Tetrahedron Vol 45, No 7, pp 1893 to 1904, 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00

BIOSYNTHETIC STUDIES OF MARINE LIPIDS 16.[1]

DE NOVO STEROL BIOSYNTHESIS IN SPONGES. INCORPORATION AND TRANSFORMATION OF CYCLOARTENOL AND LANOSTEROL INTO UNCONVENTIONAL STEROLS OF MARINE AND FRESHWATER SPONGES

Russell G. Kerr, Ivan L. Stoilov, Janice E. Thompson and Carl Djerassi*

Department of Chemistry, Stanford University, Stanford, CA 94305

(*Received in USA 2 September* 1988)

ABSTRACT: Cycloartenol and lanosterol, the sterol precursors of photosynthetic and nonphotosynthetic organisms respectively, were transfromed into the 4,4,14-demethyl sterols in most but not all, of a variety of marine and freshwater sponges. Symbiotic cyanobacteria found in many sponges are apparently not involved in the observed biosynthesis. We conclude that while some sponges obtain sterols from the diet, the majority synthesize them *de novo*.

INTRODUCTION

Sponges have furnished a bewildering array of sterols with unprecedented side chains and nuclei.[2] These sterols are frequently, but not always, accompanied by conventional ones. Figure 1 illustrates some of the unusual side chains as well as some common (L, S, N) and uncommon (J, K) sterol nuclei encountered among sponges. Sponges belong to the phylum Porifera and are generally considered to be the most primitive member of the animal kingdom[3] in the subkingdom Metazoa. However, due to their extremely simple cellular organization (e.g. lack of differentiated tissues and lack of a nervous system), many workers now place sponges into their own subkingdom, the Parazoa.[3] In recent years, extensive research in our group,[4] as well as in others,[5] has focused on the mechanism of sterol side chain generation or nuclear ring-A modification. However, the primary biosynthetic origin of these sterols remains unknown, and their physiological function as cell membrane constituents is not yet clearly understood.[6]

The common biosynthetic pathway up to the stage of the synthesis of the linear polyenic precursor squalene, which both plants and animals share, has been successfully used as a keystone for a common, monophylogenetic origin of all eucaryotes. Dichotomy at the point of squalene cyclization has been established as a distinguishing feature of photosynthetic and nonphotosynthetic organisms.[7] In the former group, cycloartenol (10) is the primary polycyclic compound while in the latter, lanosterol (9) is formed. Evidence for this comes from the isolation of cycloartenol in many forms of plants[7] and from various incorporation experiments in which radiolabeled acetate and mevalonate were transformed into cycloartenol and thence into phytosterols.[7(c),8] There are a few scattered reports of the isolation of lanosterol in photosynthetic systems, such as the Poinsettia plant,[9] and even some reports of lanosterol acting as a precursor of sterols in plants. Evidently, the reason for this latter result is that cycloartenol is transformed into lanosterol.[10] Cycloartenol has not

been observed in a nonphotosynthetic organism,[11] while lanosterol is frequently isolated.[7(a),(d)] Experiments with rat liver (a commonly-used nonphotosynthetic tissue) demonstrated the conversion of labeled lanosterol to cholesterol, while the conversion of cycloartenol to cholesterol was not observed.[7(d)]

