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STUDIES OF AUSTRALIAN SOFT CORALS—XXXV

THE TERPENOID CHEMISTRY OF SOFT CORALS AND ITS IMPLICATIONS

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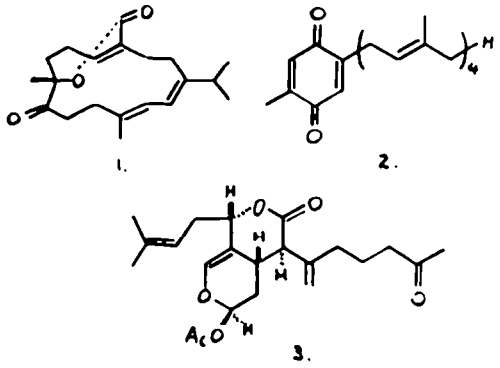
Abstract—The *in vivo* incorporation of mevalonolactone specifically into the terpene portion of a sesquiterpene hydroquinone is reported for the soft coral *Sinularia capillosa*. *Eudesma*-4,7(11)-*diene*-8β-*ol* (**20**) and the corresponding 8-keto derivative (**21**) have been isolated from the soft coral *Nephthea* species for the first time. The aeolid nudibranch *Phyllodesmium longicirra* was collected when feeding on the soft coral *Sarcophyton trocheliophorum*, and the known diterpene trocheliophorol (**25**) was found to be concentrated in the cerata of the nudibranch. The absolute stereochemistry of trocheliophorol (**25**) has been rigorously determined, and shown to be that previously assigned on spectroscopic grounds.

INTRODUCTION

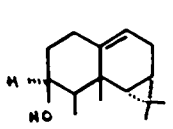
Soft corals. Soft corals are a group of colonial invertebrates belonging to the Phylum Coelenterata (Cnidaria), Class Anthozoa, Subclass Octocorallia.[1] Octocorals derive their name from the fact that each autozooid polyp of a colony has *eight* pinnate tentacles which can be used to capture small food particles usually in the form of marine zooplankton. Soft corals are thus carnivores, and the presence of the eight pinnate tentacles is the most reliable field test that the organism being collected is an octocoral.[2] Within the Octocorallia, the major distinctions are between the orders Alcyonacea or soft corals and the Gorgonacea or horny corals, although other orders, e.g. *Stolonifera* and *Heliopora* (blue hard coral) do exist. This article deals with the chemistry of Alcyonacean soft corals.

Symbiosis. The picture so far presented of soft corals is overly simplified. As well as being capable of ingesting small zooplankton,[3] soft corals contain within their tissues symbiotic algae or zooxanthellae which are capable of photosynthesis. Thus a soft coral can live for long periods of time in filtered sea water in the presence of sunlight, but many corals will die if deprived of light.[4] The zooxanthellae are believed to be the symbiotic form of the dinoflagellate alga *Symbiodinium microadriaticum* Freudenthal (= *Gymnodinium microadriaticum* = *Zooxanthella microadriatica*).[5] Because many soft corals were found to contain terpenes and because terpenes are usually plant metabolites, an early suggestion was made that soft coral terpenes may have been produced by zooxanthellae. Evidence is now building up which suggests that the algae are not responsible for terpene biosynthesis in soft corals. They provide the energy for this synthesis, but axenic cultures of zooxanthellae from terpene producing gorgonians yielded only sterols (and lipids).[6] Furthermore, aposymbiotic corals, i.e. those which have no zooxanthellae, have been reported to contain or elaborate terpenes.[7]

Terpenes. Alcyonarian soft corals have produced a vast range of terpenoid metabolites[8,9] including sesquiterpenes and diterpenes. The volatile sesquiterpene hydrocarbon distributions in some common soft coral species have shown value as taxonomic makers.[10] By far the most common terpenes reported from soft corals belong to the diterpene class,[8,9,11] and of these, cembranoid diterpenes are probably the most common. As chemical research moves from the widely distributed Family Alcyoniidae; (in which cembranoid diterpenes are the norm); to the less common Nephtheidae and Xeniidae, one encounters fewer cembranoid systems, as e.g. **1**,[12] but more often other diterpenes, as e.g. **2** from Nephtheidae[13] and **3** from Xeniidae.[14]



Role of terpenes? The range and variety, and in many cases the sheer quantity of terpenes in soft corals led a number of chemists to ask the question why they are there. As examples, the aristolane-based sesquiterpene alcohol **4** and ketone **5** were present in 3 and 1.5% of the weight of the soft coral *Lemnalia humesi after freeze-drying*. These compounds were visible as crystals on the dried coral tissue, and their volatility was such that this reported percentage was probably only a small proportion of the amount actually in the coral before freeze-drying.[15] A second pair of examples relate to the presence of the cembranolide diterpene **6** in 5% of the dry weight of the soft coral *Lobophytum crassospiculatum*[16] and the compound isosarcophytoxide **7** isolated as 3% of the dry weight of the soft coral *Sarcophyton* sp.[17]



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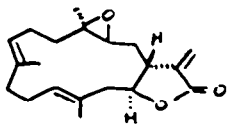

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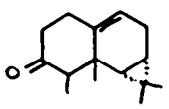

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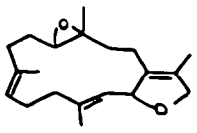
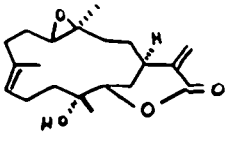


7.

