to close the gap and explain the regiochemical and stereochemical results, the theory of such interactions, particularly with respect to 1,3-dipolar cycloadditions, has not been adequately developed to allow one to reach any firm conclusions at this time. Other factors including those discussed above may also play a significant role.

The main thrust of the work that has been described in this Account was experimental. The results that have been presented on the regio- and stereochemical outcome of cycloadditions of difluoroallene and fluoroallene will be pertinent to any further theoretical rationale that is presented to explain the effect of substituents on this broad class of reactions. Moreover it should be evident that appropriately substituted allenes, with their perfectly aligned allylic bonds, constitute ideal substrates for use in testing the theories of π-facial selectivity.

Sponge Phospholipids†

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Sponges are simple cell aggregates that have thrived since the Cambrian Age in virtually all aquatic environments: marine and freshwater, from the equator to the poles, from shallow waters to enormous depths.[1] What is special about these ancient cells and their boundaries? What adaptive biochemical features exist in these oldest of the metazoans?

At a time when cell membrane biochemistry is gaining widespread attention, the discovery of well over 100 unprecedented sterols in sponges[2-5] followed by over 50 phospholipids[6] with unique structures and biosynthetic origins seems to have mystified further the riddle of membrane sterol–phospholipid interaction.[7-14] Are these sponge lipids indeed cell membrane components? What is their functional significance? In this Account we will attempt to unify the diverse findings on sponge phospholipids of the diacylglycerol type from comparative and evolutionary perspectives. Ether phospholipids, such as the plasmalogens, will not be considered.

Background

Our interest in sponge phospholipids is traceable to the earlier research on marine sterols.[2-5] In contrast to the monotony of terrestrial sterols (Figure 1), marine sterols show complex variations in both the tetracyclic nucleus and the side chain (Figures 2 and 3). The sterol mixtures of some sponges are composed entirely of the unique A-nor and 19-nor nuclei;[3] in others the isooctyl side chain of cholesterol is extended by alkyl branches and three-membered rings at various positions (Figure 3).[3-5] These structural oddities have attracted intensive biosynthetic studies.[2] The deliberate production of special lipids has, however, made even more puzzling their biological role in the organisms.

Sponges typically contain mixtures of the 24-alkyl sterols found in plants and cholesterol. However, a significant number of sponges possess substantial amounts of novel sterols, which prompted our early speculation[5a] that they were cell membrane components. Cholesterol is a membrane stabilizer:[8-13] it maintains the integrity of animal cell membranes by reducing the effective area of phospholipids (known as the condensing effect) and adjusting the flexibility and permeability of the latter. To achieve these ends the sterol molecule in general should have a rigid planar nucleus, a 3β-hydroxy group, C-18 and C-19 angular methyls, 17β and 20R configurations, and a C_6–C_{10} side chain (Figure 1).[5,8,9,14] Some sponge sterols contradict this rule (Figures 2 and 3):[5,9] the A-nor stanols have no 3β-hydroxyl function; the 19-nor stanols have no methyl group at C-19; and sterols with extended side chains interact poorly with the phospholipids in mam-

Carl Djerassi is Professor of Chemistry at Stanford University. His chemical autobiography, *Steroids Made It Possible*, appeared as Vol. 4 in the series Profiles, Pathways, and Dreams, J. I. Seeman, Editor (American Chemical Society; Washington, DC, 1990) and contains more information than any casual reader may possibly desire.

Wai-kwan Lam received his B.Sc. and M.Sc. degrees from the University of Hong Kong and the University of London, respectively. He had nearly completed the requirements for his Ph.D. at Stanford University when he met an untimely death.

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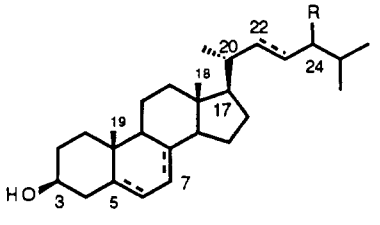

R = H	Δ^5	cholesterol	(animals)
= Me	$\Delta^{5,7,22}$	ergosterol	(yeast)
= Et	Δ^5	sitosterol	(plants)
= Et	$\Delta^{5,22}$	stigmasterol	(plants)

Figure 1. Generalized terrestrial sterol structure.

