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NATURAL PRODUCT CHEMISTRY OF THE MARINE SPONGES

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Abstract—A systematic search for constituents of marine sponges has yielded over one hundred new compounds, most of them with unique structural features. A broad survey of the field is presented and certain topics, particularly those closely related to recent work done in our own laboratory on sesquiterpenoids, are discussed in more detail.

INTRODUCTION

In the context of the recent increased interest in the chemistry of marine organisms, the sponges, very primitive multicellular animals, have also received attention leading to the discovery of many novel molecules. Since Bergmann's pioneering work[1] on the fatty acids and sterols in sponges over a hundred different compounds have been isolated, mostly in the last 5–6 yr.

When I started to prepare this lecture I had the choice of either presenting a summary review, or selecting only certain topics. It seemed to me best to choose the former. This would give a general picture of the sponge-derived natural products and should help to focus attention on the structural relationship between compounds isolated from different species, and allow us to see if metabolites so far isolated from sponges also occur in other marine phyla and/or terrestrial organisms.

With your permission, I will attempt to discuss in more detail the very recent results from our own Laboratory, particularly those concerning sesquiterpenoids.

In this lecture, the known natural products from sponges have been grouped in accordance with their probable biosynthetic origins, and I will discuss bromo-compounds, terpenes, compounds of mixed biogenesis, and sterols in that order. A brief mention of some miscellaneous compounds will be also made. Fatty acids and pigments are excluded as very little has been published on these topics since they were last reviewed in 1968.[2,3]

BROMO-COMPOUNDS

About a hundred naturally occurring organobromo-compounds have been so far described, and only one of these was not isolated from a marine organism.[4] Thus these compounds, which belong to such diverse chemical classes as phenols, pyrroles, indoles, sesquiterpenes, diterpenes, and polynuclear heterocycles, appear to be characteristic of the marine environment. They have been found especially in algae.[5,6] Several brominated monoterpenes, sesquiterpenes, and diterpenes have also been extracted from the digestive gland of molluscs of the genus *Aplysia*, but experiments revealed that the chemical constituents of the digestive gland depended on the algal diet of the individual *Aplysia*,[7] and there now seems no reason to doubt that the brominated terpenes from *Aplysia* are derived from red algae such *Laurencia*, a common food of the sea hare. So, besides the algae, the richest source of bromo-compounds appears to be the sponges.

Sponges of the family Verongidae have provided a series of antibiotics and other closely related compounds which may be considered as metabolites of 3,5-dibromotyrosine. Figure 1 lists their structures. The first two members of the series were isolated from the methanolic extracts of *Verongia fistularis* and *V. cauliformis* by Sharma and Burkholder.[8–10] The failure to convert I into II by reacting with methanol under various conditions allowed the authors to assume that the ketal II was a genuine natural product and not an artifact generated during the extraction. Recently Andersen and Faulkner[11] have isolated from the ethanolic extracts of an undescribed species of *Verongia* the dienone I and the mixed ketal III, which latter was revealed to be a mixture of diastereoisomers (two methoxy signals in the 220 MHz NMR). This suggested that the ketal was not a natural product and led the authors to propose that the dienone I, the dimethoxyketal II and the mixed ketal III may all be derived from a single intermediate, such as an arene oxide (XI), by 1,4 addition of water, methanol, or ethanol during the extraction process. The recent work of Kasperek *et al.*,[12] showing that acid-catalyzed addition of methanol to 1,4-dimethylbenzene oxide give 4 - methoxy - 1,4 - dimethyl - 2,5 - cyclohexadienol, was quoted by the authors in support of their arguments.

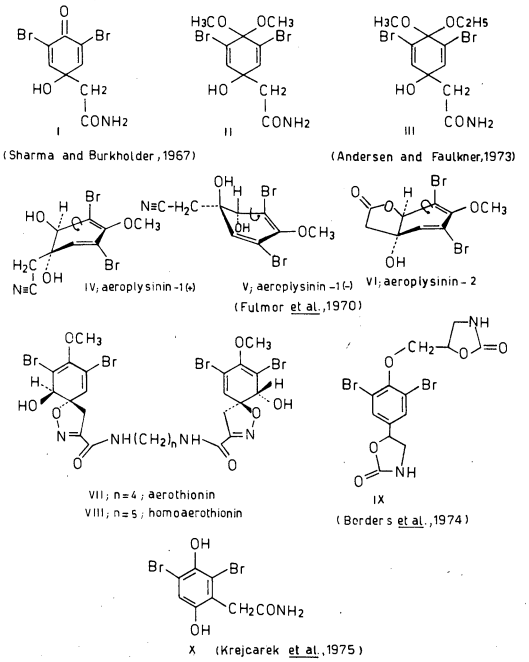
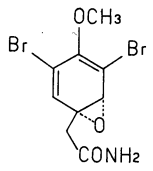


Fig. 1. Tyrosine-derived bromo-compounds.



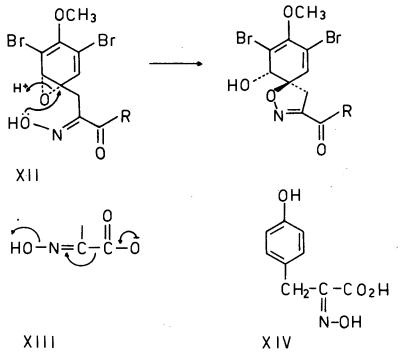
XI

Aeroplysinin-1 (IV), the nitrile component, was first isolated as the dextrorotatory isomer in our laboratory from *Verongia aerophoba*,[13] which also contains the dienone I, the lactone VI,[14] and the more complex VII and VIII.[15] Fulmor *et al.*[16] isolated the laevorotatory antipode of aeroplysinin-1 from the closely related sponge *Ianthella ardis*, for which they propose the absolute configuration as shown in V on the basis of combined chemical, c.d. and NMR data. The absolute structure of both antipodes as shown in IV and V have now been firmly established by two independent X-ray studies.[17,18]

The occurrence of each enantiomorph in different genera of the same class of invertebrates is most unusual.

Aeroplysinin-1, which possesses, besides antibacterial properties, antitumor activity, is the first example of a naturally occurring 1,2 - dihydroarene - 1,2 - diol and it could be biosynthesized *via* an arene oxide[19] in agreement with the stereochemistry.

The two more complex brominated metabolites (VII and VIII) obtained from *V. aerophoba* were also isolated by Moody and Thomson from *V. thiona*, and accordingly named aerothionin and homoaerothionin. Structural elucidation of these two spirocyclohexadienylisoxazoles was the result of a collaborative effort between Professor Thomson's laboratory in Aberdeen and our own group in Naples.[15] I cannot specify in detail the arguments leading to the structures VII and VIII but I must add that the structure of aerothionin, the major component of both sponges (*ca.* 10% in *V. aerophoba*), has now been confirmed by X-ray crystallographic analysis (J. Clardy, personal communication), which also revealed the relative stereochemistry (O–H and O–N *trans*). The spiro systems in VIII and VIII could arise in various ways including nucleophilic attack by an oxime function on an arene oxide as shown in XII (Fig. 2). Following suggestions that nitriles may be derived *in vivo* from α-amino-acids by way of α-keto and α-oximino-acids,[20] we speculated that the oxime (XII, R = OH) could be also a likely precursor of the nitrile aeroplysinin-1 (IV and V), as indicated in XIII. We now have obtained good support for this hypothesis by isolating from a marine sponge *Hymeniacidon sanguinea* the oximinopyruvic acid XIV.[21]



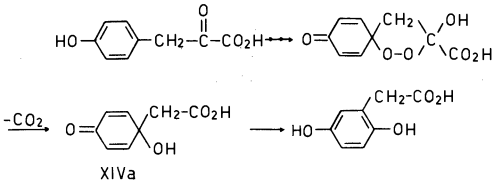
XII XIII XIV

Fig. 2. Biogenetic hypothesis for the formation of the spiro system in aerothionin and of the nitrile function in aeroplysinin-1.

The compound IX listed in Fig. 1 has been isolated from *Verongia lacunosa*[22] and it is unique in that it appears to be the first bromo-compound containing 2-oxalidone rings isolated from a sponge. The latest addition to this group is X isolated from *V. aurea* by the Rinehart's group (Illinois).[23] It represents a major departure from the previously reported *Verongia* brominated metabolites in which the aliphatic side chain remains in the *para* position relative to the hydroxyl group flanked by bromine atoms. An analogy for such a rearrangement of the tyrosine skeleton is available, however, in the conversion of 4-hydroxyphenylpyruvic acid into 2,5-dihydroxyphenylacetic (homogentisic acid), catalyzed by an enzyme classified as a mono-oxygenase.[24] Interestingly a nonenzymic pathway shown in Fig. 3 for the conversion of 4-hydroxyphenylpyruvic into homogentisic acid *via* the quinol XIVa, itself formed most probably *via* a cyclic peroxide, has been very recently described.[25] This could also offer an alternative plausible explanation for the biogenetic formation of the dienone I (Fig. 1).

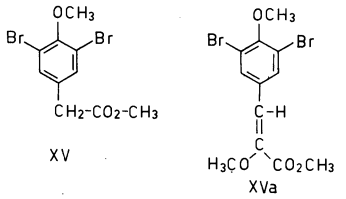
Beyond these speculations, all the *Verongia* brominated metabolites seem fairly obviously biosynthesized from 3,5-dibromotyrosine, itself found in sponge proteins,[26] and presumably the central C_4N_2 and C_5N_2 chains of aerothionin (VII) and homoaerothionin (VIII) are derived from ornithine and lysine, respectively.

Quite surprisingly *V. aerophoba* failed to incorporate radioactivity from {U-^{14}C}-L-tyrosine into aerothionin (VII), aeroplysinin-1 (IV) and the dienone (I); inactive aerothionin (VII) was also isolated when the animals were fed with {U-^{14}C}-L-ornithine and {CH_3-^{14}C} methionine.[27] However the sponge utilized these aminoacids for the synthesis of fatty acids. A very slow rate of biosynthesis might account for these results. A dietary origin for these compounds can be also suspected and in this connection the recent report of the isolation of the brominated esters XV and XVa (after methylation) from hydrolyzed extracts of the red alga *Halopytis incurvus*[28] seems relevant. So it appears possible that the brominated compounds isolated from sponges, like the bromo-terpenes from molluscs, were originally fabricated by algae. In addition I must emphasize that all the Verongidae, like many other sponges, have large amounts of symbiontic bacteria and blue-green algae in their tissues.[29]



XIVa

Fig. 3. Nonenzymic pathway for the conversion of 4-hydroxyphenylpyruvic acid to homogentisic acid (I. Saito *et al.*, 1975).



XV XVa

Fig. 4. Bromo-compounds (after methylation) from the hydrolyzed extracts of the red alga *Halopytis incurvus* (J. M. Chantraine *et al.*, 1973).

