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MARINE BROWN ALGAE OF FAMILY CYSTOSEIRACEAE: CHEMISTRY AND CHEMOTAXONOMY

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Key Word Index—*Cystoseira*; *Cystophora*; *Acrocarpia*; *Bifurcaria*; *Caulocystis*; *Halidrys*; *Landsburgia*; Fucales; Phaeophyceae; Cystoseiraceae; diterpenoids; meroditerpenes (tetraprenyltoluquinols); 1,4-naphthoquinone derivatives; chemotaxonomy.

Abstract—The algal family Cystoseiraceae (order Fucales, class Phaeophyceae) includes the genera *Acrocarpia*, *Acystis*, *Bifurcaria*, *Bifurcariopsis*, *Carpoglossum*, *Caulocystis*, *Coccophora*, *Cystophora*, *Cystoseria*, *Halidrys*, *Hormophysa*, *Landsburgia*, *Myriodesma*, *Scaberia* and *Stolonophora*. Among these *Cystoseira* and *Cystophora*, the richest in species, are the most representative of the family. *Cystoseira* is a genus of worldwide distribution with about 80% of the species occurring along the Mediterranean and adjoining Atlantic coasts, while *Cystophora* is limited to the coasts of Australia and New Zealand. Members of the genus *Cystoseira* generally synthesise tetraprenyltoluquinols. Compounds of the same structural type have also been isolated from species belonging to the genera *Halidrys* (northern coasts of England), *Bifurcaria* (coasts of the Galapagos Islands), and *Cystophora*. Some species of these last two also elaborate linear diterpenoids as do some *Cystoseira* species. Tetraprenyltoluquinols have never been found in *Caulocystis* and *Acrocarpia*, which instead accumulate acetogenins. Finally, *Landsburgia* is the sole genus which produces naphthoquinone derivatives. Phytochemical studies of the secondary metabolites of the remaining genera are not reported. The chemistry of secondary metabolites and their distribution in the family Cystoseiraceae will be illustrated, and some taxonomic implications will be discussed.

God said, "Let the waters under heaven come together into a single mass, and let dry land appear." And so it was. God called the dry land 'earth' and the mass of waters 'seas', and God saw that it was good. Evening came and morning came: the third day.

(*Genesis,1. 9,10-13*)

INTRODUCTION

Within the past twenty years various research groups have shown an increasing interest in marine natural products chemistry. This area is particularly attractive not only because a small percentage of the over 200 000 species supposed to be present in the seas and oceans (by contrast with terrestrial organisms) have been chemically investigated, but also because of the fascinating structural peculiarity and unusual molecular arrangements of many marine compounds [1].

Up to 1972 only 210 compounds had been isolated and characterized (Fig. 1) [2–4], whereas over 6000 new structures have been identified between 1973 and 1994 confirming this considerable development in marine chemistry (Fig. 2) [5–20]. The reason for this uninterrupted interest can undoubtedly be ascribed to an awareness of the great contribution that chemical data can give to exploring important pharmacological properties [21–33] or some fascinating phenomena at the interface between chemistry and biology (see for example the explosion of marine ecology [34–44]). However, it is appropriate to point out that the above-mentioned attention would not achieve such results without the power of modern chromatographic and spectroscopic techniques with which it is possible to purify an organic extract and to determine the chemical structure of a new compound with just a few grams of starting material and a few milligrams of purified substances, respectively.

Even though the hope of obtaining new molecules from the sea for therapeutic use has been below expectation, and the current interest in marine ecology seems to have diminished since it has diverted towards other more

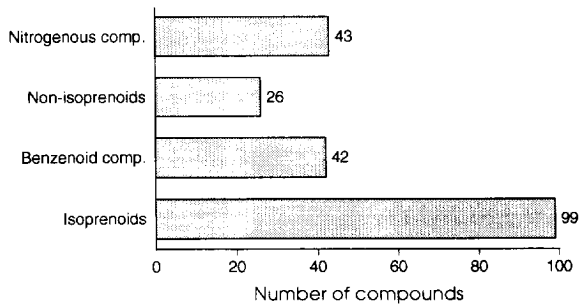


Fig. 1. Marine metabolites (total = 210 compounds) recorded up to 1972, grouped on the basis of their structure.

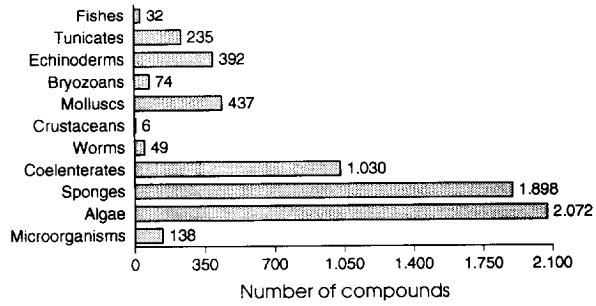


Fig. 3. Phyletic distribution of marine metabolites (total = 6363 compounds) reported in the period 1973–1994.

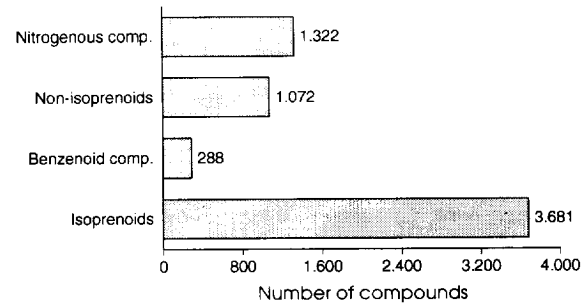


Fig. 2. Marine metabolites (total = 6363 compounds) reported in the period 1973–1994, grouped on the basis of their structure.

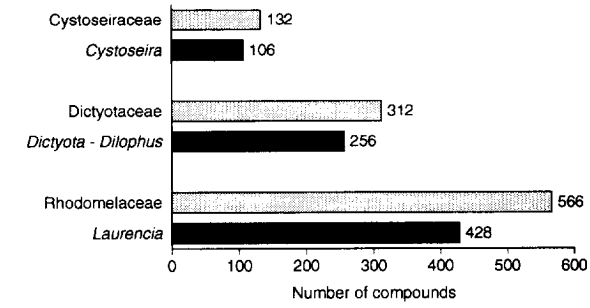


Fig. 4. Metabolites from algal families Cystoseiraceae, Dictyotaceae and Rodhomelaceae (total = 1010 compounds) together with those from their most representative genera *Cystoseira*, *Dictyota* and *Dilophus*, *Laurencia* (total = 790 compounds) reported in the period 1973–1994.

fashionable (also more financed) research fields, the great number of secondary metabolites isolated from marine organisms has certainly brought forward both the discovery of new biosynthetic pathways occurring only in a few taxa or the revision of known ones [45–47], and also the utilization of chemical data as useful support for taxonomic studies [48–60]. Perhaps chemotaxonomy is the one area which can better utilize the enormous amount of chemical information gathered on marine organisms up to now. Notwithstanding this, apart from the pioneering chemotaxonomic attempt of Bergmann's group on Porifera essentially based on the sterol patterns [48], only two significant examples are reported in the literature; in 1983 Berquist and Wells used chemical data, in conjunction with systematic arrangements based on structural, reproductive and histological criteria, to review the classification of Porifera [49], and more recently Stonik and Elyakov have utilized secondary metabolites from echinoderms for chemotaxonomic purposes [50]. However, I believe the use of secondary metabolism products as taxonomic markers may prove to be extremly helpful in the chemotaxonomy of algae [51–61].

Algae, possibly due to their ease of collection, are the marine organisms that have been most subject to study (Fig. 3). Of all the algae, the genus *Laurencia* of Rhodomelaceae and the two genera *Dictyota* and *Dilophus* of the Dictyotaceae are the most studied, but an important contribution has also been provided by Cystoseiraceae (Fig. 4) involving mainly Mediterranean and Australian research teams. Over 130 compounds have been isolated from algae of this family, and their structures have been determined essentially by physical methods. These compounds range from acetogenins to linear or variously cyclized and functionalized terpenoids. Even if few of the isolated metabolites displayed interesting biological activity, apart from some recent encouraging antioxidant and radical scavenger properties [62], the chemical data up to now accumulated from Cystoseiraceae can be used to clarify some phylogenetic aspects of this important family of brown algae, and constitute a good example of how the phytochemical studies can be used for the above-mentioned taxonomic purposes.

DISCUSSION

This paper first gives a general view of the family Cystoseiraceae and the geographical distribution of its genera; then deals with the results of phytochemical studies, discussing the chemical data obtained separately on the individual genera with more emphasis on those of *Cystoseira* and *Cystophora*, the two richest in species and the most widely studied. Finally, on the basis of phytochemical studies, it proposes a complete and de-

tailed biogenetic scheme for *Cystoseira* meroditerpenoids, suggests a separation of the *Cystoseira* species into chemical groups showing the close relationships between this chemical classification and those based on morphological information, and concludes with some considerations that permit a hypothesis for the chemical classification also for the other Cystoseiraceae genera, although less investigated than *Cystoseira*.

Present and past distribution of Cystoseiraceae

In order to understand the present-day distribution of Cystoseiraceae (order Fucales, class Phaeophyceae), it is necessary to briefly analyse the evolution and diffusion of Fucales.

It has been suggested that Fucales (along with Laminariales, Desmarestiales and Sporochnales) originated in the late Mesozoic (Jurassic) somewhere in Australasia and that they differentiated and evolved from the Miocene to the Pliocene. The present-day geographical distribution of species and genera reflects in part subsequent changes in the relative positions of the continents and in the earth's climate, and in part the result of evolutionary processes as an adaptive response to the environment [63–65].

The six families in Table 1 are currently recognized in Fucales. Some of these were probably in existence prior to the land mass split in the late Cretaceous (80 million years ago), while it has also been suggested that both the more widespread Fucalean genera, as well as the Australian species, were formed successively from simple chromophyte ancestors that were distributed over the tropical and subtropical Pacific and Tethys oceans. Thus, when between the Cretaceous and Oligocene the enlargement of the split formed the Paleoatlantic and Paleoindian oceans, the Cystoseiraceae family gave rise to various genera according to the biogeographic phenomenon of vicariance [66]. Among these, *Cystoseira* is distributed through the subtropics but with its centre of speciation in the Mediterranean, while *Cystophora* is confined to the Australian hemisphere, particularly to South Western Australia.