Each of these examples implies considerable synthetic activity on the part of the soft coral in making compounds for which no role had been determined. Tursch *et al.*[8] in their review of coelenterate chemistry suggested that the terpenes many of which were ichthyotoxic, had both a defensive role and an antifeedant–feeding deterrency role. We followed this suggestion by an extensive series of studies on the ichthyotoxicity of aqueous soft coral extracts that contained terpenes and found that only about 50% of common exposed soft corals were toxic.[18] An extension of this study to feeding deterrency showed that half of the non-toxic soft coral extracts possessed significant antifeedant activity.[19] Furthermore we have shown an inverse correlation between physical defence and toxicity.[19] We have thus added extensive statistical support to the preliminary studies and suggestions of the Belgian workers.

A further implication of the presence of large quantities of toxic terpenes in soft corals is that they should be released into the water column surrounding the soft coral colony and provide a competitive advantage against any organism growing in the vicinity of the soft coral. We have been able to demonstrate the presence of toxic terpenes in the water surrounding several soft corals by an underwater sampling device developed for the purpose.[20] Furthermore, we have recorded numerous field examples which show that toxic soft corals retard the growth of neighbouring hard corals; we have also carried out experiments to verify this allelopathic effect: the deleterious effects of water-borne terpenoid toxins on neighbours.[21] Further extensions of this work have shown that certain soft coral toxins, esp. flexibilide (**8**) from *Sinularia flexibilis*[22] and the diterpene **9** from *Lobophytum pauciflorum*,[23] can kill the scleractinian (hard) corals *Acropora formosa* and *Porites andrewsii* at between 2 and 5 ppm.[24] Other experiments with sublethal amounts of **8**, **9** and the furanoquinol **10** from *Sinularia capillosa* showed significant effects photosynthesis and respiration in the hard coral *Acropora formosa*.[25]



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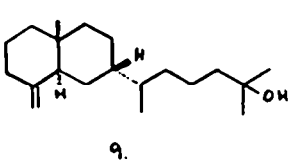

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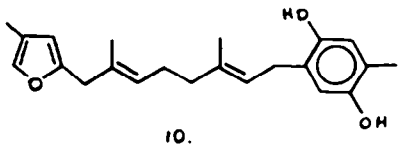
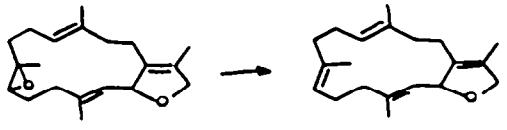


10.

It thus seems clear that the presence of terpenes provides a significant evolutionary advantage to soft corals both in defense and competition. Preliminary surveys of a limited number of hard corals show that, although they are rich in lipids, they do not contain appreciable quantities of terpenes. Both hard and soft corals are able to calcify; hard corals produce elaborate aragonitic skeletons while soft corals use calcitic sclerites to make the colony more rigid, to defend the polyps, to strengthen the cortical layer, to prevent collapse of the hydrostatic canals and for a range of other uses only now becoming evident. It appears that the older[26] octocorals adopted chemical defense as a strategy; only two members of the subclass have a hard calcareous skeleton, *Heliopora* sp. and *Tubipora musica*. All the rest are relatively soft and fleshy. Hard coral polyps put much energy into production of their skeletons, which provide a substantive defensive attribute.

Predator-prey relationships. As is common in biological systems, especially those as diverse as the Great Barrier Reef, specialist feeders can ingest large quantities of otherwise toxic metabolites in order to gain the benefits of an exclusive food source.[27] The mollusc *Ovula ovum* has such a relationship with soft corals, and we have recently reported the ingestion and transformation of the toxic terpene sarcophytoxide **11** into the less toxic 7,8-deoxysarcophytoxide **12**.[28]



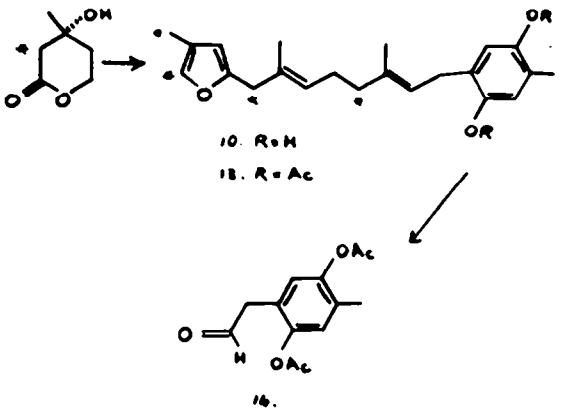
11. 12.

With this overview of the nature and origin of terpenes in soft corals and their likely roles in reef ecology, we will present some of our recent results in these respective areas.

RECENT RESULTS

Terpene biosynthesis. In the course of experiments to determine the contributions made by each partner in the soft coral–zooxanthellae symbiosis, we obtained rather high incorporation of mevalonolactone in a quinolated sesquiterpene **10** using an intact soft coral, and demonstrated the specificity of this incorporation into the sesquiterpene portion of the molecule by degradation.

Incubation of small colonies of *Sinularia capillosa*, freshly collected from Magnetic Island, with 2-^{3}H mevalonolactone and sodium [^{14}C]bicarbonate afforded, after extraction and chromatography, the crystalline furanoquinol **10**.[25] This had a specific activity of 2600 dpm/mg of tritium, and no C-14 label was associated with the sesquiterpene **10**. The combined lipid components from other fractions of the chromatography contained a total of 10^7 dpm of C-14, confirming considerable photosynthetic activity. Subsequent incubations showed wide variations in specific activity of the furanoquinol **10**, the highest value obtained was 50,000 dpm/mg. Based on this



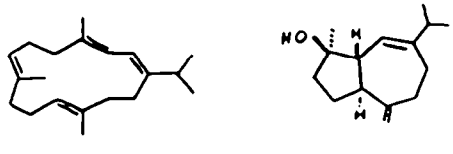
Scheme 1.

figure approximately 1% of the available tritium was incorporated into the sesquiterpene **10**.