Before leaving this subject I should like to show you the list of sponges, collected in different seas and oceans, which have been reported to contain tyrosine-derived bromo compounds (Table 1). So these compounds seem to be confined to the *Verongidae* family and to the closely related *Ianthella genus*; this has received support from a recent survey for the presence of the above group of compounds covering 33 more species representative of 17 families and 9 orders of the class Desmospongiae.[30]

Table 1. Sponges in which dibromotyrosine-derived compounds have been reported[30]

Sponges
Order Dictioceratida
Family Verongidae
V. aerophoba
V. archeri
V. cauliformis
V. fistularis
V. thiona
V. sp.
V. lacunosa
V. aurea
Family Disideidae
Ianthella ardis
Ianthella sp.

Another group of bromocompounds are the biogenetically related bromopyrroles isolated from two *Agelas* and three Axinellida. Fig. 5 lists their structures. The simple dibromopyrroles (XVI–XVIII) have been isolated from *Agelas oroides*,[31] which also contains, in much larger amounts (2–3% of dry sponge), the more complex oroidin, to which the structure XIX has been definitively assigned after the synthetic work of Garcia *et al.*[32] Oroidin has also been recently found in *Axinella damicornis* and *A. verrucosa*.[30] The polycyclic dibromophakellin (XX) and 4-bromophakellin (XXI) closely related to oroidin, and showing broad spectrum antimicrobial activity, have been isolated from *Phakellia flabellata* (Axinellidae) by Sharma and Burkholder.[33] The complete structure of dibromophakellin (XX), the major component of the sponge, was established by X-ray analysis of a single crystal of the monoacetyl derivative. The latest addition to this group is the new antibiotic 4 - bromopyrrole - 2 - carbonylguanidine (XXII), isolated from an unidentified species of *Agelas*.[34] The occurrence of related bromopyrroles from two *Agelas* (order Poecilosclerida) and three Axinellida

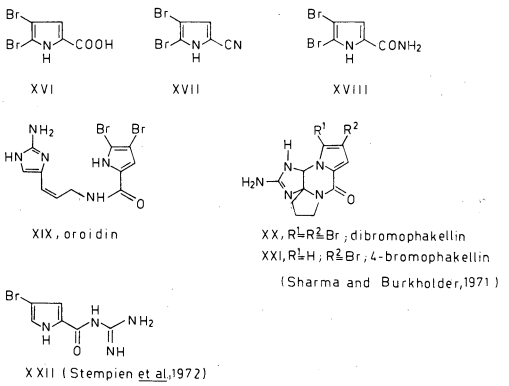


Fig. 5. Bromopyrroles from sponges.

seems to indicate a relationship between *Agelas* and some *Axinellida*. In this connection it is important to note that Bergquist and Hartmann[35] observed an anomalous amino-acid pattern in the *Agelas* genus with respect to those of other Poecilosclerida and found it difficult to differentiate *Agelas* from typical Axihellidae.

In Fig. 6 the remaining bromo-compounds so far isolated from sponges are shown. The two brominated phenoxyphenols, active against both gram-neg. and gram-pos. organisms, have been found in *Disidea herbacea*,[36] while the two new antibacterial bromoindole metabolites have been isolated from the Caribbean sponge *Polyfibrospongia maynardii*.[37]

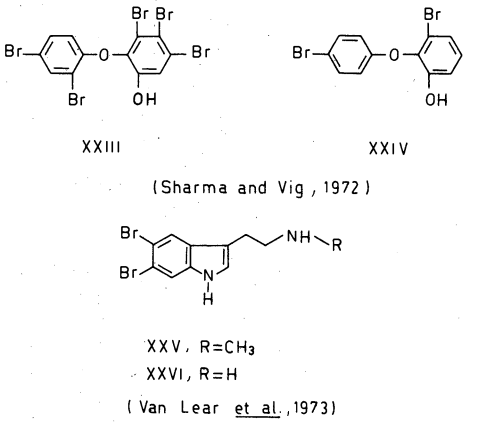


Fig. 6. Miscellaneous bromo-compounds from sponges.

TERPENES

Terpenes are amongst the most widespread groups of natural products. They are mainly of fungal and plant origin, but they have also been isolated from insects and marine animals. In marine organisms they have been reported to date from algae, coelenterates, molluscs and sponges. The distribution among marine phyla may be even smaller, as stated by Faulkner and Andersen in their recent excellent review which appeared in *The Sea*,[38] since there is evidence that algal symbionts are the true sources of terpenoid compounds isolated from coelenterates and that the terpenoids from molluscs are derived from ingested algae.

Sponges have provided terpenes in large amounts, most of them possessing unique structural features without parallel in terrestrial sources.

Furan rings occur frequently, although hitherto nearly all known furanoterpenes were plant products. In this group the linear furanoterpenes containing 21 carbon atoms are the most intriguing compounds from the biogenetic point of view. More recently we have encountered an interesting assortment of furanoid sesquiterpenes, and I would like to give you a brief account of some work on these compounds. Furthermore, sesterterpenes are relatively abundant in sponges in contrast with their very limited distribution elsewhere.[39] Even more unusual are terpenes bearing an isonitrile function, a very rare feature in nature. At this time neither monoterpenes not triterpenes have been reported from sponges sources, apart from squalene which was found in *Ircinia spinosula*[40] and *I. muscarum*.[41]

Sesquiterpenes

Now let me discuss the sesquiterpenes. *Disidea pallescens* has provided ten new sesquiterpenes[42] (Fig. 7).

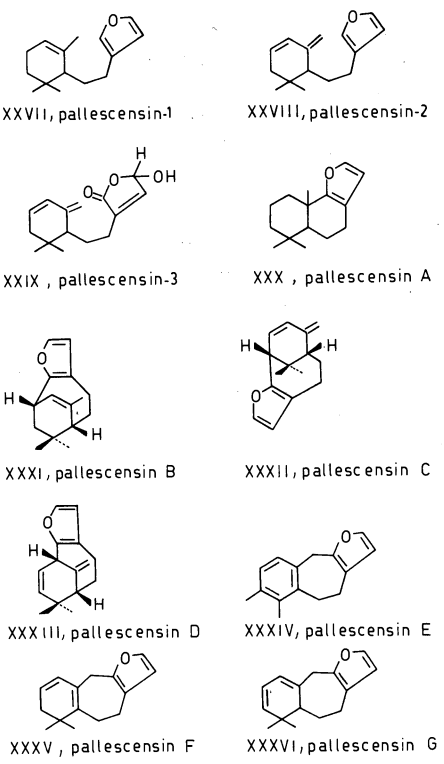


Fig. 7. Furan sesquiterpenes from the sponge *Disidea pallescens.*

These include three of a mono-cyclofaresane type, pallescensin-1 (XXVII), –2 (XXVIII) and –3 (XXIX), and seven, pallescensins A–G (XXX–XXXVI), closely related, having a 2,3-disubstituted furan ring and two additional cycles. Lack of material and the instability of most of them prevented extensive chemical investigation and the structural assignments are mainly based on spectral grounds, biogenetic considerations, and interrelation between them.

U.V. and NMR spectra (Fig. 8) clearly revealed the presence in pallescensin-2 of a β-substituted furan ring isolated from a conjugated diene system. The NMR pattern due to the olefinic protons was readily assigned to a 1,3-disubstituted conjugated butadiene system. The downfield vinyl-H (δ 5.94) is clearly an internal hydrogen of the conjugated system and the 10 Hz coupling indicates a *cis* double bond. Hydrogenation yielded two dihydro-derivatives and the major one is the 1–4 addition product (XXVII); in the mass spectrum a significant *m/e* 162 fragment corresponding to the elimination of isobutene by the retro-Diels–Alder process, supported the presence in its structure of a 4,4 - dimethylcyclohex - 1 - ene ring. The structure XXVIII, proposed for pallescensin-2, fits with all data and its mass spectrum, which is marked by intense "McLafferty type" fragments at *m/e* 122 and 94 (Fig. 8), added confirmatory evidence. The structures of pallescensin-1 (XXVII) and pallescensin-3 (XXIX) in which latter the furan ring is modified as a γ - hydroxy - α,β - butenolide, were determined by spectral data and interrelations with pallescensin-2 (XXVIII) shown in Fig. 8.

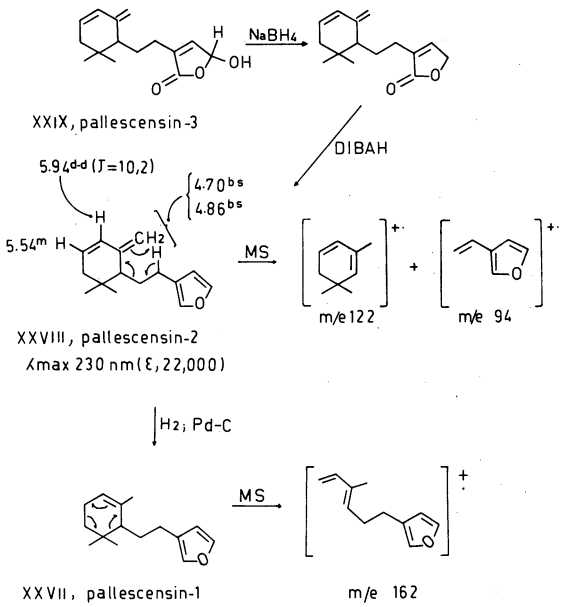


Fig. 8. Structures of pallescensin-1, -2 and -3. Chemical shifts are in ppm from TMS and coupling constants in Hz.

Pallescensin A (XXX), which showed NMR characteristics indicative of three *tert*-methyl groups and a 2,3-substituted furan ring (Fig. 9), was also interrelated with pallescensin-1 (XXVII). The latter, on treatment with BF_3–etherate, yielded a tricyclic compound identical with XXX in GLC, SiO_2–$AgNO_3$ TLC, and mass spectrometry.

The cage like structures of pallescensins B–D (XXXI–XXXIII) were essentially based on a detailed analysis of the NMR data summarized in Figs. 10 and 10a, together with decoupling experiments.

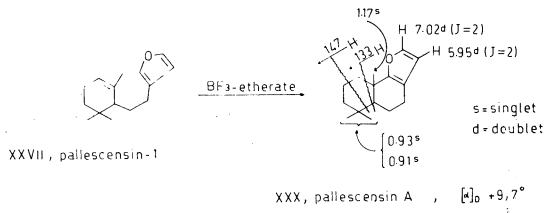


Fig. 9. Conversion of pallescensin-1(XXVII) to pallescensin-A(XXX).