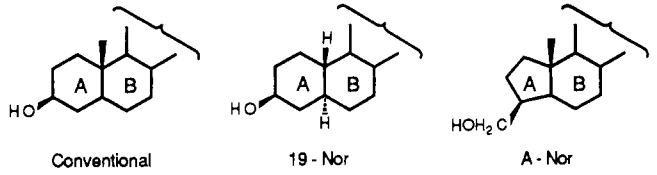

Figure 2. Generalized sponge sterol nuclei.

malian type model membranes systems.[14] If complex side chains are located in the hydrophobic region of the phospholipid bilayer, then do the phospholipids of sponges, in particular the fatty acyl components, display complementary structural novelties?

Chemical Diversity of Sponge Phospholipids

A. The Demospongic Acids. Studies over the past two decades have yielded surprising results. Litchfield and collaborators reported[15-20] high levels (34–79% of total) of C_{24}–C_{34} fatty acids in numerous sponge species as noted initially by Bergman and Swift.[21] These exceptionally long chain fatty acids, mostly polyunsaturated, were dubbed "demospongic acids".[20] Of special interest is the 5*Z*,9*Z* diene pattern of the many C_{24}–C_{28} acids studied (e.g., **1** and **2** in Figure 4). These molecules contrast sharply with their familiar C_{14}–C_{22} counterparts where the methylene-interrupted unsaturation pattern is common (e.g., $\Delta^{9,12,15}$-18:3, linolenic acid).[13] The demospongic acids, notably, were found mostly in the amino phospholipids phosphatidylethanolamines (PE) and phosphatidylserines (PS),[18] whereas the short-chain fatty acids predominated in the common "animal phospholipids",[13] the phosphatidylcholines (PC).

Our venture into the phospholipid field began with the marine sponge *Aplysina fistularis*,[22] whose principal sterols are aplysterol and its 25,26-dehydro analogue (Figure 3). The phospholipid acyl components of this sponge comprise 85% conventional C_{14}–C_{20} acids (some branched, as is common among bacteria) and 15% C_{27}–C_{30} demospongic types. The latter, again mainly in PE and PS, all possess the unusual 5*Z*,9*Z* diene system and have an additional methyl branch or double bond at the ω7 carbon (e.g., **3** in Figure 4).

Molecular models of one of the major demospongic acids in *A. fistularis*, 22-methyl-5,9-octacosadienoic acid (22Me-$\Delta^{5,9}$-28:2), suggested[22] that if the 5*Z*,9*Z* double bonds were stacked upon each other in the cell membrane to achieve maximum π overlap, the 22-methyl branch would sterically interfere with the terminal methyl groups of the sterol side chain. In yeast, a disordering of the phospholipids is caused by the C-24 methyl group of ergosterol.[8,9] Apparently this disordering effect plays a compensatory role in fluidizing the cell membrane of yeast and some plants where highly unsaturated fatty acids are lacking.[9] In the case of sponges the accumulation of C_{24}–C_{34} acyl chains in the membrane would reduce its flexibility and render membrane-associated reactions more difficult than with their standard C_{14}–C_{22} counterparts. So is the steric interference a means to restore membrane fluidity in the sponge? Or is the sponge membrane distinct in structure and function as Litchfield and Morales speculated?[18]

Such conjectures prompted us to undertake a systematic investigation of the phospholipid fatty acids of various sponges. Some of the remarkable features of demospongic acids (Figure 4) include the presence of cyclopropyl,[25] α-acetoxy,[26] or α-methoxy[27,28] functions (of *R* configuration) in C_{22}–C_{30} acyl chains; methyl branches at various positions;[28-31] the unique 6-bromo-$\Delta^{5,9}$ moiety of C_{26}–C_{27} acids with or without methyl branching;[6,32] and the abundance of numerous odd-chain (C_{21}–C_{27}) analogues.[33] Common among the unsaturated acids is again the 5*Z*,9*Z* diene pattern, although some long-chain monoenes have been identified (e.g., 19-Me,24-Me-Δ^5-25:1, **8** in Figure 4).[29] The Δ^5 double bond, incidentally, is unusual and exists mainly in the C_{16}–C_{18} fatty acids of marine invertebrates[29,34-36] and some plants.[37-39] Recently the structural range of demospongic acids has been widened further by Carballeira and co-workers, who observed, in addition to the above structural features,[40-42] the

