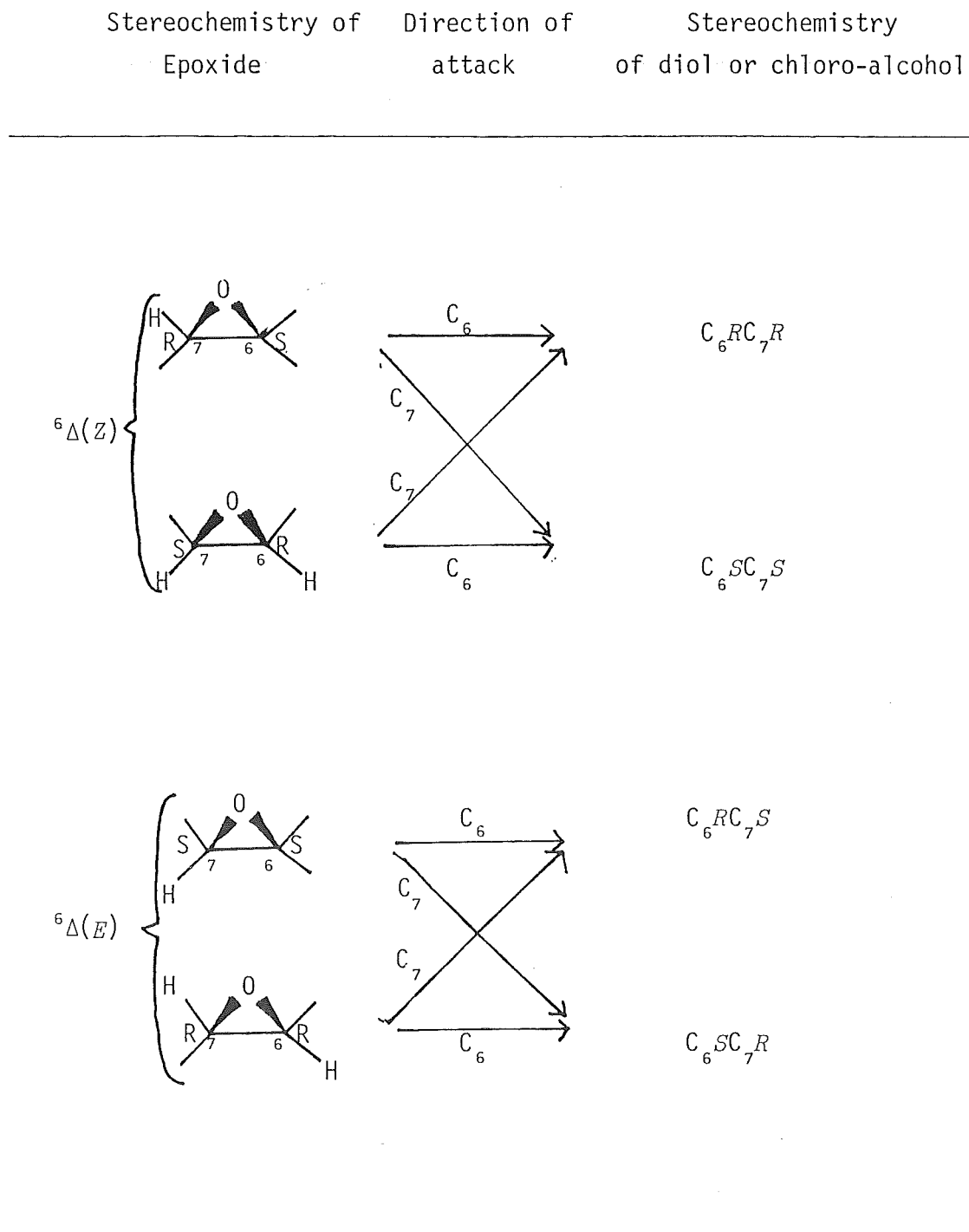


Figure 13: Stereochemical results of trans epoxide opening by water or chloride.

Table 15: Stereochemistry, ether ring size and side chain length for some of the *Laurencia* acetylenic cyclic ethers.

	Chirality						Ether Ring Size	Length of Side Chains
	C ₆	C ₇	C ₉	C ₁₀	C ₁₂	C ₁₃		
Dehydrobromolaurefucin (64)	R	R	R	+	*	R	8	6,2
Acetyllaurefucin (51a)	R	R	R	R	S	R	8	6,2
Laurefucin (51)	R	R	R	R	S	R	8	6,2
Laurencin (48)	R	R	*	*	S	R	8	6,2
Deacetyllaurencin (48a)	R	R	*	*	S	R	8	6,2
Poiteol (66)	R	R	R	S	S	R	8	6,2
Chlorofucin (65)	S	S	S	S	S	R	8	6,2
Laurepinnacin (73)	R	R	*	*	R	R	8	6,2
Chondriol (60)	R	R	*	*	*	*	8	5,3

Chondrin (62)	<i>S</i>	<i>R</i>	*	*	*	*	8	5,3
Laureatin (49)	<i>S</i>	<i>S</i>	<i>R</i>	<i>R</i>	<i>R</i>	<i>S</i>	8	5,3
Isolaureatin (50)	<i>S</i>	<i>S</i>	<i>S</i>	<i>S</i>	<i>R</i>	<i>S</i>	8	5,3
Laurenyne (58)	<i>S</i>	<i>S</i>	*	*	<i>S</i>	*	8	5,3
Cis-isodihydrorhodophytin (59)	<i>S</i>	<i>S</i>	*	*	<i>S</i>	*	8	5,3
Epoxyrhodophytin (63)	<i>S</i>	<i>S</i>	<i>R</i>	<i>S</i>	*	*	8	5,3
Rhodophytin (61)	<i>S</i>	<i>S</i>	*	*	*	*	8	5,3
Venustin A (68)	<i>R</i>	<i>R</i>	<i>S</i>	<i>R</i>	*	*	8	5,3
Venustin B (69)	<i>R</i>	<i>R</i>	*	*	*	*	8	5,3
Laurencienyne (57)	<i>S</i>	<i>S</i>	<i>S</i>	<i>S</i>	<i>R</i>	<i>S</i>	8	5,3
Isoprelaurefucin (53)	<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>	<i>S</i>	7	6,3
Isolaurepinnacin (74)	<i>R</i>	<i>R</i>	*	*	<i>S</i>	<i>S</i>	7	6,3
Obtusenyne (56)	<i>S</i>	<i>S</i>	*	*	<i>S</i>	<i>S</i>	9	5,2
Laureepoxide (54)	<i>S</i>	<i>R</i>	<i>S</i>	<i>R</i>	<i>S</i>	<i>R</i>	5	9,2

⁺ Not determined

* Not applicable, due to the presence of a double bond

permit stereochemistry to be controlled simply by the geometry of the ${}^6\Delta$ double bond.

Other features of the acetylenic cyclic ethers provide an indication of the mechanism of formation of the ether ring. The predominant size of the ether ring is eight membered and this is usually the only ring in the molecule. These eight membered ring compounds comprise two types:

- (i) those with side chains of six and two carbons,
- (ii) those with side chains of five and three carbons.

Type (i) may be considered the result of a cyclisation *via* oxygen between C_7 and C_{13} of a straight chain precursor. Similarly Type (ii) is a product of a C_6 -O- C_{12} cyclisation. The seven and nine membered ether ring compounds involve C_7 -O- C_{12} and C_6 -O- C_{13} cyclisation respectively. Laureepoxide (54), which has a five membered ether ring, involves a C_{10} -O- C_{13} cyclisation.

Thus the reactive sites for all but one of the cyclisations are C_6C_7 and $C_{12}C_{13}$. A likely mechanism for this reaction is a bromonium ion induced cyclisation. This has been suggested previously^{65,73} and is strongly supported by the presence of a bromine substituent at C_{12} or C_{13} in all but two examples. These exceptions are dehydrobromolaurefucin (64) and laurenyne (58). These two acetylenes contain ${}^{11}\Delta$ and ${}^{13}\Delta$ double bonds respectively which could be the result of post cyclisation elimination of HBr.

The bromonium ion cyclisation is illustrated in Figure 14. The cyclisation may be concerted or the epoxide may be opened prior to cyclisation as has occurred with the laurediols.

If this cyclisation involves a ${}^{12}\Delta$ double bond then the $C_{12}C_{13}$ positions should be saturated upon completion of the reaction. However there are a number of examples (chondriol (60), rhodophytin (61), chondrin

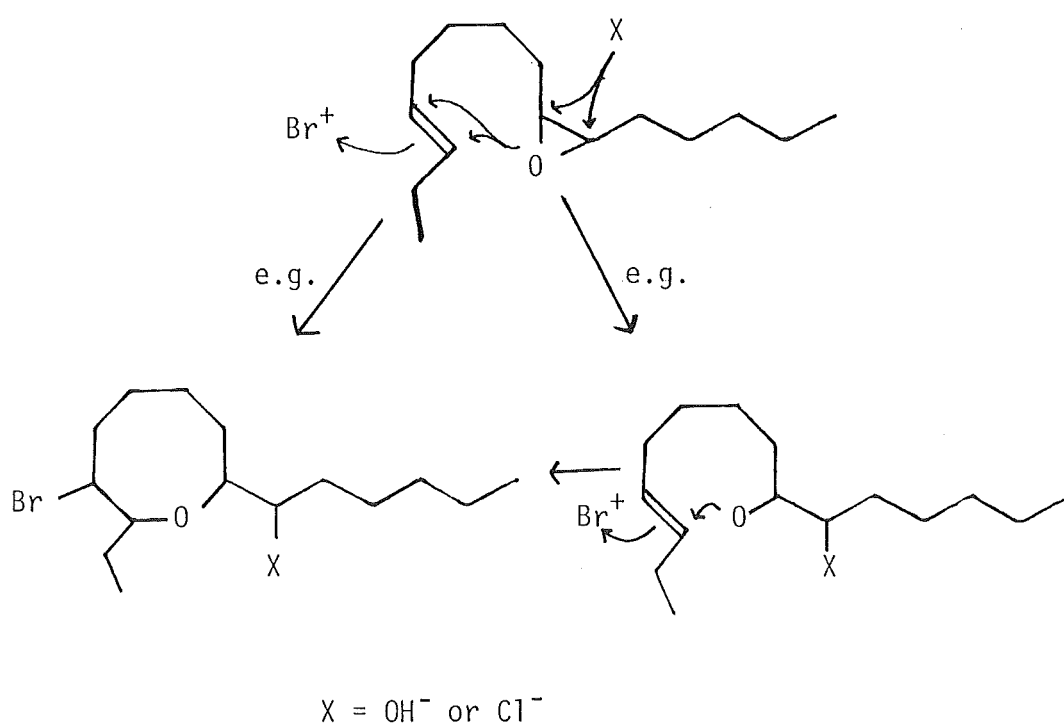


Figure 14: Proposed bromonium ion induced cyclisation to create the ether ring of the *Laurencia* acetylenic cyclic ethers.

(62), epoxyrhodophytin (63), venustin A (68) and venustin B (69)) where a $^{12}\Delta$ double bond is still contained in the molecule. It is proposed that cyclisation in these cases may be to a 12-yne grouping rather than 12-ene.

An assumption made by Kurosawa et. al.⁵⁰ during the early work on these compounds was that from a biogenetic viewpoint the relative stereochemistry between C_{12} and C_{13} was *erythro*. This would imply opposite chirality at C_{12} and C_{13} and proved useful in assigning the stereochemistry of the molecules they isolated (in particular laureatin (49), iso-laureatin (50), laurefucin (51) and isoprelaurefucin (53)). The resulting assignments were confirmed by other evidence and this assumption has proved consistent (See Table 15) in all but three cases: obtusenyne (56), laurepinnacin (73) and isolaurepinnacin (74).

It has already been noted that in the presumed precursors to these molecules, the laurediols, have a $^{12}\Delta(E)$ double bond and laurencenyne a $^{12}\Delta(Z)$ double bond. Each of these olefins have two faces: the $^{12}\Delta(E)$ double bond has $C_{12}siC_{13}si$ and $C_{12}reC_{13}re$ faces and the $^{12}\Delta(Z)$ double bond has $C_{12}siC_{13}re$ and $C_{12}reC_{13}si$ faces. Figure 15 shows the resulting stereochemistries of the cyclising oxygen attack of the proposed mechanism as it occurs from above the page onto these double bonds. The stereochemical results are quite specific and allow the construction of Table 16 which gives the type of double bond attacked, the direction and face of attack for the appropriate compounds. From Table 16 it can be seen that the $C_{12}reC_{13}re$ face attack onto a $^{12}\Delta(E)$ double bond is remarkably consistent apart from the three exceptions mentioned earlier.

Figure 15 contains only thirteen of the compounds in Table 15. The six compounds which retain a $^{12}\Delta$ double bond are not amenable to this type of analysis. In dehydrobromolaurefucin (64) the stereochemistry has

	$^{12}\Delta$ Double bond	Direction of Attack	Resulting Stereochemistry
(E)		C_{12} C_{13}	$C_{12}SC_{13}R$ $C_{12}RC_{13}S$
(E)		C_{12} C_{13}	$C_{12}RC_{13}S$ $C_{12}SC_{13}R$
(Z)		C_{12} C_{13}	$C_{12}RC_{13}R$ $C_{12}SC_{13}S$
(Z)		C_{12} C_{13}	$C_{12}SC_{13}S$ $C_{12}RC_{13}R$

Figure 15: Stereochemical results of attack alternatives for the bromonium ion cyclisation (oxygen attack from above the page).

