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Minor and Trace Sterols in Marine Invertebrates

VI. Occurrence and Possible Origins of Sterols Possessing Unusually Short Hydrocarbon Side Chains[1,2]

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Sterols with biosynthetically unusually short side chains (fewer than eight carbon atoms expected for primary squalene cyclization products) have been identified in the extracts of numerous marine invertebrates. The structures of the short side chain and conventional side chain sterols have been determined for various species of Porifera and Coelenterata. Sterol structures were determined by comparison of their mass spectra and gas chromatographic retention times with those of authentic or synthetic samples. Evidence is presented supporting the natural occurrence of these compounds in the tissues of the marine invertebrates as opposed to formation by degradative processes during sample handling or laboratory work-up. The short side chain sterols were found to possess predominantly the androst-5-en-3β-ol nucleus with C-17 alkyl side chains ranging from zero to six carbon atoms. Concentrations of short side chain sterols range from trace levels to over 5% of the sterol mixture in various species. The possible origins of these short side chain sterols are evaluated in the light of current knowledge of sterol function, biosynthesis, dealkylation, microbial degradation, and autoxidation. Known sterol autoxidations are reviewed, and possible singlet oxygen and free radical mechanisms of sterol side chain autoxidation (at physiological temperatures) which may lead to sterols with shortened hydrocarbon side chain are suggested. The possible autoxidative generation of short side chain sterols from known marine sterols by the suggested mechanisms is evaluated through application of the REACT computer program. Predicted short side chains are tabulated for each parent marine sterol side chain and then compared with the compositions of the actual sterols found in the marine extracts examined. The possible natural environmental or *in vivo* autoxidative formation of the short side chain marine sterols is supported by these evaluations.

INTRODUCTION

Marine organisms have been shown to be extremely rich sources of unusual sterols. These marine sterols—the subject of numerous recent reviews (*1–3*) and an intense area of ongoing structural organic research (*e.g.*, *4–6*)—raise many questions concerning their modes of biosynthesis and the effect of their unusual structures in cellular membrane functions. Very little is known about marine sterol biosynthesis and essentially nothing is known about marine sterol function.

Over the past several years one of our primary interests has been the discovery, structure elucidation, and synthesis of sterols with unusually massive side chains [*i.e.*,

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[1] Dedicated with admiration and affection to Professor William S. Johnson—a former teacher and present friend and colleague.

[2] For the previous paper in this series, see Ref. (*128*).

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C_{30} sterols with 11 carbon atom side chains (*7–9*)] which are unique to marine sources and can be the major component in complex marine sterol mixtures (*10*). However, the presence of sterols with unusually short side chains ($<C_8$ expected for squalene cyclization products) in marine extracts as minor consitituents (usually less than 5% of the sterol mixture) was suspected as early as 1964 (*11*). The discovery of a series of C_{26} sterols [24-nor-cholesta-5,22-dien-3β-ol (*12*), 24-nor-5α-cholest-22-en-3β-ol (*13*), asterasterol (the $\Delta^{7,22}$ analog) (*14*), 24-nor-cholest-5-en-3β-ol (*15*), and 24-nor-5α-cholestan-3β-ol (*16*)] in the 1970's and subsequent surveys of various marine species (*17–20*) and even sea water (*21*) have firmly established the existence and wide distribution of these uniquely marine sterols. The existence of C_{26} sterols clearly raises many intriguing biosynthetic questions. Various biosynthetic mechanisms have been proposed for their production, involving demethylation (*5*, *22*) and even a nonsqualene precursor (*23*).

We became interested in the structural compositions of minor and trace components of marine sterol mixtures primarily with regard to the detection of active C_{30} sterol biosynthetic intermediates; however, the minor and trace sterol analysis scheme we developed (*10*, *24*) made possible detection of a series of unusually low molecular weight sterols.[4] One of these unusual sterols (MW = 370) was shown to contain the unexpected 19-nor-Δ^5 steroid nucleus and a conventional C_8 "cholesterol" side chain (*6*). However, the remaining low molecular weight sterols were suspected to contain side chains unexpectedly ranging from zero to six carbon atoms.

We now present structural identifications and syntheses for a number of these unusual sterols which do in fact contain unexpectedly short side chains, and a detailed evaluation of their possible origins along with evidence supporting a wide distribution of such sterols among marine Coelenterata and Porifera.

The well known *chemical definition* of a sterol specifies a steroid nucleus, a single hydroxyl substituent at C-3 and a C-17 hydrocarbon side chain; however, the intense interest in membrane function over the past several years has resulted in numerous biophysical and biological studies which have now evolved a *functional definition* of a sterol as a membrane constituent (*29–33*). The functional definition requires that a sterol: (i) reduce the effective molecular area of membrane phospholipid molecules (known as the sterol condensing effect); (ii) mobilize phospholipid fatty acid chains for phospholipids in the ordered gel state, but reduce mobility of fatty acid chains for phospholipids in the fluid liquid crystalline state; and (iii) reduce nonionic permeability for phospholipids in the liquid crystalline state, but increase nonionic permeability for phospholipids in the ordered gel state. The net effect of these sterol activities is to increase membrane fluidity and stability and to modify membrane permeability in a way essential to eukaryotic life. A general biological requirement would seem to be that a "sterol" must support life in "sterol"-deficient mutants. There is some evidence that even some nonsteroids may "functionally" be sterols (*30*). This situation parallels in some ways the case of nonsteroidal compounds possessing estrogenic activities (*34*).

Conversely, not all chemically defined sterols are sterols in a functional sense.

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[4] Since then several reports have appeared concerning the presence of similar compounds containing pregnane skeletons, hydrocarbon side chains, and 20(21) double bonds in other marine organisms (*25–27*). Earlier a report (*28*) appeared on the detection of a C_{22} steroid believed to be 20-methyl-pregna-5,20(22)-dien-3β-ol, but the physical constants do not correspond to the synthetic product described below.

Biophysical and biological studies (*29–33*) have shown that for a chemically defined sterol to be a sterol in the functional sense it must possess (i) a planar steroid nucleus (this would eliminate the common 5β configuration or other forms which distort the alternating *trans-anti* stereochemical configuration of the planar steroid nucleus); (ii) a 3β-hydroxyl group (eliminating 3α-epimers); and (iii) a hydrocarbon side chain possessing at least five carbon atoms (*35*) but preferably eight to nine (*36*). This final requirement for sterol activity indicates that the short side chain sterols presented in this paper should not be sterols in the functional sense.[5] Although these compounds may not function as cellular membrane constituents, they do occur widely in marine invertebrates. This occurrence results in a series of perplexing questions concerning the functions and origins of these short side chain compounds. We will consider these questions with respect to processes known to result in shortening of sterol side chains; however, evidence for the existence and structures of these short side chain sterols is presented first.