Because only the sesquiterpene portion of the furanoquinol **10** would be expected to derive from mevalonate (Scheme 1,* indicates the sites of tritiation), it was decided to confirm the specificity of this incorporation. A sample of [^{3}H]furanoquinol **10** was diluted with cold material and acetylated to give a diacetate with a specific activity of 160 dpm/mg. The diacetate **13** was degraded with osmium tetroxide–sodium metaperiodate in dioxan[29] to afford after chromatography, the aldehyde **14** with specific activity 4.6 dpm/mg. Were the label uniformly distributed over all H atoms in the furanoquinol **10**, the aldehyde should have a specific activity of 46 dpm/mg; if distributed as *per* Scheme 1, the value should be zero.

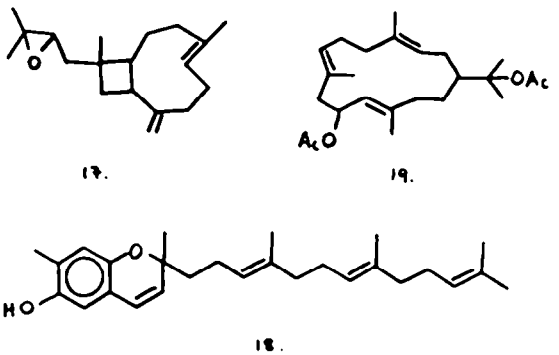
The experiment confirms the *in vivo* incorporation of mevalonolactone into terpenes in soft corals, and shows that in molecules of mixed biosynthetic origins, radioactivity derived from mevalonolactone is localised in the terpene portion of the molecule.

Novel sesquiterpenes from a Nephthea *species*. The genus *Nephthea* appears to be one of the more versatile in the field of terpene biosynthesis. Thus from one specimen of *Nephthea chabrolii*, we isolated the known cembranoid diterpene, isoneocembrane-A (= cembrane-C) **15** and a novel guaiane-based sesquiterpene **16**.[30] The cooccurrence of sesquiterpenes and diterpenes in the same species is not common.[8]



15 16

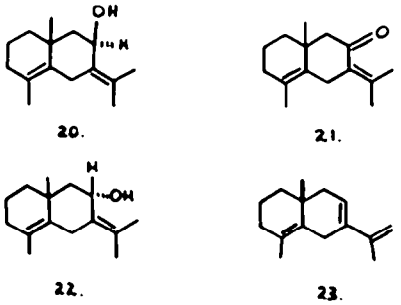
Another *Nephthea* species contained caryophyllene-based diterpenes (e.g. **17**)[31] of a type previously reported from the genus *Xenia*.[32] From another *Nephthea* species we isolated several tetraprenylbenzoquinone derivatives (e.g. **2,18**)[13] with the unusual 2,5-dialkyl substitution pattern previouly restricted to terrestrial isolates.[33] Finally, specimens of *Nephthea brassica* have afforded ten different cembranoid diterpenes, five of which (e.g. **19**) were novel at the time they were reported.[34]



17. 19.

18.

Examination of the soft coral *Nephthea sp.*,† which was one of the toxic soft corals tested in an earlier study,[18] revealed the presence of two novel sesquiterpenes: an alcohol **20** and the corresponding ketone **21**.



20. 21.

22. 23.

The molecular formula of the alcohol **20**, $C_{15}H_{24}O$ required four double bond equivalents and the ^{13}C NMR spectrum contained four singlets in the range 120–135 ppm for sp^2 C atoms. The molecule was bicyclic; the presence of an alcohol function was indicated by the IR spectrum (ν_{max} 3350 cm^{-1}) and shown to be secondary by the presence of a doublet at 67.6 ppm and a one proton multiplet ($W_{h/2}$ = 8 Hz) at δ 4.84. This proton was clearly allylic. The ^{1}H NMR spectrum of **20** contained four Me resonances (δ1.70, 1.80, 1.85 and 1.36), the first three were clearly vinylic, but the fourth had to be assigned to an allylic bridgehead Me. This would be expected to resonate at *ca* δ1.0. In the ketone **21**, the most shielded methyl resonated at δ1.1; when the ketone **21** was reduced to give an inseparable mixture of **20** and its epimer **22**, the bridgehead methyl in the latter resonated at δ0.98. Clearly, the 1,3-diaxial relationship between the alcohol and the bridgehead Me in **20** deshields the Me. This phenomenon is not uncommon and many examples exist in the steroid field.[35] This provides very strong evidence for the stereochemistry proposed for structure **20**. The ^{1}H NMR spectrum of the alcohol **20** contained an *AB* pattern (δ2.80, 3.42, J = 16 Hz) which was attributed to a pair of doubly allylic methylene protons. The signal at δ4.84 collapsed to a sharp singlet ($W_{h/2}$ = 5 Hz) on irradiation at δ1.93. This same irradiation simultaneously reduced long range couplings from the more upfield broad doublet of the *AB*

†Tentative classification: Registered sample number G12654 Queensland Museum, Gregory Terrace, Brisbane 4000 Qld., Australia

quartet at δ2.80. The ^{1}H NMR spectrum of the ketone **21** contained a corresponding *AB* quartet at δ3.04, 3.40 (J = 18 Hz) and a sharp two proton singlet at δ 2.30 ascribed to the methylene now α to the ketone. Two methyl resonances (δ 2.12, 1.90) were now on a conjugated enone system and the remaining vinyl Me resonated at δ 1.70. These spectral characteristics were accommodated by a eudesmane-based sesquiterpene (e.g. **20**).