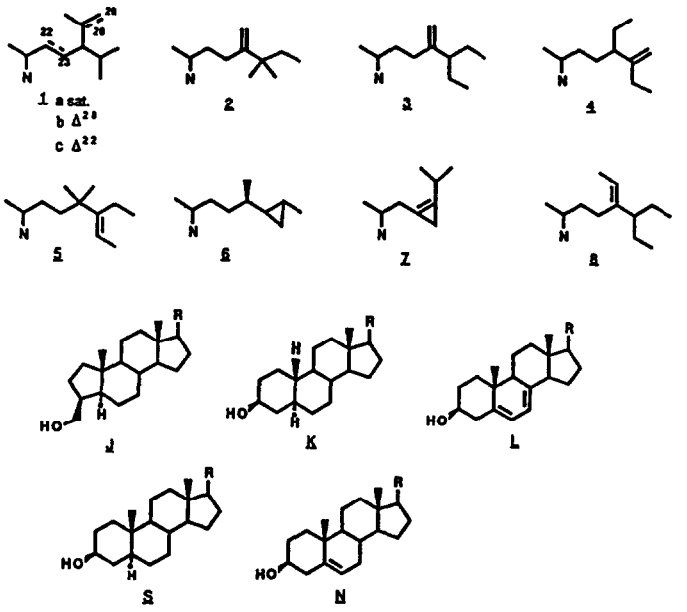


Figure 1

There are a few possible sources of the unprecedented sterols found in sponges. First, they could be synthesized *de novo* by the sponge; results concerning this route are, as yet, inconclusive.[7(h),(j),(k)] Minale and coworkers[5] reported evidence that did not favor *de novo* sterol biosynthesis in the sponges *Verongia aerophoba*, *Axinella verrucosa*, *A. polypoides* and *Calyx nicaeensis*, while our laboratory has observed small but measurable amounts of incorporation of mevalonate into the Δ^5 sterols of *Aplysina fistularis*[12] and a significant incorporation of this precursor into the predominant sterols of *Xestospongia testudinaria*.[13] Minale *et al.* proposed[5] that the *Axinella* and *Calyx* species obtain sterols from their diet. The poor incorporation (or even complete lack of incorporation) of mevalonate into some of these sponges may well be due to technical difficulties associated with feeding a water-soluble precursor to the filter-feeding sponges.[14]

A second possible source of sterols is from one or more of the symbionts associated with many sponges,[3(b)] such as the cyanobacteria[15] of the genera *Aphanocapsa* and *Phormidium* These organisms are photosynthetic and are found both extra- and intra-cellularly in many sponges. Other photosynthetic symbionts found in sponges include zooxanthellae and zoochlorellae.[3(b)] There has been some controversy concerning the existence and biosynthesis of sterols in these symbionts.[7(e),16] It now appears that sterols do occur in cyanobacteria[17] although it is still not known with any certainty whether or not they are capable of sterol biosynthesis. Nevertheless, it is conceivable that the cyanobacteria as well as zooxanthellae and zoochlorellae are somehow involved in the biosynthesis of the unusual sterols found in sponges

Table 1

Sponge	Location[a]	Steroid nucleus	Recovered activity in free sterols (dpm) Cycloartenol (4.4×10^7 dpm)	Lanosterol (4.4×10^7 dpm)	Symbiotic eucaryotes Photosynthetic	Nonphotosynthetic
MARINE SPECIES						
Family: Axinellidae						
Phakellia sp. (AIMS-RA 11)[b]	GBR	J	cold	cold	absent[c]	unknown
Axinella verrucosa	Med.	J	cold	cold	absent[d]	present[d]
Axinella polypoides	Med.	K	cold	cold	absent[d]	present[d]
Pseudaxynissa sp. A (AM-4989)[b]	GBR	L	cold	6×10^5	present[e]	present[e]
Pseudaxynissa sp. B (AM-4988)[b]	GBR	N	2×10^6	2×10^5	present[e]	present[e]
Family: Tethyidae						
Tethya aurantia californiana	Calif.	N	8×10^4	6×10^5	absent[e,f]	present[e]
Family: Jaspidae						
Jaspis stellifera	GBR	N	cold	3×10^5	present[c]	present[c]
Family: Adociidae						
Petrosia ficiformis	Med.	N	cold	2×10^5	present[d]	present[d]
Family: Petrosiidae						
Xestospongia muta	PR	N	8×10^5	7×10^5	present[g]	present[h]
Xestospongia muta (endoderm only)	PR	N	2×10^5	8×10^5	absent[i]	present[h]
Xestospongia sp. (AIMS-RA 35)[b]	GBR	N	2×10^6	2×10^6	present[e]	present[e]
Family: Clathriidae						
Microciona prolifera	Calif.	S & N	5×10^4	5×10^5	absent[e]	absent[e]
Family: Halichondriidae						
Ciocalypta sp	Hawaii	L	cold	cold	unknown	unknown
Family: Verongiidae						
Aplysina fistularis	Calif.	N	3×10^5	6×10^5	absent[e]	present[e]
FRESHWATER SPECIES						
Family: Spongillidae						
Ephydata fluviatilis	Stan. 1	N	1×10^6	8×10^6	unknown	unknown
Eunapius fragilis	Stan. 2	N	2×10^6	2×10^6	unknown	unknown

[a]GBR = Great Barrier Reef; Med = Mediterranean Sea; PR = SW coast of Puerto Rico; Calif. = California coast; Stan 1 = Lake Lagunita, Stanford Univ.; Stan. 2 = Searsville Lake, Stanford Univ.

[b]Australian Museum (AM) and Australian Institute of Marine Sciences (AIMS).

[c]C.R. Wilkinson, Australian Institute of Marine Sciences, personal communication

[d]See reference no. 21.

[e]Conclusions based on electron microscopy, see Experimental Section

[f]Macroscopic alga always penetrate the surface tissues of the sponge and thus may contribute to the observed biosynthesis.

[g]See reference no. 30.

[h]D. Santavy, University of Maryland, personal communication.

[i]The outer ectoderm, containing cyanobacteria, was removed with a knife.