EXPERIMENTAL

Analytical glc was performed using a Hewlett–Packard 402 high-efficiency chromatograph equipped with 1.8 × 4 mm i.d. U-shaped columns packed with 3% OV-25 or OV-17 on 100/120-mesh GCQ (Applied Science, Inc.) and helium carrier at a 100 ml/min flow. Injection block and detector temperatures were both 290°C. OV-25 column temperatures for natural products and synthetic work were 256 and 265°C, respectively. OV-17 column temperature was 276°C. The standard 402 flame ionization detector was used throughout this work. Gas chromatograph retention times (R_f) are reported relative to cholesterol ($R_f = 1.00$). Low resolution mass spectra were measured either on an AEI MS-9 spectrometer operated by Mr. R. Ross or on a Varian Associates MAT-44 spectrometer. Combined gas chromatography–mass spectrometry (gc–ms) was performed using either the MAT-44 instrument in "GC" mode equipped with a 2.2 m × 2-mm i.d. eight-turn spiral column packed with either 3% SP 2250 (1 : 1 phenyl–methylsilicone) on 100/120-mesh Supelcoport (Supelco, Inc.) or 3% OV-101 on GCQ (Applied Science, Inc.) column temperature 270° or a Hewlett–Packard 7610A gas chromatograph equipped with 3 m × 2-mm i.d. U-shaped column (3% OV-17 on GCQ, column temperature 260°C) and interfaced with a Varian MAT 711 double-focusing spectrometer (equipped with an all-glass Watson–Biemann dual stage separator and a PDP-11/45 computer for data acquisition and reduction) operating at a resolution of 1000 to 5000. Nuclear magnetic resonance (nmr) spectra were measured on a Varian Associates T-60 or XL-100 spectrometer using deuteriochloroform as the solvent and tetramethylsilane as the internal reference. Infrared (ir) spectra were recorded on a Perkin/Elmer 700 infrared spectrophotometer. Optical rotations were determined for chloroform solutions using a Perkin–

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[5] One limitation to this conclusion is that the membrane constituents of marine invertebrates are in some cases very different from the phospholipid bilayer systems used in model studies [e.g., the C_{24}–C_{30} fatty acids of Porifera (*37*)], and before firm conclusions concerning the functionality of the short side chain sterols can be drawn, studies must be performed using the membrane systems in which these sterols are found.

Elmer 141 polarimeter. Melting points were determined on a Thomas–Hoover "Uni-Melt" capillary melting point apparatus and are uncorrected. High-pressure liquid chromatography (hplc) was performed using a Haskel Model 28303 pump, 7000-psi Valco loop injection valve and Waters Associates dual cell differential refractometer as specified earlier (*10*, *38*). Free hydroxy sterols were separated using either a Waters Associates 30 cm × 4-mm i.d. μ Bondopak C_{18} "reversed phase" column (or two such columns in series) and a methanol–water (92:8), mobile phase at 1000 to 2000 psi pressures and 0.8 to 2.0-ml/min flow rates or the higher capacity Whatman Partisil M9 10/50 ODS-2 column using a pure methanol mobile phase at pressures less than 1500 psi.

NATURAL PRODUCTS ANALYSIS

General methods of sterol fractionation involving column and thin-layer chromatography over various conventional and argentic adsorbents, fractional digitonin precipitations, and hplc are described in detail in a previous communication in this series (*10*). Gorgonians *Plexaura homomalla* and *Pseudoplexaura porosa* are from the Caribbean, sponges *Damiriana hawaiiana* and *Callyspongia plicifera* are from Hawaiian waters, the sponge *Luffariella* spp. is from the Australian barrier reef, and the gorgonian *Murecia californica* is from the coast of Southern California. The extraction procedures used with the Caribbean gorgonians were described previously (*10*), as were those for the Hawaiian sponges (*39*). *C. plicifera* was freeze-dried prior to low temperature extraction. *M. californica* was extracted by two separate procedures using samples collected 5 months apart in an effort to detect variations in short side chain sterol compositions as a function of sample handling, laboratory work-up, and season of collection. Both samples were collected in shallow water off San Diego, California. Sample No. 1 was collected in February 1976, air-dried in a hood for several days, ground in mortar and pestle, and extracted by reflux in a Soxhlet extractor (72 hr) using acetone. The extract was reduced in volume under reduced pressure and was fractionated as described earlier (*10*), yielding sterols as 0.4% of dry tissue weight. Sample No. 2 was collected in June 1976, and air mailed alive immediately in containers packed in ice and filled with oxygenated sea water. This fresh living sample was divided into two parts. The first was treated in the same manner as sample No. 1, yielding sterols as 0.38% of the tissue dry weight. The second part, 60 g of fresh tissues, was extracted immediately using the Bligh and Dyer procedure (methanol–chloroform–water) (*40*). The $CHCl_3$ layer was immediately filtered and evaporated under vacuum at room temperature, yielding 2.1 g of residue, from which the sterols were isolated at room temperature as the digitonides using a large excess of digitonin (*41–42*) (analytical, peroxide-free solvents used throughout). Isolated sterols were analyzed immediately by gc (OV-25). The entire procedure required approximately 3 to 4 hr. *Both methods of sample preparation produced sterol mixtures of identical composition.*

Short side chain sterols from the extracts were identified by comparison of their mass spectra and gc retention times (pertinent data in Table 1) to those of authentic samples (available in our laboratory[6]) or synthetic samples described below. Conventional C_{26}–

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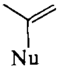
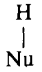
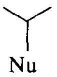
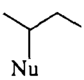
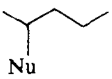
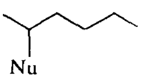
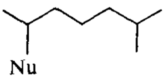
[6] We wish to thank Dr. E. P. Oliveto (Hoffman–LaRoche) for a generous gift of both the 17α and β epimers (*44*) of **IX** and Dr. A. D. Tait (Cambridge University) for a generous gift of **VI** (*45*).

C_{30} side chains are all well-known structures and were determined by mass spectra–gc retention time comparisons to authentic samples (previous reports of sterol compositions of ***P. homomalla*** and ***P. porosa*** (*10*), ***M. californica*** (*43*), and ***D. hawaiiana*** (*38*), have been made).

TABLE 1

MASS SPECTRA/GC RETENTION DATA OF SHORT SIDE CHAIN STEROLS WITH Δ^5-3β-HYDROXY STEROID NUCLEUS[a]

Structure No.	(R) side chain	Fragments containing side chain				Fragments lacking side chain					Retention times OV-25
		M^+	*A*	*B*	*C*	*V*	*W*	*X*	*Y*	*Z*	
Unsaturated side chains											
XV		272 (100)	133 (68)	161 (78)	187 (51)	213 (—)	231 (—)	255 (—)	273 (—)	271 (—)	0.16
IX		300 (78)	161 (29)	189 (9)	215 (23)	213 (61)	231 (37)	255 (2)	273 (—)	271 (4)	0.24
X		300 (71)	161 (33)	189 (17)	215 (9)	213 (10)	231 (—)	255 (1)	273 (—)	271 (11)	0.26
VII		314 (100)	175 (8)	203 (4)	229 (30)	213 (28)	231 (13)	255 (5)	273 —	271 (4)	0.39
Saturated side chains											
XII		274 (74)	135 (65)	163 (100)	189 (49)	213 (4)	—	—	—	—	0.17
VIII		302 (87)	163 (60)	191 (100)	217 (59)	213 (20)	231 (14)	255 (11)	—	—	0.25
VI		316 (100)	177 (46)	205 (82)	231 (64)	213 (22)	231 (64)	255 (18)	273 (14)	—	0.34
V		330 (100)	191 (18)	219 (55)	245 (37)	213 (24)	231 (15)	255 (21)	273 (17)	—	0.44
IV		344 (100)	205 (22)	233 (78)	259 (43)	213 (32)	231 (19)	255 (24)	273 (18)	—	0.58
II		358 (100)	219 (18)	247 (60)	273 (46)	213 (22)	231 (16)	255 (18)	273 (46)	—	0.68
XVI		386 (100)	247 (10)	275 (50)	301 (29)	213 (20)	231 (13)	255 (16)	273)16)	—	1.00

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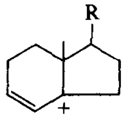
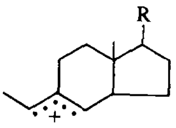
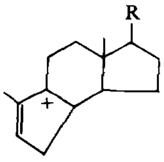
[a] Relative intensities in parentheses.