In order to prove that a eudesmane skeleton was involved, elimination of the OH group in **20** was achieved, and a conjugated diene resulted **23**. Proton loss had occurred from one of the exocyclic isopropylidine Me groups and not from the endocyclic methylene adjacent to the OH group. The elimination product contained three vinyl protons: a methylene group at δ4.87, 5.05 and a conjugated vinyl proton at δ 5.87. An *AB* quartet at δ 2.84, 3.51 (J = 16 Hz) indicated the persistence of the doubly allylic methlene group. Three Me resonances (δ 1.95, 1.68 and 1.02) were assigned to a Me on the conjugated diene system, the vinyl Me and a bridgehead Me.

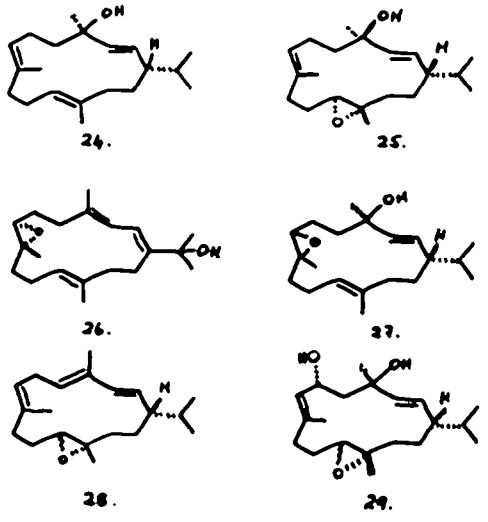
Hydrogenolysis of the alcohol **20** afforded a mixture which was analysed by GC-MS. The two major products were identical by GC-MS with the products from hydrogenation of β-selinene, thus confirming the presence of the eudesmane ring system in **20** and **21**.

Predator-prey relationships

Phyllodesmium longicirra and Sarcophyton trocheliophorum. Stephane La Barre, the ecologist in our group, chanced to observe several aeolid nudibranchs, *Phyllodesmium longicirra* eating the soft coral *Sarcophyton trocheliophorum* in one of the bays on Orpheus Island, Palm Island Group, about 80 km northwest of Townsville. The predators and prey were transported alive and intact to the University where they were maintained in contact for several days. The nudibranchs continued to eat the soft coral although they did not thrive in captivity, and lost a number of cerata, which are the long extensions of the bodywall of the animal. These were preserved frozen and subjected to chemical analysis after freeze-drying. Several days later, more cerata were shed, and it became obvious that the two specimens were dying. One was preserved as a museum specimen while the second was subjected to freeze-drying and chemical analysis. TLC analysis of the dichloromethane extracts of the cerata, the bodywall and the soft coral (Fig. 1) revealed that the terpenoid constituents in the cerata were derived from the coral: the two extracts were qualitatively identical by TLC. However, the extracts of bodywall and cerata were made on identical weights of tissue, clearly the terpenes derived from the soft coral are concentrated in the cerata.

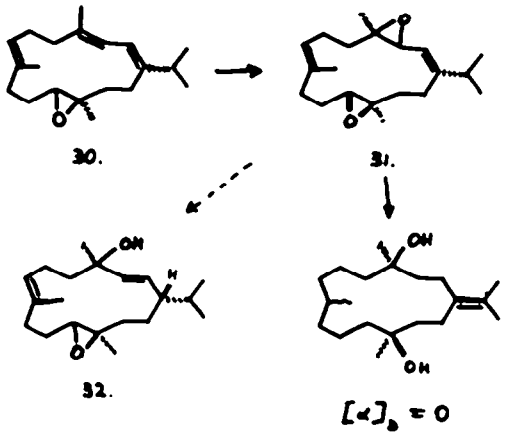
This finding is consistent with suggestions in the literature. Some aeolids feed extensively and perhaps exclusively on soft corals and their colour and shape often make them well camouflaged when on their soft coral prey. Most aeolid nudibranchs feed on hydrozoan or anthozoan coelenterates and store the stinging nematocysts from the coelenterates in sacs at the tips of their cerata—dorsal outgrowths of the bodywall containing the digestive gland. The nematocysts can be discharged by the aeolid in defence when attacked by a predator. However, the alcyonarian feeding aeolids do not store nematocysts, and Rudman[36] has suggested that a glandular terminal sac may contain defensive substances derived from the soft-corals. The cerata are shed when the animal is distressed, and if tasted by a potential predator, the presence of soft coral toxins would render the ceras unpalatable (if not toxic), and would deter the would-be predator from attacking the whole aeolid.

The major metabolites in the soft coral and in the nudibranch were shown to be (+)-thunbergol **24**,[37] a (+)-epoxythunbergol **25**, and the known diterpene alcohol **26**.[38] Because there were two possible epoxythunbergols, **25** and **27**, it was necessary to determine which compound was present. Reductive elimination of the epoxide group using a Zn–Cu couple[39] afforded (+)-thunbergol **24**, while elimination of the tertiary alcohol function in **25** afforded **28**. Compound **28** was subjected to double resonance experiments, which showed that the newly generated vinyl proton was adjacent to a doubly allylic methylene group. The epoxythunbergol was thus the known compound (+)-trocheliophorol **25**.[40] In three earlier reviews, this structure was incorrectly drawn,[8,38,41] and although it was correctly specified in the primary source,[40] the absolute configuration about the epoxide group was not determined.