The occurrence of nonphotosynthetic, symbiotic bacteria in demosponges has been demonstrated by Vacelet[18] and by Wilkinson.[19] As with the cyanobacteria, these bacteria occur both extra- and intra-cellularly, and since some seem to contain sterols, they may transport them to the host sponge.

The abundance of exogenous sterols, particularly in phytoplankton,[20] could provide an alternative source of the sterols found in sponges. Obtaining sterols from their diet is in agreement with the classification of sponges as filter feeders.[3(a)] Dietary sterols could be incorporated intact into the sponge cell membranes, or after side chain modification via SAM bioalkylation,[7(a),(c)] with or without nuclear modification.

Due to the primitive nature of sponges and their uncertain position in the evolutionary hierarchy and the variety of possible biosynthetic sources of their sterols, we decided to investigate the incorporation and subsequent transformation of radiolabeled lanosterol (9) and cycloartenol (10). This study was performed with a variety of Demospongiae[3] (the largest of four classes of sponges) containing a wide range of conventional and unconventional sterols, belonging to different families and from different environmental and geographical regions. Also, sponges with and without photosynthetic and nonphotosynthetic symbionts were examined to determine the role of these organisms in sterol biosynthesis.

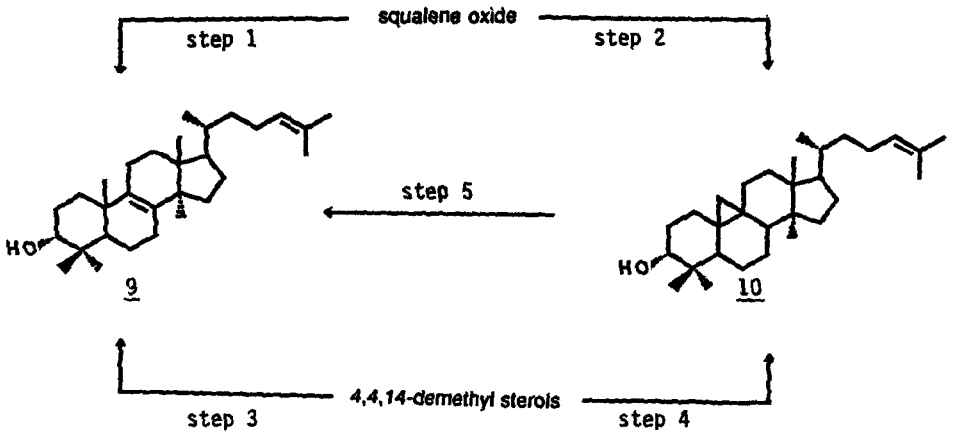
RESULTS AND DISCUSSION

Results of the incorporations of tritium-labeled lanosterol (9) and cycloartenol (10) into sterols in a variety of families of sponges belonging to the class Demospongiae are summarized in Table 1. In each case the precursor was taken up by the sponge and either transformed into the 4,4,14-demethyl sterols or recovered unchanged. Entries marked "cold" in Table 1 are therefore significant as they reflect the sponge's inability to metabolize the precursor, rather than the lack of incorporation of the triterpene into the sponge cells. In the majority of the sponges in Table 1, both cycloartenol and lanosterol were equally effectively transformed into the sterols of the sponge, while in three of the sixteen sponges, only lanosterol was metabolized. In the remaining four, neither precursor was metabolized. These latter sponges evidently obtain their sterols from their diet.

It is evident from Table 1 that there is no relationship between sponges in which cycloartenol and lanosterol were efficiently transformed into 4,4,14-demethyl sterols and their geographical location. Thus, the temperature of the water and the available diet for sponges of one particular region is not a factor. There also appears to be no correlation between the ability of sponges to metabolize cycloartenol and lanosterol, and the family to which the sponge belongs (for example, see *Axinellidae* in Table 1).

The transformation of both lanosterol (9) and cycloartenol (10) into the 4,4,14-demethyl sterols in a number of sponges suggests the unprecedented cyclization of squalene oxide to 9 and/or 10 (see Scheme 1). As mentioned in the introduction, the cyclization of squalene oxide to cycloartenol has not been observed in the animal kingdom. This lends support to the now generally-accepted placement of the Porifera within the distinct subkingdom Parazoa, although in three of the sixteen sponges, only lanosterol, the animal sterol precursor, was metabolized.

The 4,4,14-demethyl sterols in Scheme 1 could be produced by two parallel routes, one proceeding via lanosterol and the other through cycloartenol (step 1 followed by step 3 and



Scheme 1

step 2 followed by step 4). It is also possible, however, that cycloartenol is transformed into the final sterol product via lanosterol (steps 2, 5, and 3). This has been shown[9,10] to operate in the Poinsettia plant, as discussed earlier.