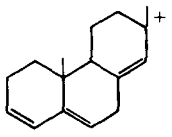
TABLE 1—*continued*

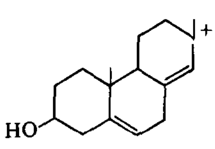
Ion structures proposed in the recent literature (*53–57*)

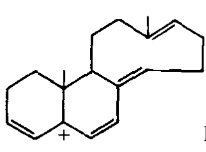

A (*m/e* 134 + R)

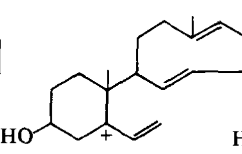

B (*m/e* 162 + R)

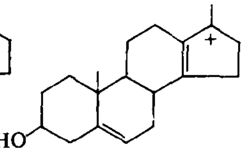

C (*m/e* 188 + R)


V (*m/e* 213)


W (*m/e* 231)


X (*m/e* 255)


Y (*m/e* 273)


Z (*m/e* 271)

SYNTHESES[7]

Sterols **V** (*46*), **IV** (*47*), and **II** (*48*) were each synthesized by reaction of the appropriate phosphorane with the aldehyde (3α,5-cyclo-6β-methoxy-5α-pregnan-20-carboxaldehyde) formed by ozonolysis (at −78°C) of (22*E*)-3α,5-cyclo-6β-methoxy-5α-stigmast-22-ene (*49*). The unsaturated (Δ^{22}) *i*-methyl ethers thus formed were subsequently hydrogenated over platinum oxide to afford side chain saturated *i*-methyl ethers, which upon acid hydrolysis (*51*) gave the required 3β-hydroxy-Δ^5-sterols **II**, **IV**, and **V**. 20-Methyl-5,20-pregnadien-3β-ol (**VII**) was prepared from pregnenolone in order to resolve a discrepancy in the physical constants reported for this compound.

*20-Methyl-5,20-pregnadien-3β-*ol (**VII**).

Treatment of pregnenolone with methylenetriphenylphosphorane in benzene (*52*) and chromatography of the crude reaction product gave **VII** as needles from methanol, mp 134–135°C; nmr 5.30 ppm (1H, m, C=CH), 4.64 (1H, d, $J = 2$ Hz) and 4.80 (1h, d, $J = 2$ Hz) ($C{=}CH_2$), 3.50 (1H, m, C*H*OH), 1.83 (3H, s, $CH_3{-}C{=}C$), 1.00 (3H, s, C-19 methyl), 0.60 (3H, s, C-18 methyl); $[\alpha]^{20}_D$ −64° (*c* 1.00); mass spectrum (MS-9): *m/e* 314 (M^+, 100%). Lit. (*50*): mp 133–134°C; $[\alpha]^{25}_D$ −66.4°.

(*20S*)-*3α,5-Cyclo-5α-pregnan-20-carboxaldehyde* (*49*).

(22*E*)-3α,5-Cyclo-6β-methoxy-5α-stigmast-22-ene was treated with ozone, as described previously. The crude product was chromatographed (silica gel, 10% ether in hexane, fractions collected under nitrogen) to afford the aldehyde product as a white solid, mp 82–83°C ir ν_{max} ($CHCl_3$) 1723 cm^{-1}; nmr 9.59 ppm (1H, d, $J = 4$ Hz, H–C=O), 3.33 (3H, s, OCH_3), 2.73 (1H, m, $-CH{-}OCH_3$), 1.12 (3H, d, $J = 8$ Hz, C-21 methyl), 1.05 (3H, s, C-19 methyl), 0.77 (3H, s, C-18 methyl); $[\alpha]^{20}_D$ +42.5° (*c* 0.55); mass spectrum (MAT-44): *m/e* 344 (M^+, 25%), 289 (100).

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[7] The phrase "work-up by ether (hexane) extraction" indicates that the reaction mixture was poured into water and thoroughly extracted with ether (hexane). Combined ether (hexane) extracts were then washed successively with saturated $NaHCO_3$ solution (as necessary), water, and saturated NaCl solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Preparation of steroids **II**, **IV**, **V** *from (20S),3α,5-Cyclo-5α-pregnan-20-carboxaldehyde*

A typical procedure is exemplified by that used for the conversion of (20*S*),3*α*,5-cyclo-6*β*-methoxy-5*α*-pregnan-20-carboxaldehyde into **IV**. A stirred suspension of ethyltriphenylphosphonium bromide (0.95 g, 2.5 m*M*) in anhydrous tetrahydrofuran (30 ml) was treated, under a nitrogen atmosphere at 20°C, with *n*-butyllithium (1.75 ml of 1.66 *M* solution in hexane, 2.5 m*M*). After 1 hr, a solution of (20*S*),3*α*,5-cyclo-6*β*-methoxy-5*α*-pregnan-20-carboxaldehyde (0.8 g, 2.3 m*M*) in anhydrous tetrahydrofuran (10 ml) was added, and the reaction mixture was stirred for 48 hr. Work-up by ether extraction gave a product which was purified by column chromatography (silica gel, 10% ether in hexane) to afford a mixture of (22*E*) and (22*Z*)-3*α*,5-cyclo-6*β*-methoxy-5*α*-chol-22-ene as an oil; nmr 5.5–5.0 ppm (2H, m, H–C=C–H), 3.33 (3H, s, $-OCH_3$), 2.76 (1H, m, *H*–C–OCH_3); mass spectrum (MAT-44): *m/e* 356 (M^+, 25%), 301 (100). The 22*E* and 22*Z* olefins (560 mg) in ethyl acetate (50 ml) were hydrogenated at 20°C and atmospheric pressure over platinum oxide (80 mg) for 20 hr. The reaction mixture was filtered through Celite and the filtrate was concentrated to give 3*α*,5-cyclo-6*β*-methoxy-5*α*-cholane (560 mg) as an oil; nmr 3.33 ppm (3H, s, O–CH_3), 2.73 (1H, m, *H*–C–OCH_3), 1.03 (3H, s, C-19 methyl), 0.90 (3H, d, $J = 6$ Hz, C-21 methyl), 0.72 (3H, s, C-18 methyl); mass spectrum (MAT-44): *m/e* 358 (M^+. 28%), 303 (100). The *i*-methyl ether (500 mg) in aqueous *p*-dioxane (8 ml of 10%) containing *p*-toluenesulfonic acid (50 mg) was heated under reflux for 1 hr. Work-up by ether extraction gave chol-5-en-3*β*-ol (**IV**) (400 mg) as needles from methanol, mp 131–132°C. Lit. (*47*): 127–130°C: ir ν_{max} ($CHCl_3$) 3625, 3560, 3400, 1460, 1375 cm^{-1}; nmr 5.33 ppm (1H, m, C=CH), 3.50 (1H, m, C*H*–OH), 1.00 (3H, s, C-19 methyl), 0.90 (3H, d, $J = 6$ Hz, C-21 methyl), 0.71 (3H, s, C-18 methyl; $[\alpha]_D^{20}$ −42.5° (*c* 0.90); mass spectrum M^+ at *m/e* 344.31110 (100%), calcd for $C_{24}H_{38}O$, 344.30991; gc, one peak, $R_f = 0.53$.

24-Norchol-5-en-3β-ol (**V**). Prepared in an analogous manner to that described above, **V** was obtained as needles from methanol, mp 135–136°C; ir ν_{max} ($CHCl_3$) 3625, 3560, 3400, 1460, 1375 cm^{-1}; nmr 5.33 ppm (1H, m, C=CH), 3.50 (1H, m, C*H*OH), 1.00 (3H, s, C-19 methyl), 0.90 (3H, d, $J = 6$ Hz, C-21 methyl), 0.70 (3H, s, C-18 methyl); $[\alpha]_D^{20}$ −5.5° (*c* 1.00); mass spectrum: M^+ at *m/e* 330.29191 (100%), calcd for $C_{23}H_{38}O$, 330.29225; gc showed one peak with $R_f = 0.44$.

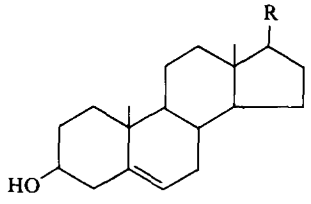
26,27-Dinorcholest-5-en-3β-ol (**II**). Compound **II** was obtained as plates from methanol, mp 136–137°C; ir ν_{max} ($CHCl_3$) 3625, 3560, 3400, 1460, 1375 cm^{-1}; nmr 5.33 ppm (1H, m, C=CH), 3.50 (1H, m, C*H*OH), 1.00 (3H, s, C-19 methyl), 0.91 (3H, d, $J = 6$ Hz, C-21 methyl), 0.70 (3H, s, C-18 methyl); $[\alpha]_D^{20}$ −39° (*c* 0.9); mass spectrum: M^+ at *m/e* 358.32121 (100%), calcd for $C_{25}H_{42}O$, 358.31887; gc showed one peak with $R_f = 0.68$.