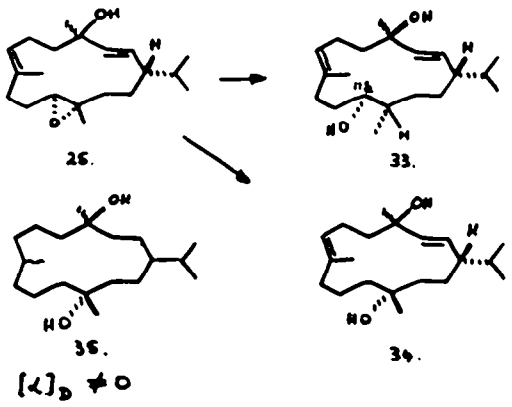
In a subsequent report, Kashman[42] assigned absolute configuration to (+)-trocheliophorol as shown in structure **25**. On the basis of ^{13}C NMR spectroscopic comparison with a diterpene diol epoxide **29** possessing many features in common with trocheliophorol **25**, the epoxide and tertiary alcohol oxygens were placed on opposite sides of the cembrane ring. Because we had recently observed a stereospecific autoxidation of the cembranoid diterpene **30** yielding the epoxy derivative **31**[43] with the two epoxide rings on the same side of the cembrane ring, we thought that this process might offer an alternative route to the formation of trocheliophorol **25**. Thus biological reductive opening of the allylic epoxide **31** should afford a "trocheliophorol" **32** in which the epoxide and tertiary alcohol groups were on the same side of the molecule (Scheme 2).

We decided to test this possibility, using the protocol developed by Tursch.[39] Thus reduction of tro-



Scheme 2.

perimentally confirmed Kashman's stereochemical assignment for (+)-trocheliophorol as **25**.



cheliophorol **25** in boiling THF afforded a secondary **33** and tertiary alcohol **34** from the epoxide. Horeau determination of the absolute configuration of the secondary alcohol indicated the 11*S*-configuration. The ditertiary diol **34** was hydrogenated over Pt to afford the saturated ditertiary diol **35**, which remained optically active. If trocheliophoral had structure **32**, the saturated ditertiary diol **35**, would be optically inactive. This result and the 11*S*-configuration for the secondary alcohol **33** ex-

CONCLUSIONS

Even after almost a quarter of a century of intensive research which commenced with the pioneering work of Leon Ciereszko,[4] octocorals continue to produce a range of novel constituents. These compounds have been shown to exert a range of effects on living organisms, and serve defensive and competitive roles in the survival and growth of soft corals. Certain predators appear capable of ingesting soft coral terpenes without ill effect, and use soft

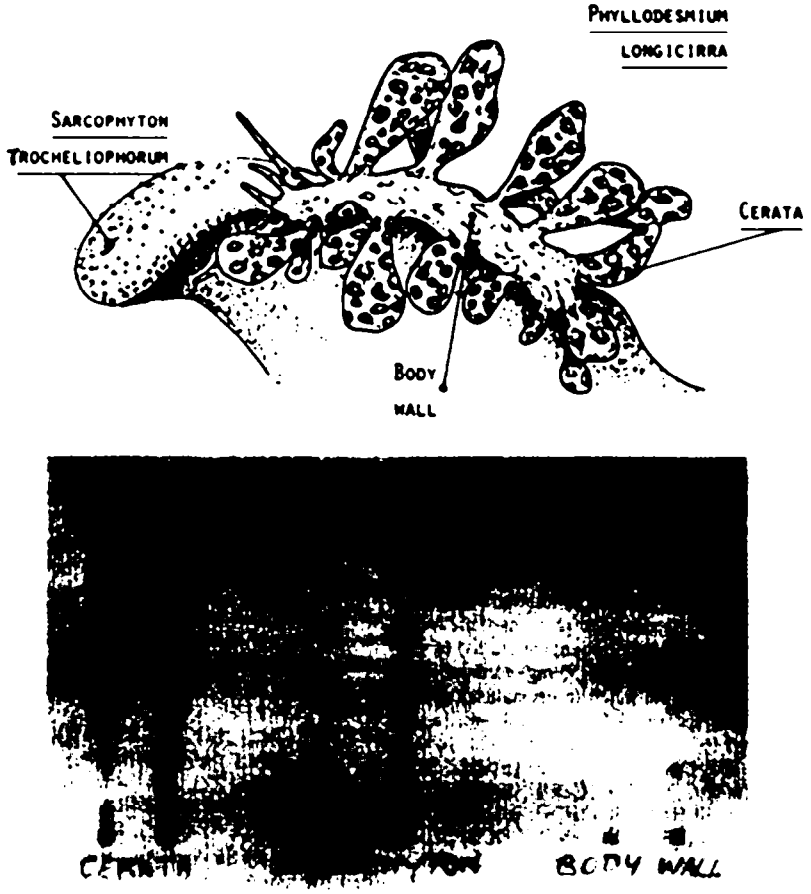


Fig. 1.

corals as a food source. The toxic terpenes in the ingested soft coral tissue serve as stored defensive substances in specialist predators. Preliminary biosynthetic experiments on soft corals suggest that mevalonic acid is also the specific precursor of terpenes in these coelenterates.