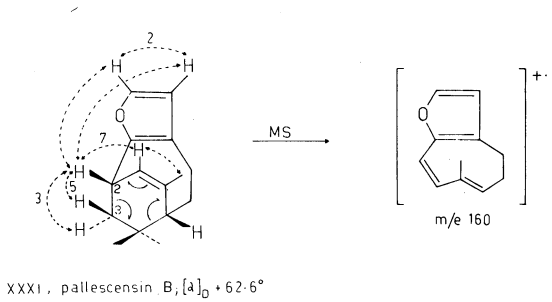
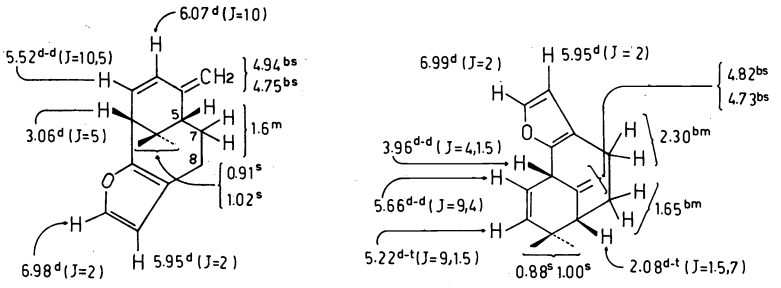


Fig. 10. Proton interactions in pallescensin B detected by decoupling; coupling constants in Hz.

In the mass spectrum of pallescensin B (Fig. 10) the significant *m/e* 160 fragment was interpreted as originating by elimination of isobutene by the retro-Diels–Alder process and suggested the presence of a dimethylcyclohexene ring. The sequence of protons in the six-membered ring was determined by decoupling, and the detected proton interactions are indicated in Fig. 10. Irradiation at δ 3.40 (H-2) sharpened both the furanoid protons, reduced the olefinic broad doublet at δ 5.83 into a broad singlet and also the signal at δ 1.60 (2H; H_2 at C-3)



XXXII, pallescensin C; $[\alpha]_D$ + 42.4°

XXXIII, pallescensin D; $[\alpha]_D$ + 45.3°

Fig. 10(a). Chemical shifts (ppm from TMS) and coupling constants (J = Hz) of pallescensin C and D in C_6D_6.

into a singlet. Thus a quaternary carbon must be close to the C-3 carbon. The vinyl methyl at δ 1.80 was found to be "long-range" coupled with the olefinic proton. The spectral data, coupled with the isoprene rule and the molecular formula, $C_{15}H_{20}O$, which requires in addition to the furan ring two more cycles, makes it possible to write the structure XXXI.

As expected the olefin was unreactive towards osmium tetroxide, *m*-chloroperbenzoid acid, and hydrogenation in different conditions. Moreover, CrO_3-pyridine oxidation left pallescensin B unchanged.

The analysis of the NMR data of pallescensin C and D together with double resonance experiments gave the sequence of all the H atoms. As shown in Fig. 10a all the protons of pallescensin D resonate as well-separated signals. In the spectrum of pallescensin C protons on C-8 and C-5 overlap and form a large broad multiplet spread between δ 2.3–1.9, but irradiation at δ 1.6, the centre of the multiplet due to the C-7 methylene protons, transformed the δ 2.3–1.9 multiplet into a simpler signal from which emerged a clearly visible ABq (J = 16 Hz) and a broad singlet, and this gave the $-CH_2CH_2CH-$ sequence for the remaining protons of the molecule.

Pallescensin E–G (XXXIV–XXXVI) are the remaining sesquiterpene constituents of the sponge *Disidea pallescens* and Figs. 11 and 12a list some data on which the assignments of their structures are based.

The cisoid diene chromophore in pallescensin G was evident from the UV spectrum which also suggested the presence of a furan ring isolated from the diene. Additional evidence for the presence of a cisoid diene in an asymmetrical environment came from c.d. measurements (strong negative Cotton effect). The furan ring is 2,3-disubstituted as indicated by the NMR (1H doublets at δ 7.17 and 6.09; J = 2 Hz) and the vinyl-H signals appear in C_6D_6 as an ABX pattern {ν_A 5·75 (H-2), ν_B 5.58 (H-1), ν_X 5.30 (H-3) ppm; J_{AB} 4.5 Hz, J_{AX} 9 Hz, J_{BX} 1.5 Hz}. The NMR spectrum also displayed signals for an isolated methylene group (ABq at δ 3.49, J_{AB} 17 Hz) between the furan ring and the diene system, and also two *tert*-methyl groups (0.99 and 1.05 ppm). Decoupling experiments, which also revealed long-range proton interactions depicted in Fig. 11, were particularly informative and allowed us to propose the structure XXXVI.

The optically inactive isomeric pallescensin F, which gave spectral data in accord with the structure XXXV, was interrelated with pallescensin G by showing the identity (NMR, MS and GLC) of its 1,4-hydrogenation product with the major dihydroderivative (XXXVII) of pallescensin G. Significantly XXXVII gave in the mass spectrum a strong peak at m/e 160 corresponding to elimination of isobutene from the dimethylcyclohexene ring.

The alternative structures XXXVIII and XXXIX for pallescensins F and G, which could fit with most of the NMR data, are unlikely on biogenetical grounds.

We also tentatively suggested for pallescensin G the absolute configuration shown in Fig. 12.

The negative Cotton effect observed for pallescensin G suggested that the diene chromophore is twisted in the form of a left-handled helix. Since irradiation at H-5 left unchanged the shape of the H-3 olefinic signal, a quasi-

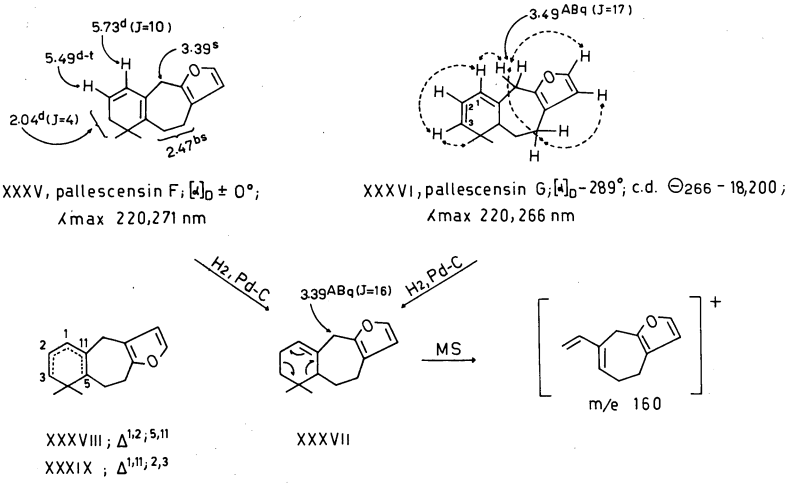


Fig. 11. Structures of pallescensin G and F; ---- long range proton interaction detected by decoupling.

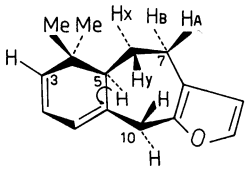


Fig. 12. Absolute configuration of pallescensin G (XXXV17; c.d. $\theta_{266} - 18{,}200$.

axial orientation is required for H-5 (in the case of a quasi-equatorial orientation, H-5 should be in a W relationship with H-3 and one should expect couplings, even small, between them). On this basis, we tentatively propose for pallescensin G the absolute configuration shown with R-chirality at the sole asymmetric center C-5. Furthermore from inspection of the Dreiding models of the two possible conformers, one is unreal because of the steric hindrance between one *tert*-Me and H_2C-7, and for the more favourable one the coupling constants H_A–H_X, H_A–H_Y, H_B–H_X and H_B–H_Y calculated from the corresponding dihedral angles by applying the Karplus equation accord with the observed values.

Figure 12a summarizes the spectral data of pallescensin E, the benzenoid component of this group. An accurate analysis of the small long-range couplings, detected by decoupling, was also in this case the key argument which favoured the structure XXXIV for pallescensin E.

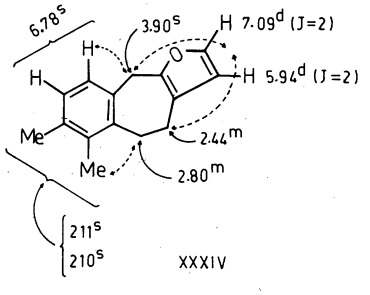


Fig. 12(a). Chemical shifts (ppm from TMS) of pallescensin E (C_6D_6); ---- long range proton interaction detected by decoupling.

Pallescensins A–G represent new skeletal types amongst the sesquiterpenoids, and their structures can also be rationalized biogenetically when a furanoid monocyclofarnesane intermediate is submitted to C–C cyclizations, as shown in Fig. 13, and further oxidations.

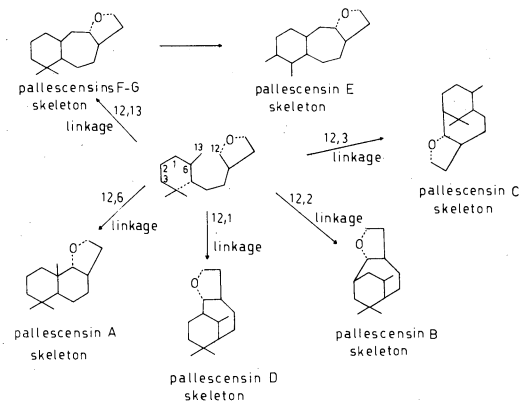


Fig. 13. Possible biogenetic scheme for the formation of pallescensins A–G.

The co-occurrence of furanoid sesquiterpenes of the monocyclofarnesane type provides good support for this suggestion.

A further group of furan sesuqiterpenes have been extracted from the sponge *Pleraplysilla spinifera*. A marked difference was found between the components of two samples collected in the Bay of Naples. However, according to the expert opinion of an authoritative zoologist, both samples were identical from a spicule analysis standpoint.

This, we believe, is an interesting finding. The two samples of the sponge show only slight morphological differences, but enough to be differentiated. I must emphasize that sponges are exceptionally difficult to classify and possibly the two samples belong to different species.

Figure 14 lists the structures of the constituents of the first sample.[43,44]

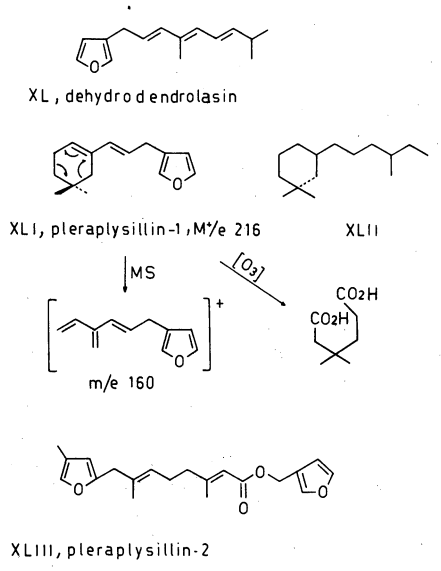


Fig. 14. Furan sesquiterpenes from *Pleraplysilla spinifera* (first sample).