RESULTS

Short side chain sterols occur in the marine extracts at concentrations too low to allow isolation and subsequent determination of various physical properties (i.e., nmr, mp, $[\alpha]_D$, etc.). Identifications are, therefore, based on correlation of mass spectral and gc retention time data with authentic or synthetic samples as specified under

Experimental. Such correlations allow unambiguous identifications in many cases because of the very characteristic mass spectrometric (*53–57*) and gas chromatographic (*58–62*) properties of sterols. An important limitation is that certain isomeric patterns of alkylation in sterol side chains are indistinguishable by gc-ms (*5*, *59*); therefore, there is some uncertainty whether the naturally occurring sterol **II** has the side chain of the synthetic compound (which is specified) or in fact has a 23-methylcholane side chain. However, there is no doubt that sterols **II**, **IV**, **V**, **VI**, **VIII**, and **XII** possess 3β-hydroxy-Δ^5-androstene nuclei, and C_6, C_5, C_4, C_3, C_2 and C_0 saturated hydrocarbon side chains at C-17. In Table 1, mass spectral fragmentations characteristic of the androst-5-en-3β-ol nucleus with retention of side chain: A ($M^+ - 85$), B ($M^+ - 111$), C ($M^+ - 138$) are observed for each short side chain sterol. The A, B, C–peak pattern is seen to "slide" across the series of mass spectra in 14 *m/e* increments as the side chains vary from C_0 to C_7. Fragments generated by loss of the C-17 side chain V (213), W (231), X (255), and Y (273) appear in all but sterol **XII** (in which the side chain is absent), these fragments becoming more important as side chains increase in length (Table 1). Spectra of **II**, **IV**, **V**, **VI**, **VIII**, and **XII** below *m/e* 145 are essentially identical (with intense peaks at *m/e* *145*, 135, 121–119, 109, *107*, 105, *95*, 93, 91, *81*, 79m *69*, *67*, *57*, and *55*—particularly intense peaks being in italics). These low mass range peaks have been shown to have multiple origins (*56*, *63*) and are therefore of little diagnostic value except that their general pattern is characteristic of many sterols. All sterols exhibit the expected M^+, M^+-15, M^+-18, and M^+-33 (methyl and water loss) peaks. The short side chain sterols with unsaturated side chains, **VII**, **IX**, and **X** exhibit somewhat different mass spectral properties, with fragmentations involving the Δ^5 system (A, B, and C) being suppressed relative to fragmentations involving loss of side chain.

It is important to note that during isolation sterols **VII** and **IX** are significantly retarded during argentic chromatographic separations owing to their terminal methylene group (*10*, *43*). Mass spectral and gc characteristics of oxygenated products **XI**, **XIII**, and **XIV** are well known (*55*), and the properties of **III** are presented elsewhere (*38*).



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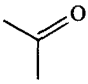

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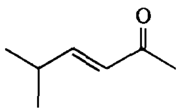

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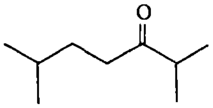
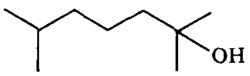


XIII R =



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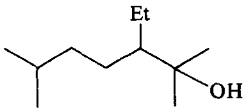


The studies with fresh *M. californica* show that short side chain sterols **V**, **IX**, and **XII** occur in the living tissues of that animal. A final interesting note is that in *P. porosa*, the short side chain sterol **VI** was found only in the steryl–fatty acid ester fraction (*10*).

DISCUSSION

The unlikely participation of smaller short side chain sterols in membrane functions was mentioned in the introduction. A nonmembrane role for these compounds is also supported by low concentrations in tissues (Table 2) seemingly too low for a structural membrane role (*30*)). This raises a question as to the function and origin of these compounds. It is premature to speculate on the function of such compounds; however, their possible origins can be evaluated in terms of our present understanding of steroid biosynthesis, dealkylation, biological degradation, and autoxidation. Each of these processes suggests the role of products derived by that process, and through such considerations some feeling for the possible functions of the short side chain sterols can be developed.

If the short side chain sterols have no physiological function, then it seems unlikely that they are formed by enzymic cyclization or dealkylation reactions. Furthermore, if the short side chain sterols are then functionless degradation products it seems unlikely that they are formed through biological or microbiological oxidative degradative processes, since those processes (*64*, *65*) invariably result in side chain oxygenated products. However, the finding that saturated hydrocarbons can be formed by *in vivo* autoxidative processes (*66*, *67*) and the discovery (*68*) that short side chain sterols as suspected autoxidation products in a 20-year-old air-aged sample of cholesterol, albeit at extremely low concentrations (less than 0.001% after the 20 year period), suggest the more likely proposition that the short side chain sterols may be *in vivo* autoxidative products. It has been suggested (*68–70*) that the short side chain sterols with or without oxygenated functionalities and low molecular weight compounds generated during the autoxidation of cholesterol are decomposition products of the 25 and 20 hydroperoxides of cholesterol which are formed by addition of ground state "biradical" oxygen to a 25 or 20 tertiary alkyl radical produced during autoxidation. These short side chain and oxygenated side chain autoxidation products, particularly cholest-5-en-3β,25-diol, are found only in cholesterol autoxidized in the crystalline state (*71–72*), perhaps because of a topochemical effect obscuring attack at the more active nuclear allylic positions relative to the side chain tertiary positions. In cholesterol solutions or dispersions only autoxidation products with nuclear modifications (*71*, *73–75*), initiated by the 5,6 double bond, are observed. Modern methods of sterol analysis (*10*) do not require crystallization of sterol fractions before subsequent analyses by physical methods (notably gc–ms). However, we have worried that the short side chain steroids isolated by us and others (*25*) may be artifacts formed by sample work-up, particularly for sterols possessing unsaturations in the side chain as is common in marine sources (Table 3), since these would be expected to be much more susceptible to side chain autoxidation. The studies using fresh *M. californica*, discussed above, have alleviated this worry, at least in the case of *M. californica*. The concentrations of short side chain sterols in extracts of several species studied (including *M. californica*) are over a thousand times higher than those found in the air-aged 20-year-old cholesterol (*68*). Our

TABLE 2

Percentage Compositions for Short and Conventional Side Chains in Marine Sterol Extracts—Sterols with Δ^5-3β-Hydroxy Nucleus

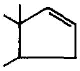
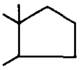
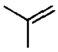
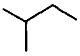
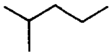
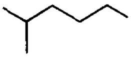
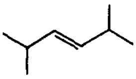
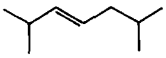
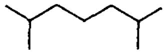
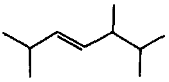
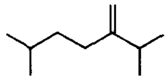
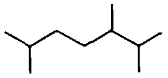
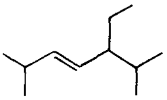
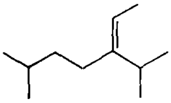
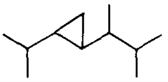
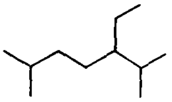
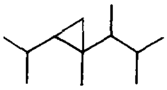
	Structure	No. or ref. letter	Coelenterata			Porifera		
			P. porosa	*P. homomalla*	*M. californica*	*C. plicifera*	*Luffariella* spp.	*D. hawaii*
C_{19}		**XV**						0.4
		**XII**	0.4	0.3	—[a]	—[a]	2.6	3.1
C_{21}		**X**	0.05					
		**IX**			2.0	—[a]		0.4
		**VIII**	0.4	0.3		—[a]	1.5	0.7
C_{22}		**VII**				—[a]	—[a]	0.8
		**VI**	0.2	—[a]		—[a]	0.5	0.2
C_{23}		**V**	—[a]		0.15			0.2
C_{24}		**IV**	—[a]			—[a]		0.5
C_{25}		**II**	—[a]					—[a]
C_{26}		A		—[a]	4			0.2
C_{27}		F		1	10	4.5	2.4	1.2
		H	6	50	44	8.9	29.7	45
C_{28}		J	11	10	16	16.9	18.4	5.9
		L	3.5	9	12			
		M	12.5	9	1	21	2.7	4.3