EXPERIMENTAL

Biosynthetic experiments on Sinularia capillosa

Collection. Soft coral colonies were collected in Geoffrey Bay on Magnetic Island 5 miles north of Townsville, North Queensland, Australia. Colonies were selected to be in the range of 50–100 g wet weight, and were removed intact from the substratum. They were stabilised in a flow-through aquarium system at the Australian Institute of Marine Science under natural shaded sunlight conditions, and obviously healthy colonies were chosen for incubation.

Incubations. Small colonies of the soft coral *Sinularia capillosa* were incubated in 100 ml beakers of seawater to which was added sodium [^{14}C]bicarbonate and 2-[^{3}H]mevalonolactone each at a final concentration of 1 μCi/ml (3.7×10^4 Bq/ml). The incubations were performed at 26–28°C under natural shaded light conditions for 20 hr, after which time each colony was washed with seawater, frozen and freeze-dried. The total CH_2Cl_2 extract showed an incorporation of 10^7 dpm of C-14 and 6.5×10^7 dpm of tritium. Rapid silica gel chromatography of the CH_2Cl_2 extract of the freeze-dried coral using increasing proportions of diethyl ether in petroleum ether afforded fractions from which the furanoquinol **10** crystallised out. The rate of incorporation of ^{3}H into **10** was extremely variable: crystalline material from different incubations ranged in specific activity from no incorporation to a more usual value of about 3000 dpm/mg, to an all time high of 50,000 dpm/mg on one occasion. In all cases, there was no ^{14}C-label in **10**.

Degradative sequence. A sample of **10** (16 mg) with specific activity 2600 dpm/mg was diluted with unlabelled furanoquinol to give 200 mg of crystalline material with specific activity of 208 dpm/mg. This was acetylated using Ac_2O/pyridine (1:1; 4 ml) at room temp overnight. After usual workup, **13** (220 mg; specific activity 160 dpm/mg of ^{3}H) was isolated by rapid chromatography on silica gel. This was dissolved in dioxane (25 ml) and treated with osmium tetroxide/sodium metaperiodate until disappearance of **13**. After workup, the mixture was subjected to rapid chromatography to give **14**: fraction 11 from the chromatography, 21.5 mg, 4.6 dpm/mg of ^{3}H. ^{1}H NMR ($CDCl_3$) δ 9.43 (1 H, *t*, *J* = 1, CHO), 6.90, 6.85 (2H, aromatic H's), 3.35 (2H, *d*, *J* = 1, benzylic-CH_2), 2.22 (3H, *s*, aromatic CH_3), 2.17, 2.12 (2 × 3H, *s*, acetate methyls); EIMS (*m/z*) 250 ($C_{13}H_{14}O_5$, 1%), 208 (30%), 166 (90%), 138 (75%), 129 (95%), 127 (90%), 83 (50%), 73 (70%), 43 (100%).

Other fractions from the chromatography: fractions 1–6: 3 mg, 110 dpm/mg; fraction 7: 1 mg, 40 dpm/mg; fraction 9: 4.5 mg; 136 dpm/mg; fractions 12–14: 13.8 mg; 60 dpm/mg were all considerably richer than fraction 11, containing aldehyde **14**, 4.6 dpm/mg.

Novel sesquiterpenes from a Nephthea *species* G. 12654

Extraction of freeze-dried soft coral (35 g) with CH_2Cl_2 afforded an organic extract (2.7 g). This chromatographed on silica gel using the usual rapid chromatography technique with light petroleum containing increasing amounts of diethyl ether as eluant. The less polar component **21** (150 mg) contained in the third fraction from the column failed to crystallise, even after repeated purification *eudesma*-4,7(11)-*dien*-8-*one*, $[\alpha]_D$ = +99° (*c* 0.2); IR (film) 1675, 1600, 1435, 1370, 1320, 1280, 1200, 1135, 1070, 1050, 985, 688, 645 cm^{-1}; UV(EtOH): 205 nm (ϵ 1400), 250 nm (ϵ 6600); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 3.40, 3.04 (2H, ABq, *J* = 18 Hz), 2.30 (2H, s), 2.12 (3H, s), 1.90 (3H, s), 1.70 (3H, s), 1.1 (3H, s); ^{13}C NMR ($CDCl_3$) 202.3 s, 143.5 s, 130.7 s, 130.1 s, 125.6 s, 55.4 t, 38.0 t, 35.5 s, 32.1 t, 29.6 t, 25.6 q, 22.9 q, 22.4 q, 18.8 q,t; EIMS (*m/z*) 218 (100%), 203 (75%), 175 (25%), 161 (40%), 157 (50%), 133 (25%), 119 (20%), 105 (25%), 91 (30%).

The more polar **20** (100 mg) contained in the fifth fraction from the column crystallised from petroleum ether, *eudesma*-4,7(11)-*dien*-8β-*ol*, m.p. 59–60°; $[\alpha]_D$ = −10° (*c* 0.15); IR (film) 3350 (broad), 1440, 1380, 1230, 1030, 960, 900, 815, 760 cm^{-1}; UV (EtOH) 212 nm (ϵ 10,000); ^{1}H NMR ($CDCl_3$, 100 MHz) δ4.84 (1H, broad singlet, $W_{h/2}$ = 8 Hz), 3.42, 2.80 (2H, ABq, *J* = 16 Hz), 1.85 (6H, s), 1.7 (3H, s); double resonance: see text; ^{13}C NMR ($CDCl_3$) 134.7 s, 133.1 s, 125.1 s, 123.7 s, 67.6 [δ4.84]d, 48.5 t, 40.1 t, 34.4 s, 32.7 t, 27.2 q, 25.5 [δ 3.41]t, 20.6 q, 19.8 q, 19.5 q, 18.5 t, t; EIMS (*m/z*) [Found M^{+} 220.186; $C_{15}H_{24}O$ requires 220.183]: 220 (100%), 205 (39%), 202 (50%), 187 (60%), 177 (95%), 123 (60%), 122 (80%), 107 (35%), 95 (30%).