Dehydrodendrolasin (XL), the major component (5% of dry sponge), is closely related to dendrolasin, the odour-substance of the ant *Dendrolasius fuliginosus*; the second component, pleraplysillin-1 (XLI), is a new type of sesquiterpene. The formation of the six-membered ring in pleraplysillin-1 is of biosynthetic interest, as it seems to arise by a C–C cyclization involving a lateral methyl group.

Its structure was deduced from spectral and degradative data: UV and NMR spectra pointed out the presence of a β-substituted furan ring isolated by a methylene from the 1,3-diene system; oxidative ozonolysis gave 3,3'-dimethyl adipic acid and the position of the double bond in the cyclohexene ring was confirmed by a strong peak at *m/e* 160 in the mass spectrum corresponding to elimination of isobutene by the retro-Diels–Alder process. The third component in *P. spinifera* is the ester pleraplysillin-2 (XLIII).

The second sample of *Pleraplysilla spinifera* contains longifolin (XLIV), previously found in a terrestrial plant,[45] and two more cyclic furan sesquiterpenes, named spiniferin-1 and -2, for which we propose the alternative structures (XLVa)–(XLVb) and (XLVIa)–(XLVIb), respectively (Fig. 15), with carbon skeletons of a new structural type.[46]

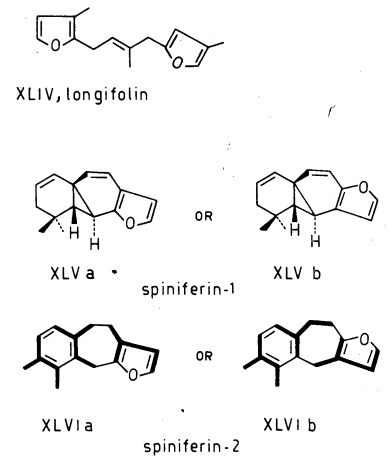


Fig. 15. Furan sesquiterpenes from *Pleraplysilla spinifera* (second sample).

Spiniferin-2 is an optically inactive oil showing UV absorptions (Fig. 16) consistent with the presence of furan and benzene rings. In the NMR spectrum the presence of a 2,3-disubstituted furan is indicated by doublets at δ 7.03 and 5.96. The benzenoid protons are seen as a singlet at δ 6.82 and two aromatic methyl groups resonate at δ 2.21 and 2.25. An isolated methylene group between the aromatic and furan rings is indicated by a low field broad singlet at δ 4.02, while a C_2 saturated chain is suggested by an A_2B_2 system with line positions at δ 2.61 and 2.92 ppm. An accurate analysis of the small long-range couplings, detected by decoupling, established that the isolated methylene is proximate to the methyl resonating at δ 2.25 (homobenzylic coupling), while the multiplet at δ 2.92 couples to the benzenoid protons (benzylic coupling). Decoupling experiments also revealed benzylic coupling between the C-6 methylene protons and both the furanoid protons and established the existence of "homobenzylic" couplings between the C-6 and C-9 methylene protons ($J_{6,9}$ 1 Hz). Smaller but observable couplings of the C-6 protons with the C-10 protons were also noted.

Oxidative ozonolysis, followed by methylation with diazomethane, afforded a dicarboxylic acid methyl ester, whose spectral properties fully agreed with the structure XLVII: the two aromatic protons now appear as an AB quartet with $J = 8$ Hz indicating an *ortho*-relationship.

Now only two alternative structures (XLVIa and XLVIb), both equally compatible with above evidence, appear to be possible for spiniferin-2. Chemical experiments directed to distinguish between them only gave untractable material.

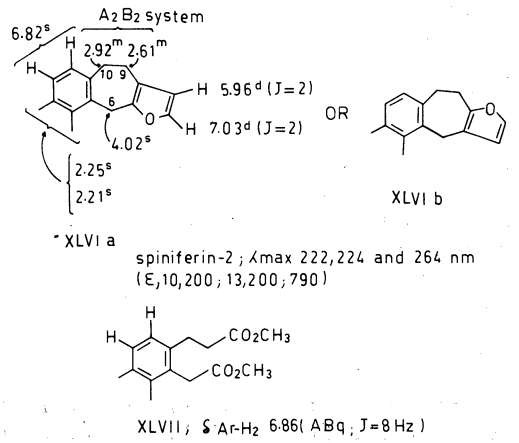


Fig. 16. Structure of spiniferin-2; Chemical shifts in ppm from TMS (CCl_4), coupling constants in Hz.

Spiniferin-1, the major furanoid constituent of the sponge, is optically active and showed UV absorptions suggesting the presence of a conjugated chromophore (Fig. 17).

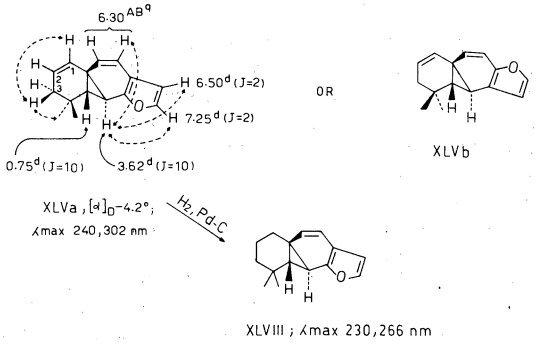


Fig. 17. Structure of spiniferin-1; Chemical shifts in ppm from TMS (CCl_4); coupling constants in Hz; ---- long range proton interaction detected by decoupling.

The two furan protons resonate at relatively low field, δ 7.25 and 6.50 (d, $J = 2$ Hz), in agreement with the presence of further unsaturation conjugated with the heterocyclic moiety (Fig. 17). The signal centred at δ 6.30 with the apparent feature of the central bands of an AB quartet is in agreement with the presence of a conjugated CH=CH. The NMR spectrum also displayed signals for two *tert*-methyl groups and an isolated $CH{=}CH{-}CH_2$ unit appearing as an ABXY system with line positions at δ 6.26 (H-1), 5.34 (H-2), 2.88 (H-3ax) and 2.02 (H-3eq) and coupling constants of 16 Hz (J 3eq, 3ax), 10 Hz (1, 2), 8 Hz (2, 3eq), 3 Hz (2, 3ax) and 3 Hz (1, 3ax). The two cyclopropane protons resonate as doublets at δ 3.62 and 0.75. All these assignments were confirmed by decoupling, which also revealed small interactions between the *ax*-methyl signal (δ 0.8) and the H-3*ax* at δ 2.88 (*trans*-methyl proton interaction) and between the cyclopropyl-H at δ 3.62 and both the furanoid protons and the vinyl-H signal at δ 6.30. The proton noise decoupled with off-resonance FT-^{13}C-NMR data of spiniferin-1 showed the presence of fifteen carbons. Eight of these were identified with four olefinic carbons with one attached proton (129.03, 123.98, 111.38, 108.31) and four furan ring carbons, two with one attached proton (139.78, 108.93) and two quaternary (152.3, 117.61). The remaining carbon atoms were identified as two quaternary (39.33, 29.40) two tertiary (33.88 split into a pair of doublets in the off-resonance spectrum), one methylene (43.98) and two methyl (30.50, 28.01) carbons. Bearing in mind the proposed alternative structures of the co-occurring spiniferin-2, the spectral data can be reasonably interpreted in terms of the alternative structures XLVa and XLVb. Hydrogenation of spiniferin-1 gave a 1,2-dihydroderivative (XLVIII) with UV absorption at 230, 266 nm in agreement with a furan chromophore conjugated with a double bond. By comparison the absorption of the parent compound (λ_{max} 240, 302 nm) is consistent with the further conjugation of the double bond in the dimethylcyclohexene ring through the spiro carbon of cyclopropane (spiroconjugation). As occurred with spiniferin-2, chemical attempts to distinguish between the two possibilities also failed.

Both pairs XLVa–XLVIa and XLVb–XLVIb appear biogenetically reasonable and they are based on carbon skeletons so far unique amongst sesquiterpenoids. Figure 18 shows a possible biogenetic scheme for the formation of both pairs starting from a *cis*-farnesyl precursor. The formation of the dimethylcyclohexane ring in XLIX through a C–C cyclization involving a lateral methyl group of the polyisoprene chain also occurs in pleraplysilin-1 (XLI), isolated from the first sample of *Pleraplysilla spinifera*.

A series of four more furano sesquiterpenes have now been obtained from the sponge *Microciona toxystila* and their structures are listed in Fig. 19. Microcionin-3 has been formulated as LII on spectral data and formation, on ozonolysis, of 2,2,6-trimethylcyclohexanone.

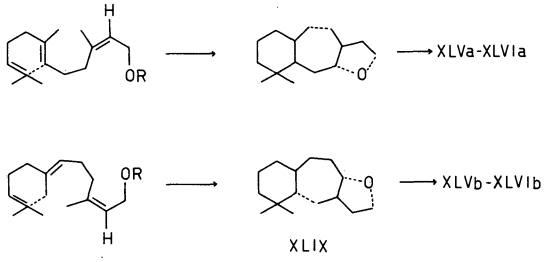


Fig. 18. Possible biogenetic scheme for the formation of spiniferin-1 and -2.

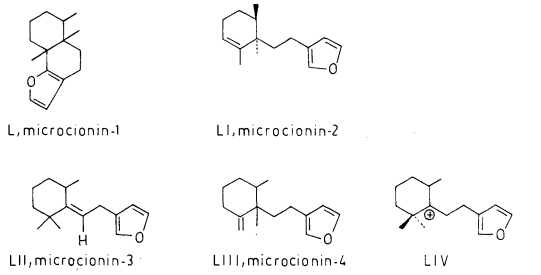


Fig. 19. Furan sesquiterpenes from *Microciona toxystila*.