Table 16: Stereochemistry, ether link numbering, $^{12,13}\Delta$ double bond geometry and face of attack in proposed cyclisation.

	Chirality		Ether Link	$^{12,13}\Delta$ Double Bond
	C ₁₂	C ₁₃		
Poiteol (66)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Deacetyl laurencin (48a)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Laurencin (48)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Acetyl laurefucin (51a)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Laurefucin (51)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Chlorofucin (65)	<i>S</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Isoprelaurefucin (53)	<i>R</i>	<i>S</i>	C ₇ -O-C ₁₂	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Laurencienyne (57)	<i>R</i>	<i>S</i>	C ₆ -O-C ₁₂	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Isolaureatin (50)	<i>R</i>	<i>S</i>	C ₆ -O-C ₁₂	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Laureatin (49)	<i>R</i>	<i>S</i>	C ₆ -O-C ₁₂	(<i>E</i>) C ₁₂ ^{re} C ₁₃ ^{re}
Laurepinnacin (73)	<i>R</i>	<i>R</i>	C ₇ -O-C ₁₃	(<i>Z</i>) C ₁₂ ^{si} C ₁₃ ^{re}
Isolaurepinnacin (74)	<i>S</i>	<i>S</i>	C ₇ -O-C ₁₂	(<i>Z</i>) C ₁₂ ^{si} C ₁₃ ^{re}
Obtusenyne (56)	<i>S</i>	<i>S</i>	C ₆ -O-C ₁₃	(<i>Z</i>) C ₁₂ ^{re} C ₁₃ ^{si}

been lost at C_{12} but at C_{13} the chirality is R which is consistent with a $^{12}\Delta(E)$ $C_{12}^{re}C_{13}^{re}$ attack. In laurenyne (58) the stereochemistry has been lost at C_{13} but the C_{12}^S chirality is an exception to the general trend. The C_{12}^S chirality of cis-isodihyrorhodophytin is another exception but as the stereochemistry of this molecule was not positively determined the conclusions on this example will be left open.

The remaining compound, laureepoxide (54) provides some interesting stereochemistry. The $C_{12}^SC_{13}^R$ chirality suggests C_{10} -O attack on C_{13} at the $C_{12}^{re}C_{13}^{re}$ face of a $^{12}\Delta(E)$ double bond, consistent with the general pattern. The $C_9^SC_{10}^R$ stereochemistries arise from the opening of a $C_9^RC_{10}^R$ epoxide at C_9 . This epoxide would be derived from a $^9\Delta(E)$ double bond.

This deduced $^9\Delta(E)$ double bond in a hypothetical precursor of laureepoxide is however, the only example of this. In the laurediols and laurencyne the $^9\Delta$ double bond has (Z) configuration and this bond is retained in many of the cyclic ethers. A few compounds are epoxides at this point (poiteol (66), epoxyrhodophytin (63) and venustin A (68)) and the epoxides are generated from $^9\Delta(Z)$ double bonds. Further, it can be seen that in the remaining compounds (See Table 15), identical chirality exists at C_9 and C_{10} which is consistent with the trans opening of a C_9C_{10} epoxide resulting from a $^9\Delta(Z)$ double bond. This evidence for epoxide formation and opening supports the proposed epoxidation of the $^6\Delta$ double bond, particularly in view of the existence of venustin B (69) and rhodophytin (61) and the corresponding epoxides venustin A (68) and epoxyrhodophytin (63) respectively.

The possibility of an internal cyclisation to convert chondriol (60) to chondrin (62) has already been suggested. In the other compounds

which contain additional ether rings (laureatin (49), isolaureatin (50), laurefucin (51), acetyl-laurefucin (51a), isoprelaurefucin (53), dehydro-bromolaurefucin (64) and chlorofucin (65)) the *threo* relationship at C₆C₇ is retained. This suggests that in these cases the cyclisation occurs from C₆-O or C₇-O to a ⁹Δ double bond (*via* a bromonium ion induced reaction for laureatin, isolaureatin and isoprelaurefucin) or C₉C₁₀ epoxide (in the case of dehydrobromolaurefucin).

Thus it would appear that the laurediols, with C₆*R*C₇*R* and C₆*S*C₇*S* stereochemistry derived from a ⁶Δ(*Z*) double bond, a ⁹Δ(*Z*) double bond and a ¹²Δ(*E*) double bond, have the appropriate characteristics to be precursors of most of the acetylenic cyclic ethers. Laurencenyne contains the ⁶Δ(*Z*) and ⁹Δ(*Z*) double bonds with a ¹²Δ(*Z*) double bond which makes it the more likely precursor of obtusenyne, laurepinnacin and isoprelaurepinnacin.

Considering now the acetylenes from *Laurencia thyrsifera* the first point to be made is that the consistency of the *threo* C₆C₇ stereochemistry in the other molecules strongly suggests that a similar *threo* relationship exists in these molecules and hence if the chirality of C₇ is *R* then the chirality of C₆ will also be *R*. The ¹¹Δ double bonds in (82a-d), (87a-d), (89a), (89b) and (92) is akin to the ¹¹Δ double bond in dehydrobromolaurefucin (64) and may be the result of HBr elimination. The presence in the same alga of (92) with a C₁₂ bromo-substituent supports this.

The concurrent existence of the diols (82a), (82b), and the diacetates (82c), (82d) has already been commented on as not unusual. However the diol functionality is previously unknown in the cyclic ethers (although it does occur in the laurediols) but is consistent with diol formation from a C₉C₁₀ epoxide. The C₉*R*C₁₀*R* stereochemistry is consistent with the identical stereochemistry observed at C₉ and C₁₀ in all the other relevant compounds.

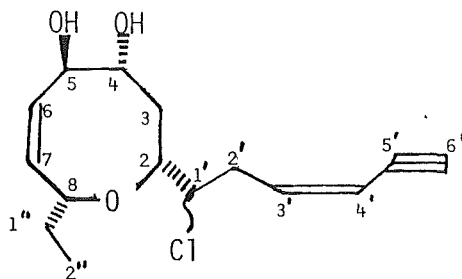
The C₆ chloro substituent in all the *Laurencia thyrsoifera* ethers is in keeping with chloride acting as a nucleophile in the opening of a C₆C₇ epoxide, and the C₁₂S C₁₃R chirality assumed for (95) is in keeping with the general trend of ¹²Δ(E) C₁₂reC₁₃re attack.

The more unusual aspects of the *Laurencia thyrsoifera* ethers are the C₁₀ ketone of (89a) and (89b) and the vinyl chlorine in (92). The C₁₀ ketone may arise from one of two routes. Rearrangement of a C₉C₁₀ epoxide could lead directly to the C₁₀ ketone. Alternatively regiospecific dehydration of the diols (82a) and (82b) could lead to the same ketone *via* the enol.

The vinylic chloride of (92) is conceivably due to the dehydration of a suitable chlorohydrin (103). Such a compound was not detected however.

APPENDIX: NOMENCLATURE

The systematic name for the diol (82a) is $[2R-(2\alpha,4\alpha,5\beta,8\alpha)]-2-[(1\xi,3Z)-1\text{-chlorohex-3-en-5-ynyl}]-8\text{-ethyl-3,4,5,8-tetrahydro-2H-oxocin-4,5-diol}$ and (82b) is the corresponding $(3E)$ isomer, with the numbering as shown in the following diagram.



The trivial name lauthisan⁹⁶ has been coined to describe the basic skeleton of the diols (82a) and (82b). Laurencin (48) and the various laurefucins could also be described as lauthisan derivatives. The numbering of the lauthisan skeleton (as shown on structure (82a)) is consistent with that used by other authors to describe the various acetylenic cyclic ethers.

Under this scheme the acetylenic cyclic ethers of *Laurencia thyrifera* would therefore be named:

(82a): $(3Z,6\xi,9R,10R)-6\text{-chlorolauthisa-3,11-dien-1-yn-9,10-diol}$.

- (82b): (3*Z*,6*ξ*,9*R*,10*R*)-6-chloro-9,10-diacetylauthisa-3,11-dien-1-yn-9,10-diol.
- (82c): (3*E*,6*ξ*,9*R*,10*R*)-6-chlorolauthisa-3,11-dien-1-yn-9,10-diol.
- (82d): (3*E*,6*ξ*,9*R*,10*R*)-6-chloro-9,10-diacetylauthisa-3,11-dien-1-yn-9,10-diol.
- (87a): (3*Z*,6*ξ*,9*R*,10*R*)-9-acetoxy-6-chlorolauthisa-3,11-dien-1-yn-9,10-diol.
- (87b): (3*E*,6*ξ*,9*R*,10*R*)-9-acetoxy-6-chlorolauthisa-3,11-dien-1-yn-9,10-diol.
- (87c): (3*Z*,6*ξ*,9*R*,10*R*)-10-acetoxy-6-chlorolauthisa-3,11-dien-1-yn-9,10-diol.
- (87d): (3*E*,6*ξ*,9*R*,10*R*)-10-acetoxy-6-chlorolauthisa-3,11-dien-1-yn-9,10-diol.
- (89a): (3*Z*,6*ξ*,9*R*,10*R*)-6-chlorolauthisa-3,11-dien-1-yn-10-one.
- (89b): (3*E*,6*ξ*,9*R*,10*R*)-6-chlorolauthisa-3,11-dien-1-yn-10-one.
- (92): (3*Z*,6*ξ*,9*R*,10*R*)-6,10-dichlorolauthisa-3,9,11-trien-1-yne.
- (95): (3*Z*,6*ξ*,9*R*,10*R*)-12-bromo-6-chlorolauthisa-3,9-dien-1-yne.

EXPERIMENTAL

The *Laurencia thyrsoifera* samples were collected as living plants from the intertidal region of Seal Reef, Kaikoura, New Zealand (Voucher No. 248071). Identification and preparation of voucher specimens was performed by Dr M.J. Parsons, D.S.I.R., Lincoln.

The *Laurencia distichophylla* samples were collected from the Echinoderm Reef of the Leigh Marine Reserve, New Zealand (Voucher No. for Sample 1: 368013). Collection, identification and preparation of voucher specimens was performed by Dr M.H.G. Munro, University of Canterbury and Dr M. Hawkes, University of Auckland.

The microanalyses were carried out by Professor A.D. Campbell and associates, University of Otago. Melting points were determined in sealed capillaries and are uncorrected.

Infra red spectra were recorded on a Shimadzu IR-27G spectrophotometer as solutions in chloroform or carbon tetrachloride. Ultraviolet spectra were recorded on a Varian Superscan 3 spectrophotometer as ethanol solutions. Optical rotary dispersion curves and $[\alpha]_D$ values were recorded on a JASCO Model ORD / UV-5 spectrophotometer.

High resolution mass spectrometry was performed on an AEI-MS902 instrument in the electron impact (EI) mode. Low resolution chemical ionization (CI) mass spectra were obtained on a Hewlett-Packard GCMS 5982A instrument using direct insertion and either methane or isobutane as the reagent gas.

Routine ^1H n.m.r. spectra were recorded on a Varian T60 spectrometer. ^{13}C n.m.r. spectra and quoted ^1H n.m.r. spectra were obtained using a Varian CFT-20 Fourier Transform NMR spectrometer. The high field ^1H n.m.r.

spectra were recorded on a Bruker HX-270 spectrometer by Dr A.J. Jones and associates at the Australian NMR Centre, Canberra, Australia. All the n.m.r. spectra were recorded for deuterated chloroform solutions unless otherwise stated. All chemical shifts are expressed as parts per million (ppm) downfield from tetramethylsilane as the internal reference and are quoted as position (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), relative integral and coupling constants (J, Hz).

Analytical thin layer chromatography (t.l.c.) was performed on 0.5 mm thick silica gel G (Stahl) plates and the chromatograms were developed by spraying with phosphomolybdic acid in ethanol (10% w/v) and baking at 100°C. Preparative t.l.c. was carried out on 200 x 200 x 1 mm plates of silica gel G (Stahl) and visualised under ultraviolet light. Column chromatography was routinely carried out using 10% water deactivated alumina (Spence; Grade H, 100/200 mesh) and occasionally on Florisil (100/200 mesh).

The gas liquid chromatography (g.l.c.) was carried out using a Varian 1400 instrument. The chromatograms displayed in Figures 2, 8 and 9 were recorded using a 3 m silylated glass column containing 3% OV17 on Chromosorb W, a flame ionisation detector and nitrogen as the carrier gas. The fatty acid ester g.l.c. analysis was carried out using a 2 m, 3% DEGS on Chromosorb W glass column.

High pressure liquid chromatography (h.p.l.c.) was performed on a Varian 5020 Chromatograph. Various columns were used including Zorbax-CN analytical and preparative columns, Zorbax-NH₂ and Zorbax-C-8 reverse phase analytical columns. Solvents employed were hexane/isopropanol for normal phase and water/methanol for reverse phase separations.

"Ether" refers to redistilled commercial diethyl ether and "pet. ether" refers to redistilled petroleum ether (50° - 70°C). All solvents were either of analytical grade (AR) or were purified and dried according to standard procedures.

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The collected alga was air dried for ten days at room temperature. It was then ground to a coarse powder and extracted in a Soxhlet apparatus with dichloromethane to produce a dark green oil [7.8 g, 2.6% yield based on dry weight of algae (300 g)].