Table 1 . Families and genera of the Fucales

Families	Genera
Cystoseiraceae	*Acrocarpia, Acystis, Bifurcaria, Bifurcariopsis, Carpoglossum, Caulocystis, Coccophora, Cystophora, Cystoseira, Halidrys, Hormophysa, Landsburgia, Myagropsis, Myriodesma, Scaberia, Stolonophora*
Fucaceae	*Ascophyllum, Fucus, Hesperophycus, Pelvetia, Pelvetiopsis, Xiphophora*
Himanthaliaceae	*Himanthalia*
Hormosiraceae	*Hormosira*
Sargassaceae	*Anthophycus, Carpophyllum, Cladophyllum, Hizichia, Oerstedtia, Sargassum, Turbinaria*
Seirococcaceae	*Axillariella, Cystosphaera, Marginariella, Phyllospora, Scytothalia, Seirococcus*

Cystoseiraceae and its two most important genera Cystoseira *and* Cystophora

Cystoseiraceae includes the 15 genera reported in Table 2, which also shows the number of species presently listed in each genus and their geographical distribution.

Cystoseira. The genus *Cystoseira* was created in 1820 by the Swedish phycologist Carl Adolph Agardh. Originally the taxon contained 37 species [67], but since then at least 114 entities have been assigned to it, until the two monographs of Valiante (1883) [68] and Sauvageau (1912) [69] put forward an organization fairly similar to that of today. Later some Mediterranean taxa were revised by Ercegovic [70]. Several new Mediterranean species were successively described by Gerloff and Nizamuddin [71, 72], but all of them are regarded by Giaccone as invalid [73, 74]. Today most taxonomists list 50 species of *Cystoseira*, of which only 14 were originally recognized by Agardh [53, 75–80].

The vicissitudes of this genus demonstrate how it is still imperfectly understood. Any uncertainties which still exist may be due to the polymorphism of some species, as well as its ongoing active speciation [77]. The eventual hybridization between species which have evolved closely together may constitute a further complication. Table 3 reports the geographic distribution of *Cystoseira* in the seas and oceans around the world according to Giaccone [79, 80], while Table 4 lists the Mediterranean species (29 in total with 19 endemic species asterisked) and Fig. 5 points out their distribution in this area.

The geographical distribution reported in Table 3 reflects the evolutionary history of *Cystoseira* genus. Its speciation process began from a simple ancestor in the late Cretaceous, around 80 million years ago, in Wegener's sea of Tethys, a deep gulf placed between

Table 2. Genera of the family Cystoseiraceae*

Genus	Number	Distribution
1. *Acrocarpia*†	2	Australia
2. *Acystis*	1	Red Sea
3. *Bifurcaria*†	3	Atlantic Ocean (South and North
4. *Bifurcariopsis*	1	South Africa
5. *Carpoglossum*	4	Australia–Guadalupe
6. *Caulocystis*†	2	Australia
7. *Coccophora*	2	Sea of Japan
8. *Cystophora*	23	Australia–New Zealand
9. *Cystoseira*†	46	See Table 3
10. *Halidrys*†	2	Atlantic, Indian and Pacific Oceans
11. *Hormophysa*	1	Australia
12. *Landsburgia*†	2	New Zealand
13. *Myriodesma*	8	Australia
14. *Scaberia*	2	Australia–Tasmania
15. *Stolonophora*	1	Guadalupe

*Adapted from M. Roberts (see ref. 77).
†The genera studied from a phytochemical point of view.

Table 3. Distribution of the *Cystoseira* species

Sea	Number of species
Indian Ocean	1
Red Sea, Persian Gulf, Eastern African coast, Carribean, Florida, Eastern Australia	2
Sea of Japan	4
Pacific Ocean (North American coasts, Sea of Okhotst)	4
Atlantic Ocean	12
Mediterranean	29*

*Nineteen species are endemic (see Table 4).

Table 4. Mediterranean species of *Cystoseira* (according to Giaccone [80])

C. abies-marina	*C. jabukae**
*C. algeriensis**	*C. mauritanica*
*C. amentacea**	*C. mediterranea**
C. barbata	*C. nodicaulis*
*C. barbatula**	*C. pelagosae**
*C. brachycarpa**	*C. sauvageauana**
C. compressa	*C. schiffneri*
*C. corniculata**	*C. sedoides**
*C. crinita**	*C. spinosa**
*C. crinitophylla**	*C. squarrosa**
*C. dubia**	*C. susanensis**
*C. elegans**	*C. tamariscifolia*
C. foeniculacea	*C. usneoides*
C. humilis	*C. zosteroides**
*C. hyblaea**	

*Endemic species.

Eurasia and Africa. 'The subsequent movement of the crustal plates gradually transformed the bottom of the Tethys into an almost landlocked sea, the Mediterranean, from time to time connected with a new ocean, the Atlantic, which was opening between America, Europe and Africa. In the course of these movements some species of *Cystoseira* remained confined to the Pacific, while others entered the Atlantic and the adjacent Mediterranean, where they began to differentiate actively. A little more than 5 million years ago, in the Cenozoic, as a consequence of its complete isolation from contiguous seas and evaporation of water, the Mediterranean turned into an immense salt plain with several hypersaline lakes and this caused the disappearance of the majority of marine organisms. When, at the beginning of the Pliocene, the waters of the Atlantic forced a passage through the Gibraltar diaphragm, *Cystoseira* species again reached the Mediterranean, where they found the ideal conditions for the speciation process which is still active today (Piattelli [58])' (Fig. 6).

Cystophora. The genus *Cystophora* was created by Agardh [81–83]. Together with *Acrocarpia* (two species) and *Caulocystis* (two species) it is the most common genus along the Australian coastline [84] having 23 species, the most part of which inhabit Southern Australia. Six species occur in New Zealand, four of which also occur in Australia while two are endemic of the New Zealand region. No species of this genus is known outside the Australian region. In fact, after its birth which may be dated back to the same period as *Cystoseira*, it remained confined to Australia where it found the most suitable environmental conditions for its evolution (Table 5).

Bifurcaria, Halidrys, Acrocarpia, Caulocystis and Landsburgia. Bifurcaria and *Halidrys* are the two genera mor-

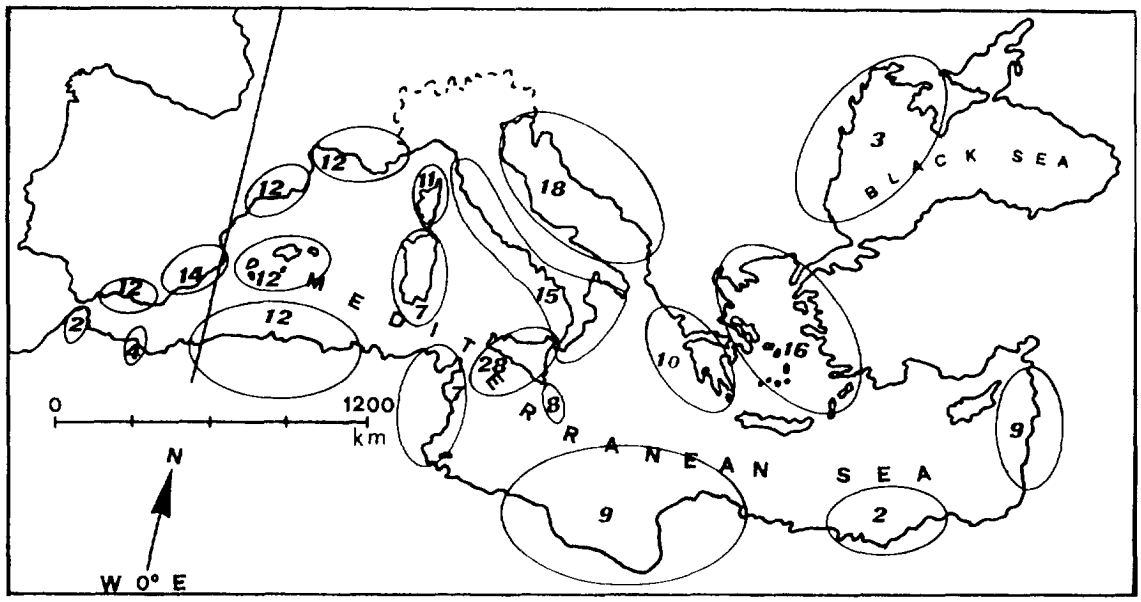


Fig. 5. Distribution of genus *Cystoseira* in Mediterranean sea (circled numbers represent the extant species in the different geographical areas).