The two double bond isomers, LI and LIII, were interrelated by showing the identity of their dihydro derivatives, while the relationship between LI and tricyclic component L has been established by converting the former to the latter, using BF_3–etherate. The structure of microcionin-2 (LI) has been deduced from NMR characteristics indicative of a β-substituted furan ring, an olefinic hydrogen and of three methyl groups, one vinyl on a trisubstituted double bond, one tertiary and one secondary, together with chemical transformations and related spectroscopic properties. Microcionin-2 has been converted into the enone LV (Fig. 20). In the NMR spectrum of this compound, recorded in the presence of Eu(fod-d_9)$_3$, the resonances of the methylene α to the ketone were resolved giving rise to an 8-line multiplet clearly constituting the AB part of an ABX system, thus confirming that C-6 is tertiary and C-5 quaternary. An indication of the relative stereochemistry at C-5 and C-6 comes from a study of Eu(fod-d_9)$_3$ induced shifts of the methyl resonances of the two diastereoisomeric epoxides LVI and LVII. The normalized ratios of 10:6.14:5.65 and 10:8.41:3.73 for the induced shifts of the C-4, C-5 and C-6 methyl groups in LVI and LVII, respectively, are consistent with the relative stereochemistry as indicated. In accordance the shift of the H-6 signal in the spectrum of

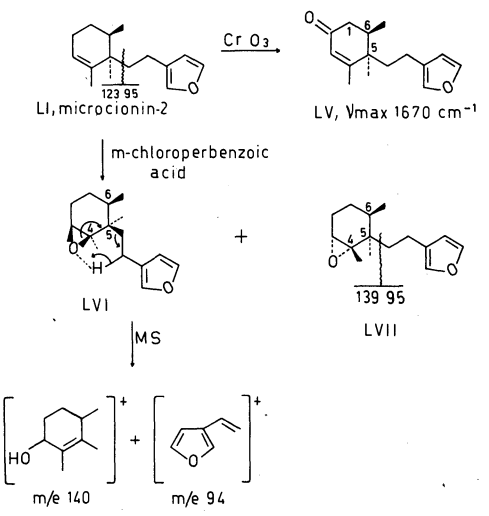


Fig. 20. Structure microcionin-2.

LVII is approximately four times greater in magnitude than that of the same signal in the spectrum of LVI. Reinforcing evidence for the structure assigned to microcionin-2 (LI) came from the mass fragmentation patterns of the two epoxides. The mass spectrum of LVI is marked by peaks at *m/e* 140 and 94 resulting from a "McLafferty type" rearrangement, in agreement with the *syn*-relationship between the epoxide ring and the alkyl chain at C-5, while LVII breaks down without hydrogen transfer giving intense ions at *m/e* 139 and 95.

The co-occurrence of microcionins-1, -2 and -4, having a rearranged skeleton, along with microcionin-3 suggests that the whole group is derived from a common biosynthetic precursor, the ion LIV (Fig. 19).

As pointed out before the isonitrile function is a very rare feature in nature. Until 1973, only xanthocillin, isolated from *Penicillium notatum*, had been described as a natural isonitrile.[47] The isonitrile function has now been found in five sponge sesquiterpenes and one diterpene. The structures of the sesquiterpenes are shown in Fig. 21. Fattorusso and his co-workers[48,49] obtained the first two, accompanied by the corresponding formamides and isothiocyanates,[50] from *Axinella cannabina*. Interestingly,

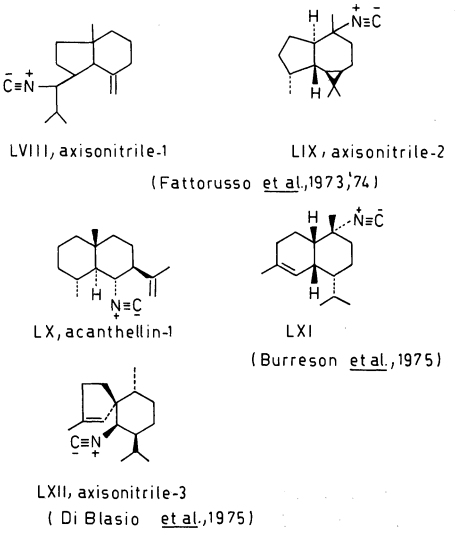


Fig. 21. Isonitrile sesquiterpenoids from sponges.

the new skeletal type of axisonitrile-1 also occurs in oppositol, a bromo-sesquiterpene recently isolated from the red alga *Laurencia subopposita*.[51]

The third isonitrile sesquiterpene in Fig. 21, which has a 4-*epi*-eudesmane skeleton, has been found in the closely related species *Acanthella acuta* (Axinellidae),[52] while the amorphane sesquiterpenoid LXI has been isolated, with its corresponding formamide and isothiocyanate, from an unrelated *Halichondria* sp. (Halichondridae) by the Scheur's group in Hawaii.[53] Co-occurrence of terpenoid isonitrile–formamide pairs is strong evidence that a formamide is the biogenetic precursor of the rare isonitrile function. The latest addition to this group is LXII, which has been isolated in the form of beautifully crystalline substance from *Axinella cannabina*.[54] This was submitted immediately to X-ray crystallographic analysis which established that it has the spiro structure LXII.

This represents the fourth type of spiro(4,5)decane-derived sesquiterpene, after the discovery of the acoranes and the sketally related enantiomeric alaskanes, and the spirovetivanes, all occurring in terrestrial sources, and the spirolaurenone, a bromine-containing sesquiterpene isolated from a marine source (Fig. 22).[55,56]

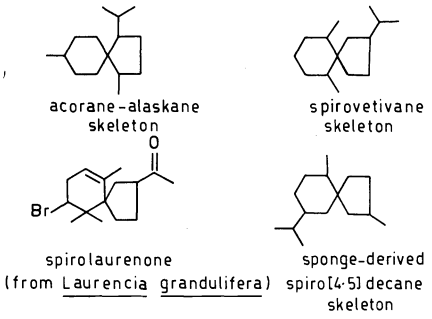
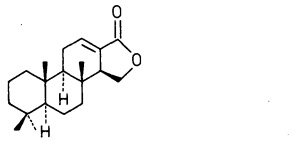


Fig. 22. Spiro[4.5]decane carbon frameworks in sesquiterpenoids.

Diterpenes

Only two examples of true diterpenoid compounds have so far been reported from sponges. One, isolated from *Spongia officinalis*,[57] proved to be the first naturally occurring compound with the carbon skeleton of isoagathic acid, the acid-catalyzed cyclization product of agathic acid, and accordingly named isoagatholactone (LXIII). The second one, elaborated by a *Halichondria* sp.,[58] is the isonitrile analogue of geranyl-linalool (LXIV), co-occurring with the corresponding formamide (LXV) and isothiocyanate (LXVI) (Fig. 23).

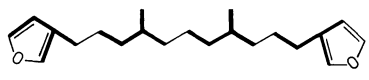


LXIV; R = $\overset{+}{N}\equiv\bar{C}$
LXV ; R = NHCHO (Burreson and Scheuer, 1974)
LXVI ; R = N=C=S

Fig. 23. Diterpenoids from sponges.

The C_{21} furanoterpenes and sesterterpenes

Among the most unusual terpenes isolated from sponges are the linear furanoterpenes containing 21 carbon atoms occurring in the genus *Spongia*. All of them possess the same carbon skeleton, LXVII and oxidation in the central chain accounts for all their differences. The individual structures are listed in Fig. 25. *Spongia nitens* contain nitenin (LXVIII) and dihydronitenin (LXIX),[59] while *Spongia officinalis* and *Hippospongia communis* both yielded furospongin-1 (LXX) as the major terpenoid compound[60] and the related compounds (LXXI–LXXV) as minor components.[61]



LXVII

Fig. 24. Carbon skeleton of the sponge-derived C_{21} furanoterpenes.

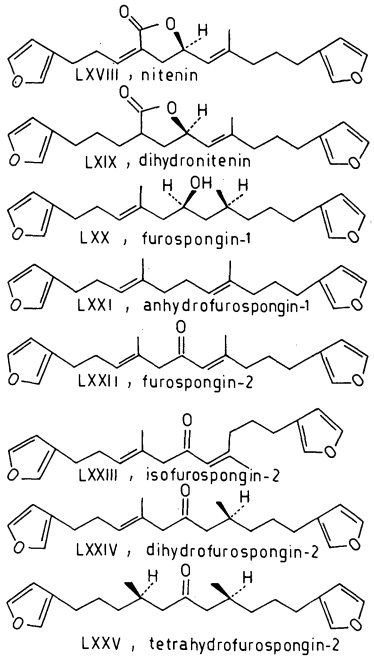


Fig. 25. C_{21} linear difuranoterpenes from sponges.

At present the biogenetic origin of these unique C-21 compounds is a matter of speculation. Radio labelling experiments using 1-^{14}C acetate in the sponge *Spongia nitens* resulted only in non-radioactive nitenin and dihydronitenin.[62] This might suggest that the sponges, like the Molluscs and Coelenterata, are unable to synthesize *de novo* terpenoids, which may be derived from the diet or may be synthesized by algae symbiotically associated with the animals.

While evidence on the biogenetic origin of these C-21 compounds is lacking, in view of the occurrence in related sponges (genus *Ircinia*) of several furanoid sesterterpenes, we prefer the idea that they are derived by degradation of sesterterpenoids to the possibility of biosynthesis by addition of a C-1 unit to a diterpenoid precursor. Figure 26 lists the structures of the linear sesterterpenes isolated from sponges. The isomeric ircinin-1 (LXXVI) and ircinin-2 (LXXVII) have been

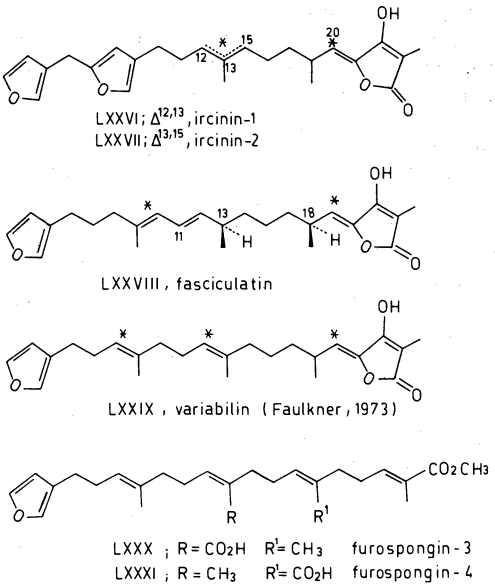


Fig. 26. Linear furanoid sesterpenes in sponges. *Stereochemistry of olefin unknown.

isolated from *Ircinia oros*,[63] while the closely related monofurano derivatives fasciculatin (LXXVIII) and variabilin (LXXIX) have been found in *I. fasciculata*[64] and *I. variabilis*,[65] respectively. Significantly, furospongin-3 (LXXX) and furospongin-4 (LXXXI), the less elaborate components of this interesting group, have been isolated from *Spongia officinalis*,[66] which also contains the C-21 furanoterpenes. Better support for our biogenetic conjecture comes from the discovery of two isomeric C-21 furanoterpenes, ircinin-3 (LXXXII) and -4 (LXXXIII) (Fig. 27) co-occurring in the sponge *Ircinia oros*[66] with the sesterterpenes, ircinin-1 (LXXVI) and ircinin-2 (LXXVII).

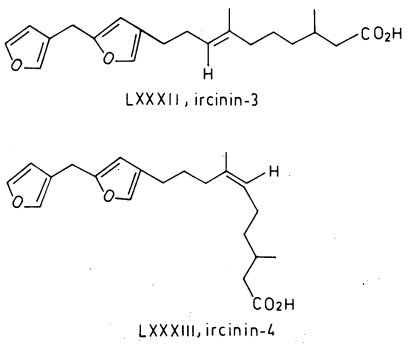


Fig. 27. C-21 furanoterpenes in *Ircinia oros*.