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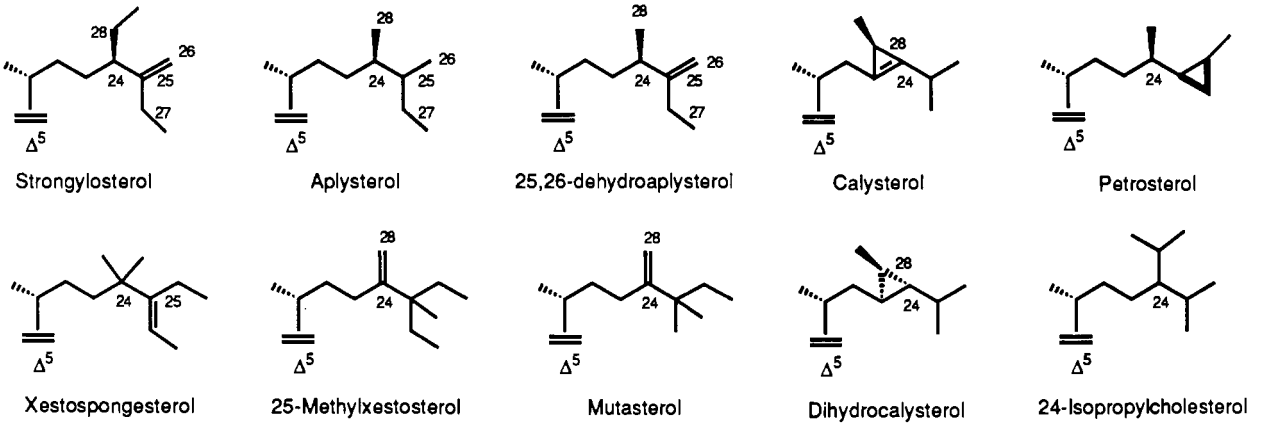
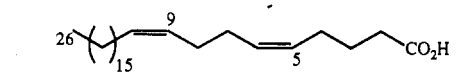
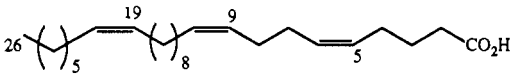


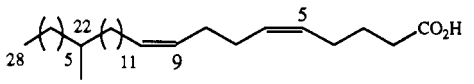
Figure 3. Examples of sponge sterol side chains.



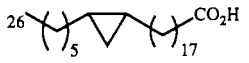
1, $\Delta^{5,9}$-26:2 (*Microciona prolifera*)



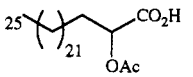
2, $\Delta^{5,9,19}$-26:3 (*Microciona prolifera*)



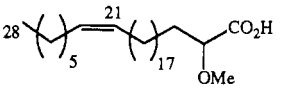
3, 22-Me-$\Delta^{5,9}$-28:2 (*Aplysina fistularis*)



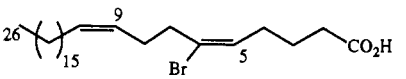
4, 19,20-Cyc-26:0 (*Calix nicaeenis*)



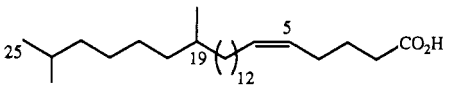
5, 2-OAc-25:0 (*Polymastia gleneni*)



6, 2-OMe-Δ^{21}-28:1 (*Higginsia tethyoides*)



7, 6-Br-$\Delta^{5,9}$-26:2 (*Hymeniacidonid Sponge*)



8, 19-Me,24-Me-Δ^{5}-25:1 (*Strongylophora durissima*)

Figure 4. Some sponge phospholipid fatty acids (demospongic acids).

α-hydroxy function[43,44] and the rare $\Delta^{6,11}$-diene system.[45] It is noteworthy that all demospongic acids identified so far predominate in the PE and PS fractions (ca. 75% vs 25% in PC).