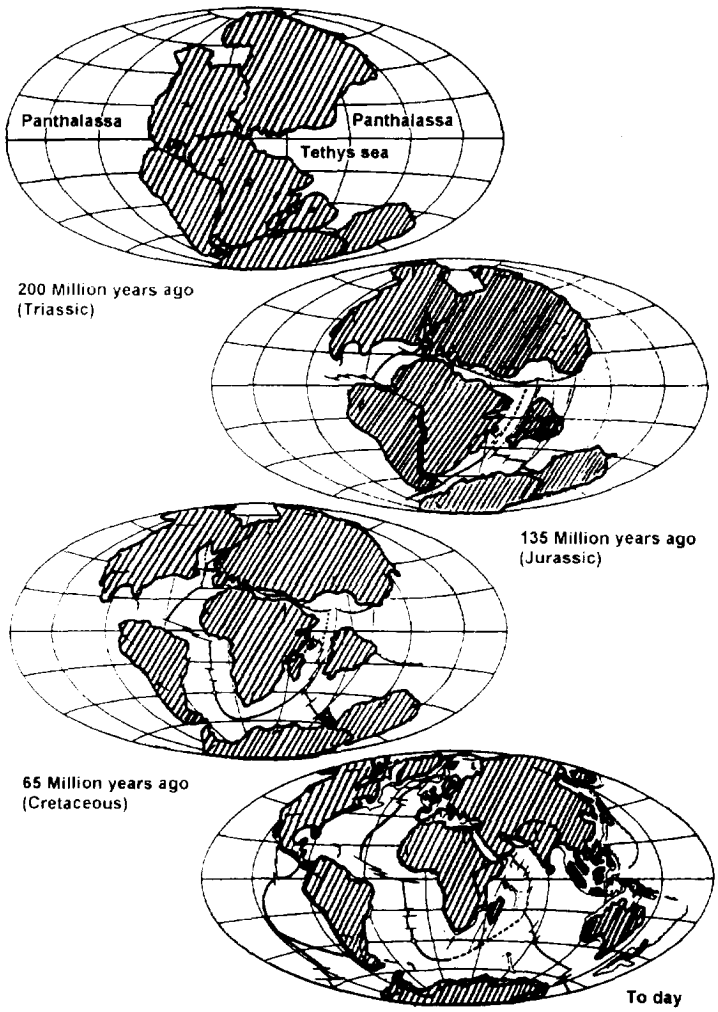


Fig. 6. The movement of the crustal plates from 200 million years ago (Triassic) to the present time, and the Wegener's sea of Tethys.

phologically closest to *Cystoseira*; they have three and two species respectively, and grow prevalently on Atlantic coasts. The genera *Acrocarpia* and *Caulocystis*, described by Areschoug in 1854 [85], were placed under *Cystophora* by most subsequent authors until, on the basis of morphological differences, Womersly separated them from *Cystophora*. They have two species each and are reported only for Australian coasts [84] (Table 5). *Landsburgia* is a genus endemic to New Zealand consisting of two distinct species: *L. quercifolia*, which grows on the North and South Islands, and *L. myricaefolia* which is endemic to the Chatham Islands [86] (Table 5).

Cystoseiraceae: phytochemical studies and chemotaxonomic results

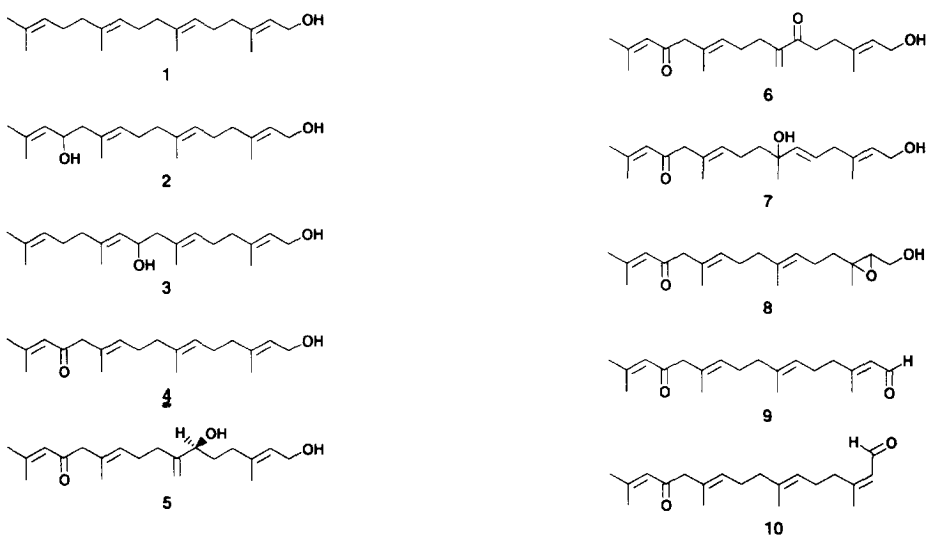
This part of the review is devoted to phytochemical studies that have been made on Cystoseiraceae, stressing those results of chemotaxonomic relevance. First of all this section takes into account the most studied genus, *Cystoseira*, followed by *Halidrys* and *Bifurcaria*, the two genera morphologically closest to it; then deals with phytochemical data from *Cystophora*, and the two genera closely related to it, namely *Caulocystis* and *Acrocarpia*, as well as *Landsburgia*, the last studied from a phytochemical point of view. The metabolites isolated and characterized from every genus are inspected from the simpler compounds to the more complex ones, allowing one to gradually construct a biosynthetic pathway so they can be logically interrelated.

Cystoseira. The earliest and simplest compounds, isolated from species belonging to the genus *Cystoseira* are geranylgeraniol '**1**' and the products of its oxidation (**2–10**) [87–93]. The quite exceptional occurrence in *C. brachycarpa* (erroneously reported as *C. crinita* [94]) of high concentrations of geranylgeraniol suggests that it has evolved the enzymatic machinery for its biosynthesis, but not yet that for its utilization. Thus the various above-mentioned oxidation products are nothing more than geranylgeraniol catabolites. The literature reports

Table 5. Species of *Cystophora*, *Acrocarpia*, *Caulocystis*, *Landsburgia* and their distribution (according to Womersley [84] and Lindauer [86])

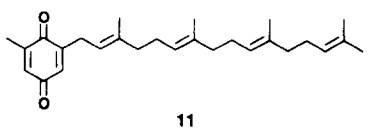
Genus and species	Distrib.	Genus and species	Distrib.
Cystophora expansa	A	*C. platylobium*	B, A, N.Z.
C. grevillei	A	*C. torulosa*	B, N.Z.
C. monilifera	A	*C. xiphocarpa*	T. only
C. moniliformis	A	*C. brownii*	C
C. polycystidea	A	*C. gracilis*	C
C. racemosa	A	*C. pectinata*	C
C. retorta	A		
C. retroflexa	A, N.Z.	*Acrocarpia robusta*	C
C. siliquosa	A	*A. paniculata*	A, B
C. subfarcinata	A		
C. botryocystis	B, A	*Caulocystis uvifera*	A
C. congesta	B, N.Z.	*C. cephalornithos*	A
C. cuspidata	B		
C. cymodoceae	B	*Landsburgia quercifolia*	N.Z.
C. intermedia	B	*L. myricaefolia*	Ch. Is.

A = Southern Australia; B = Eastern Australia (Victoria and/or Tasmania included, usually extending into South Australia and in some cases up the New South Wales coast); C = Western Australia (extending into South Australia but not reaching Victoria or Tasmania); N.Z. = New Zealand; T. = Tasmania; Ch. Is. = Chatam Islands.



the isolation of these by-products also from two other species, *C. balearica* and *C. elegans*, but the first was suggested by Giaccone to be a variety of *C. brachycarpa* [79, 80], while *C. elegans* analysed by French researchers was, in my opinion, misclassified. In this light, it should be noted that an exact botanical classification of the plant material used in chemotaxonomic studies is of prime importance. Therefore, it would be useful to promote interchanges of algae between researchers in order to verify any discrepancies in the chemical data with respect to morphological ones. Is the *Cystoseira* species collected by French authors really *C. elegans*?

The acquisition by some *Cystoseira* species of the capacity to utilize geranylgeraniol as an alkylating agent for an activated aromatic unit seems to constitute the next evolutionary step. The simplest compound supposedly produced from this reaction was isolated from *C. jabukae* as geranylgeranyltoluquinone (**11**) which is probably an isolation artifact, its natural counterpart most likely being the corresponding quinol [95]. Further transformations of geranylgeranyltoluquinol involve the oxidation of the tetraprenyl moiety and lead to about 20 compounds with a linear diterpenoid chain variously oxygenated [96–106]: compounds **12**–**21** are some representatives of the whole group and a simple inspection of the reported structures is sufficient to note that the preferred oxidation sites on the side chain, apart from the compound **12**, are the C-5, C-12 and C-15 positions [61].



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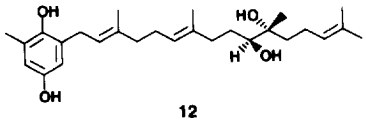

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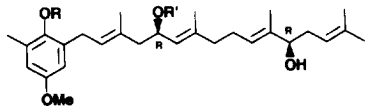
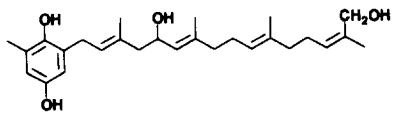


13 R = Me R' = H
56 R = H R' = MeCO



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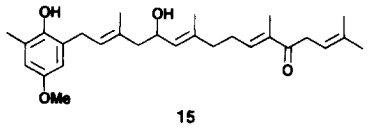

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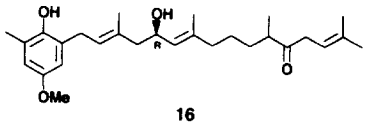

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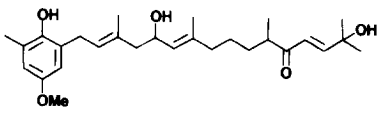

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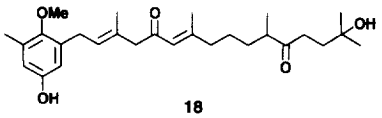

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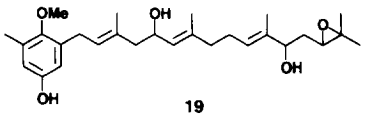

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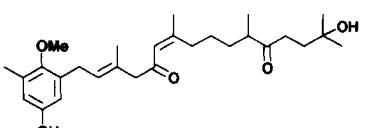

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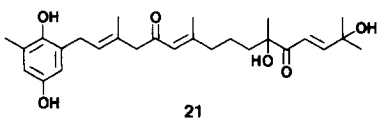