The two isomeric pairs (C_{21} and C_{25}) have structures very closely related even in the position and stereochemistry of the central double bonds. In addition, the isolation from *Ircinia spinosula*[40] of a C_{35} linear furanoterpene, LXXXIV ($n = 6$) along with the C-31 difuranoterpene (LXXXV) (Fig. 28), which are in the same biogenetic relationship as the above C_{25}–C_{21} compounds, lends further substantial support to this suggestion, since it is difficult to accept the increasing co-occurrence of such closely related structures as coincidence.

In Table 2 there is the list of sponges which have been reported to contain furanoterpenes, and you will see that these compounds have been found in three families

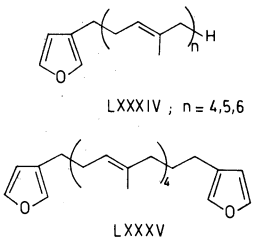


Fig. 28. Furanoid terpenes in *Ircinia spinosula*.

Table 2. Sponges in which furanoid terpenes have been reported[30]

Sponges
Order Dictioceratida
Family Spongidae
Spongia nitens
S. officinalis
S. agaricina
Hippospongia communis
Ircinia oros
I. fasciculata
I. variabilis
I. spinosula
Family Aplysillidae
Pleraplysilla spinifera sample 1
Pleraplysilla spinifera sample 2
Family Disideidae
Disidea pallescens
Order Poecilosclerida
Family Clathriidae
Microciona toxystila

belonging to the order Dictioceratides and in the unrelated *Microciona toxystila*.

Another important group of sesterterpenes with a tetracarbocyclic skeleton have been found in sponges of the genus *Cacospongia*, and in the taxonomically related *Spongia*. Their structures are shown in Fig. 29.

Scalarin (LXXXVI) from *Cacospongia scalaris* was the first compound of this group to be isolated and its structure determination was reported by Fattorusso *et al.*[67] Deoxoscalarin (LXXXVII) and scalaradial (LXXXVIII) have subsequently been isolated from *Spongia officinalis*[68] and *Cacospongia mollior*,[69] respectively, and their structures assigned on chemical interrelation with scalarin.

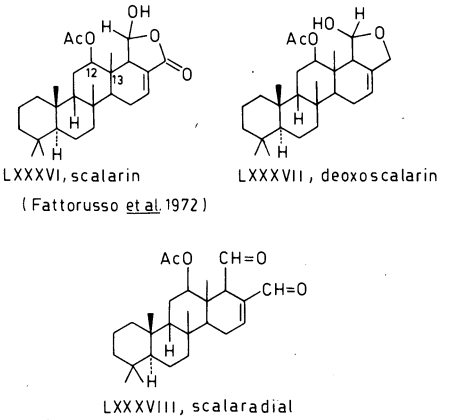
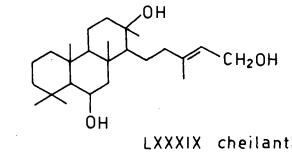
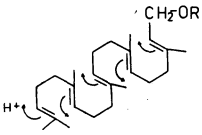


Fig. 29. Tetracarbocyclic sesterpenes in sponges.

These closely related cyclic C_{25} terpenes represent a new structure type in sesterterpenes,[70] themselves a relatively rare group of compounds. Interestingly, the sponge-derived cyclic sesterterpenes have a terrestrial representative in cheilanthatriol (LXXXIX), recently isolated from a fern *Cheilanthes farinosa*,[71] and the whole group may be derived from a geranylfarnesyl precursor (XC) by a cyclization initiated at the isopropylidene group, which is typical of triterpenes.



LXXXIX cheilanthatriol
(Khan et al.,1971)



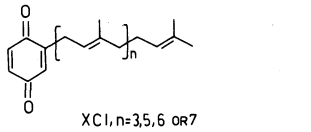
XC

Compounds of mixed biogenesis: mevolonate-benzenoid precursor

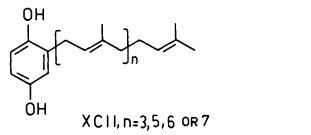
Compounds of mixed biogenesis originating partly from mevalonate and partly from a benzenoid precursor are widespread in nature. Recently two isomeric sesquiterpenoid hydroquinones, zonarol and isozonarol and the phenol taondiol with a cyclized diterpene chain have been isolated from marine sources, the algae *Dictyopteris zonarioides*[72] and *Taonia atomaris*,[73] respectively.

Sponges have also provided a series of compounds having isoprenoid skeletons linked to a benzoquinone or benzoquinol ring.

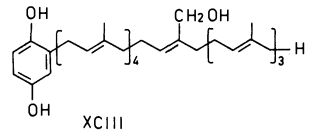
Isoprenologous 2-polyprenyl benzoquinones (XCI, n = 5, 6, 7), a novel group of terpenoid quinones, and the corresponding quinols (XCII, n = 5, 6, 7), present in the solvent extracts in much larger amounts, have been isolated from *Ircinia spinosula*,[40] which also contains the hydroxylated 2-octaprenyl quinol (XCIII) as minor metabolite. From another *Ircinia* species (*I. muscarum*), 4 - hydroxy - 3 - tetraprenylbenzoic acid (XCIV) has been isolated along with 2-tetraprenyl benzoquione (XCI, n = 3) and the corresponding quinol[41] (Fig. 30). This strongly suggests that *p*-hydroxybenzoic acid is the ring precursor as in ubiquinone biogenesis.[74]



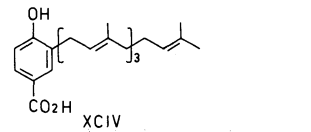
XCI, n=3,5,6 OR7



XCII, n=3,5,6 OR7



XCIII



XCIV

Fig. 30. Prenylated benzoquinones and quinols from *Ircinia* sponges.

The acetone extracts of *Halichondria panicea* have yielded[75] five new compounds, panicein-A (XCV), -B_1 (XCVI), -B_2 (XCVII), -B_3 (XCVIII) and -C (XCIX), having a sesquiterpenoid moiety linked to a benzoquinone or a benzoquinol residue, except for panicein-B_2, which is the corresponding chromenol of panicein-B_3. The structures of the paniceins are shown in Fig. 31. In the sesquiterpenoid moiety the paniceins have the uncommon feature of an aromatic ring which, notably, has been encountered already in renieratene (C) and isorenieratene (CI), arylcarotenoids found in the sponge *Reniera japonica*[76] (syn. *Halichondria panicea*), which also yielded renierapurpurin (CII)[77] and two further arylcarotenoids (CIII and CIV) with the unique feature of an acetylenic bond.[78] (Fig. 32).

A further sesquiterpenoid hydroquinone, avarol (CV), has been recently isolated from *Disidea avara*.[79] It represents the first "friedo" structure in sesquiterpenoids.

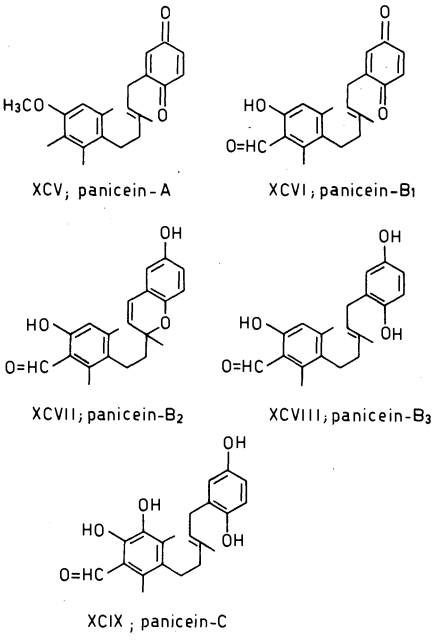


Fig. 31. Paniceins from *Halichondria panicea*.

The gross structure suggested for avarol was deduced from spectroscopic data, which were indicative for the presence in the sesquiterpenoid moiety of one olefinic proton, four methyl groups, two tertiary, one secondary and one vinyl, and a benzylic methylene linked to a saturated quaternary carbon, along with chemical transformations. The relevant chemical arguments are summarized in Fig. 33.

Oxidation with CrO_3-pyridine complex of avarol dimethyl ether gave the enone CVI, in the NMR spectrum of which H-10 and the two C-1 protons formed a clean isolated AMX system, thus confirming that C-10 is tertiary and C-5 and C-9 quaternary. The conversion of avarol dimethyl ether on treatment with acid into the tetrasubstituted olefin CVIII and dehydrogenation of both the parent compound and its acid-catalyzed rearranged product

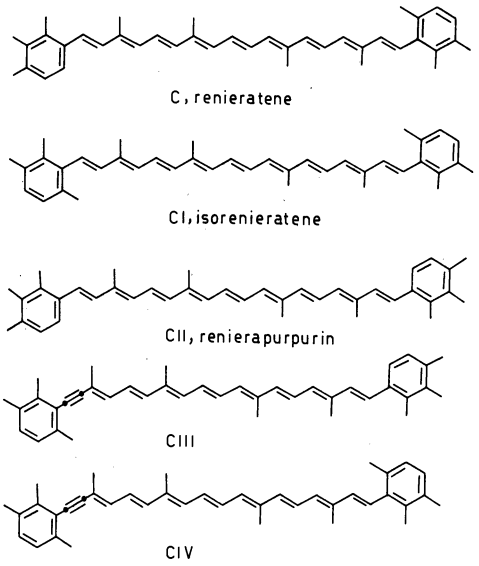


Fig. 32. Arylcarotenoids from Reniera japonica (syn. *Halichondria panicea*); (Yamaguchi, 1957, 1958, 1960; Hamasaki *et al.*, 1973).

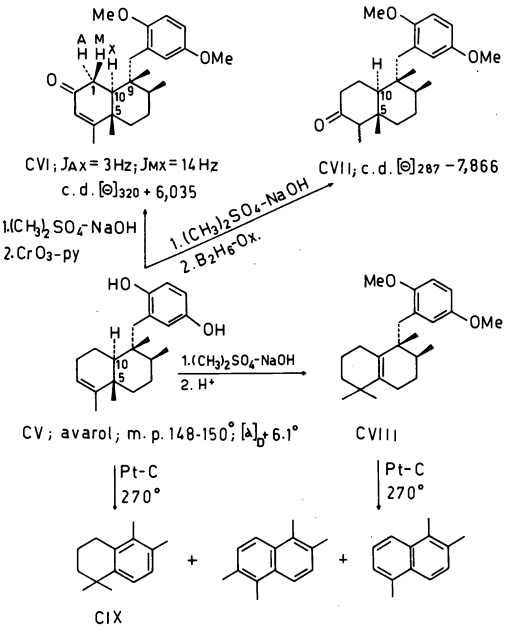


Fig. 33. Structure of avarol (CV).