Demospongic acids have been identified in marine and freshwater sponges collected from Siberia[46-49] to the Antarctic;[50] the total abundance in all cases is substantial (15–60%). Are demospongic acids complementary in structure and function to the sponge sterols? A definite answer is difficult to provide, but a few peculiar observations are worth noting. The sponge *Calyx nicaeensis* has three-membered rings in both its major sterol (calysterol, Figure 3) and one of its phospholipid fatty acids (4 in Figure 4).[25] *Strongylophora durissima* has 10 different methyl-branched long-chain fatty acids present in PE and PS, three of which are of the rare Δ^5-monoenic type (e.g., 8 in Figure 4).[29] The sterol mixture of *S. durissima* contains mostly the novel sterol strongylosterol (Figure 3) and no cholesterol. *Axinella verrucosa* and *Phakelia aruensis* contain mixtures of A-nor sterols which coexist with the $\Delta^{5,9}$-26:2 acid, which is present solely in PE and PS.[51]

The complementarity of novel sponge sterols and phospholipids is still uncertain. Although aplysterol (Figure 3) (60%) and the 22-Me-$\Delta^{5,9}$-28:2 acid (6.1%) are present in significant amounts in *A. fistularis*, there is no evidence that they interact with each other in the cell membrane, since cholesterol (11%), the short-chain fatty acids (85%), and another unusual $\Delta^{5,9,23}$-30:3 acid (5.6%) co-occur, most likely as membrane components.[22] The sterols of *Microciona prolifera* are mostly (99%) "conventional", whereas over 30% of the total phospholipid acids are of the $\Delta^{5,9}$-26:2 and $\Delta^{5,9,19}$-26:3 types.

B. Intact Sponge Phospholipids. A striking feature of sponges is the attachment of two identical demospongic acyl chains to the glycerol backbone,[52,53] in contrast to the conventional animal phospholipids which typically carry a saturated acyl group at C-1 of glycerol and an unsaturated one at C-2.[13] The relative abundance of phospholipid classes varies with sponge species, but the amino phospholipids, especially PE, are usually predominant.

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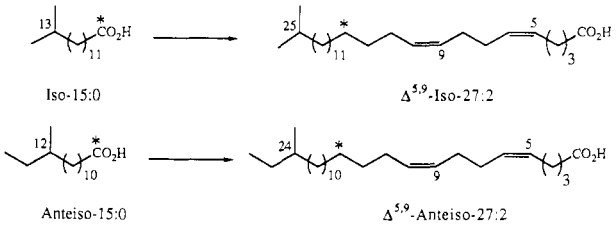
Scheme I
Incorporation of 1-^{14}C-Labeled Branched-Chain Fatty Acids into the Marine Sponge *Jaspis stellifera*



Isolation of the unstable demospongic molecular species[52,53] and structure elucidation by fast atom bombardment and tandem mass spectrometry[23,24,52,53] revealed the following unique PE molecular species: $\Delta^{5,9}$-PE(26:2,26:2); $\Delta^{5,9,19}$-PE(26:3,26:3); $\Delta^{5,9,21}$-PE-(28:3,28:3); and $\Delta^{5,9,23}$-PE(30:3,30:3). Cervonic ($\Delta^{4,7,10,13,16,19}$-22:6) and arachidonic ($\Delta^{5,8,11,14}$-20:4) acids, common among other organisms, exist in sponges as PC(22:6,22:6) and PC(20:4,20:4), respectively. Such "symmetric" phospholipids rarely exceed negligible quantities in higher organisms except for dipalmitoyl-phosphatidylcholine (DPPC), the mammalian lung surfactant.[13]

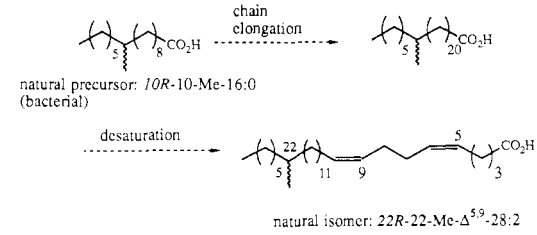
In addition to PE, PC, and PS, the other common head groups of sponge phospholipids are phosphatidylglycerol (PG)[51] and phosphatidylinositol (PI).[54,55] PG is typical of bacteria, and its high level in some sponges (usually associated with arachidonic acid, 20:4)[51] suggests either the primitive nature of the latter or the incorporation of exogenous phospholipids. PI, however, plays a distinct role in sponge cell aggregation,[54-58] the species-specific cell-recognition ability of sponges.