TABLE 2—*continued*

			Coelenterata			Porifera	
	Structure	No. or ref. letter	*P. porosa*	*P. homomalla*	*M. californica*	*C. plicifera*	*Luffariella* spp.	*D. hawaii*
C_{29}		O	1.5	3.7	1		14	3.7
		P			4.5			
		U	9.5	4.0				
		V	1.1		5	41	13	14.4
C_{30}		X	51	13				

[a] <0.001%.

findings have led us to wonder if autoxidation occurs *in vivo* in marine organisms, and, if so, whether short side chain sterols are reasonable products of such processes.

The study of *in vivo* autoxidative processes is a new area of research generating much current interest (*76*, *77*). Autoxidation of fatty acids has been shown to occur *in vivo* in mammals *fed* vitamin E (known biological antioxidant)-deficient diets, and the hydrocarbon pentane has been shown to be one of the autoxidation products. It is clear that in mammals *in vivo* autoxidation occurs normally, as is evidenced by the acculuation of lucofuscin in tissues. It is reasonable to assume that the rate of *in vivo* autoxidation may be higher for certain invertebrates.

Most specimens for marine natural products investigations are collected in intertidal, sublittoral, or coral reef areas. There are good reasons to suspect that *in vivo* autoxidation may be particularly prevalent in organisms living in these environments. Many organisms in these environments are subjected to relatively high temperatures (up to 40°C), intense sunlight, plentiful oxygen, and, in some cases, periods of atmospheric exposure (during periods of low tide). Although these organisms must contain biological antioxidants and singlet oxygen quenchers (*77*), it seems reasonable to suggest that autoxidation events must still occur to some extent. This may be particularly true for animals containing algal endosymbionts (a very common phenomenon among marine animals (*78–79*)). These animals require intense sunlight since they depend to varying extents on nutrients produced by photosynthesis in their endosymbiotic algae cells,

TABLE 3
Known Marine Sterol Side Chains[a,b]

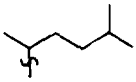
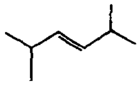
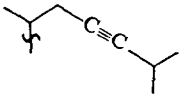
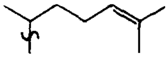
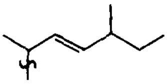
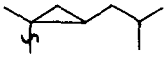
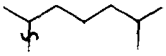
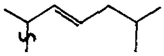
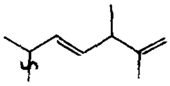
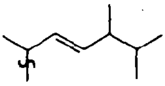
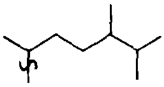
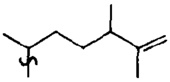
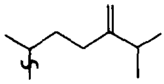
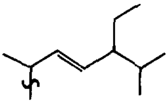
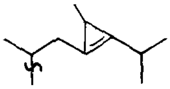
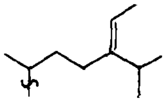
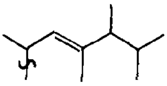
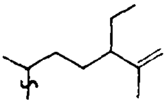
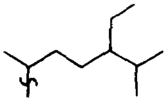
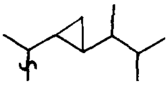
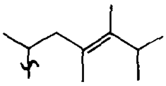
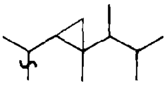
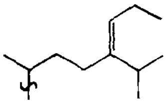
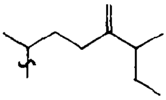
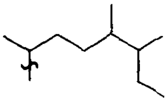
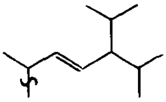
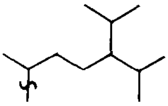
Major Constituent of Oceanic Sterol Pools	Widespread but Generally at Minor Concentrations	Limited occurrence
		B 
	A 	C 
G 	E 	D  [c]
H 	F 	I 
J 	M 	K 
L 	O 	N 
P 	R 	Q 
V 	U 	S 
X 	Y 	T 
		W 
		Z 

TABLE 3—*continued*

Major Constituents of Oceanic Sterol Pools	Widespread but Generally at Minor Concentrations	Limited occurrence
		Z′ 

[a] Side chains lettered in order of increasing molecular weight.
[b] Reactive unit used in *REACT* simulation: includes "D"-ring side chain
[c] Further studies are needed to confirm this structure.

particularly zooxanthellae. Oxygen is produced in animal tissues by the endosymbiotic algae. Indeed, considerable quantities of oxygen are actually excreted by gorgonians and coral which contain zooxanthellae (*80*). The endosymbiotic algae contain large quantities of chlorophyll, an excellent photosensitizer for production of singlet oxygen. Again it must be assumed that coral reef animals have evolved mechanisms to inhibit *in vivo* autoxidation;[8] however, as in higher animals (*77*) these protective mechanisms are probably imperfect.

The oceans are areas where natural autoxidation is expected to be an important process. One consideration is that in the oceans the ratio by mass of organic carbon in living organisms to dead particulate material (detritus) to dissolved compounds is approximately 1:10:100 (*81*). This tremendous quantity of dead organic material is directly susceptible to microbial degradation and autoxidation. The importance of this dead organic material to marine biochemical cycles and food webs was pointed out several years ago by Fox (*82*). Using conservative estimates, he calculated the total mass of suspended dead organic materials in the oceans to represent about 1.5×10^{12} tons of carbon—approximately 100 times the estimated annual productivity of land plants. The oceanic detritus is expected to contain considerable quantities of sterol material, since eukaryotic organisms generally contain 0.01 to 0.1% of their wet weights as sterols (*30*). It follows that as an upper limit the oceans contain about 1.5×10^9 tons of sterols not contained in living systems. Recent studies (*21*) suggest that the levels of oceanic sterols free from living organisms may in fact be much higher. One other important consideration in surface waters is singlet oxygen, which is known to react vigorously with unsaturated organics (*84–86*). Singlet oxygen is present at measurable levels in sea water and has been implicated in the autoxidation of organic compounds in natural waters (*87*).

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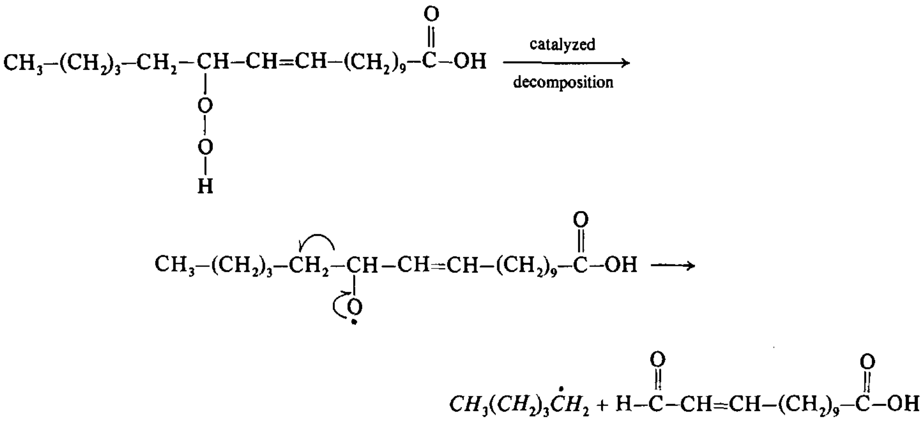
[8] In this regard it has been suggested (*83*) that the unusual 22,23—cyclopropane containing side chain structure of gorgosterol maintains the side chain in a configuration similar to that of sterols possessing a 22,23 double bond (for some as yet unknown physiological requirements), without being as readily autoxidized as the olefin. However, another explanation for the unusual structure of gorgosterol and its presence only in zooxanthellae-containing coelenterates is that the unusual side chain structure facilitates essential exchange of nutrients between endosymbiont and host by some specific modification of cell membrane permeability.