T.l.c. analysis using ether/pet. ether (1:1) as a developing solvent resolved eight components (R_f 0.83, 0.73, 0.57, 0.43, 0.37, 0.27, 0.17, 0.13).

Bulk Column Chromatography:

The crude extract (7.8 g) was chromatographed on alumina (700 g). The column was eluted with a solvent gradient system from pet. ether through dichloromethane to ether. Three major fractions were obtained:

- (i) nonpolar fraction: t.l.c. spots at R_f 0.83, 0.73 and 0.57; eluted with pet. ether (4.7 g; 60% of extract).
- (ii) midrange polarity fraction: t.l.c. spots at R_f 0.43, 0.37 and 0.27; eluted with mixtures of pet. ether and dichloromethane (1.4 g; 18% of extract).
- (iii) polar fraction: t.l.c. spots at R_f 0.17 and 0.13; eluted with dichloromethane and ether (1.7 g; 22% of extract).

Investigation of Polar Fraction:

During preparative t.l.c. attempts to isolate the acetylenic ether diols (82a) and (82b) a small quantity (10 mg) of thysiferol (43) was isolated.

ν_{\max} : 3450, 2950, 1460, 1370, 1100 cm^{-1} ; ^1H n.m.r.: δ_{H} 1.07, s; 1.10, s; 1.15, s; 1.20, s; 1.25, s; 1.35, s; 2.3, m; 3.7, m; and a series of multiplets δ_{H} 1.5 - 2.8; ^{13}C n.m.r.: δ_{C} 87.4, 86.5, 86.0, 77.6, 76.3, 76.1, 74.9,

74.4, 73.2, 72.0, 70.5, 59.0, 38.5, 37.0, 33.5, 32.4, 31.0, 28.2, 27.7, 26.6, 25.5, 23.9, 23.7, 23.4, 23.0, 22.9, 21.4, 21.1, 20.7, 20.1.

Isolation of diacetates (82c) and (82d):

A sample of the polar fraction (503 mg) was acetylated with 1:1 acetic anhydride/pyridine (2 ml) overnight at room temperature and, after dilution with water and ether extraction, gave an oil (420 mg) which was chromatographed on alumina (40 g). The diacetates (82c) and (82d), (162 mg) were eluted with dichloromethane/pet. ether (1:9).

(82c) and (82d): $[\alpha]_D^{25} - 8^\circ$ (c, 0.7 in CHCl_3); (Found: C, 61.5; H, 6.8. $\text{C}_{19}\text{H}_{25}\text{ClO}_5$ requires C, 61.8; H, 7.1%).

ν_{max} : 3300, 3040, 2100, 1740, 1350, 1100, 950, 750 cm^{-1} ; m/e 369/371 (M + 1), 333, 309/311, 249/251; $\lambda_{\text{max,EtOH}}$ 224 nm ($\epsilon = 10,000$), $\lambda_{\text{infl,EtOH}}$ 232 nm ($\epsilon = 7000$).

Isolation of individual isomers (82c) and (82d):

The isomeric diacetate mixture (120 mg) was submitted to repeated preparative t.l.c. After triple development in ether/pet. ether (1:1) the higher R_f (*Z*) isomer (41 mg) (82c) and the lower R_f (*E*) isomer (34 mg) (82d) were obtained as clear oils.

(*Z*) isomer (82c): ^1H n.m.r.: δ_{H} 1.0, t, 3 H; 1.6, m, 2 H; 2.0, m, 7 H; 2.2, m, 1 H; 2.7, m, 1 H; 2.8, m, 1 H; 3.1, d, 1 H; 3.6, m, 1 H; 3.9, m, 1 H; 4.1, m, 1 H; 5.1, m, 1 H; 5.3, m, 1 H; 5.5, m, 1 H; 5.7, m, 1 H; 6.1, m, 1 H; 6.3, m, 1 H.

(*E*) isomer (82d): ^1H n.m.r.: δ_{H} 1.0, t, 3 H; 1.6, m, 2 H; 2.0, m, 7 H; 2.2, m, 1 H; 2.7, m, 1 H; 2.8, m, 2 H; 3.6, m, 1 H; 3.9, m, 1 H; 4.1, m, 1 H; 5.1, m, 1 H; 5.3, m, 1 H; 5.6, m, 2 H; 6.1, m, 1 H; 6.3, m, 1 H.

Hydrogenation of the diacetate mixture of (82c) and (82d):

The diacetate mixture (92 mg) was hydrogenated over Pd/C (40 mg) in ethyl acetate at room temperature. Over a period of two hours 3 molar equivalents of H₂ were consumed. After filtration the product (80 mg) was submitted to preparative t.l.c. to obtain the hydrogenated product (84) (65 mg). (Found: C, 61.4; H, 8.5. C₁₉H₃₁ClO₅ requires C, 60.9; H 8.3%).
 ν_{\max} : 3040, 1740, 1350, 1100 cm⁻¹; ¹H n.m.r.: δ_{H} 1.0, t, 6 H; 2.0, s, 6 H; 3.6, m, 1 H; 3.9, m, 2 H; 5.1, m, 1 H; 5.3, m, 1 H; 5.6, m, 1 H; 6.3, m, 1 H; and a series of multiplets δ_{H} 1.5 - 2.2, 12 H.

Formation of acetonide derivatives of (82a) and (82b):

A sample of the polar fraction (245 mg) was dissolved in acetone (10 ml) and perchloric acid (70%, 60 mg) added. After fifteen minutes at room temperature 10% NaHCO₃ (10 ml) was added, the solution extracted with CH₂Cl₂ (3 x 20 ml), the CH₂Cl₂ extract dried over Na₂SO₄ and evaporated under reduced pressure to yield the acetonide mixture (214 mg). This was then chromatographed on Florisil (20 g) eluting with CH₂Cl₂ to yield (85a) and (85b) (57 mg).

ν_{\max} : 3300, 2950, 1350, 1100, 950, 880 cm⁻¹; $\lambda_{\max, \text{cyclohexane}}$ 224 nm ($\epsilon = 17,300$), $\lambda_{\text{infl}, \text{cyclohexane}}$ 233 nm ($\epsilon = 13,000$); ¹H n.m.r.: δ_{H} 3.1, d, $\frac{1}{2}$ H; 2.8, d, $\frac{1}{2}$ H; 1.4, s, 6 H; m/e 325/327 (M + 1), 267/269.

The mixture of (85a) and (85b) (57 mg) was hydrogenated over Pd/C (30 mg) in ethyl acetate at room temperature. Over a period of one hour, 3 molar equivalents of H₂ were consumed to produce (86) (56 mg).

ν_{\max} : 2950, 1360, 1150, 1050, 870 cm⁻¹; ¹H n.m.r.: δ_{H} 0.9, t, 3 H; 1.0, t, 3 H; 1.4, s, 6 H; 3.4-4.1, m, 4 H; 4.9, m, 1 H; 5.3, m, 1 H; 5.6, m, 1 H; and a series of multiplets δ_{H} 1.5-2.7, 12 H.

Isolation of the mixture of (87a), (87b), (87c) and (87d):

A sample of the polar fraction (150 mg) was submitted to repeated

preparative t.l.c. A sample of the mixture (87a), (87b), (87c) and (87d) (20 mg) was obtained as a light yellow oil.

ν_{\max} : 3600, 3300, 2950, 2100, 1740, 1350, 1100 cm^{-1} ; $\lambda_{\max, \text{EtOH}}$ 223 nm ($\epsilon = 10,600$), $\lambda_{\text{infl, EtOH}}$ 232 nm ($\epsilon = 7900$). m/e 327/329 ($M + 1$), 319, 321, 267/269; ^1H n.m.r.: δ_{H} 1.0, t, 3 H; 2.1, s, 3 H; 2.8, d, $\frac{1}{2}$ H; 3.1, d, $\frac{1}{2}$ H; ^{13}C n.m.r.: δ_{C} 170.7, 170.6, 141.5, 141.2, 140.8, 140.4, 132.9, 132.0, 131.8, 131.1, 131.0, 128.1, 128.0, 112.0, 111.8, 111.0, 110.7, 82.8, 82.5, 81.9, 81.2, 78.5, 78.4, 77.6, 77.3, 76.8, 76.5, 74.5, 74.4, 70.8, 70.4, 63.5, 63.3, 62.8, 62.7, 36.6, 36.5, 35.2, 34.7, 34.1, 33.9, 33.4, 33.2, 29.7, 28.3, 28.2, 21.2, 10.2.

Acetylation of the mixture of (87a), (87b), (87c) and (87d):

A sample of the mixture (15 mg) was acetylated with acetic anhydride/pyridine (1:1), (0.5 ml) overnight at room temperature. After workup as before a sample (10 mg) was obtained which gave infra red, ^1H n.m.r. and ^{13}C n.m.r. spectra identical to the mixture of (82c) and (82d).

Isolation of (82c) and (82d) from midrange polarity fraction:

A sample of this fraction (160 mg) was submitted to preparative t.l.c. Samples of (82c) (17 mg) and (82d) (13 mg) and also a mixture of (82c) and (82d) (38 mg) were isolated. The samples gave infra red, ^1H n.m.r. and ^{13}C n.m.r. spectra which were identical with those of the diacetates (82c) and (82d) obtained by acetylation of (82a) and (82b).

Isolation of cholesterol (88) and the enones (89a) and (89b):

A sample of the midrange polarity fraction (270 mg) was chromatographed on alumina (200 g) to yield cholesterol (88), (91 mg) and a mixture of (89a) and (89b), (103 mg). The cholesterol was recrystallised twice from methanol to give white crystals. Melting point 146.5 - 147.5°C; Literature

melting point 148°C; ^1H n.m.r.: δ_{H} 0.67, s, 3 H; 0.82, s, 3 H; 0.90, s, 3 H; 1.00, s, 3 H; 3.4, m, 1 H; 5.25, m, 1 H; ^{13}C n.m.r.: δ_{C} 140.7, 121.7, 71.8, 56.8, 56.2, 50.1, 42.3, 39.8, 39.5, 37.3, 36.5, 36.2, 35.8, 31.9, 31.7, 28.2, 28.0, 24.3, 23.8, 22.8, 22.6, 21.1, 19.4, 18.7, 11.8.

Part of the mixture of (89a) and (89b) was submitted to preparative t.l.c. to isolate the higher R_f (*Z*) isomer (84a), (22 mg) and the lower R_f (*E*) isomer (84b), (11 mg).

(89a) and (89b): $[\alpha]_{\text{D}}^{25} - 16^\circ$ (c, 0.25 in CHCl_3);

ν_{max} : 3300, 2970, 2100, 1690, 1100, 960 cm^{-1} ; $\lambda_{\text{max,EtOH}}$ 223 nm ($\epsilon = 15,500$), $\lambda_{\text{infl,EtOH}}$ 215 nm ($\epsilon = 13,400$), 233 nm ($\epsilon = 11,000$); m/e 267/269 (M + 1), 231.

(*Z*) isomer (89a): ^1H n.m.r.: δ_{H} 1.0, t, 3 H; 1.6, m, 2 H; 2.0, m, 1 H; 2.2, m, 1 H; 2.4, m, 1 H; 2.5, m, 2 H; 2.7, m, 1 H; 3.1, d, 1 H; 3.6, m, 1 H; 3.9, m, 1 H; 4.0, m, 1 H; 5.6, m, 1 H; 5.8, dd, 1 H; 5.9, dd, 1 H; 6.1, m, 1 H.

(*E*) isomer (89b): ^1H n.m.r.: δ_{H} 1.0, t, 3 H; 1.6, m, 2 H; 2.0, m, 1 H; 2.2, m, 1 H; 2.4, m, 1 H; 2.5, m, 2 H; 2.7, m, 1 H; 2.9, d, 1 H; 3.7, m, 1 H; 3.8, m, 1 H; 3.9, m, 1 H; 5.6, m, 1 H; 5.8, dd, 1 H; 5.9, dd, 1 H; 6.3, m, 1 H.

Hydrogenation of the mixture of (89a) and (89b):

A mixture of (89a) and (89b) (14 mg) was hydrogenated over Pd/C (7 mg) at room temperature in ethyl acetate. Over a period of one hour 4 molar equivalents of H_2 were taken up and after filtration the hydrogenated product (90), (12 mg) was obtained.

ν_{max} : 2950, 1700, 1450, 1080 cm^{-1} ; ^1H n.m.r.: δ_{H} 0.9, t, 6 H; 3.6, m, 3 H; and a series of multiplets δ_{H} 1.0 - 2.8, 18 H.

Isolation of (92) and (95):

A sample of the non polar fraction (200 mg) was submitted to preparative t.l.c. for preliminary separation and the partially purified fractions were completely purified by repeated reverse phase h.p.l.c. to obtain samples of (92), (19 mg) and (95), (19 mg).

(92): $[\alpha]_D^{25} - 14^\circ$ (c, 3.5 in CHCl_3); ν_{max} : 3300, 2950, 2100, 1620, 1100, 900 cm^{-1} ; $\lambda_{\text{max,EtOH}}$ 223 nm ($\epsilon = 9600$), $\lambda_{\text{infl,EtOH}}$ 214 nm ($\epsilon = 7400$), 233 nm ($\epsilon = 7400$); m/e 285/287/289 (M + 1), 249/251, 214; ^1H n.m.r.: δ_{H} 0.95, t, 3 H; 1.6, m, 2 H; 2.1, m, 1 H; 2.5, ddd, 1 H; 2.75, m, 2 H; 3.1, dd, 1 H; 3.25, m, 1 H; 3.45, m, 1 H; 4.0, m, 1 H; and a series of multiplets δ_{H} 5.4 - 6.3, 5 H.