Demospongic Acid and Phospholipid Biosynthesis

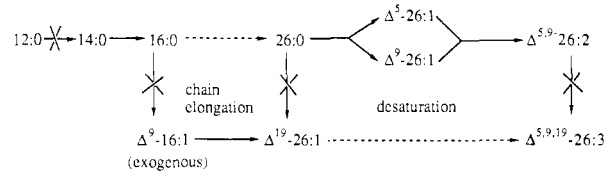
There are several possible sources of sponge fatty acids: de novo biosynthesis, dietary intake, and incorporation from symbionts, with or without further modification. Morales and Litchfield demonstrated in *M. prolifera* that the most characteristic sponge fatty acids, the $\Delta^{5,9}$-26:2 and $\Delta^{5,9,19}$-26:3 acids (Figure 4), were biosynthesized via homologation of the respective short-chain precursors, palmitic (16:0) and palmitoleic (Δ^{9}-16:1) acids, followed by desaturation.[59] De novo biosynthesis starting from acetyl-CoA was found to be negligible.[13]

Our biosynthetic studies began with the branched-chain demospongic acids. By the incorporation of synthetic 1-^{14}C-labeled 13- and 12-methylpentadecanoic acids (iso-15:0 and anteiso-15:0) into the Australian sponge *Jaspis stellifera*, it was shown[60] that the 25-methyl-5,9-hexacosadienoic acid ($\Delta^{5,9}$-iso-27:2) and its 24-methyl analogue ($\Delta^{5,9}$-anteiso-27:2) in fact originate via chain elongation of the C_{15} precursors (Scheme I). Our findings suggest that the short-chain precursors are obtained from bacteria[12] and then homologated and desaturated by the sponge.[61]

Scheme II
Biosynthesis of Chiral Branched-Chain Demospongic Acids in the Marine Sponge *Aplysina fistularis*



Scheme III
Biosynthesis of Straight-Chain Demospongic Acids in the Marine Sponge *Microciona prolifera*



We investigated the biosynthesis of 22-methyl-5,9-octacosadienoic acid (22-Me-$\Delta^{5,9}$-28:2) in *A. fistularis* to determine the role of chirality at the branching site,[61] using synthetic [1-^{14}C]-*dl*-10-methylhexadecanoic acid (10-Me-16:0) and its 10*R* and 10*S* antipodes. Surprisingly, all three acids were incorporated and transformed in vivo into the *d*/*l*-22-Me-$\Delta^{5,9}$-28:2 acid (Scheme II). No qualitative specificity between the two enantiomers was observed, although the natural acid is of the 22*R*-(−) configuration. The indiscriminate homologation of enantiomeric acyl precursors indicates that the chiral branching site, if located far enough from the carboxyl end, is not distinguished by the fatty acid synthase of the sponge. Judging from the above two biosynthetic studies,[60,61] the distal methyl branches in various demospongic acids are probably not introduced by the sponges themselves. Apparently fatty acid biosynthesis in sponges relies heavily on short-chain precursors originating from symbionts or diet: the rare 10-Me-16:0 acid, for instance, is present in the anaerobic marine sulfate-reducing bacteria which exist within *A. fistularis*.[61]

Elucidation of the complete biosynthetic pathway for straight-chain demospongic acids was our next task. For this purpose we selected the sponge *M. prolifera*, which Litchfield and co-workers used for their early biosynthetic studies.[59] Incorporation of various 1-^{14}C-labeled fatty acids confirmed[62] that the predominant biosynthetic process for the C_{26} acids proceeds via the homologation of short-chain precursors (Scheme III). The shortest saturated homologue amenable to chain elongation to the 26:0 acid was shown to be myristic (14:0) rather than the earlier assumed[59] palmitic acid (16:0). Lauric acid (12:0) was not homologated,[62] thus confirming the initial observation[59] of minimal de novo biosynthetic activity.

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The most striking aspect of demospongic fatty acid biosynthesis is the desaturation process. Desaturation of the 26:0 acyl chain was found[62] to commence at either the Δ^5 or Δ^9 loci, meaning that the second double bond may be inserted on *either* side of the existing one (Scheme III). This is contrary to the formation of polyunsaturated fatty acid formation in animals,[13] where the first double bond is normally introduced at the Δ^9 position, with the second and subsequent ones inserted between the first bond and the carboxyl terminus. Plants, however, do desaturate between the first double bond and the methyl end of the fatty acyl chain.[13]