Studies have shown (*66*) that in the presence of metal ions and hydrogen donors fatty acid hydroperoxides decompose to yield measurable quantities of hydrocarbons. These processes are suggested to mimic *in vivo* hydrocarbon production by autoxidation and are represented below, where RH is an effective hydrogen donor and $M^{(n)+}$ is a metal ion of charge n:

1.



$$CH_3{-}(CH_2)_3{-}CH_2{-}\underset{\substack{|\\ O\\ |\\ O\\ |\\ H}}{CH}{-}CH{=}CH{-}(CH_2)_9{-}\overset{\overset{O}{\|}}{C}{-}OH \xrightarrow[\text{decomposition}]{\text{catalyzed}}$$

$$CH_3{-}(CH_2)_3{-}CH_2{-}\underset{\substack{|\\ \underset{\bullet}{O}}}{CH}{-}CH{=}CH{-}(CH_2)_9{-}\overset{\overset{O}{\|}}{C}{-}OH \longrightarrow$$

$$CH_3(CH_2)_3\dot{C}H_2 + H{-}\overset{\overset{O}{\|}}{C}{-}CH{=}CH{-}(CH_2)_9{-}\overset{\overset{O}{\|}}{C}{-}OH$$

2. $$CH_3(CH_2)_3\dot{C}H_2 + RH \longrightarrow CH_3(CH_2)_3CH_3 + R\cdot$$

or

3. $$CH_3(CH_2)_3\dot{C}H_2 + H^+ + M^{(n)+} \longrightarrow CH_3(CH_2)_3CH_3 + M^{(n+1)+}$$

This mechanism can, in principle, also explain the production of short side chain sterols from side chain hydroperoxides. Unlike the volatile hydrocarbon products of *in vivo* fatty acid autoxidation, short side chain sterols formed by *in vivo* autoxidative processes would be retained initially. It also seems reasonable that short side chain sterols may be much more difficult for an organism to excrete than the accompanying more polar autoxidation products, because of the basic chemical similarity of short side chain sterols to their functional longer side chain analogs.

The study of the mechanism of hydrocarbon autoxidation under mild conditions (*88–89*) and the mechanisms of reactions of unsaturated hydrocarbons involving singlet oxygen (*84–86*) have been important areas of research for many years. Much is now known about the fundamental mechanisms of hydrocarbon autoxidation (*88–92*). Since conventional marine sterol side chains are basically C_6–C_{11} hydrocarbons, it should be possible (as a first approximation) to consider sterol side chain autoxidation by applying established mechanisms of hydrocarbon autoxidation to the set of known marine sterol side chains. Considerations of this kind would help predict the most plausible short side chain sterols which may be generated by *in vivo* autoxidation of known marine sterol side chains. Since even basic sequence hydrocarbon autoxidation mechanism can be relatively complex (e.g., Figs. 1 and 2) and since there are a large number of known marine sterol side chains (Table 3), the REACT (*93*, *94*) computer program was employed in these evaluations. We now present the results of these simulations as a prelude to subsequent experimental studies of marine sterol autoxidation.[9]

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[9] Such studies are currently in progress in this laboratory.

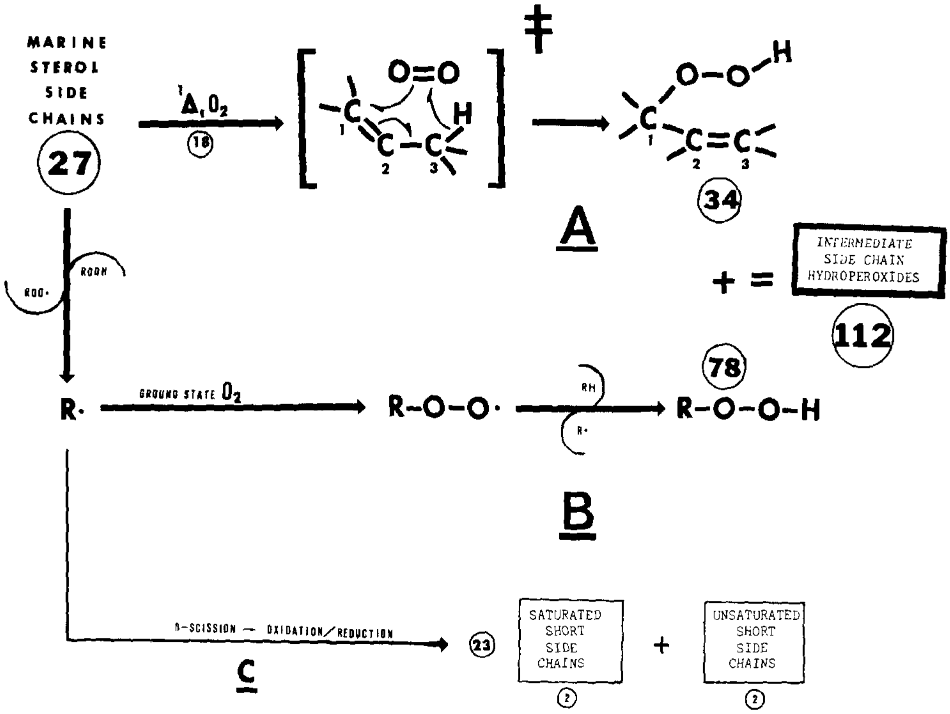

FIG. 1. Mechanisms and constraints used in REACT generation of marine sterol hydroperoxides. General: Low conversion, low-temperature processes with limited oxygen accessibility to generated radicals and in the presence or absence of oceanic or biologically important metal ions. Route *A* is singlet oxygen attack on isolated side chain double bond. The constraints are as follows: (i) C_3 must be a hydrogen-containing allylic center; (ii) if C_3 has only one bound hydrogen then search for other C_3 with two or three hydrogens available for abstraction; and (iii) oxygen attack predominently at most substituted original olefinic carbon. Route *B* is the most stable alkyl radical formation via hydrogen abstraction by selective radical, and subsequent hydroperoxide formation through the peroxy radical. The constraints are as follows: (i) vinylic, cyclopropyl, and primary radical formation not allowed; and (ii) intramolecular hydrogen abstraction by peroxy radical not allowed. Route *C* is a *β* scission of alkyl radical to form olefin and new alkyl radical. The constraints are as follows: (i) stability alkyl radical formed by scission that is greater than or equal to the stability of original alkyl radical; (ii) low yield secondary radical reaction is neglected; and (iii) effective hydrogen donor is available for quenching of side chain alkyl radicals to short alkane side chain or appropriate metal ions available to catalyze oxidation of reduction of degraded alkyl radicals to unsaturated or saturated short side chains. Note that the circled numbers are the numbers of species involved.