It is noteworthy that lanosterol (9) and cycloartenol (10) are incorporated into the unusual, highly alkylated sterols (e.g. 1, 2 and 3) typical of sponges and not just into the more conventional ones such as cholesterol. Also, the order of decrease in specific activity of sterols (1b ---> 1a ---> 1c in Table 2) is consistent with the biosynthetic pathway[4(c)] established for the side chain. A detailed examination of the incorporation of radiolabeled 9 and 10 into *Pseudaxynissa sp. B*, *Aplysina fistularis*, *Microciona prolifera* and *Tethya aurantia californiana* is presented in Tables 2, 3, 4 and 5, and leads to interesting conclusions

Table 2 Incorporation of (24-^{3}H) cycloartenol and (24-^{3}H) lanosterol in *Pseudaxynissa sp.B*

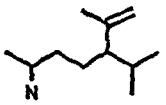
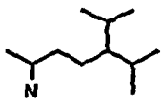
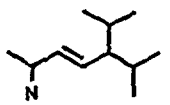
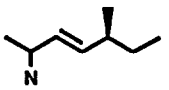
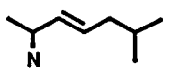
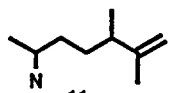
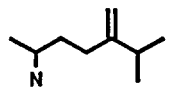
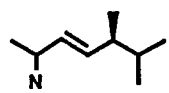
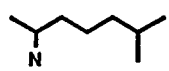
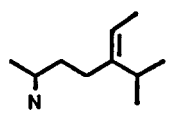
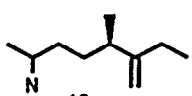
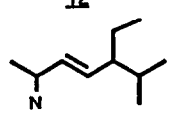
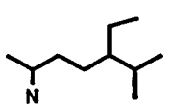
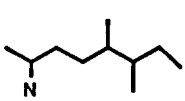
		Cycloartenol		Lanosterol		
Sterol	mg	dpm	% Incorporation	mg	dpm	% Incorporation
 1b	3	3.0×10^5	26	0.2	3.2×10^4	20
 1a	14	6.6×10^5	58	4.5	1.1×10^5	67
 1c	15	1.7×10^5	15	5.3	2.2×10^4	13

Table 2 indicates that in *Pseudaxynissa sp. B*, a sponge which only contains unconventional, highly alkylated sterols,[22] good incorporation of both precursors occurs.

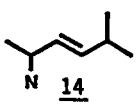
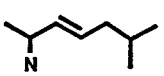
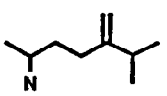
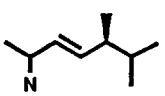
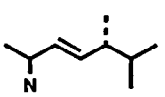
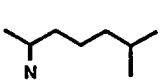
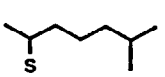
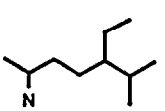
Table 3. Incorporation of (24-^{3}H) cycloartenol and (24-^{3}H) lanosterol in *Aplysina fistularis*

Sterol	Cycloartenol mg	Cycloartenol dpm	Cycloartenol % incorporation	Lanosterol mg	Lanosterol dpm	Lanosterol % incorporation
	0.2	cold	0	0.2	cold	0
	0.4	cold	0	0.9	cold	0
 11	0.2	5.6×10^4	20	0.3	1.05×10^5	18
	0.1	cold	0	0.1	cold	0
	0.3	cold	0	0.5	cold	0
	2.0	cold	0	4.4	cold	0
	0.2	cold	0	0.2	cold	0
 12	7.0	2.14×10^5	77	12.5	4.66×10^5	80
	0.2	cold	0	0.3	cold	0
	1.1	cold	0	1.9	cold	0
 13	4.2	7.2×10^3	3	5.6	1.2×10^4	2

Aplysina fistularis is an interesting sponge in that it contains unusual sterols, alkylated at C_{26} and at C_{24} (aplysterol[23] (13) and dehydroaplysterol[4(a)] (12)), as well as a variety of more conventional ones (see Table 3). In this case, cycloartenol and lanosterol were incorporated into the unusual sterols (12 and 13) but not into the others.[24] This implies that the latter sterols are obtained from the diet, while aplysterol (13) and dehydroaplysterol (12) are synthesized by the sponge.