The trienoic $\Delta^{5,9,19}$-26:3 acid is formed in the marine sponge not from the saturated precursors, as in animal systems,[13] but rather from palmitoleic acid (Δ^9-16:1), followed by desaturation at the Δ^5 or Δ^9 loci of the elongation product (Scheme III).[62] This biosynthetic sequence indicates the lack of Δ^{19}-desaturase activity in the sponge, and more importantly it reflects the low affinity of the sponge's Δ^9-desaturase enzyme for short-chain homologues like palmitic acid. Exogenous (bacterial?) palmitoleate (Δ^9-16:1) is used, instead, as the substrate for chain homologation (Scheme III).[62]

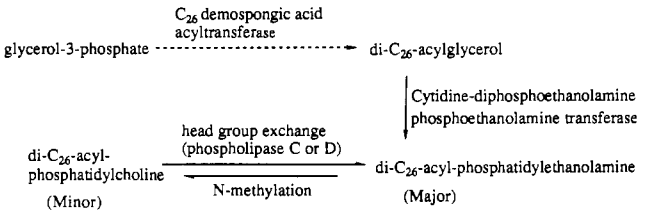
We extended our investigations to the freshwater sponges, which had been under limited study in the USSR.[46-49] A series of radiotracer experiments demonstrated[63] that Δ^{19}-desaturase activity occurs in the freshwater sponge *Ephydatia fluviatilis*. As mentioned above, desaturation between the Δ^9 locus and the methyl terminus of the fatty acid is typical of plants. Animals, including marine sponges, normally do not desaturate beyond the Δ^9 position. On the basis of their fatty acid desaturation pathways, sponges[62,63] possess both animal and plant characteristics.

The abundance of odd-chain demospongic acids in some sponge species raises the question of whether they share the same biosynthetic pattern with their even-chain counterparts. An affirmative answer was provided by our radiolabeling experiments with odd-chain precursors on a Puerto Rican sponge *Xestospongia* sp.,[64] since its $\Delta^{5,9}$-27:2 acid (>20% of total phospholipid acids) is formed mainly by homologation of the 15:0 or 17:0 acids followed by desaturation. De novo biosynthesis from propionate, which is known to be operative in higher organisms,[65] was found to be negligible.

By now a distinct pattern of demospongic acid biosynthesis has emerged. Sponges obviously possess an active homologating enzyme system acting on short-chain fatty acid precursors largely derived from exogenous sources (most likely bacteria or plankton). Fatty acid desaturation in sponges exhibits both animal and plant behavior. There is no conclusive evidence that sponges are incapable of de novo fatty acid biosynthesis, but so far the formation of short-chain acids from radiolabeled acetate has not been observed to any significant extent.

The mechanism of functionalization of sponge fatty acids is less well understood. Substituents like α-hydroxy,[43,44] α-methoxy,[27,28] α-acetoxy,[26] and bromo (C-6, vinylic)[6,32] are apparently introduced after chain elongation. The bromo function of the 6-Br-$\Delta^{5,9}$-26:2 acid,[6] for instance, has been shown in a Hymeniacidonid sponge to be introduced into the $\Delta^{5,9}$-26:2 acid in the final step of biosynthesis.

Scheme IV
Pathways of Phospholipid Metabolism in the Marine Sponge *Microciona prolifera*



The biosynthesis of intact sponge phospholipids proceeds[66] via the cytidine pathways as in other eukaryotes. The attachment of two identical acyl chains to PE appears, however, to be mediated by acyl- and phosphoethanolamine transferases specific for the demospongic acids (Scheme IV). The interconversion of phospholipid classes is appreciable, especially from PC to PE by head-group exchange and, conversely, by N-methylation. Unnatural PCs with demospongic acyl groups tend to be converted rapidly into the PE analogues after indiscriminate uptake by the sponge, thus highlighting the affinity of amino phospholipids for demospongic acids.

Cellular Studies

We recently demonstrated that demospongic acids (C_{24}–C_{34}, mostly unsaturated) and C_{24}-alkylated sterols are both found in the sponge membrane.[67-70] It must be stressed, however, that unconventional sterols are not universal among sponges. The cell membrane of *Reniera* sp., for instance, has been found to contain demospongic acids but only conventional type sterols.[69] Thus, demospongic acids do not always co-occur with novel sponge sterols. These findings point toward a highly intriguing biophysical relationship between unusual sponge membrane lipids, which we examined by using model membrane systems (vide infra).