Attempted oxidations of the alcohol **20**. Oxidations using Jones' reagent at 0°, neutral and basic oxidants involving Cr-salts, MnO_2 and aluminium isopropoxide on the allylic alcohol **20** all failed to afford detectable quantities of **21**.

Reduction of the ketone **21**. The ketone **21** (20 mg) in dry diethyl ether (10 ml) was treated with excess lithium aluminium hydride until no further ketone remained by TLC. Usual workup afforded, after silica gel chromatography, a fraction corresponding by TLC to **20**. ^{1}H NMR analysis confirmed the presence of a mixture although peaks corresponding to **20** could be identified. By subtraction of the peaks corresponding to **20**, the ^{1}H NMR spectrum of the *epi*-alcohol **22** could be obtained: δ4.46 (1H, m, CH–OH), 3.42, 2.90 (2H, ABq, *J* = 16 Hz), 1.77 (3H, s), 1.75 (3H, s), 1.73 (3H, s), 0.98 (3H, s). All attempts to separate the alcohol mixture using HPLC failed.

Elimination of the alcohol function of compound **20**. The alcohol (50 mg) in pyridine (5 ml) was heated on a steam bath with $SOCl_2$ (10 drops) for 4 hr. Usual workup and rapid silica gel chromatography afforded **23** (25 mg); an oil, $[\alpha]_D$ = +96° (*c*, 0.1); IR film 3090, 1600, 1450, 1330, 1285, 1260, 1130, 880, 815 cm^{-1}, UV (EtOH): 210 nm (ϵ 10,100), shoulder, 234 nm (ϵ 19,200), 243 nm (ϵ 13,900) shoulder; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 5.87 (1H, triplet, *J* ~ 3 Hz), 5.04 (1H, s), 4.87 (1H, s), 3.15, 2.80 (2H, ABq, *J* = 17 Hz), 1.91 (3H, s), 1.63 (3H, s), 1.00 (3H, s); ^{13}C NMR ($CDCl_3$) 143.4 s, 135.5 s, 131.9 s, 125.2 s, 123.5 d, 109.7 t, 41.8 t, 38.8 t, 33.6 s, 33.3 t, 27.7 t, 26.7 q, 20.9 q, 19.6 q,t; EIMS (*m/z*) 202 (100%), 187 (75%), 159 (35%), 146 (35%), 145 (85%), 131 (75%), 129 (40%), 105 (30%), 91 (40%). [Found M^{+} = 202.175; $C_{15}H_{22}$ requires 202.172.]

Hydrogenolysis of the alcohol **20**. The alcohol (15 mg) in EtOH (10 ml) was shaken with H_2 over 10% Pd–C. A ^{1}H NMR spectrum of the crude hydrogenolysate revealed the absence of vinyl protons, vinyl Me groups and secondary alcohols, δ 1.25 (3H, s), 0.8 (9H, d, *J* ~ 7). The mixture was subjected to gas chromatographic–mass spectrometric analysis on Carbowax 20M. Two peaks emerged on programmed elution from 50–200° at 10°/min. Peak 1, 25% 12.2 min, EIMS (*m/z*) 208 (91%), 193 (100%), 165 (64%), 137 (39%), 123 (42%), 109 (91%), 97 (33%), 96 (55%), 95 (70%), 83 (73%), 81 (61%); Peak 2, 75% 13.2 min, 208 (65%), 193 (34%), 191 (25%), 186 (20%), 165 (59%), 163 (7%), 141 (27%), 138 (20%), 137 (39%), 125 (25%), 123 (45%), 109 (100%), 97 (53%), 96 (70%), 95 (85%), 83 (67%), 81 (75%).

Hydrogenation of β*-selinene.* β-Selinene (15 mg) isolated from celery seeds was hydrogenated over 10% Pd–C in EtOH Soln (10 ml). The mixture was subjected to gas chromatographic-mass spectrometric analysis on Carbowax 20M, using a programmed elution 50–200° at 10°/min. Peak 1, 25% 12.1 min, EIMS (*m/z*) 208 (68%), 193 (86%), 165 (58%), 163 (23%), 137 (34%), 123 (35%), 109 (100%), 97 (26%), 96 (29%), 95 (78%), 83 (71%), 81 (78%); Peak 2, 75% 13.1 min, EIMS (*m/z*) 208 (100%), 193 (31%), 192 (31%), 191 (15%), 186 (17%), 165 (64%), 163 (16%), 141 (15%), 138 (27%), 137 (36%), 125 (24%), 123 (45%), 109 (87%), 97 (45%), 96 (53%), 95 (89%), 83 (67%), 81 (75%).