Microciona prolifera is an example of a sponge which only contains conventional sterols. Here, lanosterol is incorporated much more efficiently than cycloartenol and some radioactivity is encountered in every 4,4,14-demethyl sterol, except for 24-nor-22-dehydrocholesterol (14) (Table 4). This unusual class of sterols is believed to be of

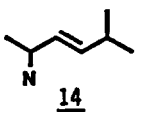
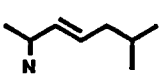
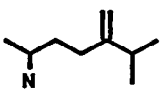
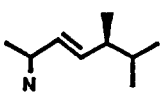
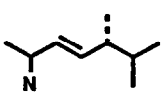
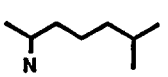
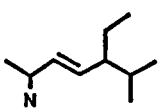
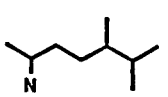
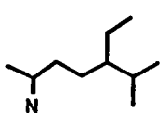
Table 4 Incorporation of (24-^{3}H) cycloartenol and (24-^{3}H) lanosterol in *Microciona prolifera*

	Cycloartenol			Lanosterol		
Sterol	mg	dpm	% incorporation	mg	dpm	% incorporation
 14	0.5	cold	0	0.3	cold	0
	1.3	4.1×10^4	10	0.7	3.8×10^5	10
	2.0	1.7×10^4	4	1.0	1.3×10^5	3
	2.9	1.7×10^4	4	1.3	1.0×10^5	3
	1.9	1.6×10^4	4	1.0	1.1×10^5	3
	15.0	2.5×10^5	60	7.0	2.34×10^6	62
	12.1	6.3×10^4	15	4.8	5.9×10^5	16
	5.4	0.9×10^3	2	3.0	1.1×10^4	3

planktonic origin[25] and the present study also points to an exogenous source for the sterol. Earlier attempts[26] to define a biosynthetic precursor have all failed.

Tethya aurantia californiana is another sponge in which only conventional sterols are found. It is similar to *Aplysina fistularis* as nonphotosynthetic symbionts are present while photosynthetic ones are not (Table 1). However, unlike *A. fistularis*, both precursors were transformed into all of the sterols of *T. aurantia* (Table 5). As was observed in *Microciona prolifera*, lanosterol is incorporated more efficiently than cycloartenol.

Table 5. Incorporation of (24-^{3}H) cycloartenol and (24-^{3}H) lanosterol in *Tethya aurantia*

	Cycloartenol			Lanosterol		
Sterol	mg	dpm	% incorporation	mg	dpm	% incorporation
 14	1	cold	0	2	cold	0
	5	21.6×10^3	3	6	4.6×10^3	1
	8	3.1×10^4	49	13	2.56×10^5	50
	3	0.9×10^3	2	4	2.5×10^3	0.5
	1	0.6×10^3	1	1	2.1×10^3	0.5
	25	2.6×10^4	42	36	2.4×10^5	47
	0.5	0.5×10^2	1	0.5	1.0×10^3	0.5
	2	0.7×10^2	1	4	21.8×10^3	1
	3	1.0×10^3	2	7	1.1×10^3	0.5

Petrosia ficiformis also merits special mention as it is the only sponge listed in Table 1 whose principal sterol is a cyclopropane-containing one, petrosterol (6).[27] The unusual course of the side chain biosynthesis has been elucidated in our laboratory,[28] but nothing is known about the earlier steps. We found both petrosterol (6) and conventional sterols such as sitosterol to be highly radioactive after incorporation of lanosterol (9). This example is interesting for another reason, namely that in this sponge, cycloartenol (10) is not employed for sterol biosynthesis.

The transformation of cycloartenol into the 4,4,14-demethyl sterols of sponges could conceivably be due to symbiotic photosynthetic symbionts such as cyanobacteria, zooxanthellae and zoochlorellae present in some sponges.[29] These organisms could transform cycloartenol into Δ^5-phytosterols and then transfer these to the host sponge. Electron microscopy of the sponges investigated allowed verification of the particular symbiont populations present in the whole sponges. Results from our laboratory and those of previous studies are summarized in Table 1. In accordance with prior investigations, most sponges were found to contain nonphotosynthetic eubacteria, although no eucaryotic symbionts of any kind were observed in the tissues of the fast-growing sponge *Microciona prolifera*. Many, but not all of the tropical sponges contained photosynthetic eucaryotes, including cyanobacteria. Cyanobacteria were observed in the external (<1.0 mm) tissues of sponges belonging to the genus *Xestospongia*, while eubacteria were found in the interior tissues. Fungi were not observed in any of the sponges examined by this laboratory. Macroscopic algae were present in the surface tissues of *Tethya aurantia californiana* as well as in the fresh water sponge *Ephydata fluviatilis*.