Another objective of our cellular studies is to identify the biosynthetic sites of demospongic phospholipids and their distribution among different cell categories. Sponges are loose aggregates of a few cell types.[1,71] Storage cells are often present, such as the gray cells or spherulous cells in some sponge species. Most crucial to our sponge lipid studies, however, are the inter- or intracellular microbial symbionts,[71] since they may contribute to the total lipid composition of the sponge or provide short-chain precursors for demospongic acid biosynthesis. Cell separation is essential, therefore, to fully characterize the unusual lipid molecules.

Different sponge cell types can be separated by the combined use of density gradients and fluorescence-

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activated cell sorting.[55,58,72-74] As expected, an uneven distribution of demospongic acids and sterols among different cell types was observed.[55,72,73] The small surface cells are rich in demospongic acids but low in sterol content as compared to the internal, metabolically active cells. The relatively high levels of short-chain acids in the metabolically active cells is most likely due to the accumulation of symbionts and engulfed food particles, which may be the ultimate supplier of precursors for demospongic acid biosynthesis. No demospongic acid or unconventional sterol, however, has been found in the cultured microbial symbionts.[72]

Radiotracer experiments with carefully separated archeocytes and choanocytes of *M. prolifera* revealed[73] fatty acid homologation occurring in both cell types. The elongation of short-chain precursors is the chief mode of demospongic acid biosynthesis in sponge cells, which require such acids for incorporation into the amino phospholipids. What is their membrane function?

Are Sponge Cell Membranes Unique?

The notion that the sponge cell membrane was unique[18] arose primarily from the steric bulk and entropy anticipated from the long-chain cis-polyenoic phospholipids. Furthermore, the abundance of PE would contribute to membrane density due to H-bonding between the ethanolamine head groups. The absence of any visible anomaly under the electron microscope challenged this speculation.[75] The currently accepted "fluid mosaic model" of animal cell membranes is a trilaminate, bilayer assembly of phospholipids (5–15 nm) integrated with cholesterol and membrane proteins.[12,13,70] The sponge membrane fulfills such morphological criteria,[75] which lead to the erroneous argument[75] that demospongic acids and the unusual sterols were not membrane components.

Demospongic phospholipids are cell membrane components, although they coexist with either the conventional or novel sponge sterols[69] and cause no visible distortion of the bilayer.[75] In order to understand the interaction between demospongic acids and sterols, we employed model membrane studies, using both isolated sponge phospholipids and their synthetic analogues. These studies led to a surprising result: the unique sponge phospholipids do not interact with sterols in cell membranes!

In our first study,[76] liposomes of either synthetic[77] $\Delta^{5,9}$-PE(26:2,26:2) or $\Delta^{5,9}$-PC(26:2,26:2) underwent an endothermic phase transition at 42 °C (typical of DPPC),[78] which was unaffected by cholesterol (up to 20 mol %). Broadening of the phase transition was minimal. The usually higher phase-transition temperature of PE (due to H-bonding between head groups) was not observed, presumably because of the overriding effect of the long acyl chains. Replacing cholesterol with *A*-norcholesterol or petrosterol (Figure 3) gave similar results: the sterol was excluded from the phospholipid bilayer.

It is striking that these two membrane lipid types, being so abundant in various sponge species, should have no mutual interaction at all in the membrane. We speculated that the 5*Z*,9*Z* diene pattern and the exceptional chain length of the acyl groups were both responsible for the exclusion of sterols from demospongic phospholipids.

To examine the first factor, liposomes containing the synthetic phospholipid $\Delta^{6,9}$-PE(26:2,26:2) or its PC analogue were prepared.[79] With unusual chain length but the conventional 6*Z*,9*Z* diene pattern, these phospholipid variants did partially incorporate cholesterol. This contrasts with the cases of $\Delta^{5,9}$-PE(26:2,26:2) and its PC analogue, where considerable broadening of the phospholipid phase-transition peak was observed with added cholesterol (5–30 mol %), thus showing the effects of unsaturation on sterol–fatty acid interactions.

The effect of chain length is more significant. Synthetic phospholipids incorporating the 5*Z*,9*Z* diene system in C_{18}–C_{24} acyl groups, namely, $\Delta^{5,9}$-PC(18:2,18:2), $\Delta^{5,9}$-PC(22:2,22:2), and $\Delta^{5,9}$-PC(24:2,24:2),[80] all were found to integrate cholesterol readily into the liposome bilayers, underscoring for the first time the critical effect of the C_{26} chain length of demospongic acids.