COMPUTER ANALYSIS

The REACT program (*93*, *94*) is written in the programming language INTERLISP. The program was initially developed as an extension to the CONGEN program (*95*) for computer-assisted structure elucidation. REACT is designed to carry out representations of chemical reactions (in this case a generalized autoxidative process, Figs. 1 and 2) on representations of chemical structure (in this case all known marine sterol side chains—Table 3). The program can be used effectively in following reactions of a single known compound or series of known compounds participating in a complex sequence

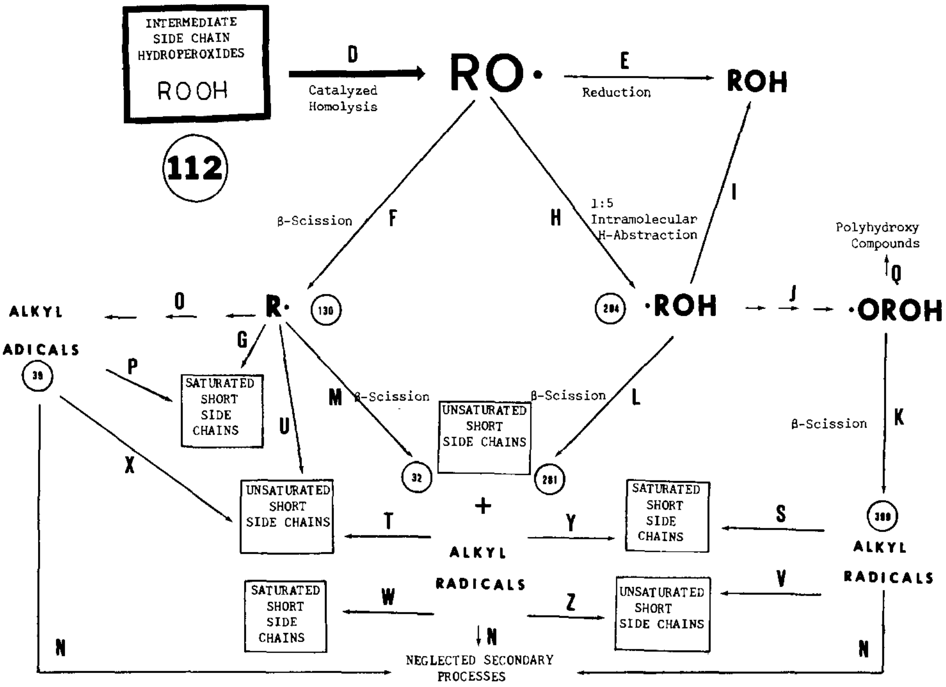

FIG. 2. Scheme for REACT decomposition of marine side chain hydroperoxides to alkoxy radical and subsequent saturated and unsaturated short side chain, polar and low molecular weight products. Note that the circled numbers are the total number of products for the specified reaction. The general assumed conditions are the same as in Fig. 1. Route *D* is alkoxy radical formation by homolysis of hydroperoxide O–O bond. Routes *E*, *I*, and *Q* are highly favored alkoxy radical quenching to the stable alcohol. Routes *F* and *K* are *β* scission of the alkoxy radical. The constraint is scission to form the most stable alkyl radical. Routes *G*, *S*, *P*, *W*, and *Y* are hydrogen abstraction by alkyl radical or metal ion-catalyzed reduction of alkyl radicals to form saturated short side chain sterols, i.e., each route represents two mechanistically separate processes which yield the same products. Routes *L* and *M* are the same as Route *C* in Fig. 1. Route *H* is a 1 : 5 intramolecular hydrogen abstraction by alkoxy radical. Route *J* is equivalent to reaction sequence BDF, and Route *O* = BDF. Route *N* represents neglected, presumably low-yield processes. Routes *T*, *U*, *V*, *X*, and *Z* represent alkyl radical oxidation to unsaturated short side chains. Processes, *F*, *K*, *L*, and *O* produce additional oxygenated products and low molecular weight side chain fragment products which are not specified, in addition to short side chain generating alkyl radicals. However, circled numbers do represent the total numbers of products involved.

of reactions. REACT runs on a Digital Equipment Corp. PDP-10 at the SUMEX computer facility, Stanford University. The program is available (to the limit of available resources) on line to interested persons over two nationwide computer networks. The authors should be contacted for information on access to the program.

Marine Sterol Side Chains

Marine sterols possessing Δ^5, $\Delta^{5,7}$, 5α-Δ^7, 5α-saturated, 5β-saturated, 19-nor-Δ^5, 5α-19-nor-saturated, 5α-$\Delta^{8(9)}$, and various C-4 methyl-containing nuclear systems are known to exist (*1–3*), and still other nuclear systems are suspected to exist but are not fully characterized (*96*). However, the real diversity of marine sterols results from the large number of uniquely marine side chain structures. We are primarily concerned with autoxidative attack on the side chain and will therefore neglect attack on the nuclear system. The well-known products of autoxidation of the most common nucleus (Δ^5) have been reviewed (*73–75*). It is perhaps pertinent to note that nuclear autoxidative attack appears to be inhibited by esterification at C-3 (*73*, *86*), although it is not known if esterification then directs autoxidative attack to the side chain. In this regard it may be important to note that the short side chain sterol **VI** was found only in the sterol ester fraction of *P. porosa.*

The known marine sterol side chain structures are presented in Table 3. Both the 24*R* and 24*S* configurations are known for side chains *J*, *M*, *O*, and *V* (*1–3*). At present only the 24*R* configuration is known for structures *R*, *U*, *W*, and *X*, and only 24*S* is known for structures *E*, *K*, and *Q* (*1–3*). The configuration at C-24 is presently unknown for *I*. Both the 24*E* and 24*Z* isomers are known for *P* (*1–3*, *20*, *39*) and *Y* (*87*), and suspected for *A* (*97*), *F* (*98*), and *J* (*99*) (although there is some controversy concerning this point (*5*). The REACT program which was used to predict the products formed from each marine sterol side chain as it is carried through the proposed autoxidation sequence (Figs. 1 and 2), cannot presently deal with stereochemical isomers. Therefore, the side chain configurations specified above are not depicted in Table 3.

The relative abundance of each side chain in terms of the estimated quantity (mass) of sterols possessing that side chain in the oceans is also given in Table 3. The estimates are based on the known sterol compositions of the various kingdoms of marine organisms and the estimated contributions of those organisms to the oceanic biomass.[10] Side chain *H* occurs as the major side chain of most Metazoa (multicellular animals) and most red algae. Side chain *G* (the direct biosynthetic precursor of *H*) is common in marine arthropods and many species of red algae. Side chain *J* occurs in diatoms (a big contributor to the oceanic biomass) and in many other marine plants and animals. Side chain *P* as the *24E* isomer is the major side chain of nearly all brown algae (the major algae of tidal areas and coastal waters (*101*)), and the *24Z* isomer occurs widely as a minor constituent of many marine invertebrates and some green algae. Side chain *V* occurs widely in many marine plants, particularly the sea grasses, which form immense beds in some areas (notably the Caribbean (*101*)). Side chain *X* occurs widely and at high concentrations in coral reef coelenterates which contain zooxanthellae. Side chain *L* also occurs at high levels in some coral reef coelenterates and many temperate water filter-feeding organisms. The importance of coral reefs to the oceanic primary production has recently been discussed by Tursch (*102*). The sterol side chains listed in the middle column of Table 3 occur widely but at minor levels in plankton, filter, and suspension-feeding animals, and in some of the organisms mentioned above. The marine sterol side chains listed in the final column of Table 3 have been reported in only a single family or species of marine organism (generally within the phylum Porifera). Consideration of the actual quantity of each specific side chain in sterols in the marine environment is important with respect to the possible quantities of the short side chain sterols they might generate (in marine food webs). Such considerations may help distinguish between dietary vs direct *in vivo* autoxidative origins for short side chain sterols found in any specific organism.

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[10] A detailed discussion of marine sterol phylogenetic and ecological distributions can be found in Ref. (*100*).