Our results show that cyanobacteria are not involved in the observed incorporation and transformation of lanosterol and cycloartenol because both precursors are transformed into the sterols of *Tethya aurantia californiana* and *Pseudaxynissa sp. B*. The latter contains cyanobacteria while the former does not. Also, *Xestospongia muta* contains cyanobacteria only in a well defined outer layer.[30] Parallel feeding experiments were performed with cycloartenol and lanosterol on sponge fragments with and without this symbiont-containing ectoderm. As can be seen in Table 1, in the sponge fragment without cyanobacteria, both precursors were incorporated and transformed into the Δ^5 sterols of the sponge to the same degree as in the sponge fragment containing cyanobacteria.

The photosynthetic zooxanthellae and zoochlorellae also appear to have no effect on the observed sterol biosynthesis as both lanosterol and cycloartenol were transformed (albeit to a different extent) into the sterols of *Microciona prolifera*, which we found to be devoid of all photosynthetic symbionts.

It is extremely unlikely that the nonphotosynthetic bacteria found in some sponges contribute to the sterol biosynthesis observed in sponges. There are only a few reported cases of sterol biosynthesis in these bacteria[31] and, as shown in Table 1, both cycloartenol and lanosterol are effectively metabolized in sponges that are free of such organisms.

CONCLUSION

Results concerning the incorporation of acetate and mevalonate are ambiguous and thus have raised doubts concerning *de novo* synthesis of sterols in sponges. By contrast, our results with lanosterol and cycloartenol are quite clear: in some sponges, lanosterol and cycloartenol are both transformed to 4,4,14-demethyl sterols. Since symbionts are not the

common denominator in Table 1, we conclude that successful lanosterol and cycloartenol incorporation can be equated to *de novo* sterol biosynthesis in sponges. Therefore, some sponges are capable of *de novo* biosynthesis and others are not. Nevertheless, even dietary precursors can be modified selectively by sponges. Thus, the first three sponges in Table 1 are incapable of *de novo* biosynthesis, but can selectively modify the nucleus to give rise[26(a)] to the unique A-nor (J) or 19-nor (K) skeletons.

EXPERIMENTAL

General: Waters HPLC equipment (M6000A and M45 pumps and R401 differential refractometers) as well as Spectra Physics pumps (SP8810) fitted with a Rheodyne sample injection valve (model 7010) were used for separation of sterol mixtures. Altex Ultrasil-Si normal-phase columns (10 mm i.d. x 25 cm) were used to separate various steroid nuclei, and further purification was achieved using two Altex Ultrasphere ODS columns (10 mm i.d. x 25 cm) connected in series. The purity of HPLC fractions was checked by GC using a Carlo Erba model 4160 instrument with FID (HP Ultra 2 capillary column, 0.32 i.d. x 25 m with 0.52 film thickness). Low resolution mass spectra were recorded with a Ribermag R-10-10 quadrupole instrument in either DI or GC-MS mode; 400 MHz ^{1}H NMR spectra were obtained on a Varian XL-400 spectrometer. Radioactivity was determined using a Beckman LZ7500 liquid scintillation counter.

[24-^{3}H] Lanosterol and cycloartenol: These sterols were prepared as described previously.[1]

Incorporation experiments: *Phakellia sp.*, *Pseudaxinyssa spp.*, *Jaspis stellifera* and *Xestospongia sp.* were collected at a depth of 14-18m at John Brewer Reef, central portion Australian Great Barrier Reef; *Aplysina fistularis* was collected from 1m at Casa Cove, La Jolla, California; *Tethya aurantia californiana* was collected from 10m at Monterey Bay, California; *Microciona prolifera* was collected at 0.1m at San Francisco Bay, California; *Xestospongia muta* was collected at 30m, off the southwest coast of Puerto Rico; *Ciocalypta* was collected from 7m at Oahu, Hawaii; *Axinella verrucosa*, *Axinella polypoides* and *Petrosia ficiformis* were collected from 12m, 40m and 7m respectively at the Gulf of Naples, Italy; *Ephydata fluviatilis* was collected from 0.1m at Lake Lagunita, Stanford, California; and *Eunapius fragilis* was collected from 1m at Searsville Lake, Jasper Ridge Biological Preserve, Stanford, California. Specimens were selected that lacked surface algal fouling, although it is not possible to find *Tethya aurantia californiana* and *Ephydata fluviatilis* without algal epibionts that have penetrated the surface tissues.

The radiolabeled precursors were administered to the sponges as described previously.[32]

Electron microscopy: Electron microscopy was performed according to Lawson *et al.*[33]

ACKNOWLEDGEMENTS

We thank E. Evans, G. Smith, and J. Fromont for assistance with incorporation experiments; F.C. Thomas for assistance with electron microscopy; M. Poirrier (University of New Orleans) for the identification of the freshwater sponges; W.H. Fenical (Scripps Institution of Oceanography), the Australian Institute of Marine Sciences and G. Sodano (Instituto per la Chimica di Molecole di Interesse Biologico, Italy) for provision of facilities; and the National Institutes of Health (grants no. GM06840 and GM28352) for

financial assistance.