(95): $[\alpha]_D^{25} + 60^\circ$ (c, 0.5 in CHCl_3); ν_{max} : 3300, 2950, 2100, 1450, 1150, 1090, 1060, 950 cm^{-1} ; $\lambda_{\text{max,EtOH}}$ 222 nm ($\epsilon = 15,000$), $\lambda_{\text{infl,EtOH}}$ 231 nm ($\epsilon = 11,400$); m/e 331/333/335 (M + 1), 295/297, 251/253; ^1H n.m.r.: δ_{H} 0.97, t, 3 H; 1.60, m, 1 H; 1.96, m, 1 H; 2.31, m, 1 H; 2.50, m, 2 H; 2.78, m, 1 H; 2.94, m, 1 H; 3.14, dd, 1 H; 3.20, m, 1 H; 3.47, m, 1 H; 3.52, m, 1 H; 4.05, dt, 1 H; 4.10, dt, 1 H; 5.60, m, 1 H; 5.90, m, 1 H; 5.95, m, 1 H; 6.13, m, 1 H.

Hydrogenation of (92):

A sample of partially purified (92), (45 mg) was hydrogenated over Pd/C (20 mg) in ethyl acetate at room temperature, until 3 molar equivalents of H_2 had been taken up. Examination of the filtered product (35 mg) by h.p.l.c. revealed a number of products, one of which (93), (5 mg) was isolated.

ν_{max} : 2950, 1650, 1460, 1090, 1060, 920 cm^{-1} ; ^1H n.m.r.: δ_{H} 0.87, t, 3 H; 0.88, t, 3 H; 5.7, t, 1 H; and a series of multiplets δ_{H} 1.2 - 3.9, 19 H.

Hydrogenation of (95):

A sample of (95), (10 mg) was hydrogenated over Pd/C (5 mg) in ethyl acetate at room temperature. The reaction was stopped after 15 minutes, at which time the major product was (100), (8 mg).

(100): $[\alpha]_D^{25} + 55^\circ$ (c, 0.4 in CHCl_3); ν_{max} : 2950, 1450, 1080, 1050 cm^{-1} ;
 ^1H n.m.r.: δ_{H} 0.93, t, 6 H; 3.5, m, 2 H; 4.0, m, 2 H; 5.9, m, 2 H; and a series of multiplets δ_{H} 1.1 - 2.6, 14 H.

This material was not purified but was rehydrogenated over Pd/C (5 mg) until 4 molar equivalents of H_2 were taken up, to produce, after filtration, (98), (8 mg).

(98): $[\alpha]_D^{25} + 40^\circ$ (c, 0.35 in CHCl_3); ν_{max} : 2950, 1460, 1360, 1090, 1060 cm^{-1} ; ^1H n.m.r.: δ_{H} 0.86, t, 3 H; 0.95, t, 3 H; 3.55, m, 2 H; 3.95, m, 2 H; and a series of multiplets δ_{H} 1.2 - 2.6, 18 H; m/e 339/341/343 (M + 1), 303/305, 259/261, 233.

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The three samples of algae were kept separate during extraction. The wet seaweed was blended with methanol and left to stand for 18 hours. Filtration produced the methanol extract. The solid residue was then extracted in a Soxhlet apparatus with dichloromethane to produce a second extract. Both extracts were identical by t.l.c.* and g.l.c. and so were combined to produce one extract for each sample.

Analysis of Extracts of Samples 2 and 3:

The combined extract (5.1 g) was chromatographed on alumina (500 g) to produce several fractions (combined weight 3.4 g). The bulk of the material was relatively non polar and was eluted with dichloromethane/pet. ether (1:19).

Isolation of (101):

This major component of the extract was isolated as a clear oil (2.0 g). $[\alpha]_D^{25} - 42^\circ$ (c, 2.5 in CHCl_3); ν_{max} : 3600, 3500, 2950, 1615, 1610, 1500, 1460, 1280, 1150, 950 cm^{-1} ; $\lambda_{\text{max,EtOH}}$ 212 nm ($\epsilon = 22,200$), 278 nm ($\epsilon = 3400$), 284 nm ($\epsilon = 3200$); EI ms: m/e 294/296 (M^+), 279/281, 215 (Found 294.06145, $\text{C}_{15}\text{H}_{19}\text{BrO}$ requires 294.06197); ^1H n.m.r.: δ_{H} 1.24, d, 7 Hz, 3 H; 1.46, s, 3 H; 2.25, s, 3 H; 5.15, bs, 1 H, D_2O ; 6.0, d, 2 Hz, 1 H; 6.57, bs, 1 H; 6.65, d, 7 Hz, 1 H; 7.17, d, 7 Hz, 1 H; and a series of multiplets from δ_{H} 1.6 - 3.2, 5 H; ^{13}C n.m.r.: δ_{C} 160.1, s; 153.3, s; 138.0, s; 128.7, s; 128.2, d; 121.3, d; 118.2, d; 101.3, d; 52.0, s; 39.2, d; 39.2, t; 31.0, t; 26.8, q; 20.6, q; 19.1, q.

* It was found that the t.l.c. spots corresponding to cuparene-type molecules would sometimes be a purple colour instead of the more usual black, after development with phosphomolybdic acid.

Formation of acetate (101a):

A sample of (101), (152 mg) was acetylated with 1:1 acetic anhydride/pyridine (1 ml) for 5 hours at room temperature. After workup in the usual manner (101a), (162 mg) was obtained. (Found: C, 60.9; H, 6.4; $C_{17}H_{21}BrO_2$ requires C, 60.5; H, 6.3%); $[\alpha]_D^{25} - 6^\circ$ (c, 5.5 in $CHCl_3$); ν_{max} : 2950, 1770, 1630, 1505, 1350, 1190, 1020, 900 cm^{-1} ; $\lambda_{max,EtOH}$ 212 nm ($\epsilon = 8800$), 267 nm ($\epsilon = 630$), 275 nm ($\epsilon = 630$); CI ms: m/e 337/339 (M + 1), 295/297, 257; EI ms: Found m/e 336.0780, $C_{17}H_{21}BrO_2$ requires 336.0725; 1H n.m.r.: δ_H 1.1, d, 7 Hz, 3 H; 1.4, s, 3 H; 2.3, s, 6 H; 6.0, d, 2 Hz, 1 H; 6.8, bs, 1 H; 6.9, bd, 7 Hz, 1H; 7.2, d, 7 Hz, 1 H; and a series of multiplets from δ_H 1.5 to 3.2, 5 H; ^{13}C n.m.r.: δ_C 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.

Conversion of (101) to isoaplysin (23):

A sample of (101), (102 mg) was stirred with trifluoroacetic acid (2 ml) for 30 minutes. After water dilution, ether extraction and neutralisation with aqueous $NaHCO_3$ (10%) a brown oil was obtained (69 mg). This was submitted to preparative t.l.c. to obtain (23), (42 mg). This conversion was also found to occur spontaneously over several months on storage in solution at $4^\circ C$. The spectral characteristics of (23) were identical to those quoted for isoaplysin²⁴ i.e. $[\alpha]_D^{25} - 45^\circ$ (c, 5.2 in $CHCl_3$); ν_{max} : 2950, 1620, 1595, 1500, 1270, 1260, 950 cm^{-1} ; $\lambda_{max,EtOH}$ 221 nm ($\epsilon = 7800$), 281 nm ($\epsilon = 3400$), 285 nm ($\epsilon = 3500$), 291 nm ($\epsilon = 3300$); EI ms: m/e 294/296 (M^+), Found m/e 294.06105, $C_{15}H_{19}BrO$ requires 294.06197; 1H n.m.r.: δ_H 1.12, d, 6 Hz, 3 H; 1.51, s, 3 H; 2.28, s, 3 H; 3.50, d, 12 Hz, 1 H; 3.65, d, 12 Hz, 1 H; 6.58, bs, 1 H; 6.65, d, 8 Hz, 1 H; 6.80, d, 8 Hz, 1 H; and a series of multiplets from δ_H 1.2 - 2.3, 5 H; ^{13}C n.m.r.: δ_C 158.7, s; 138.1, s; 132.9, s; 122.2, d; 121.4, d; 109.2, d;

97.1, s; 55.4, s; 43.6, d; 42.6, t; 34.5, t; 31.5, t; 22.8, q; 21.4, q; 13.7, q.

Isolation of α -bromocuparene (13), debromoisolaurinterol (21), debromoaplysin (7) and the polar non halogenated compound (24):

A sample of the non polar fraction (700 mg) was submitted to preparative t.l.c. and h.p.l.c. From this were isolated samples of α -bromocuparene (13), (60 mg), debromoisolaurinterol (21), (120 mg), debromoaplysin (7), (24 mg) and the polar non halogenated compound (24) (10 mg), along with additional (101), (240 mg).

α -bromocuparene (13): ν_{\max} : 2950, 1520, 1480, 1460, 1395, 1380, 1080, 1020 cm^{-1} ; $\lambda_{\max, \text{EtOH}}$ 220 nm ($\epsilon = 26,500$), 253 nm ($\epsilon = 150$), 259 nm ($\epsilon = 220$), 265 nm ($\epsilon = 300$), 274 nm ($\epsilon = 280$); EI ms: m/e 280/282 (M^+), 200, 185, Found 280.07884, $\text{C}_{15}\text{H}_{21}\text{Br}$ requires m/e 280.08270; ^1H n.m.r.: δ_{H} 0.61, s, 3 H; 1.08, s, 3 H; 1.40, s, 3 H; 2.30, s, 3 H; 4.05, dd, 8, 8 Hz, 1 H; 7.05, s, 4 H; and a series of multiplets δ_{H} 1.5 - 2.7, 4 H; ^1H n.m.r. (CCl_4): δ_{H} 0.60, s, 3 H; 1.06, s, 3 H; 1.40, s, 3 H; 2.28, s, 3 H; 4.0, dd, 8, 9 Hz, 1 H; 7.0, s, 4 H; and a series of multiplets δ_{H} 1.0 - 2.7, 4 H; ^{13}C n.m.r.: δ_{C} 144.0, s; 135.4, s; 128.2, d; 128.2, d; 127.3, d; 127.3, d; 62.0, d; 48.5, s; 47.5, s; 36.5, t; 33.2, t; 25.1, q; 22.5, q; 20.9, q; 20.7, q.

Debromoisolaurinterol (21): ν_{\max} : 3640, 3500, 2950, 1640, 1620, 1505, 1360, 1280, 1240, 1160, 1130, 950, 910 cm^{-1} ; ^1H n.m.r.: δ_{H} 1.20, d, 7 Hz, 3 H; 1.45, s, 3 H; 2.30, s, 3 H; 4.91, d, 2 Hz, 1 H; 5.05, d, 2 Hz, 1 H; 5.54, s, 1 H; 6.65, bs, 1 H; 6.70, bd, 8 Hz, 1 H; 7.20, d, 8 Hz, 1 H; and a series of multiplets δ_{H} 1.0 - 3.0, 5 H; ^{13}C n.m.r.: δ_{C} 165.7, 153.8, 137.7, 130.1, 127.7, 121.4, 118.8, 106.6, 49.9, 39.3, 37.8, 31.3, 27.9, 21.1, 20.7.

Debromoaplysin (7): ν_{\max} : 2950, 1620, 1595, 1500, 1270, 1260, 1120, 1010, 950 cm^{-1} ; EI ms: m/e 216 (M^+), 201, Found m/e 216.15328, $C_{15}H_{20}O$ requires m/e 216.15140; ^1H n.m.r.: δ_{H} 1.11, d, 6 Hz, 3 H; 1.28, s, 3 H; 1.32, s, 3 H; 2.27, s, 3 H; 6.50, bs, 1 H; 6.60, bd, 8 Hz, 1 H; 6.90, d, 8 Hz, 1 H; and a series of multiplets δ_{H} 1.6 - 2.5, 5 H; ^{13}C n.m.r.: δ_{C} 158.8, 137.8, 133.4, 122.6, 120.7, 109.5, 98.8, 54.0, 46.1, 42.6, 31.2, 23.5, 21.4, 20.0, 13.1.

Polar non halogenated compound (24): ν_{\max} : 2950, 1620, 1505, 1397, 1240, 1160, 1040 cm^{-1} ; $\lambda_{\max, \text{EtOH}}$ 206 nm ($\epsilon = 7800$), 278 nm ($\epsilon = 450$), 283 nm ($\epsilon = 500$); EI ms: m/e 216, 201, Found m/e 216.14772, $C_{15}H_{20}O$ requires m/e 216.15140; ^1H n.m.r.: δ_{H} 0.90, s, 3 H; 0.97, s, 3 H; 1.25, s, 3 H; 2.25, s, 3 H; 4.10, bd, 1 H; 6.45, bs, 1 H; 6.58, bd, 8 Hz, 1 H; 6.95, d, 8 Hz, 1 H; and a series of multiplets from δ_{H} 1.5 - 2.5, 4 H; ^{13}C n.m.r.: δ_{C} 152.3, 136.8, 130.4, 124.0, 120.6, 115.8, 86.1, 45.7, 41.3, 41.0, 30.0, 20.9, 20.4, 18.1, 14.8.