Extraction of Sarcophyton trocheliophorum. The freeze-dried soft coral (7 g) was extracted with CH_2Cl_2 to give an organic extract (800 mg) which was chromatographed on silica gel to give, in order of increasing polarity, (+)-**24**,

20 mg $[\alpha]_D$ + 65° (*c*, 0.12) [lit. + 74°] with other physical properties (esp. IR and ^{1}H NMR) identical with the literature reports;[37] (+)-**25**, 200 mg $[\alpha]_D$ + 61.2° (*c*, 0.16) [lit. + 55°], other spectral properties (esp. IR, ^{1}H NMR, and ^{13}C NMR) identical with the literature reports;[40] and the diterpene alcohol **26**, 50 mg, contaminated with sterol. The compound was identified by superimposition of the ^{1}H NMR spectrum of **26** with an authentic sample.[38]

Zinc–copper couple reduction of (+)-*trocheliophorol* **25**. The diterpene **25**, 10 mg, in absolute EtOH (10 ml) was heated under reflux with a Zn–Cu couple prepared with Zn (250 mg) and 4% $CuSO_4$ soln.[39] The product obtained after rapid silica gel chromatography was identical with (+)-**29**, 2 mg, $[\alpha]_D$ + 83.5° (*c*, 0.2) [lit. + 74°].[37]

Tosic acid catalysed elimination of (+)-*trocheliophorol* **25**. The diterpene **25**, 20 mg, in $CDCl_3$ (0.5 ml) was heated with a crystal of *p*-toluenesulfonic acid in an NMR tube until the appearance of a doublet at δ6.2 (J = 16 Hz). The reaction was quenched and rapidly chromatographed on silica gel to give (2*E*, 4*E*, 7*E*, 1*S*, 4*R*, 11*S*, 12*S*)-11,12-*epoxycembra*-2,4,7-*triene* **28**, 5 mg, an oil, $[\alpha]_D$ = + 100° (*c*, 0.1); IR 1600, 1570, 1450, 1380, 1275, 1120, 1070, 970 cm^{-1}; UV 210 (27,840), 228 (34,566), 240 (25,920), 275 (4800) nm; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 6.15 (1H, d, J = 16), 5.5 (1H, m), 5.2 (1H, dd, J = 7.7, 16), 5.1 (1H, m), 3.0 (1H, br.m), 2.5 (1H, m), 2.84 (1H, dd, J = 1.3, 6.5), 1.55 (3H, s), 1.18 (3H, s), 0.88 (3H, d, J = 6.5), 0.82 (3H, d, J = 6.5). Double resonance: irradiation at δ 1.4 collapsed the double doublet at 2.84 to a singlet; high power irradiation at δ 5.2 sharpened the signals at 3.0 and 2.5 to broadened doublets (J = 15 Hz), and collapsed the doublet at δ 6.2 to a singlet; high power irradiation at δ 2.5–3.0 collapsed the multiplets at 5.5 and 5.1 to broad singlets. EIMS (*m/z*): 288 (95), 245 (30), 227 (35), 205 (30), 277 (40), 259 (60), 249 (100), 119 (80), 107 (75), 105 (60%).

Lithium aluminium hydride reduction of (+)-*trocheliophorol* (**25**). The diterpene **25**, 40 mg, in dry THF (10 ml) was heated under reflux with excess lithium aluminium hydride for 12 hr. Usual workup and rapid silica gel chromatography afforded (2*E*, 7*E*, 1*S*, 4*R*, 11*S*)-*cembra*-2,7-*diene*-4,11-*diol* (**33**), (7 mg), an oil; $[\alpha]_D$ = −83° (*c*, 0.7); IR (film) 3380, 1460, 1380, 1370, 980 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 5.68, (1H, d, J = 16), 5.35 (1H, t, J ~ 6), 5.2 (1H, dd, J = 8, 16), 3.3 (1H, m), 1.55 (3H, s), 1.36 (3H, s), 0.9 (3H, d, J = 7), 0.75 (3H, d, J = 7). EIMS (*m/z*) 308 (1%), 290 (100), 247 (100), 229 (10), Horeau determination on 5 mg of sample gave a rotation of −0.118° (84% reaction, 55% optical purity). The alcohol had the 11*S* absolute configuration. Subsequent fractions contained mixtures of **33** and **34**. Rechromatography enabled the ditertiary diol **34** to be isolated free of **33**.

The analytical sample: (2*E*, 7*E*, 1*S*, 4*R*, 12*S*)-*cembra*-2,7-*diene*-4,12-*diol* **34**, 30 mg, m.p. 73° (phase change), then 95–97°; $[\alpha]_D$ = −39° (*c*, 0.18); IR (film): 3360, 1505, 1360, 1120, 1080, 975 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 5.6 (1H, d, J = 16), 5.35 (1H, dd, J = 7.6, 16), 5.2 (1H, m); 1.45 (3H, d, J = 1.5), 1.33 (3H, s), 1.15 (3H, s), 0.88 (3H, d, J = 6.5); *EIMS* (*m/z*) 308 ($C_{20}H_{36}O_2$, 0.5%), 290.259 ($C_{20}H_{34}O$ requires 290.261, 80%), 272 (30), 257 (20), 247 (40), 230 (35), 149 (60), 137 (100), 121 (80), 95 (80), 93 (70).

Hydrogenation of the ditertiary diol **34**. The diol **34** (10 mg) in dry MeOH (15 ml) was shaken with H_2 over Adam's catalyst for 30 min. The catalyst was removed by filtration, and the product filtered through a small silica gel column to give the saturated **35** (7 mg), m.p. 194–6°, $[\alpha]_D$ = −39° (*c*, 0.06). IR (Nujol) 3350 (br.), 930, 910, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.2 (6H, s), 0.88 (9H, d, J = 7); EIMS (*m/z*) 312 (0%), 294 (80), 279 (60), 276 (70), 43 (100). ORD $[\alpha]_{589\,nm}$ − 39°, $[\alpha]_{578}$ − 12°, $[\alpha]_{546}$ − 10°, $[\alpha]_{436}$ − 15°, $[\alpha]_{365}$ − 23°.

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