REFERENCES

1 For part 15 in this series, see Cordeiro, L.M.; Kerr, R.G.; Djerassi, C. *Tetrahedron Lett.* **1988**, *29*, 2159.

2. (a) Djerassi C. *Pure Appl. Chem.* **1981**, *53*, 873; (b) Ikekawa, I. In *Sterols and Bile Acids* (Danielson, H.; Sjovall, J.; eds.); Elsevier: Amsterdam, 1985; chapter 8.

3. (a) Bergquist, P.R. *Sponges*; University of California: Berkeley and Los Angeles, 1978; (b) Simpson, T.L. *The Cell Biology of Sponges*; Springer-Verlag: New York, 1984.

4. (a) Catalan, C.A.N.; Thompson, J.E.; Kokke, W.C.M.C.; Djerassi, C. *Tetrahedron* **1985**, *41*, 1973; (b) Proudfoot, J.R.; Catalan, C.A.N.; Djerassi, C. *Tetrahedron Lett.* **1986**, *27*, 423; (c) Stoilov, I.L.; Thompson, J.E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4147; (d) Stoilov, I.L.; Back, T.G.; Thompson, J.E.; Djerassi, C. *Ibid.* **1986**, *42*, 4156; (e) Stoilov, I.L.; Thompson, J.E.; Djerassi, C. *Tetrahedron Lett.* **1986**, *27*, 4821; (f) Stoilov, I.L.; Thompson, J.E.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235; (g) Cho, J.-H.; Thompson, J.E.; Stoilov, I.L.; Djerassi, C. *J. Org. Chem.* **1988**, *53*, 3466.

5. (a) De Rosa, M.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol.* **1973**, *45B*, 883; (b) De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 408; (c) De Rosa, M.; Minale, L.; Sodano, G. *Ibid.* **1975**, *31*, 758; (d) Minale, L.; Ricco, R.; Scalano, O.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C. *Ibid.* **1977**, *33*, 1550.

6. (a) Nes, W.R. *Lipids* **1974**, *9*, 596; (b) Bloch, K.E. *CRC Crt. Rev. Biochem.* **1983**, *14*, 47; (c) Carlson, R.M.K.; Tarchini, G.; Djerassi, C. In *Frontiers of Bioorganic Chemistry and Molecular Biology (IUPAC)* (Anachenko, S.; ed.); Pergamon: Oxford, 1980; p. 211.

7. (a) Nes, W.R.; McKean, M.L. *Biochemistry of Steroids and Other Isopentoids*; University Park: Baltimore, 1977; chapter 7; (b) Rilling, H.C.; Cheyet, L.T. In *Sterols and Bile Acids* (Danielson, H.; Sjoval, J.; eds.); Elsevier: Amsterdam, 1985; chapter 1; (c) Lederer, E. *Quart. Rev.* **1969**, *23*, 453; (d) Gibbons, G.F.; Goad, L.J.; Goodwin, T.W.; Nes, W.R. *J. Biol. Chem.* **1971**, *246*, 2967; (e) Nes, W.R. In *Isopentenoids in Plants -- Biochemistry and Function* (Nes, W.D.; Fuller, G.; Tsai, L.; eds.); Dekker: New York, 1985; (f) Harrison, D.M. *Nat. Prod. Rep.* **1985**, *2*, 525; (g) Bonsal, S.K.; Knoche, H.W. *Phytochemistry* **1980**, *19*, 1240; (h) Goad, L.J. *Pure Appl. Chem.* **1981** *51*, 837; (i) Ponsinet, G.; Ourisson, G. *Phytochemistry* **1968**, *7*, 89; (j) Barrow, K.D. In *Marine Natural Products, Chemical and Biological Perspectives* (Scheuer, P.J.; ed.); Academic: New York, 1983; vol. 5; (k) Voogt, P.A. *Netherlands J. Zool.* **1976**, *26*, 84.

8. (a) Rees, H.H.; Goad, L.J.; Goodwin, T.W. *Tetrahedron Lett.* **1968**, 723; (b) Benveniste, P.; Hirth, L.; Ourisson, G. *Phytochemistry* **1966**, *5*, 45; (c) Benveniste, P.; Hirth, L ; Ourisson, G. *Ibid.* **1968**, *7*, 31.