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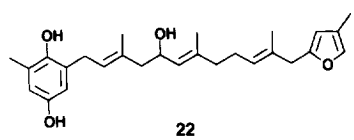
Some of the oxidation products, for instance glycol (**12**) isolated from *C. hyblaea* [97] and successively both from *C. crinita* and *C. ercegovicii* (now *C. schiffneri* [80]) [94], seem to be dead ends in the metabolic pathway, while others prelude further manipulation. One of these manipulations is the cyclization of the terminal isoprene unit to form a furan ring; the co-occurrence in *C. spinosa* var. *squarrosa* (now *C. squarrosa* [80]) of the diol (**14**) and their metabolites with the terminal furan ring (**22–24**) may be explained in Scheme 1: by introducing a carbonyl function at position 13, followed by hemiketal formation and subsequent dehydration, diol (**14**) could give compound **22** in which the terminal isoprene unit easily converts into a furan ring [96]. Another possibility is that offered by compound **19** which, by nucleophilic attack of the alcoholic hydroxyl on the oxirane ring, would afford a metabolite having a terminal tetrahydrofuran ring like compound **25** isolated from *C. zosteroides* [102]. A third possibility is that of a hypothetical compound **26** which, by simple transformations reported in Scheme 2, would afford both compound **21** and the two geometrical isomers, usneidol E (**27**) and usneidol Z (**28**) which for dehydration give usneidone E (**29**) and usneidone Z (**30**), reported from *C. usneoides* [107–109]. The same two geometrical isomer alcohols, **27** and **29**, have been recently isolated also from *C. stricta* (now *C. amentacea* var. *stricta* [80]) [110]. Among all the oxidation products, the two main geometrical isomers **18** and **20** isolated from *C. sauvageauana* [103], and more recently from *C. dubia* [105], are of great metabolic consequence since they contain both a conjugated and an unconjugated carbonyl function and are structurally suitable for further transformations. In fact, the C-11 proton, activated by the unconjugated carbonyl, can be removed to produce a carbanion, which can further attack the β-carbon of the conjugated system by the Michael mechanism (Scheme 3). This would lead to the formation of a pentatomic carbocyclic structure, as observed in metabolites (**31–34**) [106, 111, 112] which still contain two unconjugated carbonyl functions that can play a very significant role in the biosynthesis of more complex *Cystoseira* compounds.



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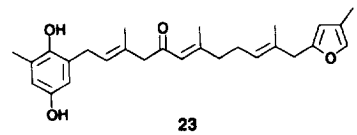

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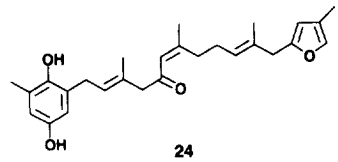

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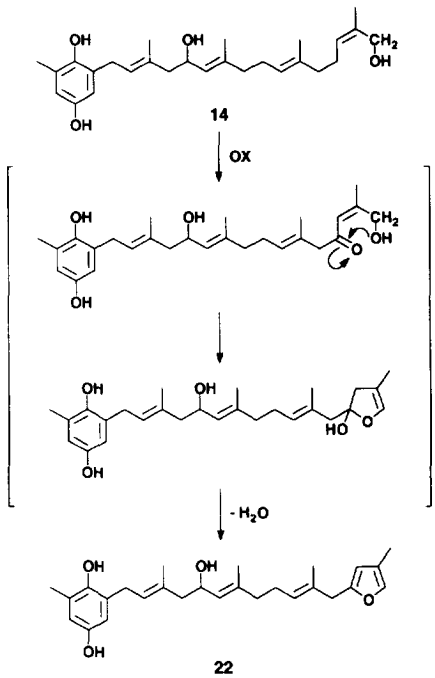
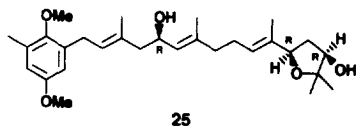
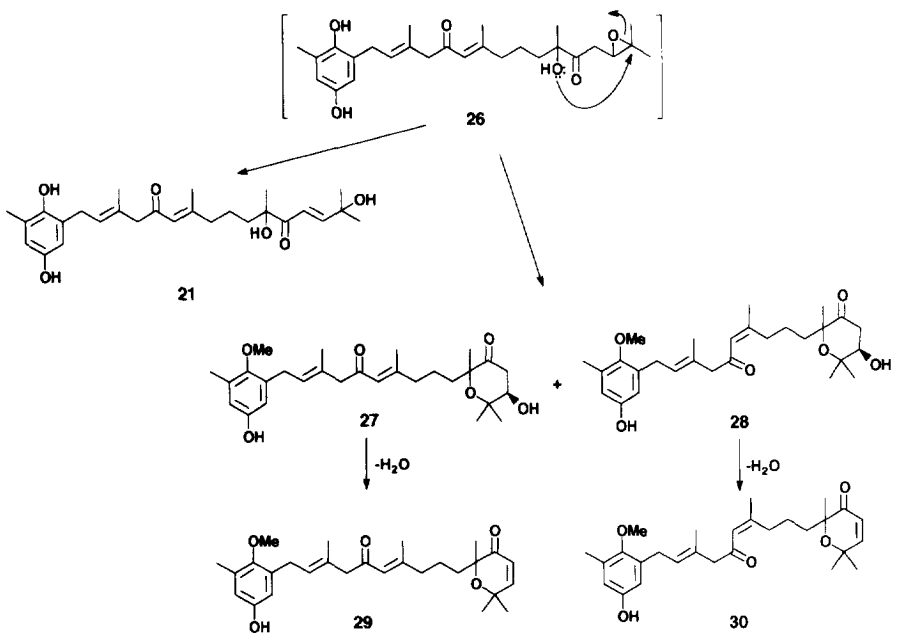


Scheme 1.

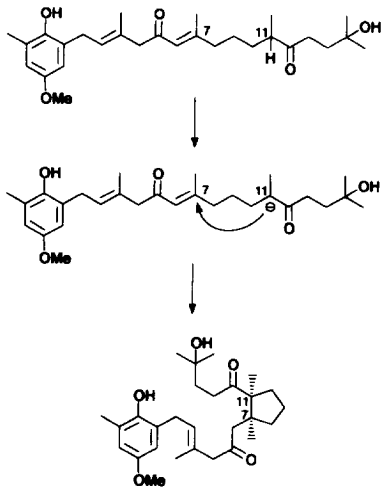


Diketones **31**–**33**, in fact, can undergo an intramolecular aldol condensation, from which many secondary metabolites isolated from *C. amentacea* var. *stricta* seem to be derived. In principle, since the molecules in question each have three activated carbonyl positions, three different pathways may be foreseen for this reaction (Scheme 4). Condensation between C-5 and C-13 produces the formation of a bicyclo[4.3.0]nonane ring system, while C-4 and C-12 bond formation yields the same bicyclic system, but with a different substitution pattern. The third type of aldol condensation, namely that involving C-6 and C-12, gives rise to a bicyclo[3.2.0]heptane ring system. The first reaction takes place in Nature leading to compounds **35** and **36** isolated from *C. algeriensis* and *C. platyramosa* (now *C. spinosa* [80]) [104, 111, 113] or to *cyclo*-1-demethylcystalgerone **37** recently reported from *C. baccata* [114] or to C-3 epimers **38a** and **38b** obtained from the same alga [115], while the second had never been observed in Nature until the recent publication of clareanone (**39**) a new meroditerpenoid isolated from a *Cystoseira* sp. [116]; however this type of aldol reaction could be produced *in vitro* in acidic conditions [117]. The third type of condensation explains the occurrence in *C. amentacea* var. *stricta* of balearone (**40**) [118, 119] and its *cis*-isomer **41** [120].

Among the three types of the above-reported aldol reaction, the first is probably the more favourite thermodynamic process, while, in order for the other two pathways to take place, a very important role is played by diketo-epoxide **33** since it contains both the terminal epoxidic function and the β-hydroxyl formed from the aldol condensation suitable to yield clareanone (**39**) or balearone (**40**) with a second step of reaction. This last could constitute the driving force of the process and provides a key to explain the presence of these more complex metabolites found only in those *Cystoseira* spe-



Scheme 2.



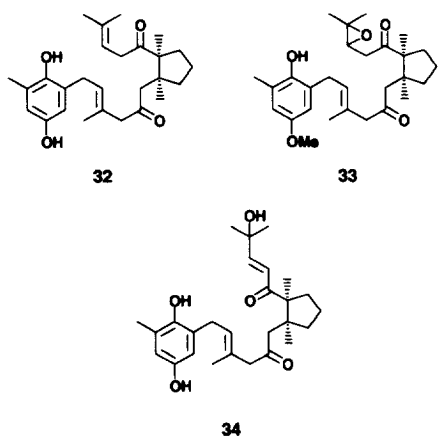
31

Scheme 3.

cies also accumulating the diketo-epoxide **33**. According to this interpretation, compound **33** becomes the second key metabolite in the biosynthetic pathway of *Cystoseira* meroditerpenoids, the first being compound **18** and its *cis* counterpart **20**.

Furthermore, the diketo-epoxide **33** through enolization of the carbonyl at position 5 (Scheme 5) could evolve in two different ways: the nucleophilic attack of the enolic hydroxyl on the carbonyl carbon at position 12 would result in the formation of strictaepoxide (**42**) [121], a hemiketal isolated from *C. stricta* var. *amentacea* (now *C. amentacea* var. *amentacea* [80]) having an oxabicyclo [4.3.0]nonane ring system, whose further elaboration would yield compounds **43–45** [122], while nucleophilic attack on C-14 would give a metabolite recently isolated from *C. tamariscifolia*, tamariscolone (**46**), containing an oxabicyclo[4.3.0]nonane ring system [123].

This latter, in the presence of acid (the acidity of $CDCl_3$ used as solvent in NMR spectroscopy is sufficient), and according to Scheme 6 undergoes a reaction which is an acid-catalysed aldol condensation followed by attack of the tertiary hydroxyl on the positively charged carbon to give a strictaketal **47** [123]. Since this compound, along with its *cis*-isomer **48** have been isolated in *C. amentacea* var. *stricta* [120, 124, 125], the aldol reaction reported in the aforesaid Scheme 6 could explain its biosynthetic formation from diketo-epoxide, *via* tamariscolone, in the living alga.