Isolation of cholesterol (88):

The midrange fraction (200 mg) was chromatographed on alumina (200 g). From this a sample of cholesterol (40 mg) was obtained and recrystallised from methanol. Melting point 146 - 147°. Literature melting point 148°.

Analysis of the Extract of Sample 1:

The crude extract (4.2 g) was chromatographed on alumina (400 g) to give fractions that had a combined weight of 1.9 g. The bulk of the material consisted of allolaurinterol (17), (1.2 g).

Allolaurinterol (17): ν_{\max} : 3650, 2950, 1650, 1610, 1490, 1450, 1390, 1360, 1230, 1150, 880 cm^{-1} ; EI ms: m/e 294/296 (M^+), 279/281, Found m/e 294.06054, $C_{15}H_{19}BrO$ requires 294.06197; ^1H n.m.r.: δ_{H} 0.71, d, 7 Hz, 3 H;

1.21, s, 3 H; 2.28, s, 3 H; 2.95, q, 7 Hz, 1 H; 4.9, bs, 1 H; 4.95, bs, 1 H, D₂O; 5.0, bs, 1 H; 6.65, s, 1 H; 7.25, 1 H, s; and a series of multiplets δ_{H} 1.5 - 2.6, 4 H; ¹³C n.m.r.: δ_{C} 157.9, 152.6, 136.3, 134.1, 131.9, 118.2, 115.5, 106.6, 48.5, 34.7, 27.9, 25.9, 22.3, 19.6.

The allolaurinterol cyclised spontaneously over several weeks to filiformin (29).

Filiformin (29): ¹H n.m.r.: δ_{H} 0.74, d, 7 Hz, 3 H; 1.30, s, 3 H; 1.36, s, 3 H; 2.25, s, 3 H; 6.57, s, 1 H; 7.17, s, 1 H; and a series of multiplets δ_{H} 1.5 - 2.5, 5 H; ¹³C n.m.r.: δ_{C} 152.3, 136.3, 130.1, 128.3, 117.4, 114.3, 85.3, 46.2, 44.7, 42.1, 37.2, 22.9, 22.4, 20.3, 7.3.

Isolation of Isolaurinterol (11):

A sample of the non polar fraction was chromatographed by h.p.l.c. A sample of isolaurinterol (11), (20 mg) was obtained, along with filiformin (29), (150 mg) and a cuparene-type compound (4 mg).

Isolaurinterol (11): ν_{max} : 3650, 3500, 3000, 1640, 1610, 1170, 910 cm⁻¹; ¹H n.m.r.: δ_{H} 1.20, d, 7 Hz, 3 H; 1.45, s, 3 H; 2.31, s, 3 H; 4.75, d, 2 Hz, 1 H; 5.05, d, 2 Hz, 1 H; 5.5, s, 1 H; 6.65, s, 1 H; 7.4, s, 1 H; and a series of multiplets δ_{H} 1.5 - 3.0, 5 H; ¹³C n.m.r.: δ_{C} 165.0, 153.0, 137.2, 132.8, 131.3, 120.5, 115.6, 107.0, 49.8, 39.2, 37.7, 31.3, 27.8, 22.3, 21.1.

Cuparene-type compound: ν_{max} : 2950, 1720, 1260, 1210, 910 cm⁻¹; $\lambda_{\text{max,EtOH}}$ 206, 232, 292, 298 nm; ¹H n.m.r.: δ_{H} 1.05, d, 7 Hz, 3 H; 1.45, s, 3 H; 2.30, s, 3 H; 3.75, bd, 5 Hz, 2 H; 6.6, bs, 1 H; 7.1, s, 1 H; 7.2, s, 1 H; ¹³C n.m.r.: δ_{C} 137.1, 126.4, 110.8, 100.3, 64.0, 54.6, 42.4, 31.7, 29.7, 23.1, 22.9, 13.8.

Isolation of Cholesterol (88) and Acetylenic Compound:

Preparative t.l.c. and h.p.l.c. of the midrange fractions resulted in the isolation of cholesterol (88) and an acetylenic compound (14 mg). The cholesterol was recrystallized from methanol to give white crystals. (20 mg). Melting point: 146 - 147°C. Literature melting point 148°C.

Acetylenic compound: ν_{\max} : 3650, 3350, 2950, 2100, 1215, 990, 650, 620 cm^{-1} ; $\lambda_{\max, \text{EtOH}}$ 238, 258 nm; ^{13}C n.m.r.: δ_{C} 141.1, 139.7, 134.0, 127.1, 123.9, 108.3, 83.5, 71.7, 35.2, 29.2, 27.4, 22.5, 14.0.

REFERENCES

1. Martin, D.F., "Marine Chemistry", Marcel Dekker Inc., New York, 1970, Vol. 2, p.1.
2. Wilson, T.R.S., "Salinity and the Major Elements of Seawater". In "Chemical Oceanography", 2nd edition, J.P. Riley and G. Skirrow (ed.), Academic Press, New York, 1975, Vol. 1., p. 365.
3. Harvey, H.W., "The Chemistry and Fertility of the Sea", Cambridge University Press, 1955, p.4.
4. Faulkner, D.J., and Anderson, R.J., "Natural Products Chemistry of the Marine Environment". In E.D. Goldberg (ed.), "The Sea", John Wiley and Sons, New York, 1974, p. 679.
5. Bold, H.C., and Wynne, M.J., "Introduction to the Algae", Prentice Hall Inc., New Jersey, 1978.
6. Williams, P.J. le B., "Biological and Chemical Aspects of Dissolved Organic Material In Seawater". In "Chemical Oceanography", 2nd edition, J.P. Riley and G. Skirrow (ed.), Academic Press, New York, 1975, Vol. 2, p. 301.
7. Thomson, R.H., J. Indian Chem. Soc., 1978, 55, 1209.
8. Faulkner, D.J., Tetrahedron, 1977, 33, 1421.
9. Fenical, W., J. Phycol., 1975, 11, 245.
10. Scheuer, P.J. (ed.), "Marine Natural Products", Academic Press Inc., New York, 1978, Volume 1, Chapters 2 and 3; Volume 2, Chapter 4. Volume 3, 1980. Volume 4, 1981.

11. Suzuki, M., Furusaki, A., Kurosawa, E., Tetrahedron, 1979, 35, 823.
12. Sims, J.J., Lin, G.H.Y., Wing, R.M., Tet. Letters, 1974, 3487.
13. Suzuki, T., Chem. Letters, 1980, No. 5, 541.
14. Howard, B.M., Fenical, W., J. Org. Chem., 1977, 42, 2518.
15. Kazlauskas, R., Murphy, P.T., Wells, R.J., Daly, J.J., Oberhänsli, W.E., Aust. J. Chem., 1977, 30, 2679.
16. Yamamura, S., Hirata, Y., Tetrahedron, 1963, 19, 1485.
17. Irie, T., Suzuki, M., Hayakawa, Y., Bull. Chem. Soc. Jpn., 1969, 42, 843.
18. Suzuki, M., Kurosawa, E., Bull. Chem. Soc. Jpn., 1979, 52, 3352.
19. Irie, T., Yasunari, Y., Suzuki, T., Imai, N., Kurosawa, E., Masamune, T., Tet. Letters, 1965, 3619.
20. Irie, T., Suzuki, T., Yasunari, Y., Kurosawa, E., Masamune, T., Tetrahedron, 1969, 25, 459.
21. Suzuki, T., Suzuki, M., Kurosawa, E., Tet. Letters, 1975, 3057.
22. Irie, T., Fukuzawa, A., Izawa, M., Kurosawa, E., Tet. Letters, 1969, 1343.
- 23.(a) Suzuki, M., Kurosawa, E., Tet. Letters, 1976, 4817.
(b) Suzuki, M., Kurosawa, E., Bull. Chem. Soc. Jpn., 1979, 52, 3349.
24. Suzuki, M., Kurosawa, E., Tet. Letters, 1978, 2503.
25. Kazlauskas, R., Murphy, P.T., Quinn, R.J., Wells, R.J., Aust. J. Chem., 1976, 29, 2533.

26. Wratten, S.J., Faulkner, D.J., *J. Org. Chem.*, 1977, 42, 3343.
27. Izac, R.R., Sims, J.J., *J.A.C.S.*, 1979, 101, 6136.
28. Izac, R.R., Drage, J.S., Sims, J.J., *Tet. Letters*, 1981, 1799.
29. McMillan, J.A., Paul, I.C., Caccamese, S., Rinehart, K.L., *Tet. Letters*, 1976, 4219.
30. Waraszkiewicz, S.M., Erickson, K.L., *Tet. Letters*, 1974, 2003.
31. Fenical, W., *Phytochemistry*, 1976, 15, 511.
32. Selover, S.J., Crews, P., *J. Org. Chem.*, 1980, 45, 69.
33. Howard, B.M., Fenical, W., *Tet. Letters*, 1976, 41.
34. Faulkner, D.J., *Phytochemistry*, 1976, 15, 1993.
35. González, A.G., Darias, J., Martín, J.D., Pérez, C., *Tet. Letters*, 1974, 1249.
36. Pettit, G.R., Herald, C.L., Allen, M.S., Von Dreele, R.B., Vanell, L.D., Kao, J.P.Y., Blake, W., *J.A.C.S.*, 1977, 99, 262.
37. Capon, R., Ghisalberti, E.L., Jefferies, P.R., Skelton, B.W., White, A.H., *Tetrahedron*, 1981, 37, 1613.
38. Sims, J.J., Lin, G.H.Y., Wing, R.M., Fenical, W., *Chem. Comm.*, 1973, 470.
39. Howard, B.M., Fenical, W., *J. Org. Chem.*, 1978, 43, 4401.
40. Howard, B.M., Fenical, W., *Tet. Letters*, 1978, 2453.
41. Blunt, J.W., Hartshorn, M.P., McLennan, T.J., Munro, M.H.G., Robinson, W.T., Yorke, S.C., *Tet. Letters*, 1978, 69.

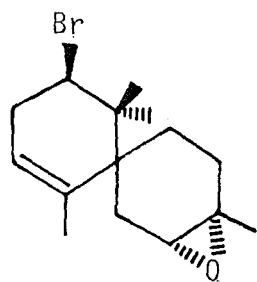
42. Carter, G.T., Rinehart, K.L., Li, L.H., Kuentzel, S.L., Connor, J.L., Tet. Letters, 1978, 4479.
43. Howard, B.M., Fenical, W., Arnold, E.V., Clardy, J., Tet. Letters, 1979, 2841.
44. Fenical, W., Schulte, G.R., Finer, J., Clardy, J., J. Org. Chem., 1978, 43, 3628.
45. Schmitz, F.J., Hollenbeak, K.H., Vanderah, D.J., Tetrahedron, 1978, 34, 2719.
46. (a) Irie, T., Suzuki, M., Masamune, T., Tetrahedron, 1968, 24, 4193; (b) Forbes Cameron, A., Cheung, K.K., Ferguson, G., Monteath Robertson, J., J. Chem. Soc. (B), 1969, 559.
47. (a) Irie, T., Izawa, M., Kurosawa, E., Tetrahedron, 1970, 26, 851; (b) Kurosawa, E., Furusaki, A., Izawa, M., Fukuzawa, A., Irie, T., Tet. Letters, 1973, 3857.
48. (a) Fukuzawa, A., Kurosawa, E., Irie, T., Tet. Letters, 1972, 3; (b) Furusaki, A., Kurosawa, E., Fukuzawa, A., Irie, T., Tet. Letters, 1973, 4579.
49. Kurosawa, E., Fukuzawa, A., Irie, T., Tet. Letters, 1972, 2121.
50. Kurosawa, E., Fukuzawa, A., Irie, T., Tet. Letters, 1973, 4135.
51. Fukuzawa, A., Kurosawa, E., Tet. Letters, 1980, 1471.
52. Fukuzawa, A., Kurosawa, E., Tet. Letters, 1979, 2797.
53. King, T.J., Imre, S., Oztunc, A., Thomson, R.H., Tet. Letters, 1979, 1453.