CVIII, affording 1,2,5,6 - tetramethylnaphthalene and 1,2,5-trimethylnaphthalene along with a major amount of tetralin CIX, eventually confirmed the structure CV without stereochemical implications. The stereochemistry of avarol remains for discussion and now let me make a brief mention of this work.

The magnitude of the coupling constants between H-10 and H-1*ax* and H-1*eq* in the enone CVI indicated that the compound has either the *trans*-AB ring fusion or the *cis*-fusion in the conformation with H-10 axial. Since the deduction of absolute configuration from the Cotton effects of α,β-unsaturated ketones is known to be fraught with difficulties, c.d. measurements were also performed on the ketone CVII, prepared by hydroboration-oxidation of avarol dimethyl ether. On the assumption of a *trans*-ring junction the strong negative Cotton effect would lead to the assignment of absolute configuration as indicated ($5\beta,10\alpha$ series). Even in the *cis*-clerodane diterpenoids series the enones corresponding to CVI are known to prefer a "steroid like" conformation with H-10 equatorial, so a *cis*-AB ring fusion could not be excluded for avarol. An useful approach to solve this was the application of the nuclear magnetic resonance shift reagent Eu(fod-d_9)$_3$ to the study of the diastereoisomeric epoxides CX and CXI, which also provided evidence for the stereochemistry at C-9.

For the purpose of comparison it was convenient to normalize the induced shifts to give a value of 10.0 to the lowest field methyl signal and the results are shown in Fig. 34. The comparison of changes of the chemical shifts of 4-Me and 5-Me protons of both diasteroisomers clearly revealed the relative stereochemistry between the oxirane ring and the angular methyl group; in the major compound CXI, in which the oxirane ring and the angular methyl are *anti* each to the other, the H-10 proton is more strongly shifted towards lower field than the epoxide proton, and this is only consistent with a *syn*-relationship to the epoxide ring. α- and β-epoxides of friedel-3-ene produce patterns for H-10 and 4- and 5- methyl protons very similar to these produced by CX and CXI, respectively. Furthermore the "normalized" induced shifts of the benzylic methylene and 9-methyl protons are consistent with the stereochemistry as indicated.

The observations that with boron trifluoride the α-epoxide (CXI) gives exclusively the $\Delta^{5,10}$ olefin while the β-epoxide (CX) furnishes the $\Delta^{5,10}$ (CXII)- and $\Delta^{5,6}$ (CXIII)-olefins in approximatively equal amounts, provide confirmatory evidence for the α-orientation of H-10. *Trans*-clerodane diterpenoid 3,4-epoxides are known to behave similarly on BF_3 exposure and McCrindle and Nakamura[80] offered a plausible explanation, which follows. In the case of the α-epoxide (CXI), as the epoxide ring opens, the C-3 oxygen function is suitably disposed to remove the C-10 proton (CXV or related species) in an intramolecular process, forming the tetrasubstituted olefin. In the case of the β-epoxide (CX) either the C-10 or

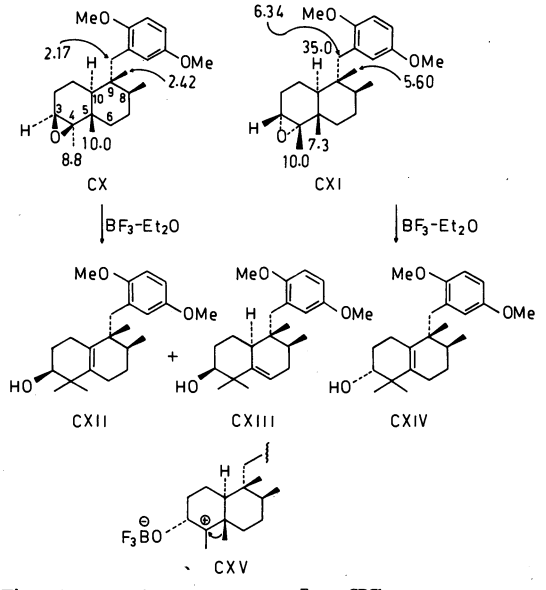


Fig. 34. Normalized shifts ($\Delta = \delta^{Eu} - \delta^{CDCl_3}$) induced by Eu (fod-$d_9$)$_3$ in the spectra of $3\beta,4\beta$- and $3\alpha,4\alpha$-epoxides of avarol dimethyl ether.

the C-6α-proton, both of which are *trans*-antiparallel to the C-5 methyl, is removed in an intermolecular reaction. Now the stereochemistry at C-8 remains to be assigned and this was established with the aid of ^{13}C-NMR spectroscopy, which also confirmed the stereochemistry at C-5, C-9 and C-10. The ^{13}C-NMR spectra of avarol dimethyl ether and its dihydro derivative were compared with those of some model compounds of known stereochemistry shown in Fig. 35.

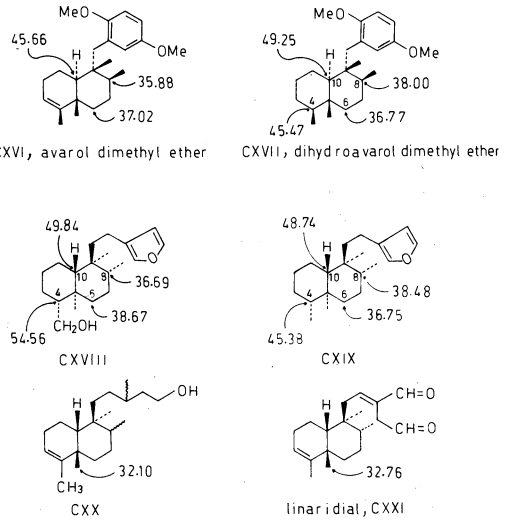


Fig. 35. ^{13}C-NMR data of avarol, dihydroavarol and some model compounds.

The compounds with a *cis*-AB-ring junction produced ^{13}C-NMR spectra clearly distinct from those produced by the *trans*-models and particularly diagnostic, as expected, was the chemical shift of the angular methyl group which in the former is at least 12 ppm further downfield than that in the latter. Now the comparison of the ^{13}C NMR spectra of dihydroavarol dimethyl ether and the clerodane diterpenoid CXIX revealed them to have nearly identical signals for the methyl and decalin carbons; particularly diagnostic for the equatorial orientation of the 8-methyl group are the shifts of the carbons C-6, C-8 and C-10, which are heavily dependent on the C-8 stereochemistry. The methine carbons could be easily distinguished by the strong β effect of the OH substituent deshielding C-4 in CXVIII, and by considering the endocyclic homoallylic effect exhibited by C-10 in CXVI. This leaves the doublet ranging from 35.88 to 38.48 to the C-8 carbon. The C-6 methylene carbon was assigned on a methyl-substituent parameter calculation and because its shift is nearly invariant in the spectra of all four compounds. As pointed out before avarol represents the first "friedo" structure in sesquiterpenoids and it can be conceived as derivable from a farnesy precursor by cyclization to an intermediate cation involving a drimane skeleton, followed by a "friedo" rearrangement and finally deprotonation. Assuming the sequence of 1,2-shifts of methyl groups and hydrogen atoms be concerted, the stereochemistry of the intermediate cation should be as shown in CXXII (Fig. 36). Interestingly the sponge *Disidea pallescens*, in addition to being a rich source of furanoid sesquiterpenes (Fig. 7), has also yielded a chromansesquiterpenoid, *ent*chromazonarol,[81] with the absolute configuration CXXIII biogenetically related to that of avarol. It is worthy of mention that the brown alga *Dyctyopteris undulata*[82]

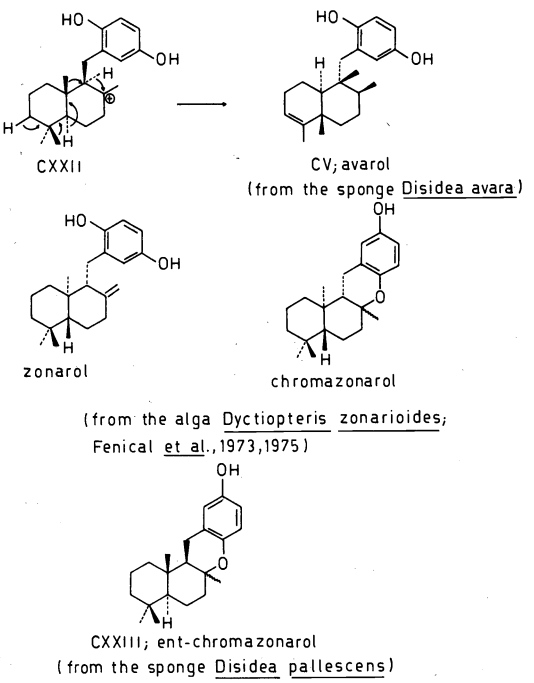
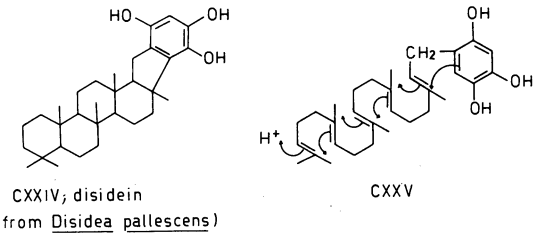


Fig. 36. Relationship between the sponge-derived avarol and ent-chromazonarol and the algal zonarol and chromazonarol.

contains the antipodal isomer, chromazonarol, along with its phenolic isomer, zonarol.

The biosynthesis of antipodal terpenoids by different organisms is of considerable interest.

Disidea pallescens also contains in larger amounts disidein, having a sesterterpenoid moiety linked to a hydroxyhydroquinone residue. It has been isolated as the sodium calcium salt of the disulphate. For the free phenol the structure CXXIV, with a carbocyclic system of the scalarin type (see Fig. 29), has been proposed[83] as the more probable one, which is also well explained from the standpoint of its biogenesis. In fact, we may imagine that



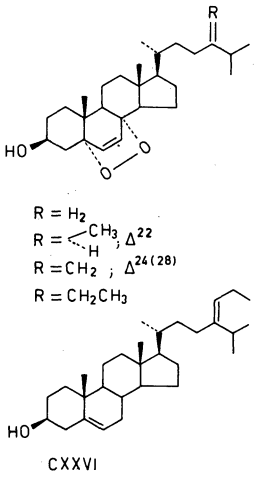
CXXV undergoes an essentially synchronous process for ring formation if H^+ is furnished at C-3.