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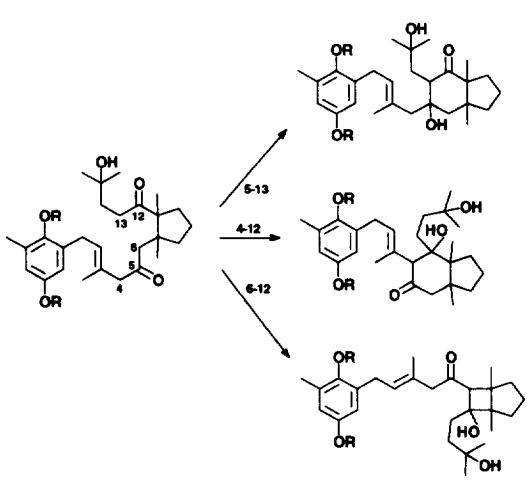
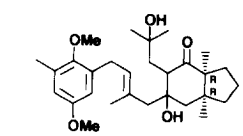


Scheme 4.



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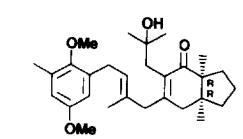

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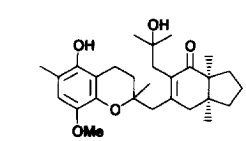

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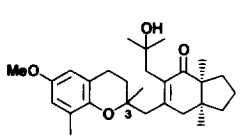

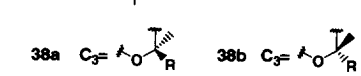

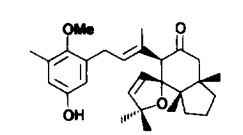

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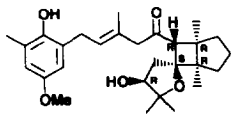

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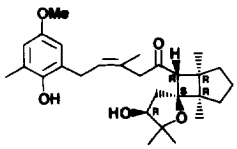

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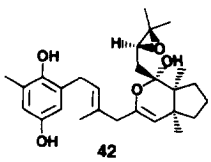

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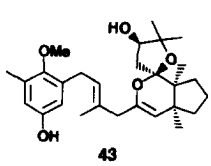

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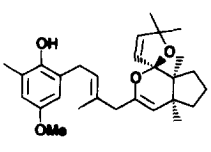

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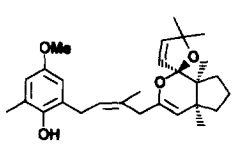

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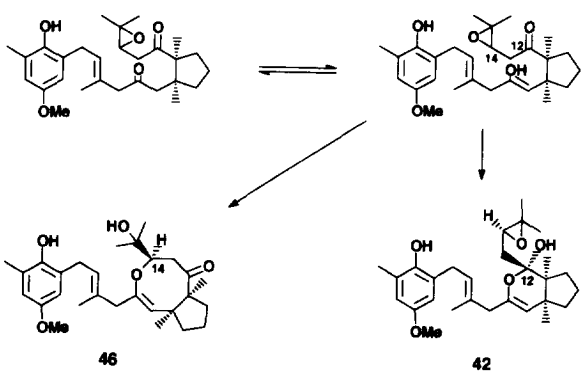

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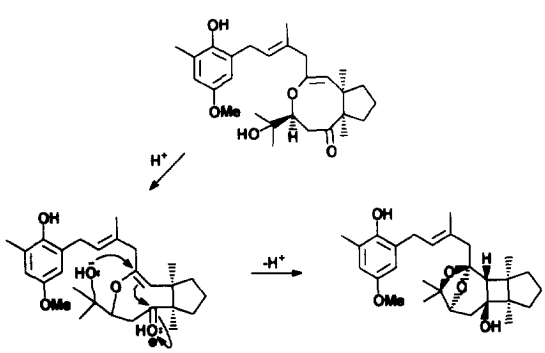
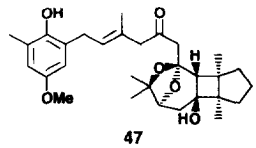
Scheme 5.



Scheme 6.



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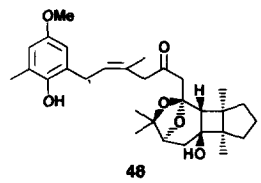

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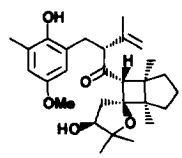

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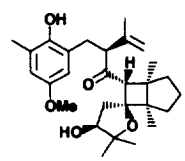

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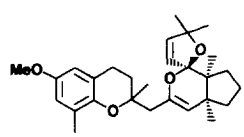

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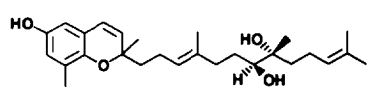

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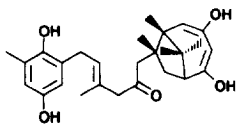
The irregular terpenoids neobalearone (**49**) and epi-neo-balearone (**50**) isolated from *C. amentacea* var. *stricta*, are among the most recent secondary metabolites from *Cystoseira* species [126]. These compounds can hypothetically derive from the attack of an activated form of geranyllavandulol on an aromatic precursor, followed by modifications possibly catalysed by the same enzyme systems that are involved in the biosynthesis of the regular diterpenoid counterpart, balearone (**40**).

Along with all the above-mentioned compounds in *Cystoseira* chromane and chromene derivatives have also been cited, e.g. **51** and **52** [122, 127, 128]. With regard to compound **51**, the authors observe that it occurs as a mixture of epimers at C-3 and suggest it is a possible artifact or perhaps a compound formed in living alga through a non-enzymatic reaction [122].

Aside from the already noted metabolites two interesting classes of rearranged meroditerpenoids, namely mediterraneols and cystoseirols, have been isolated from *C. mediterranea* by some French authors [115, 129–134].

Mediterraneols are characterized by a bicyclo[4.2.1] nonane ring, while the diterpenic moiety of cystoseirols is cyclized to give an oxabicyclo[5.4.1]dodecane ring. Compounds **53**, mediterraneol A, and **54**, cystoseirol D, are two examples of these unusual secondary metabolites, the others being mediterraneol B (C-7 empimer of A), mediterraneols C and D (chromane derivatives of A and B, respectively), cystoseirol E (*cis*-isomer at C-2 of cystoseirol D), cystoseirols B and C (mixture of chromane isomers at C-3 of D) and, lastly, the first of cystoseirols reported, cystoseirol A (**55**), also present in the living alga as mixture of epimers at C-3. These compounds have been reported from *C. stricta* and *C. tamariscifolia* as well, but we never succeeded in isolating them from any of the above three species. We found no explanation of why this should be so, therefore, firmly convinced of the possibility of using the *Cystoseira* secondary metabolites as taxonomic markers and, consequently, of their invariability as regards the sites or the seasons of collection, we tried to evaluate how much the above-mentioned factors could influence the metabolic pattern of different species. For that purpose we collected numerous samples of several *Cystoseira* species and, after careful examination of extracts, we ascertained that the presence/absence of secondary metabolites within each species is nearly invariant (apart from the total amount per dry weight of the alga) with respect to the aforesaid factors [117]. In contrast with these experimental data, the three Sicilian *Cystoseira* species seem to accumulate metabolites different to those of the same species collected on the French coasts. However, regarding cystoseirols, I would like to note a curious coincidence. The original ^{1}H NMR spectrum of cystoseirol A (**55**) kindly donated by the French authors, shows perfect alignment with that of the chromane derivative of cystoketal (**51**) suggesting that the two compounds are the same, though without stating which of two is the correct structure (Fig. 7).



Mediterraneol A

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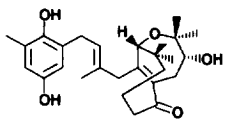

Cystoseirol D

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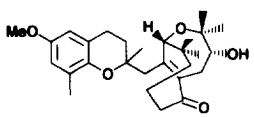


Cystoseirol A

55

Mediterraneols and cystoseirols, being rearranged meroditerpenoids, are the only metabolites which do not fit the biosynthetic transformations traced in this general survey and outlined in Fig. 8, if one does not consider the still open question regarding the structure of the above-mentioned cystoseirols.

Chemotaxonomic relationships. The great number of phytochemical data, up to now collected from *Cystoseira* genus, permits the drawing of some important conclusions regarding the taxonomic relationships. In this way, Piattelli subdivided *Cystoseira* collected along the Sicilian coasts by the chemical composition of each species into seven groups and, after careful comparison of the arrangements of the Mediterranean *Cystoseira* species in the morphologically based groups proposed by Giaccone [76], found considerable agreement between the two types of classification, chemical and morphological [17, 58]. The same type of Piattelli's analysis has been approached by Valls for *Cystoseira* species collected from the French Mediterranean coast and the Atlantic coast of Morocco. On the basis of his chemical data, compared with those reported by Amico for the Sicilian *Cystoseira* species, he proposes to classify species of the genus *Cystoseira* into three broad chemical groups, the third of which being subdivided into three other subgroups [60]. Also Valls notes the close relationships between his chemical classification and those based on morphological considerations [76, 77]. Nevertheless he concludes that 'a reliable chemotaxonomic study of the genus *Cystoseira* should involve not only the determination of the characteristic chemical structures of the species, but also the biosynthetic process which led to their metabolism in alga', suggesting a confirmation of every biogenetic hypothesis with *in vivo* labelled experiments of biosynthesis. With this in mind we have been growing some Mediterranean *Cystoseira* species in uni-algal culture, verifying that in these conditions the pattern of secondary metabolites is nearly the same of those of the corresponding wild algae, but we have not yet started tracer experiments [135].

Agreeing with Piattelli and Valls that the chemical and morphological data are closely related, I thought it appropriate, after a careful analysis of their two different arrangements into chemical groups, to combine them together with all the most recent chemical data, in order to obtain a new almost definitive chemical classification. Table 6 reports the proposed chemical groups of *Cystoseira* species showing a very good accordance, apart from *C. schiffneri* which requires further investigation, with those obtained by Giaccone in a different manner [53, 76, 79, 80], thus validating diterpenoids as taxonomic markers within the genus *Cystoseira*.