54. Howard, B.M., Schulte, G.R., Fenical, W., Solheim, B., Clardy, J., Tetrahedron, 1980, 36, 1747.
55. Caccamese, S., Azzolina, R., Duesler, E.N., Paul, I.C., Rinehart, K.L., Tet. Letters, 1980, 2299.
56. Falshaw, C.P., King, T.J., Imre, S., Islimyeli, S., Thomson, R.H., Tet. Letters, 1980, 4951.
57. (a) Fenical, W., Sims, J.J., Radlick, P., Tet. Letters, 1973, 313;
(b) Fenical, W., Gifkins, K.B., Clardy, J., Tet. Letters, 1974, 1507.
58. Fenical, W., Norris, J.N., J. Phycol., 1975, 11, 104.
59. Fenical, W., J.A.C.S., 1974, 96, 5580.
60. Howard, B.M., Fenical, W., Hirotsu, K., Solheim, B., Clardy, J., Tetrahedron, 1980, 36, 171.
61. White, R.H., Hager, L.P., Phytochemistry, 1978, 17, 939.
62. Suzuki, M., Kurosawa, E., Chem. Letters, 1980, 1177.
63. Suzuki, M., Kurosawa, E., Tet. Letters, 1981, 3853.
64. Kigoshi, H., Shizuri, Y., Niwa, H., Yamada, K., Tet. Letters, 1981, 4729.
65. Fukuzawa, A., Masamune, T., Tet. Letters, 1981, 4081.
66. Waraszkiewicz, S.M., Sun, H.H., Erickson, K.L., Finer, J., Clardy, J., J. Org. Chem., 1978, 43, 3194.
67. (a) Waraszkiewicz, S.M., Erickson, K.L., Tet. Letters, 1975, 281;

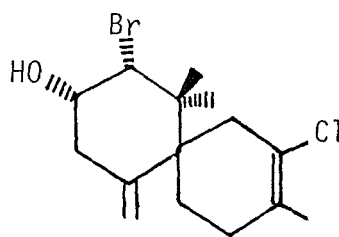
- (b) Waraszkiewicz, S.M., Erickson, K.L., Tet. Letters, 1976, 1443;
- (c) Waraszkiewicz, S.M., Erickson, K.L., Finer, J., Clardy, J., Tet. Letters, 1977, 2311; (d) Sun, H.H., Waraszkiewicz, S.M., Erickson, K.L., Tet. Letters, 1976, 585.
68. Sims, J.J., Fenical, W., Wing, R.M., Radlick, P., J.A.C.S., 1971, 93, 3774.
69. Sims, J.J., Fenical, W., Wing, R.M., Radlick, P., J.A.C.S., 1973, 95, 972.
70. Faulkner, D.J., Stallard, M.O., Ireland, C., Tet. Letters, 1974, 3571.
71. McDonald, F.J., Campbell, D.C., Vanderah, D.J., Schmitz, F.J., Washecheck, D.M., Burks, J.E., van der Helm, D., J. Org. Chem., 1975, 40, 665.
72. Vanderah, D.J., Schmitz, F.J., J. Org. Chem., 1976, 41, 3480.
73. Kinnel, R.B., Dieter, R.K., Meinwald, J., Van Engen, D., Clardy, J., Eisner, T., Stallard, M.O., Fenical, W., Proc. Natl. Acad. Sci. U.S.A., 1979, 76, 3576.
74. Irie, T., Suzuki, M., Kurosawa, E., Masamune, T., Tet. Letters, 1966, 1837.
75. Caccamese, S., Hager, L.P., Rinehart, K.L. Setzer, R.B., Botanica Marina, 1979, 22, 41.
76. Le Cocq, C., Lallemand, J., J.C.S. Chem. Comm., 1981, 150.
77. Shoolery, J.N., "Interface", Varian NMR Users Newsletter, 1981, Vol. 8.

78. Wehrli, F.W., Wirthlin, T., "Interpretation of Carbon-13 NMR Spectra", Heyden, London, 1978, p. 76.
79. Yorke, S.C., M.Sc. thesis, University of Canterbury, 1977.
80. Parsons, M.J., personal communication.
81. Scott, A.I., "Interpretation of the Ultraviolet Spectra of Natural Products", Pergamon Press, Oxford, 1964.
82. Haasnoot, C.A.G., De Leeuw, F.A.A.M., Altona, C., Tetrahedron, 1980, 36, 2783.
83. Jankowski, K., Bélanger, J., Söler, F., Zamojski, A., Org. Magn. Reson., 1979, 12, 544.
84. Pouchert, C.J., Campbell, J.R., "The Aldrich Library of NMR Spectra", 1974, Vol. 10, Spectrum 100D.
85. Blunt, J.W., Stothers, J.B., Org. Magn. Reson., 1977, 9, 439.
86. Ackman, R.G., "Gas-Liquid Chromatography of Fatty Acids and Esters". In Lowenstein, J.M. (ed.), "Methods in Enzymology", Vol. XIV, Academic Press, New York, 1969, p. 329.
87. Fawcett, R.W., Harris, J.O., J. Chem. Soc., 1954, 157, 2673.
88. Pesch, E., Friess, S.L., J.A.C.S., 1950, 72, 5756.
89. Williams, D.H., Fleming, I., "Spectroscopic Methods in Organic Chemistry", McGraw-Hill, England, 1973, Chapter 1.
90. Irie, T., Suzuki, M., Kurosawa, E., Masamune, T., Tetrahedron, 1970, 26, 3271.

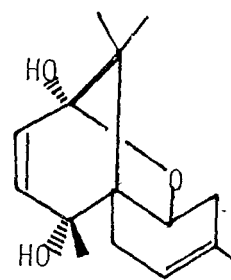
91. Howard, B.M., Nonomura, A.M., Fenical, W., Biochemical Systematics and Ecology, 1980, 8, 329.
92. Packter, N.M., "Biosynthesis of Acetate Derived Compounds", John Wiley and Sons, London, 1973.
93. Lehninger, A.L., "Biochemistry", Worth Publishers Inc., 1975.
94. Geissman, T.A., Crout, D.H.G., "Organic Chemistry of Secondary Plant Metabolism", Freeman, Cooper and Company, San Francisco, California, 1969.
95. Professor Sir Ewart Jones, Chemistry in Britain, 1966, 2, 6.
96. Blunt, J.W., Lake, R.J., Munro, M.H.G., Yorke, S.C., Aust. J. Chem., 1981, 34, 2393.



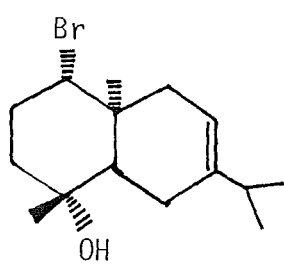
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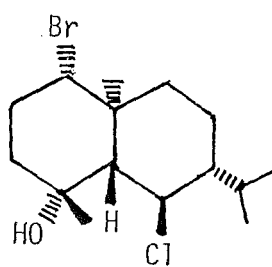
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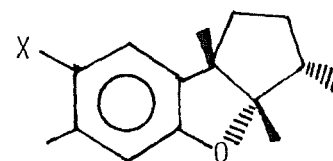
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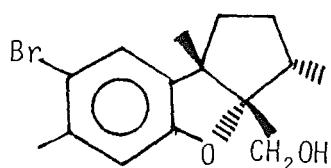
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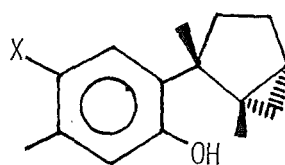
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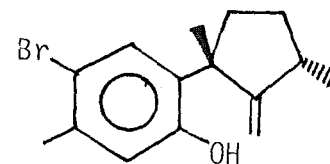
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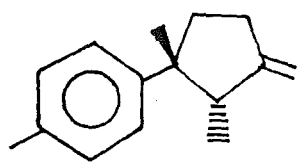
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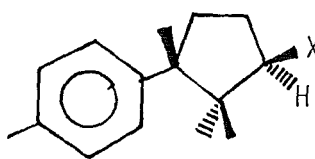
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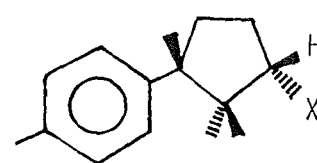
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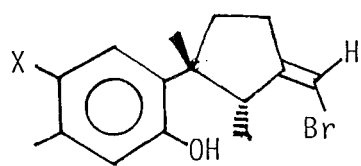
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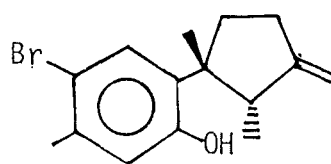
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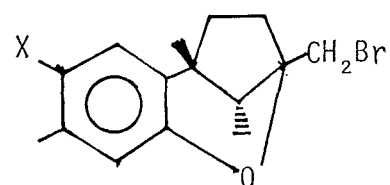
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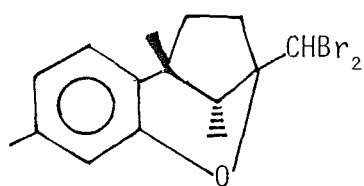
(15) X=H (16) X=Br



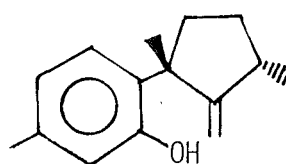
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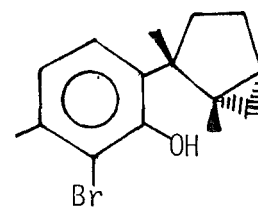
(18) X=Br (20) X=H



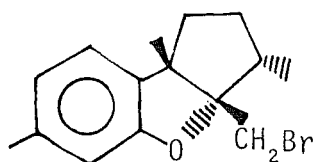
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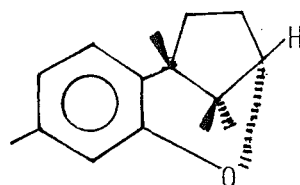
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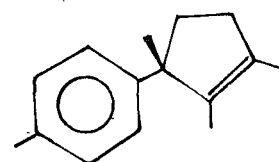
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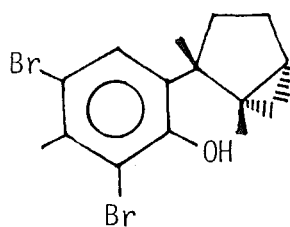
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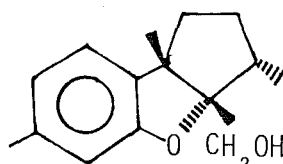
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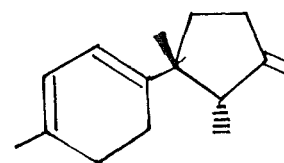
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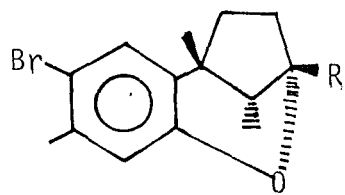
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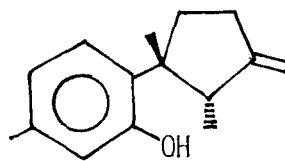
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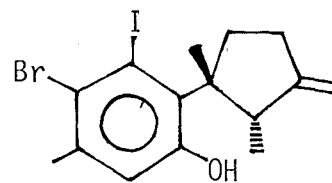
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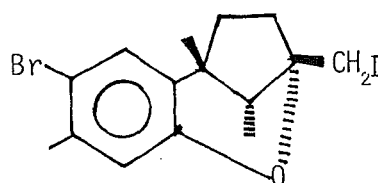
(29) R=CH₃ (30) R=CH₂OH.