STEROLS

Now let me consider briefly the recent results on the sterols from sponges. Since the extensive work of Bergmann on the sterols of invertebrates, it has been recognized that, in the Animal Kingdom, the sponges contain the greatest variety of sterols. The elegant work of Bergmann and his co-workers resulted in the isolation of a number of new sterols, but Bergmann himself was aware that much of the data accumulated probably referred to sterol mixtures. Thus most of the earlier work now requires revision with the aid of more sophisticated chromatographic techniques.

Since 1972 modern reinvestigations of the sterols of sponges have began to appear, confirming the complexity of sterol composition in this phylum and announcing the discovery of sterols of completely new types.

The reexamination of the sponges *Cliona celata* and *Hymeniacidon perleve* by Erdman and Thomson[84] resulted in the isolation of three new marine stanols: 24 - norcholesta - 22 - en - 3β - ol, 22-dehydrocholestanol and 24-methylenecholestanol. This is the first occurrence in sponges of a sterol with twenty-six carbon atoms (24-nor) which are widespread in marine invertebrates and to date have been reported in five marine phyla, the Coelenterates, the Echinoderms, the Molluscs, the Tunicates and the Sponges. An analysis of the sterols of *Axinella cannabina* by the Fattorusso's group revealed the presence of fifteen components, C_{27-29} Δ^7,$\Delta^{8(9)}$ and $\Delta^{5,7}$-sterols[85] accompanied by a mixture of 5,8-peroxides, from which the two major compounds, ergosterol peroxide and 5,8 - epidioxycholesta - 6,22 - dien - 3β - ol, have been isolated.[86] Sheikh and Djerassi[87] in an examination of five sponges for their steroid content, have also succeeded in isolating a series of 5α,8α-peroxides, listed in Fig. 37, from *Tehya aurantia* which also contains Z - 24 - propylidene - cholest - 5 - en - 3β - ol (CXXVI), previously encountered in the scallop *Placopecten magellanicus*.[88] The same authors have also found in *Stelleta clarella* a series of Δ^4-3-ketones, the first occasion on which any α,β-unsaturated steran-3-ones have been detected from marine sources.

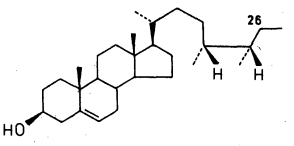


CXXVI

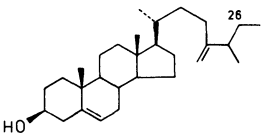
Fig. 37. 5α,8α-Peroxides of sterols and the C-30 sterol from the sponge *Tethya aurantia* (Sheikh and Djerassi, 1974).

Even more unexpected is the isolation by our research group of aplysterol (CXXVII) and 24,28 - didehydro-aplysterol (CXXVIII), the first examples of 26-alkylation in steroid biosynthesis (Fig. 38). The structure of 26 - methyl - 24 - methylenecholesterol was suggested for 24,28 - didehydroaplysterol from spectroscopic data along with degradative work[89] and confirmed by synthesis,[90] while the structure of 24,26 - dimethylcholesterol, suggested for aplysterol on spectral evidence and interrelation with didehydroaplysterol,[89] has been confirmed by single-crystal X-ray diffraction studies of its *p*-iodobenzoate, which also revealed the stereochemistry of the side-chain to be 24R, 25S as shown in CXXVII.[90]

These unique sterols appear to be confined to the *Verongia* genus: in an examination of 25 sponges for their sterol composition, using GLC and mass spectrometry we



CXXVII ; aplysterol



CXXVIII ; 24,28-didehydroaplysterol

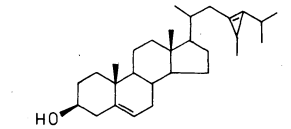
Fig. 38. 26-Methyl sterol from *Verongia* genus.

have found that all *Verongia* species examined contained as principal components the two new sterols (Table 3), and they proved to be absent in all the remaining species examined.[91] This study also confirmed the complexity of the sterol pattern in this phylum; interestingly, the C_{26} sterols have been found in very low yield in almost all the species examined.

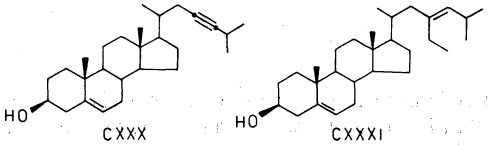
A further remarkable side-chain alkylation pattern has been found in calysterol, the principal sterol component of the sponge *Calyx nicaensis*. The structure CXXIX assigned to this sterol which includes the unique feature of a cyclopropene ring in the side-chain, has been recently proposed on the basis of spectral data and chemical degradations by Fattorusso and co-workers.[92] The same group has also discovered two further "unusual" sterols, CXXX and CXXXI, from *Calyx nicaensis*.[92] The occurrence of CXXX, the first example of an acetylenic functionality in a steroid, might be an answer to the problem of the biochemical precursor of the unique calysterol itself and the gorgonian sterols having a 22,23-cyclopropane ring. Figure 39 lists the structures of the *Calyx* sterols.

Table 3. Sponges in which aplysterol and 24,28-didehydroaplysterol have been found[91]

Sponges	Source	% of the total sterol content
Ordr Dictioceratida		
Family Verongidae		
V. aerophoba	Naples	70%
V. archeri (hard)	Jamaican N. Shore	60
V. archeri (soft)	British Virgin Islands	60
V. fistularis	Bermuda	67
V. thiona	La Jolla (California)	78



CXXIX ; calysterol



CXXX CXXXI

Fig. 39. Sterols with unusual side-chain alkylation patterns from *Calyx nicaensis*; (Fattorusso *et al.*, 1974, 1975).

Modifications of the sterol nucleus has also been found in sponges.

The total sterol content of *Axinella polypoides* is a mixture of stanols having a 19-norcholestanol nucleus carrying conventional saturated and mono-unsaturated C_7 (24-nor), C_8, C_9 and C_{10} side-chains[93] (Fig. 40).

Axinella verrucosa contains a series of stanols with a new 3β - hydroxy - A - nor - 5α - cholestane nucleus carrying conventional C_8, C_9 and C_{10} side-chains[94] (Fig. 40). Interestingly in this sponge the usual sterols are also absent.

These examples indicate that the sponges may be a source of further new types of sterols and a careful reinvestigation of marine sponges for their sterol content is clearly warranted.

Whereas our knowledge about the sterols present in sponges has rapidly increased in the last few years, our insight into the metabolism of sterols in these animals is still meagre.

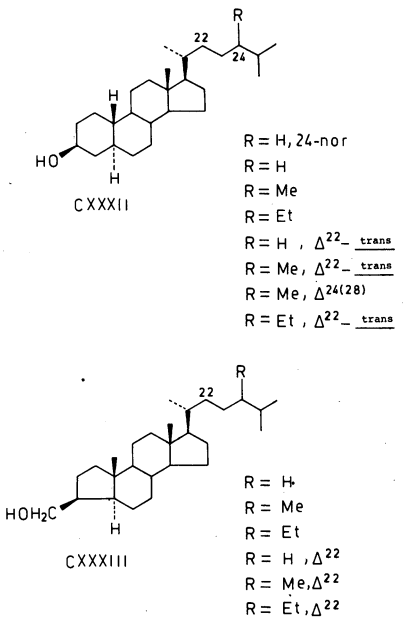


Fig. 40. Stanols with modified tetracyclic nuclei in sponges.

Recent results from our laboratory have indicated that *Verongia aerophoba* failed to incorporate either 1-^{14}C-acetate or 2-^{14}C-mevalonate into aplysterol (CXXVII) and 24(28)-didehydroaplysterol (CXXVIII). A radiolabelling experiment using CH_3–^{14}C methionine in the sponge *V. aerophoba* also resulted in nonradioactive CXXVII and CXXVIII.[27]

A similar situation arose with *Axinella polypoides* and *A. verrucosa*, the sponges containing the 19-nor-stanols and the hydroxymethyl - A - nor - steranes, respectively, when they were fed with labelled acetate. However the two sponges have been shown capable of converting very efficiently cholesterol to 19 - nor - cholestanol and 3β - hydroxymethyl - A - nor - cholestane,[95] respectively. So, it appears that in *A. polypoides* and *A. verrucosa*, the sterols cannot originate from *de novo* biosynthesis but arise by modification of dietary sterols. A similar conclusion is possibly applicable to the sponge *Verongia aerophoba*.

Clearly, much work is required before any definite conclusions can be drawn about sterol metabolism in sponges.

MISCELLANEOUS COMPOUNDS

Finally, I would like to remind you of the extensive work of Ackermann on amines and, more generally, on products associated with amino-acid metabolism in marine invertebrates including sponges.[96]

I am sorry to be unable, because of lack of time, to go through this excellent contribution. However, a brief summary of nitrogen compounds in Porifera has appeared in 1970 from Ciereszko.[97] I can just report here the latest additions to this miscellaneous group (Fig. 41).

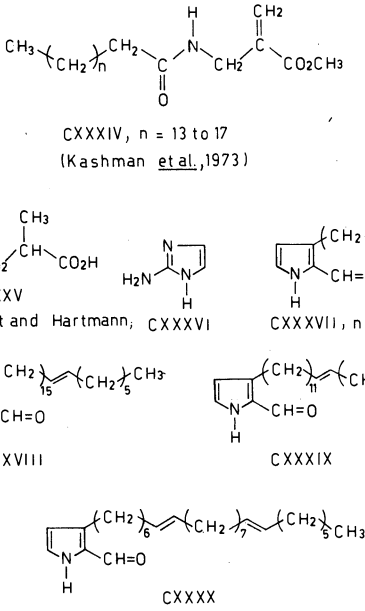


Fig. 41. Some miscellaneous compounds recently isolated from sponges.

A series of *N* - acylated - 2 - methylene - β - alanine methyl esters (CXXXIV) has been found in *Faciospongia cavernosa*, in remarkably high concentrations, by Kashman *et al.*[98] It is interesting to note that the rare 2 - methyl - β - alanine (CXXXV) was found in several sponges by Bergquist and Hartmann.[35]

The simple 2-aminoimidazole (CXXXVI), a metabolite possibly associated with arginine metabolism, has been obtained from *Reniera cratera*.[99]

A novel group of compounds characterized by saturated, mono and diunsaturated long alkyl chains linked at position 3 of a pyrrole-2-aldehyde moiety (CXXXVII–CXXXX) has been isolated from *Oscarella lobularis*, which also yielded a series of corresponding pyrrole-2 carboxylic acids and methyl esters.

Acknowledgements—I have the fortune of being associated with a group of enthusiastic colleagues, Drs G. Cimino, S. De Stefano, R. Riccio and G. Sodano, whose ability, generous efforts and stimulating ideas have made this work possible. I would like to express my special thanks to Professor E. Fattorusso, who was associated with our group in the earlier phase of this project. I am also indebted to Professors E. Lederer and R. H. Thomson for advice and interest in this work. The cooperation of the Zoological Station (Napoli) in the collection of sponges is gratefully acknowledged.

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