Supposing that biological evolution parallels biochemical evolution, i.e. that species capable of synthesizing metabolites which require greater chemical manipulation and therefore more complex enzyme systems should be regarded as more evolved than those which accumulate simpler metabolites, then the secondary metabolite biosynthetic pathway of *Cystoseira* outlined above Fig. 8 shows the evolution of the genus, and fur-

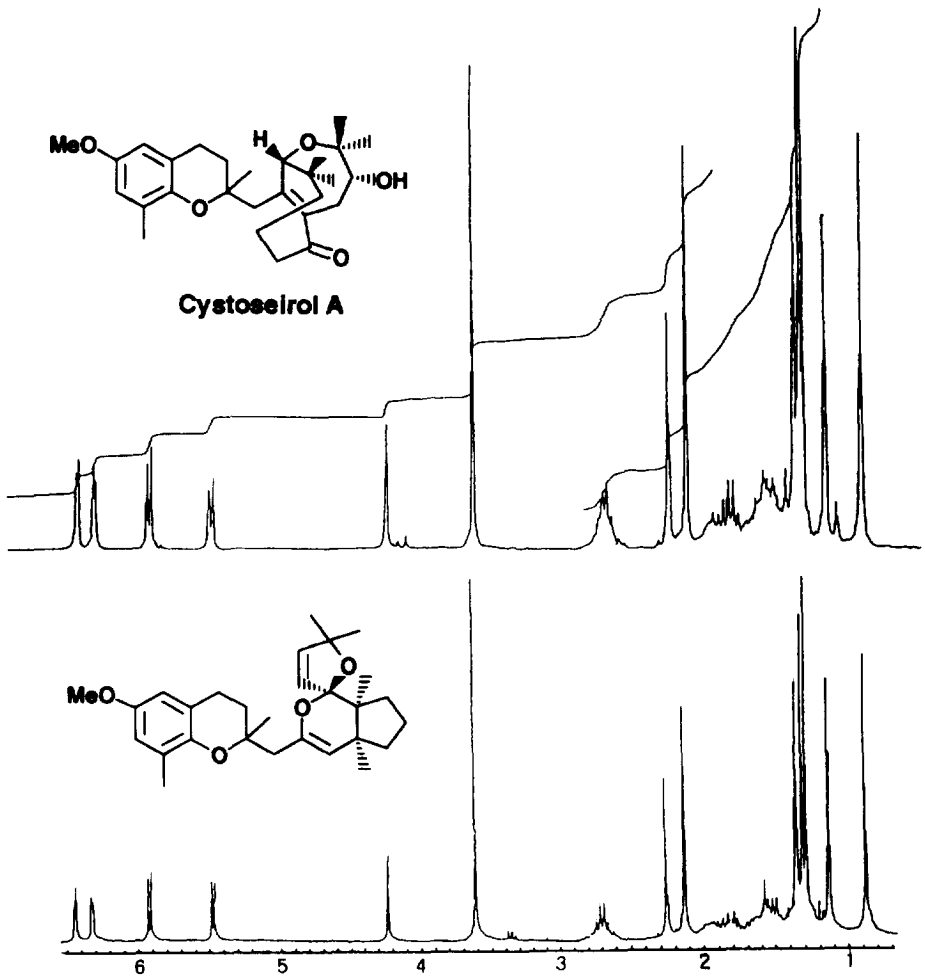


Fig. 7. ^{1}H NMR spectra of Cystoketal chromane and Cystoseirol A.

thermore its close agreement with the possible phylogenetic tree traced on the basis of morphological evidence also confirms the phylogenetic value of chemical data (Fig. 9).

Even though the above itemized biosynthetic pathway has been strengthened in some key points by *in vitro* reactions, namely the intramolecular Michael reaction on compounds **18** and **20** or the subsequent aldol condensation (a sort of Robinson annulation) giving bicyclononane skeleton, the biogenetic proposal for meroditerpenes from the genus *Cystoseira* summarized in Fig. 8 is purely speculative and must be further validated. Another confirmatory step could be determining the absolute configuration of some key metabolites to establish they are really interconnected through stereocontrolled enzymatic reaction. To this end we have recently tackled the unsolved problem of the absolute configuration of some *Cystoseira* metabolites, as well as that of the configuration of compounds containing more centres in a flexible structure (linear meroditerpenoids, e.g. **13**) [136]. By applying a recent and more reliable modification of the Mosher's method for the elucidation of the absolute configuration of secondary alcohols [137–138], we obtained results which agreed well with the biogenetic hypothesis thereby taking a further step towards demonstrating the stereoselectivity of some *in vivo* transformations of *Cystoseira* meroditerpenoids, and consequently the hypothesized pathway. (The exact *R* or *S* configurations at the chiral centres of the compounds studied from this point of view are reported in the formulae.)

Secondary metabolites of Cystoseira as chemotaxonomic markers. Using the secondary metabolites of *Cystoseira* as markers, results of a certain botanical importance or taxonomic relevance were obtained such as the identification of a new species, the characterization of a natural hybrid and the distinction of two species hitherto considered synonymous. The first result of botanical interest was the identification of a short length of the infralittoral fringe along the Southern Sicilian coastline, in which, *C. amentaceae* var. *stricta*, the species usually dominant at that level, is completely replaced by a morphologically and chemically different species. This seaweed was recognized as a new species, *C. hyblaea* [139]; the limited confines of its distribution area indicate that

Table 6. Chemical groups of Mediterranean *Cystoseira* species compared with Giaccone's morphological groups

	Chemical groups							
					Group V			
Giaccone's groups	Group I	Group II	Group III	Group IV	[a]	[b]	Group VI	Group VII
Group I							*C. amentacea* var. *amen.* *C. amentacea* var. *stricta*	*C. tamariscifolia* *C. mediterranea*
Group II		*C. brachycarpa* *C. balerica*	*C. dubia* *C. hyblaea* *C. barbatula* *C. crinita* *C. sauvageauana*					
Group III				*C. squarrosa* *C. zosteroides* *C. usneoides*	*C. jabukae* *C. elegans*	*C. algeriensis* *C. spinosa* *C. baccata*		
Group IV	*C. compressa* *C. humilis* *C. susanensis* *C. barbata*		*C. schiffneri*					

Chemical groups: Group I = no lipophilic secondary metabolites; Group II = linear diterpenoids; Group III = linear meroditerpenoids; Group IV = tetrahydrofurane, furane and pirane ring; Group V = cyclic meroditerpenoids (a = cyclopentane ring; b = bicyclo[4.3.0]nonane ring system); Group VI = Bicyclo[3.2.0]heptane ring system; Group VII = Rearranged meroditerpenoids.

Giaccone's groups: Group I = *Cystoseira ericaefolia*; Group II = *C. crinito-selaginoides*; Group III = *C. spinifero-opuntioides*; Group IV = *C. discors-abratanifolioides*.

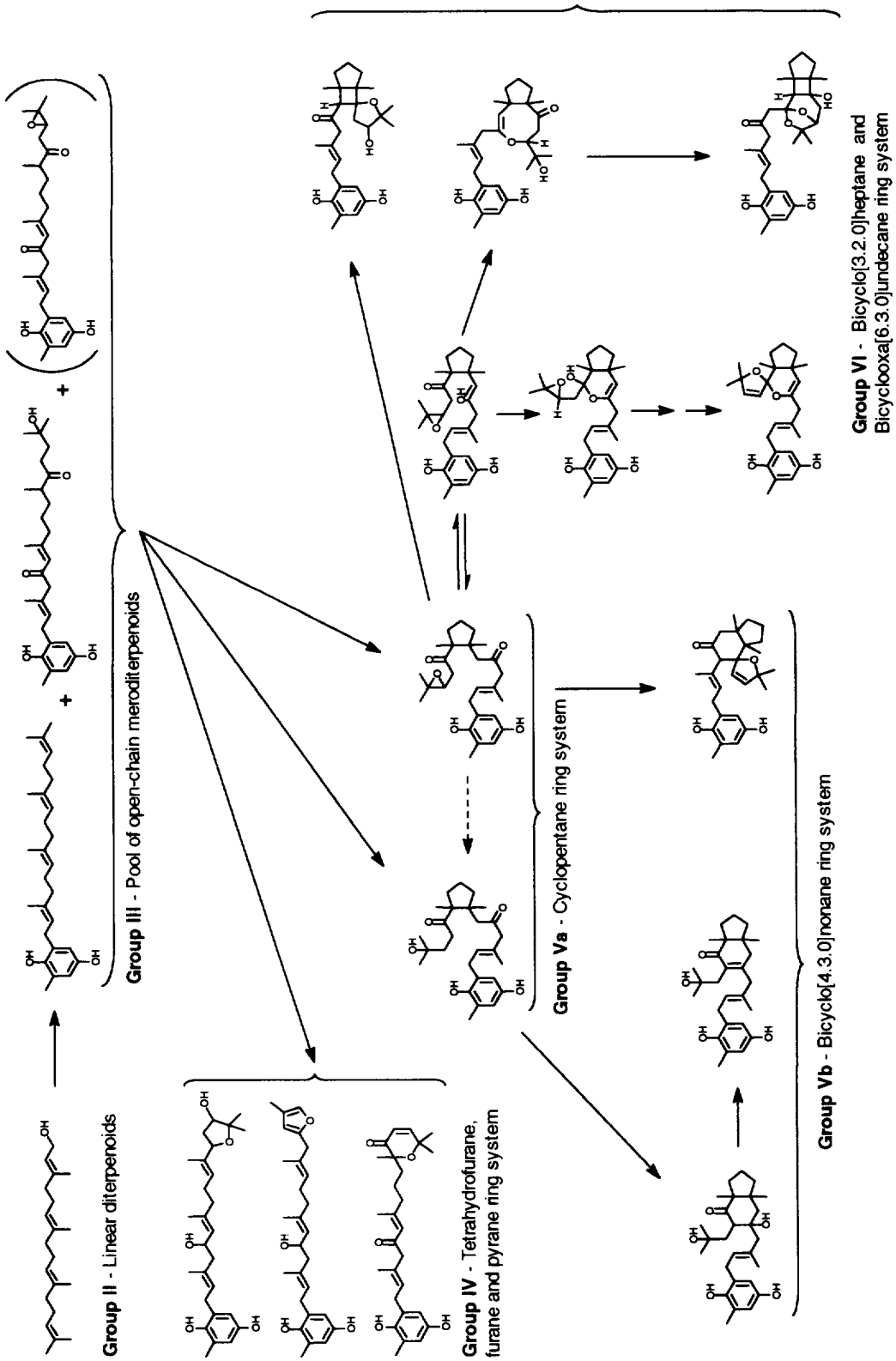


Fig. 8. Hypothetical biosynthetic pathway of *Cystoseira* metabolites.