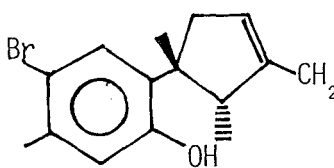
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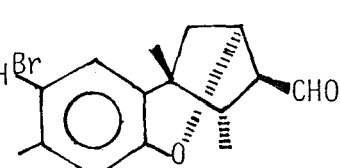
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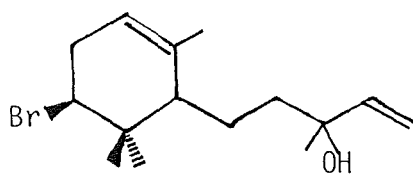
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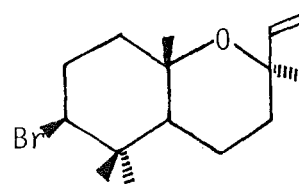
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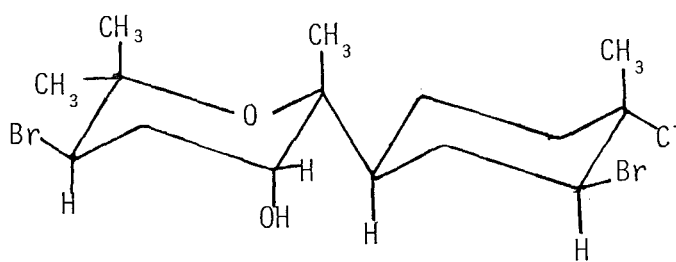
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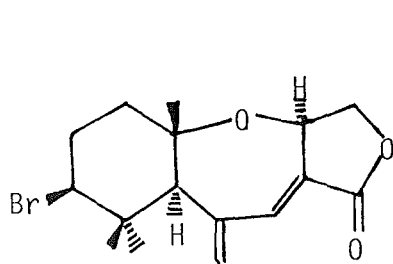
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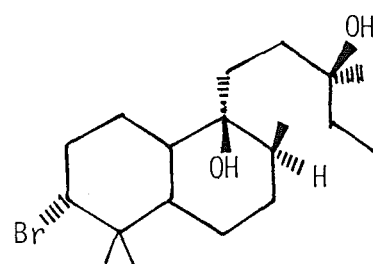
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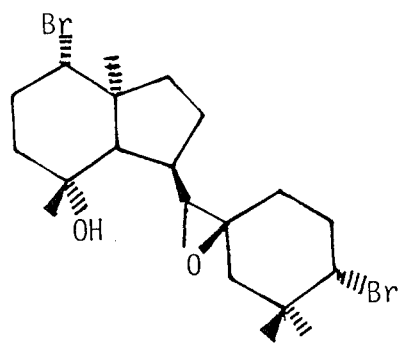
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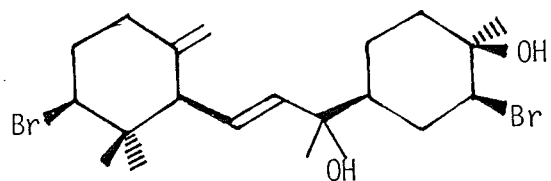
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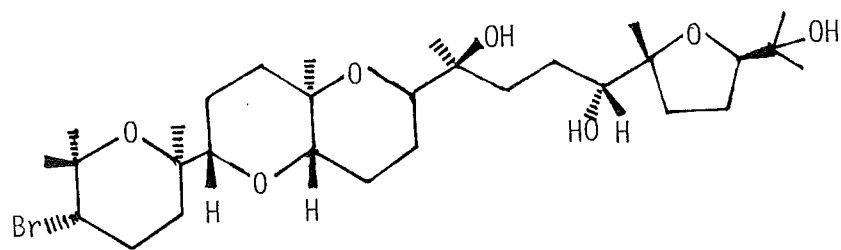
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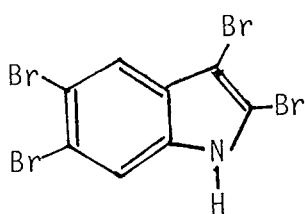
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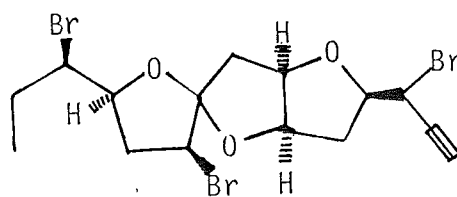
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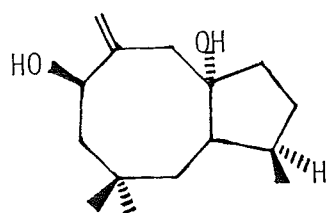
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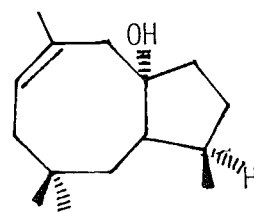
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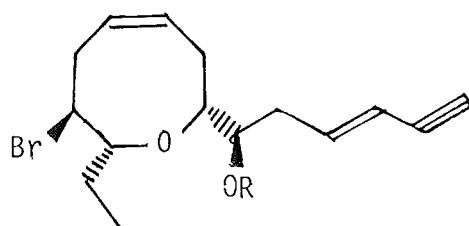
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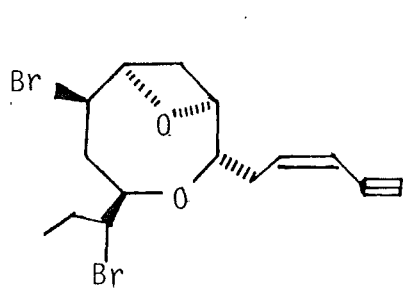
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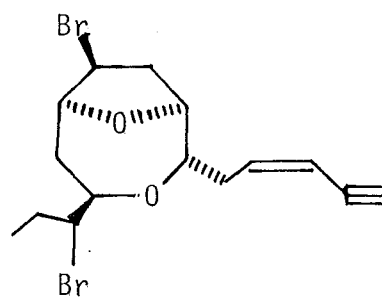
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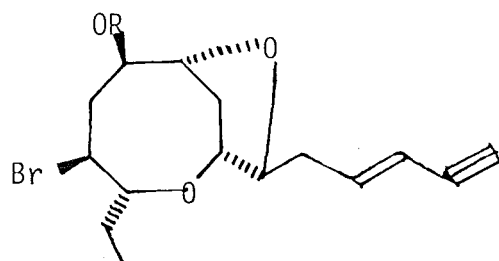
(48) R = Ac (48a) R = H



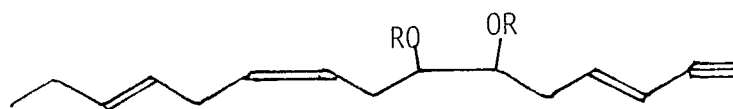
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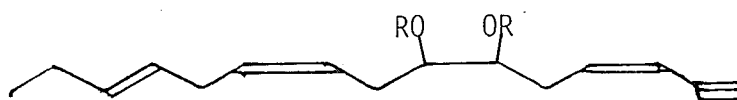
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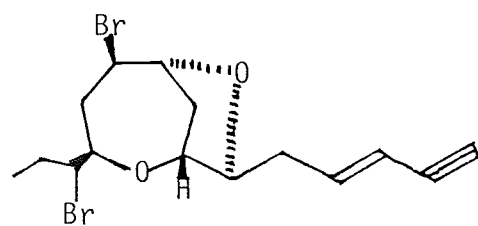
(51) R = H (51a) R = Ac



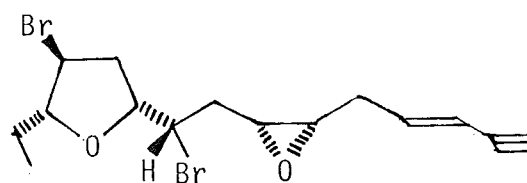
(52a) R = H (52c) R = Ac



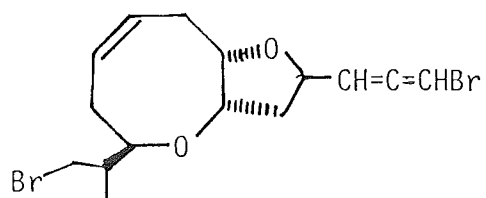
(52b) R = H (52d) R = Ac



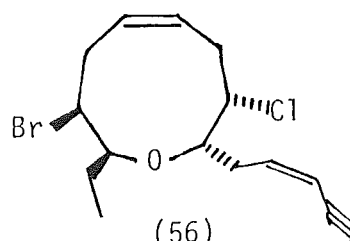
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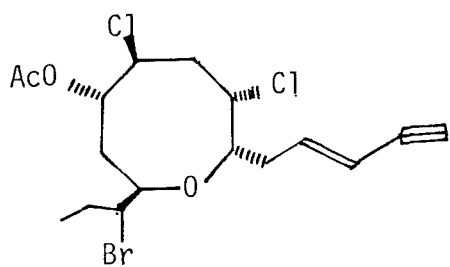
(54)



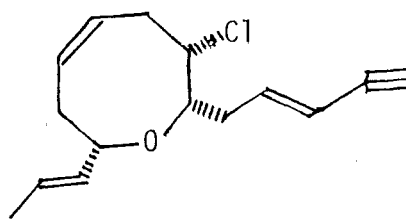
(55)



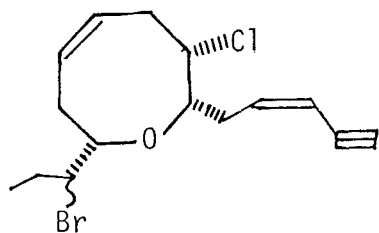
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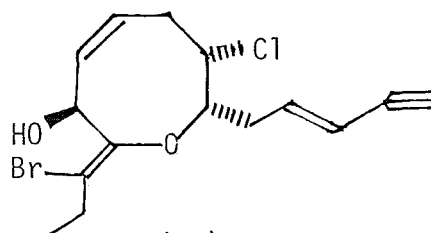
(57)



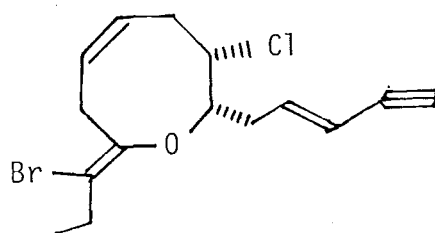
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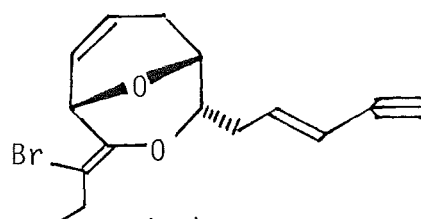
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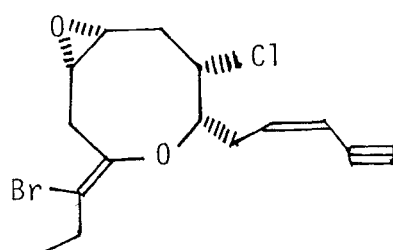
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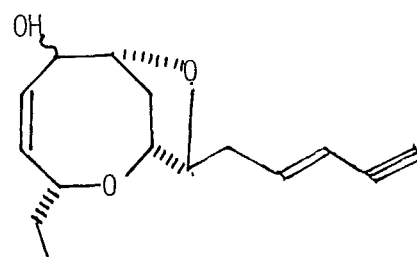
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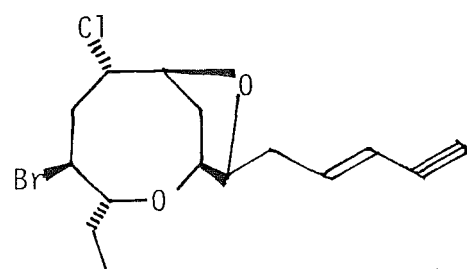
(62)



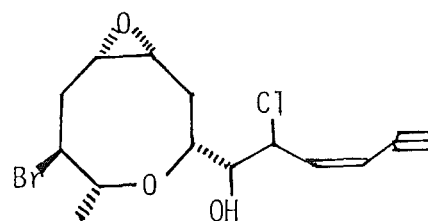
(63)



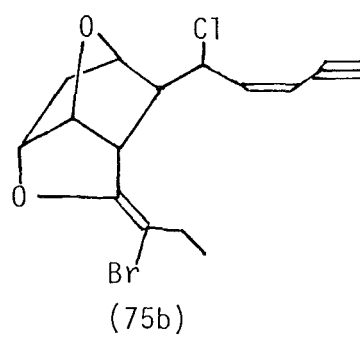
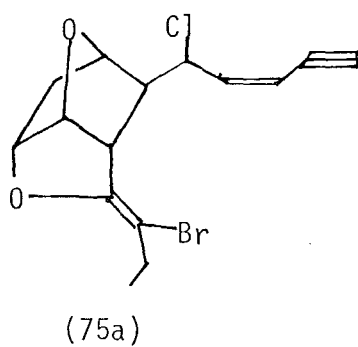
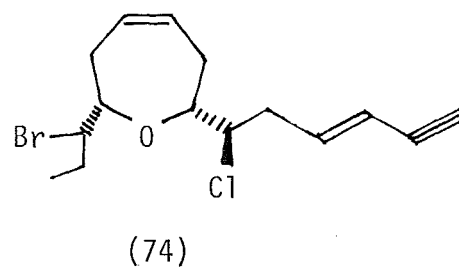
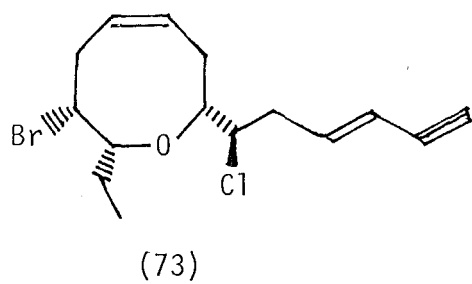
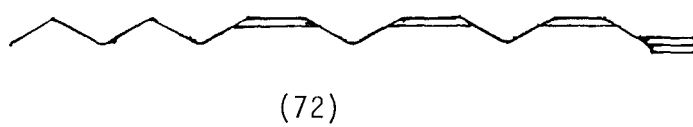
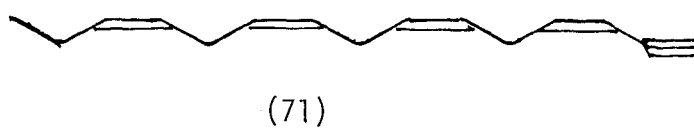
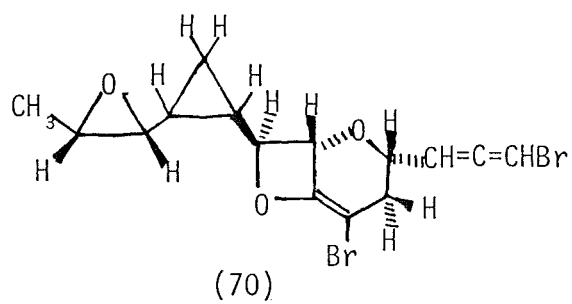
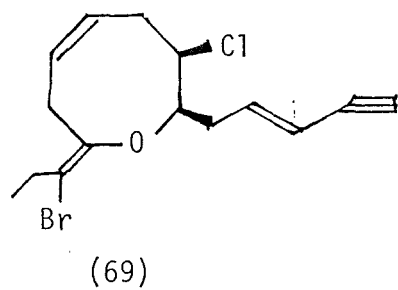
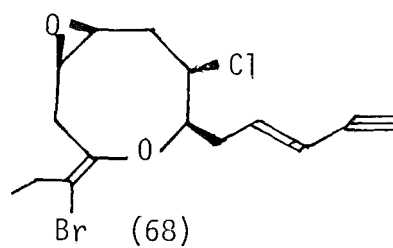
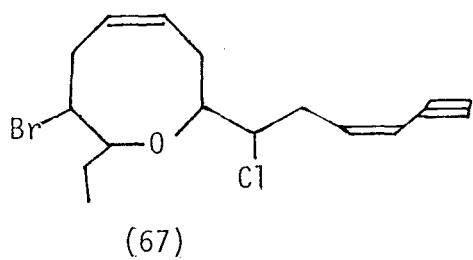
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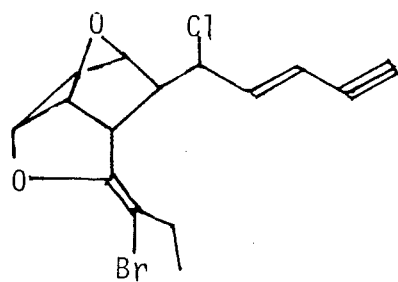