9. Sekula, B.C.; Nes, W.R. *Phytochemistry* **1980**, *19*, 1509.

10. Ponsinet, G.; Ourisson, G. *Phytochemistry* **1968**, *7*, 757.

11 Cycloartenol has been detected in the starfish *Asterias rubens* but it is likely of dietary origin (ref. 7(c)). Also, in two amoebae of the genus *Naeglera*, evidence suggests that sterols are synthesized via cycloartenol (Raedersdorff, D.; Rohmer, M. *J. Biochem.* **1987**, *164*, 421).

12. Wunsche, L.; unpublished result in this laboratory.

13. Stoilov, I.L.; Thompson, J.E.; Djerassi, C. *Tetrahedron Lett.* **1986**, 4821.

14. The ability of sponges to "extract" labeled sterols and not mevalonate from sea water may be due to the difference in solubility of these precursors; mevalonate is much more soluble in water than are sterols. Whether or not sponges are capable of extracting

dissolved material is an area of controversy.
15. Kockova-Knatochilova, A. In *Biotechnology* (Rehm, H.-J.; Reed, G.; eds.); Weinheim-Verlag Chemie: Deerfield Beach, Florida, 1981; chapter 1.
16. Reitz, R.C.; Hamilton, J.G. *Comp. Biochem. Physiol.* **1968**, *25*, 401.
17. DéSouza, N.J.; Nes, W.R. *Science* **1968**, *162*, 363.
18. Vacelet, J. *J. Micros. Biol. Cell.* **1975**, *23*, 271.
19. Wilkinson, C.R. *Mar. Biol.* **1978**, *49*, 169.
20. Boutry, J.L.; Saliot, A.; Barbier, M. *Experientia* **1979**, *35*, 1541.
21. Wilkinson, C.R. In *Endocytobiology, Endosymbiosis and Cell Biology* (Schwemmler, W.; Schenk, H.E.A.; eds.); Walter de Gruyter: New York, 1980; pp. 553-563.
22. (a) Tam Ha, T.B.; Kokke, W.C.M.C.; Thompson, J.E.; Proudfoot, J.R.; Djerassi, C. *Steroids* **1985**, *45*, 263; (b) Hofheinz, W.; Oesterhelt, G. *Helv. Chim. Acta.* **1979**, *62*, 1307; (c) Li, X.; Djerassi, C. *Tetrahedron Lett.* **1983**, 665.
23. De Rosa, M.; Minale, L.; Sodano, G. *Comp. Biochem. Physiol.* **1973**, *45B*, 823.
24. Radioactivity was also found in codisterol (11). This has been shown[4(a)] to be the precursor of dehydroaplysterol (12) and aplysterol (13).
25. Boutry, J.L.; Bordes, M.; Barbier, M. *Biochem. System. Ecol.* **1976**, *4*, 201; (b) Gagosian, R.B. *Limnol. Oceanogr.* **1976**, *21*, 702.
26. (a) Minale, L.; Sodano, G. In *Marine Natural Products Chemistry* (Faulkner, D.J.; Fenical, W.H.; eds.); Plenum: New York, 1977; pp. 87-109; (b) Kobayashi, M.; Mitsuhashi, H. *Steroids* **1975**, *26*, 605; (c) Djerassi, C.; Theobald, N.; Kokke, W.C.M.C.; Pak, C.S.; Carlson, R.M.K. *Pure Appl. Chem.* **1979**, *51*, 1815.
27. (a) Mattia, C.A.; Mazzarella, L.; Puliti, R.; Sica, D.; Zollo, F. *Tetrahedron Lett.* **1978**, 3953; (b) Ravi, B.N.; Kokke, W.C.M.C.; Delseth, C.; Djerassi, C. *Ibid.* **1978**, 4379.
28. Proudfoot, J.R.; Catalan, C.A.N.; Djerassi, C. *Tetrahedron Lett.* **1986**, 423.
29. In most cases, the presence or absence of cyanobacteria was determined by electron microscopy. The appearance of an absorption characteristic of chlorophyll in the UV/vis spectrum of the acetone extract of a sponge was also used for this purpose as described in reference 28.
30. Wilkinson, C.R. *Science* **1987**, *236*, 1654.
31. (a) Bird, C.W.; Lynch, J.M.; Pirt, F.J.; Reid, W.W.; Brooks, C.J.W.; Middleditch, B.S. *Nature* **1971**, *230*, 473; (b) Bouvier, P.; Rohmer, M.; Benveniste, P.; Ourisson, G. *J. Biochem.* **1976**, *159*, 267.
32. Carballeira, N.; Thompson, J.E.; Ayanoglu, E.; Djerassi, C. *J. Org. Chem.* **1986**, *51*, 2751.
33. Lawson, M.P.; Thompson, J.E.; Djerassi, C. *Lipids* **1988**, *23*, 741.