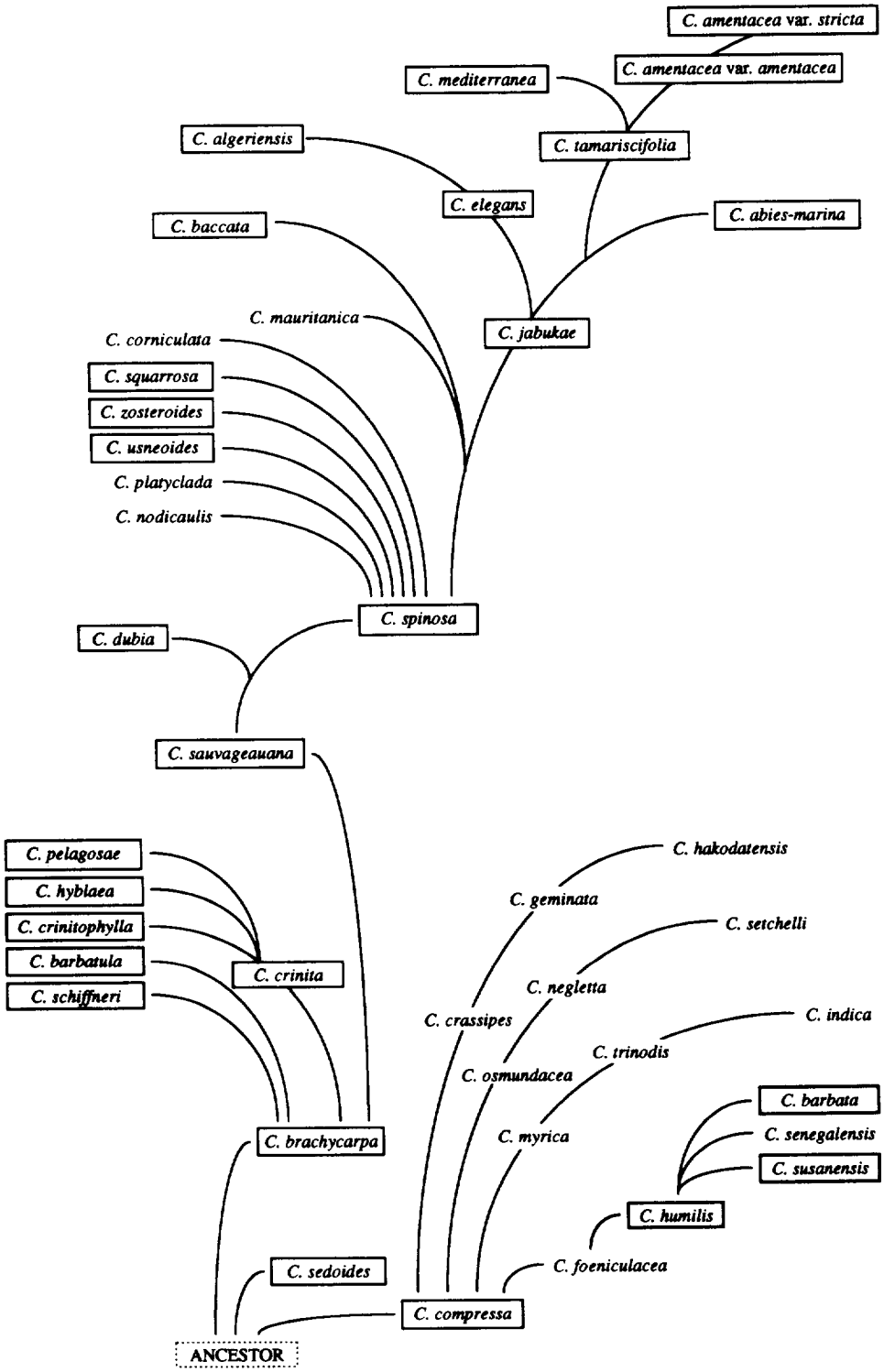


Fig. 9. Possible phylogenetic relationships of the species of *Cystoseira* according to Giaccone [53, 75, 79, 80] (the boxes indicate the species studied from a phytochemical point of view).

this species has differentiated very recently, thus confirming the opinion of Ercegovic about the genetic plasticity of the genus *Cystoseira* [70, 77].

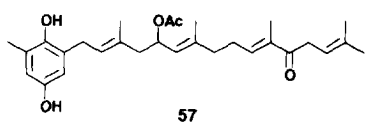
The second result, taxonomically more relevant, regards the occurrence in nature of interspecific hybridization within the genus *Cystoseira*. Field observations of a mixed population of *C. elegans* and *C. algeriensis* revealed the presence of some individual plants whose

general appearance was intermediate to the two species, occasionally resembling one or other of them more closely. This suggested that these plants could be the natural hybrid *C. elegans* × *C. algeriensis*. Since studies of the chemistry of secondary metabolites from higher plants have been used to establish the existence in Nature of hybrids, on the basis that in general the chemical composition of a hybrid is the sum of that of its parents [140–143], we investigated the chemical constituents of the suspected hybrid and found that, as expected, the profile of secondary metabolites in its lipid fraction is essentially a composite of that of the putative parent species [112]. This result permits us to extend the utilization of chemical markers as a criterion for interspecific hybridization to algae as well.

The last result of taxonomic significance regards *C. barbatula* and *C. barbata*. Of these two species, *C. barbatula*, described in 1860 by Kützing from samples collected in the Bay of Naples, has been quoted by several taxonomists (Valiante [68], Hamel [144], Ercegovic [70]) as a synonym of *C. barbata* J. Agardh. Since we obtained samples of two algae closely corresponding to Agardh's description of *C. barbata* [67] and the other to *C. barbatula* as described by Kützing, we considered a comparison of their chemical composition as useful in resolving the problem. The analysis of *C. barbatula* afforded the five new compounds (**56–60**) [99], which are closely related to those previously isolated from *C. zosteroides* [102], *C. sauvageana* [103] and *C. adriatica* var. *intermedia* (now *C. spinosa* var. *compressa* [80]) [98], whereas the pattern of *C. barbata* secondary metabolites was simpler than that of *C. barbatula* and yielded α-tocopherol and a single tetraprenyltoluquinol, namely (**12**) previously isolated from *C. hyblaea* [97]. The different patterns observed suggested they are both distinct species as successively confirmed from morphological observations [80].

Bifurcaria *and* Halidrys

Bifurcaria and *Halidrys* are the two genera morphologically closest ot *Cystoseira* and grow prevalently on Atlantic coasts. The simple linear diterpenoids **61–69**, along with compounds **2**, **4** and **8** previously reported from *Cystoseira brachycarpa* [91], are secondary metabolites isolated from *Bifurcaria bifurcata* [145–152], while the species of the same genus, *B. galapagensis* afforded bifurcarenone [153], (**70**), the first meroditerpenoid isolated from Cystoseiraceae, whose *trans*-stereoisomer, **34**, we later found in *Cystoseira* together with numerous other related metabolites [106]. The structure of bifucarenone has been successively revised, as a result of its total synthesis [154].



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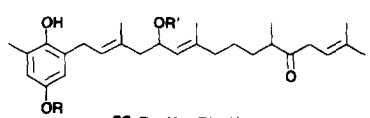
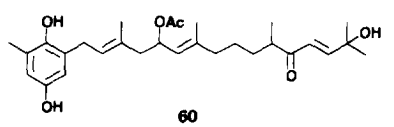


58 R = H R' = H
59 R = H R' = MeCO



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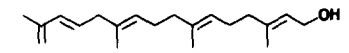

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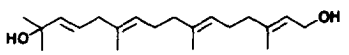

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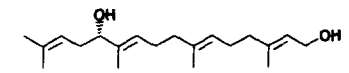

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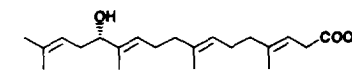

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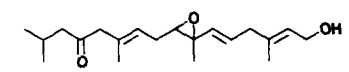

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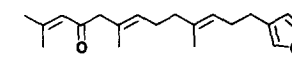

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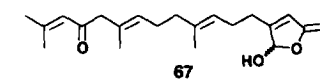

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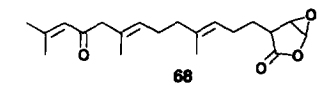

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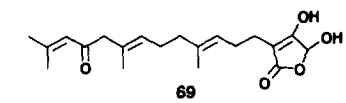

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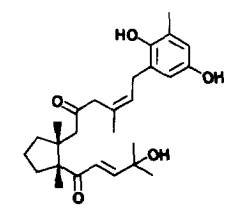
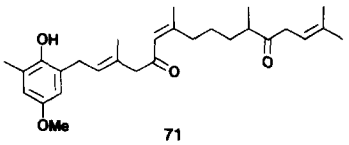


Bifurcarenone
70

Halidrys siliquosa, the only species studied of this genus, provided the six monomethyl hydroquinols with oxygenated diterpene side chains **71–75** [155] together with compound **16** which has also been found in *Cys-*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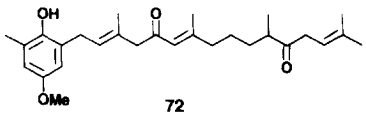
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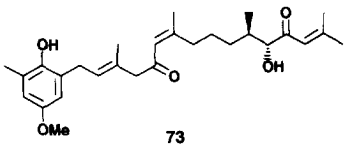
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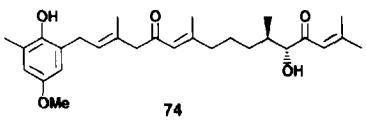
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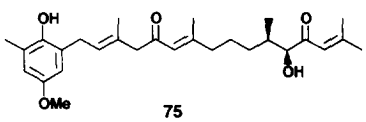
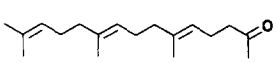

75

toseira zosteroides [102], confirming the morphological affinity of this alga to the *Cystoseira* species.