(65)

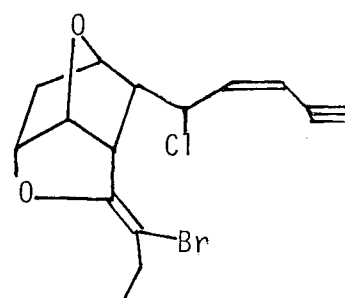


(66)

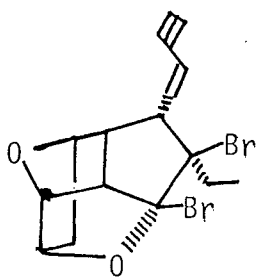




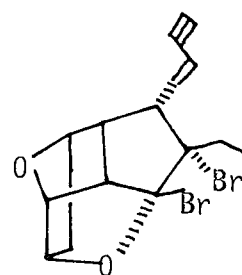
(75c)



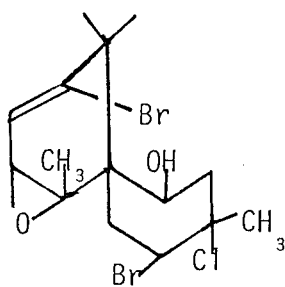
(75d)



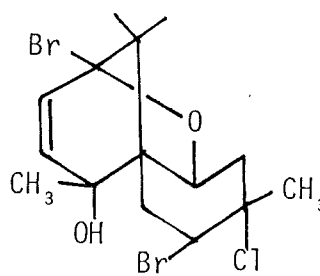
(75e)



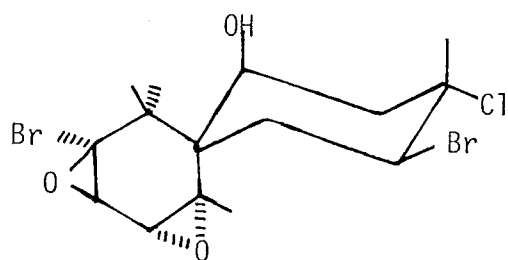
(75f)



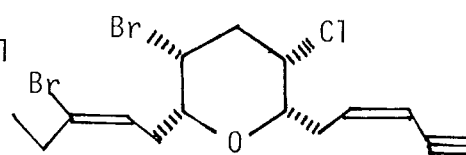
(76)



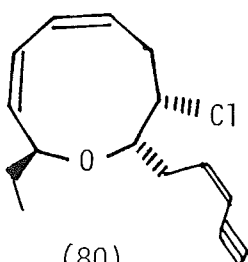
(77)



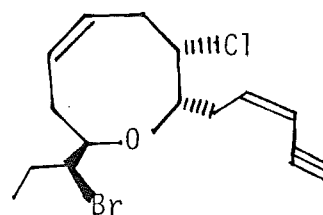
(78)



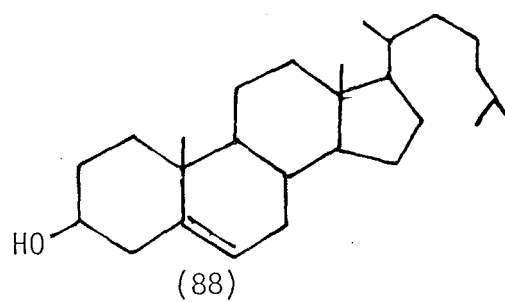
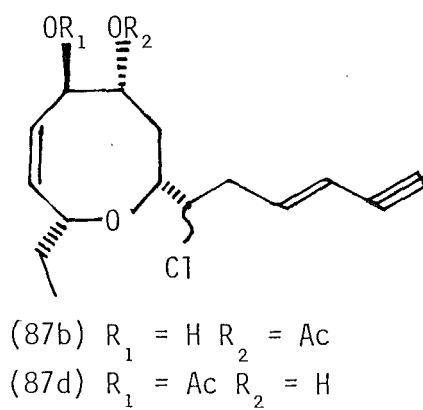
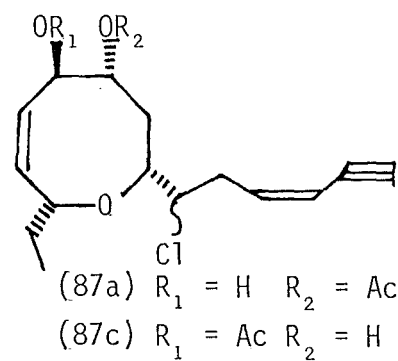
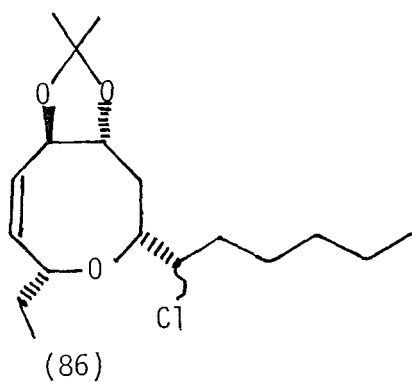
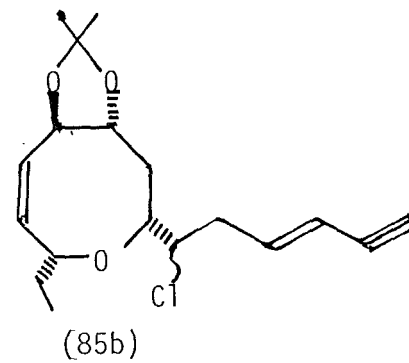
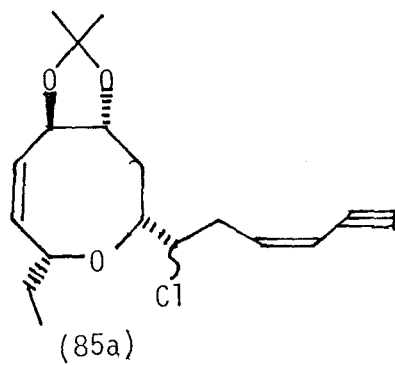
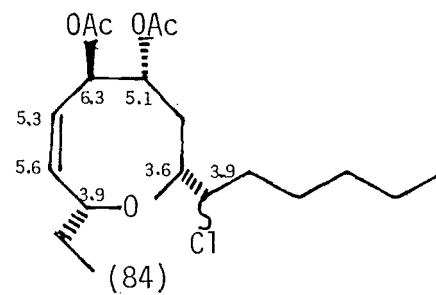
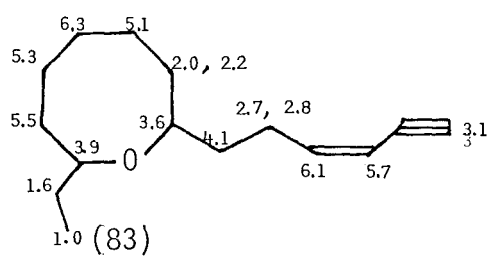
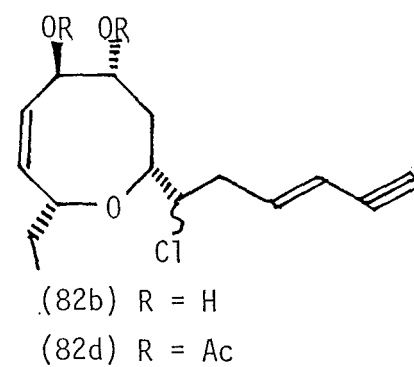
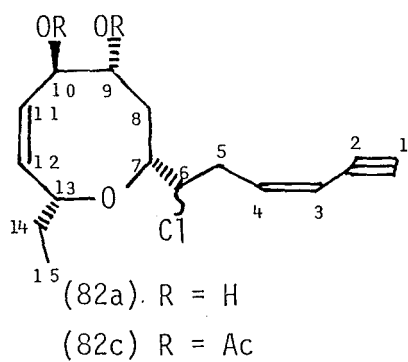
(79)

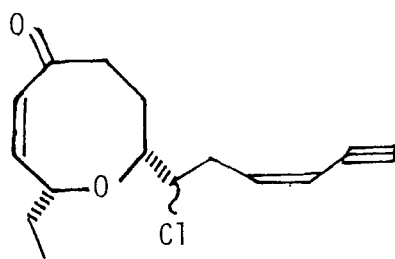


(80)

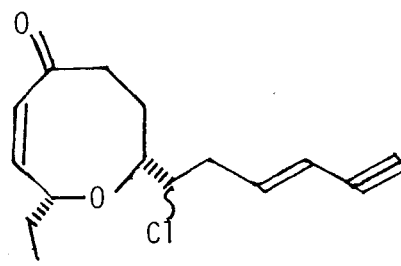


(81)

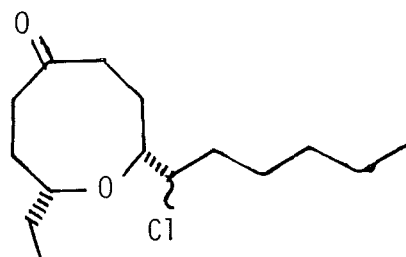




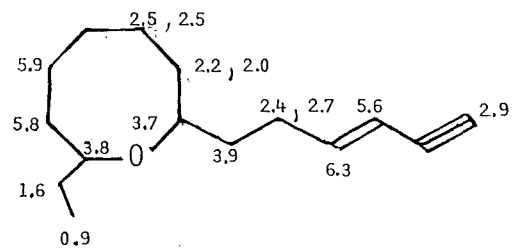
(89a)



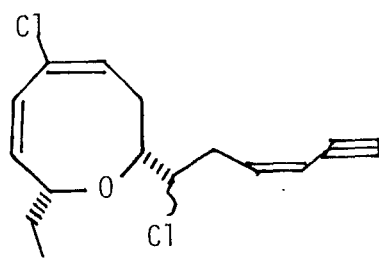
(89b)



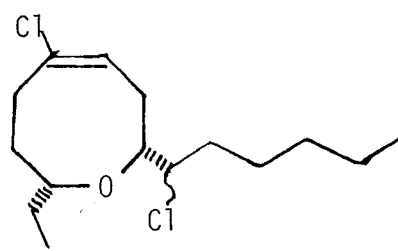
(90)



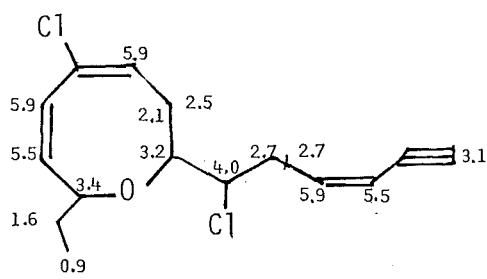
(91)



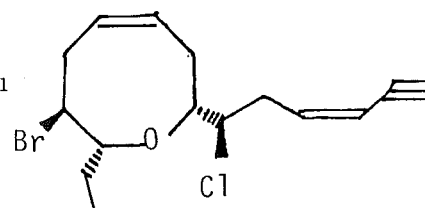
(92)



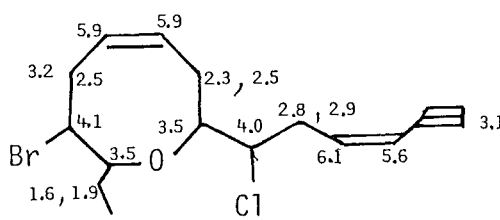
(93)



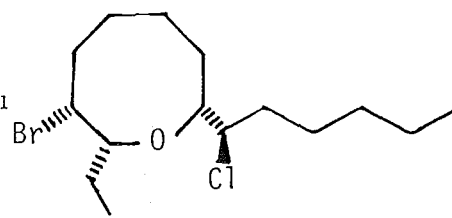
(94)



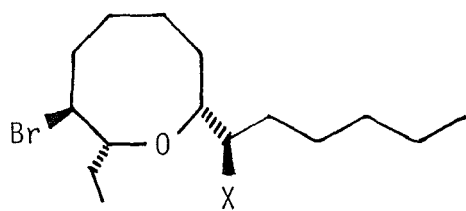
(95)



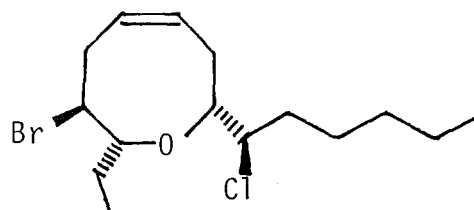
(96)



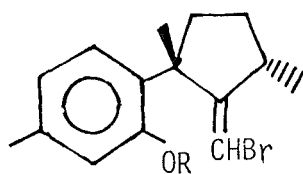
(97)



(98) X = Cl (99) X = OH

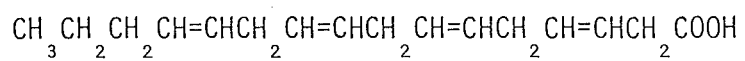


(100)

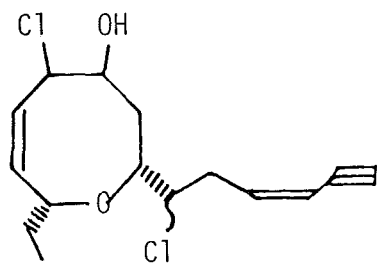


(101) R = H

(101a) R = Ac



(102)



(103)