The thermotropic properties of several monoenoic C_{24}-acyl phospholipids were also examined.[81] A chain length of below C_{26} was selected to display only the effect of double-bond location. Both Δ^{5}-PC(24:1,24:1) and Δ^{9}-PC(24:1,24:1) behaved like $\Delta^{6,9}$-PC(26:2,26:2), with partial incorporation of cholesterol, whereas in the case of Δ^{15}-PC(24:1,24:1), cholesterol interacted with the saturated acyl regions just as with PC(16:0,16:0). Judging from these preliminary results, both the C_{26} chain length and the 5*Z*,9*Z* diene pattern of demospongic acids were critical to the exclusion of sterols from the sponge phospholipid bilayer.

In our most recent model membrane study,[82] a number of novel marine sterols were found to interact readily with the conventional phospholipids PC(16:0,16:0) and PC(18:0,Δ^{9}-18:1). These phospholipids also interact with the demospongic type $\Delta^{5,9}$-PC(26:2,26:2). Do sterols only integrate the short-chain fatty acids in sponge membranes? Is the interaction between demospongic acids and sponge sterols mediated by the short-chain fatty acids?

Evolution in the oxygen-rich atmosphere has dictated the role of sterols in adjusting the thickness, cohesive strength, and fluidity of eukaryotic membranes.[83] In prokaryotes and some lower organisms (e.g., *Tetrahymenia*), the place of sterols is taken by analogous molecules[12,13] or their phylogenetic precursors.[84] The

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existence of novel sponge sterols that do not interact with demospongic phospholipids strongly implicates some other membrane molecules participating in lipid interaction, the most likely candidates being the membrane proteins.[12,13,83,84]

We recently identified[85] a number of conventional (mainly C_{16}–C_{18}) fatty acids covalently linked to intrinsic proteins of marine sponge membranes. Is it probable that these lipoproteins are interacting with the sterols or demospongic acids? In view of the relatively rare occurrence of acylated membrane proteins and their obscure biological roles,[12,86] new insights into these molecules may well be gained from further studies with sponge proteins.

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Concluding Remarks

The sponge cell membrane is unique in terms of its lipid diversity and lack of interaction of its phospholipids with sterols. The biological significance of these characteristics is unknown. They may be the result of neutral mutations[9] or perhaps represent molecular fossils. In any event, the sponge membrane allows a sponge to adapt to the precarious aquatic environment, with its fluctuating temperature, oxygen content, and osmolarity. Further investigation of the components of this distinct membrane will deepen our understanding of cell membrane function in general.

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Controlling Electrochemical Catalysis with Surfactant Microstructures

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Surfactants are amphiphilic ions or molecules with charged or polar head groups and long hydrocarbon tails. Two of their properties are useful in electrochemistry: adsorption at interfaces and aggregation into supramolecular structures. In the early part of this century, adsorption of surface-active compounds on Hg electrodes was found to suppress unwanted convection.[1] Surfactants became widely used for this purpose. Micelles (Figure 1) are examples of surfactant aggregates. They are formed from soluble surfactants above a critical micelle concentration (CMC). Solutes can bind at the micelle–water interface (Stern layer) and in hydrophobic regions just below this interface.[2] In 1952, Proske was the first to use micelles to solubilize nonpolar organic compounds in water for electrochemical measurements.[3]

The past 10–15 years saw many new applications of surfactants in electrochemistry. For example, ion radicals produced at electrodes were stabilized by coulombic and hydrophobic interactions with micelles.[3] Reducible and oxidizable probes began to be used to measure diffusion in surfactant media.[4,5] Microstructures of amphiphiles adsorbed on electrodes were investigated.[6] Coatings of functional amphiphiles were prepared on electrodes by Langmuir–Blodgett (LB) methods.[7]

Scheme I

$$P + e \rightleftharpoons Q \qquad E^\circ \text{ (at electrode)} \qquad (1)$$

$$ArX + Q \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ArX^{\bullet -} + P \qquad (2)$$

$$ArX^{\bullet -} \xrightarrow{k} Ar^\bullet + X^- \qquad (3)$$

$$Ar^\bullet + Q \longrightarrow P + Ar^- \qquad \text{(fast)} \qquad (4)$$

$$Ar^- + (H^+) \longrightarrow ArH \qquad \text{(fast)} \qquad (5)$$

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