Cystophora

Despite being very rich in species, the data on compounds isolated from the genus *Cystophora* are much less ample than those on *Cystoseira*, since only seven of the 23 *Cystophora* species were considered from a phytochemical point of view. Only polyenes, alkenylresorcinols, phloroglucinols and polyphenols have been reported from *C. torulosa*, *C. congesta*, *C. scalaris* and *C. monilifera*, whereas *C. expansa* and *C. platylobium* contain δ-tocotrienol as the major lipid-soluble metabolite [156–159]. The sole species with metabolites other than acetogenins is *C. moniliformis*, which furnished some variously functionalized and cyclized farnesylacetone derivatives **76–88** [160, 161]. This agrees with the placing of this alga as one of the most developed species of this genus (Fig. 10) [84]. Compounds of this chemical type had previously been described from the brown alga *Sargassum micracanthum* and the author had suggested that these metabolites were derived from tocotrienols, present in other *Sargassum* species [162]. Prenyltoluquinol derivatives have also been isolated from *Cystophora*, but unfortunately the species of origin is not reported and therefore the results are taxonomically unusable [163]. However, it should be more morphologically evolved than *C. moniliformis*.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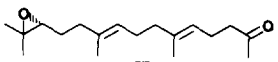
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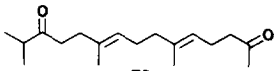
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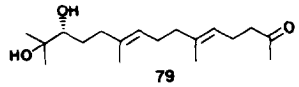
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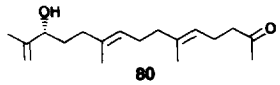
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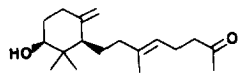
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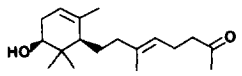
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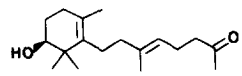
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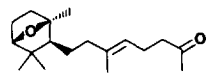
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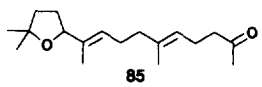
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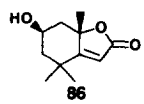
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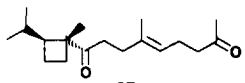
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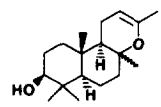

88

Acrocarpia *and* Caulocystis

The separation of the genera *Acrocarpia* and *Caulocystis* from *Cystophora* on the basis of morphological differences is fully supported by chemical data, since the secondary metabolite pattern of the seven *Cystophora* species analysed to date was distinctly different from that of

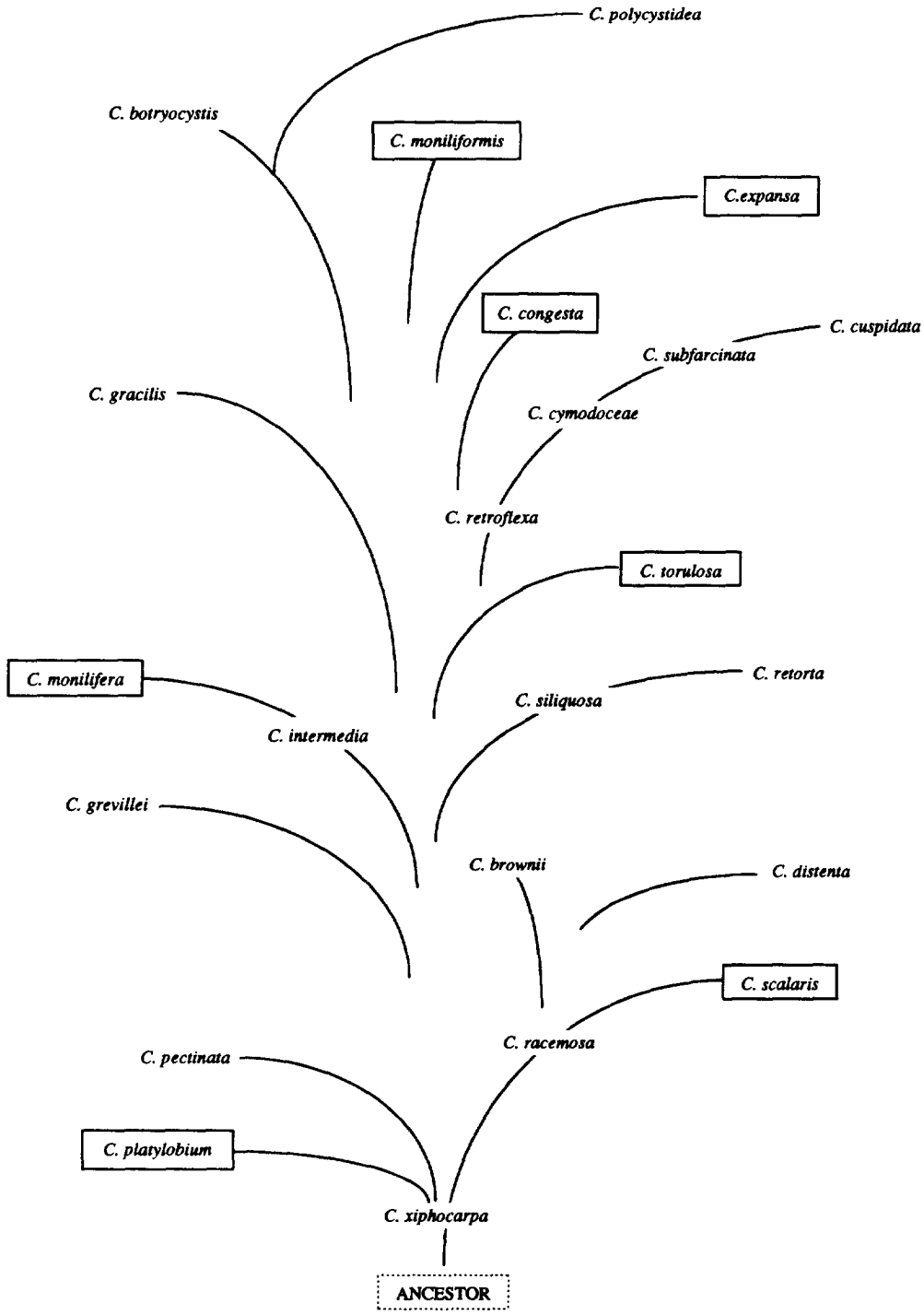
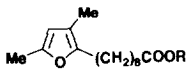


Fig. 10. Possible phylogenetic relationships of the species of *Cystophora* according to Womersley [84] (the boxes indicate the species studied from a phytochemical point of view).

Acrocarpia and *Caulocystis*. In fact, the dominant secondary metabolites of *A. paniculata* are two furanoid ethyl esters **89** and **91** [164], which are probably artifacts of the methanol/H_2O extraction procedure (the natural counterparts being the corresponding acids **90** and **92**), while the prevalent compound isolated from *C. cephalornithos* is salicylic acid with other simple acetogenins **93–96** [165-166].

Comparison of the secondary metabolite patterns found in the brown alga genus *Cystophora* with those found in *Caulocystis* and *Acrocarpia* are consistent with all three being distinct but related genera.



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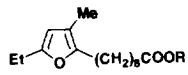
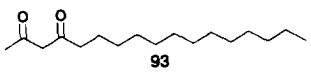
90 R = H



91 R = Et
92 R = H



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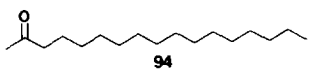
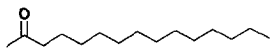
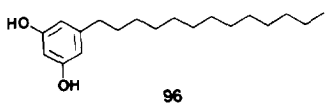


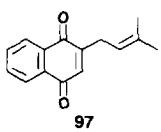
94



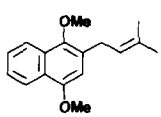
95



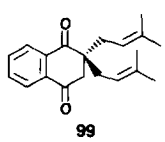
96



97



98



99

Landsburgia

Apart from a report on carotenoid content [167], the major metabolite from *L. quercifolia* is deoxylapachol (**97**), a cytotoxic and antifungal 1,4-naphthoquinone isolated along with minor amounts of 1,4-dimethoxy-2-(3-methyl-2-butenyl)-naphthalene (**98**) and 2,3-dihydro-2,2-bis(3-methyl-2-butenyl)-1,4-naphthalene-dione (**99**) [168]. These compounds constitute the sole example of naphthoquinone metabolites isolated from a Cystoseiraceae.

CONCLUSIONS

Within the genus, the chemical data, closely agreeing with the morphological data, proved that species with the most complex metabolites are also the most evolved. Thus, as regards the *Cystoseira* genus, *C. amentacea* var. *stricta* which elaborates the more complex meroditerpenoids is more evolved than for example *C. sauvageauana*, which in turn is more evolved than *C. brachycarpa*. Furthermore, *C. sauvageauana*, accumulating compounds **18** and **20**, constitutes an important phylogenetic and metabolic crossing.

As for the *Cystophora* genus, only one species, namely *C. moniliformis*, seems to be more evolved than the others, since it accumulates terpenoids instead of acetogenins. Chemical data also support the morphological data for the genera considered morphologically closest to the above two. In fact *Halidrys* and *Bifurcaria* produce metabolites related to those of *Cystoseira*, while *Acrocarpia* and *Caulocystis* accumulate simple acetogenins as do nearly all the *Cystophora*. Thus, if one accepts as an evolutionary criterion the capacity to synthesize more or less complex terpenoids, then it may be said that *Cystoseira* is the most evolved genus; *Halidrys* and *Bifurcaria* follow, and then *Cystophora*, of which only one species, in agreement with morphological data, is more evolved than the others, without even taking into account the unclassified species reported in ref. 163. Support for this hypothesis might come from a study of the homogeneity of the protein sequences which, analogously with that found by Margoliash *et al.* for cytochrome *c*, could provide significant phylogenetic correlations between Cystoseiraceae [169–173].

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