Autoxidative Sequence

The generalized scheme for sterol side chain autoxidative degradation (Figs. 1 and 2) follows the sequences proposed for low-temperature, low conversion, restricted oxygen hydrocarbon autoxidations in the presence (*103*, *104*) or absence (*89*, *105*, *106*) of catalytic quantities of biologically important metal ions; however, our computer program considers only those processes which generate degraded alkyl radicals and disregards processes which lead exclusively to oxygen-containing products. The program considers those routes which lead to the most chemically reasonable degraded alkyl radicals. Saturated short side chain sterol formation by quenching of these alkyl radicals through abstraction of a hydrogen atom from an efficient donor or by a reduction involving a naturally occurring metal ion is considered. Unsaturated short side-chain sterol formation form degraded alkyl radicals by an oxidation involving a naturally occurring metal ion or by β scission of the alkyl radicals is also considered. In the presence of even small quantities of oxygen, the formation of peroxy radicals would appear to be greatly favored; however, there is now considerable precedent for hydrocarbon production during lipid autoxidations (*67*, *107*, *108*), notably *in vivo* autoxidations (*66*, *109*, *110*), and the role of oxygen diffusivity in such processes may be an important consideration (*111*).

The 27 marine sterol side chains (Table 3) are considered to undergo two possible initial attack processes (Fig. 1, routes A and B), both of which lead to intermediate hydroperoxides. Route A involves attack of singlet oxygen on the side chain double bond system; therefore, only the 18 side chains (Table 3) possessing an unsaturation can participate in this route. The attack process is considered to proceed via the six-membered cyclic "ene" mechanism (*86*) resulting in a concomitant double bond shift and abstraction of an allylic hydrogen by the oxygen, resulting in hydroperoxide formation at the most substituted original olefinic carbon. The well-known stereochemical requirements of this reaction (*84–86*) are considered by REACT by favoring the abstraction of a hydrogen on an allylic carbon possessing two or three hydrogens. Route B involves attack of a selective radical initiator (such as a peroxy radical (*112*)) on all 27 marine side chains. Radical–double bond additions to the 18 unsaturated side chains are not considered for reasons discussed earlier. The process is envisioned to involve abstraction of a hydrogen atom to form the most stable alkyl radical. The alkyl radical reacts with ground state molecular oxygen to form a peroxy radical (intermolecular hydrogen abstraction by the alkyl radical, allowed in later steps, simply regenerates the starting side chain). Intramolecular hydrogen abstraction by peroxy radicals predominantly via 1:4 or 1:5 isomerizations have been reported (*106*); but at the temperatures, and under the conditions considered it seems reasonable to neglect isomerization of this relatively selective radical (*112*) in favor of direct hydroperoxide formation by intermolecular hydrogen abstraction.

The initial attack results in the formation of 34 hydroperoxides by Route A (singlet O_2) and 78 hydroperoxides by Route B (radical process). Catalyzed homolytic scission of the weakest bond (O–O) results (Fig. 2, Route D) in the formation of equal numbers of alkoxy radicals. Under the conditions we are considering, particularly in the presence of common cellular functionalities and metal complexes (*113*), it seems reasonable that formation of alkoxy radicals would be a major mechanism of hydroperoxide decomposition. The alkoxy radicals can undergo several reactions (*114*, *115*). First, because we are allowing a ready supply of easily abstractable hydrogen atoms, the predominant reaction must be formation of the alcohol (Route E). This route clearly terminates in stable hydroxyl-containing side chains and therefore is not considered further. However, these considerations do indicate that hydroxylated side chains should be the major product of the side chain autoxidation processes. Hydroxylated side chains are in fact found in these extracts (*38*), although apparently not at the high relative concentrations expected. The higher polarity of the side chain hydroxylated species, coupled with the demonstration that these compounds are toxic (*116*, *117*), could possibly account for a more rapid differential removal of these hydroxylated side chain-containing compounds relative to the short hydrocarbon side chain-containing compounds. The second competing reaction expected for the alkoxy radicals is the well-known β scission (Route F) process, resulting in formation of an alkyl radical (R•) and a stable ketone or aldehyde. The alkyl radical forms a stable short side chain sterol by either intermolecular hydrogen abstraction or by a reduction involving a naturally occurring metal ion (both processes represented by Route G). The alkyl radicals formed by Route F could also react with oxygen to form a peroxy radical which could react in the same manner discussed above; however, the steady state concentration of these alkyl radicals is very low, and therefore the concentrations of peroxy radicals formed in a second reaction step are presumably negligible, as would be the concentrations of any short side chain sterols formed by those processes.

It is well known that the very reactive alkoxy radical can undergo 1:5 and to a much lesser extent 1:4 intramolecular hydrogen abstraction to yield a hydroxyl-containing alkyl radical (Route H) (*114*, *118*). These alkyl radicals can abstract a hydrogen atom (Route I) to become a hydroxyl-containing side chain (same products as Route E), or react with oxygen to form a peroxy radical which goes through the reaction sequences similar to those just described to yield short side chain sterols as one of the products (Routes J → K → S).

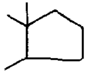
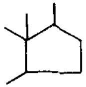
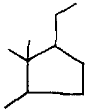
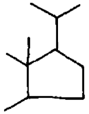
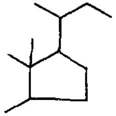
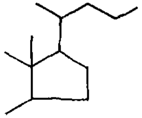
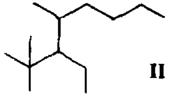
In addition to alkyl radical reductions which lead to saturated short side chains (Routes G, P, S, W, and Y) alkyl radical oxidations involving metal ions which lead to unsaturated short side chains are considered (Routes T, U, V, X, and Z). Also, as an alternate mechanism of unsaturated short side chain formation, the products of the energetically less favorable β scission of intermediate alkyl radical (Routes C, L, and M) were considered. Precedent does exist (*119*) for low-temperature β scission of alkyl radicals, notably in depolymerization reactions. The β scission of intermediate alkyl radicals to form "shorter" alkyl radicals and olefins is energetically less favorable than the previously described processes in solution. However, in living tissues, when parent alkyl radicals are still in membrane bilayers, the energetics of such a cleavage will probably change, possibly allowing the reaction to occur to some extent. The short side chain sterols predicted by REACT are considered in the next section.

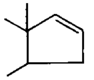
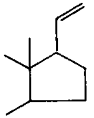
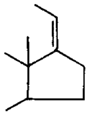
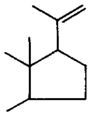
Simulation Results

The pertinent short side chain products predicted for the action of the autoxidation sequences (Figs. 1 and 2) upon the marine sterol side chains (Table 3) are presented in Table 4. Every saturated short side chain can in principle be accounted for by a primary β scission of the unisomerized alkoxy radical. The numbers of processes which can generate each side chain by both primary β scission and β scission following

TABLE 4

REACT Results: Possible Degradative Mechanisms[a] Applicable to Parent Marine Sterol Side Chains (A–Z′)[b]

Short side chain products		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Z′	Total number of processes
	**XII**	2 7	2 4		6 8 10	2 7	2 7	3 6	2 6	2 7	2 7	4	5 6[c]	2 4 6	4 11	2 7	3 6[c]	4	2	3 5 9 11	5 6[c]	2 4[c] 6 8 10	2 4 6	2 4 6	2 4 8	3 6[c]	2 7	2 4 6	65
		Not a *REACT* product *and* not found in marine extracts																											0
	**VIII**	1	10			1	1	11	4	1	1	5 10	3 4 11	4 10		1	4 11	5 10		7	3 4 11	2 8	4 10	4 10	2	4 11	1	4	35
	**VI**		8						10			3 8		8				3 8		1			8	8					10
	**V**		2						8			2 5		2 8				2					2 8	2 8				8	23
	**IV**								2																				1
	**II**													2										2					2

	**XV**	5 13	13 15	8	5 7	5 13 7	14	13	5 7 13	5 7 13	15		13 15	6 15	5 7 13	14	15	5 13	5 14		6 8 13 15	13 15	13 15	6 8 13 15	14	5 7 13	13 15	39/12[d]
	**IX**	12			12	12	6	15	12	12		6 14 15	15		12	6 15			5	6 14 15	13 8	15	15	13	6 15	12	15	21/6[d]
	**X**	12			12	12		15	12	12		14 15	15		12	15				14 15	13 8	15	15	13	15	12	15	21/0[d]
	**VII**		8	10[c]							14 8		8				14 8		12			8	8					9/2[d]

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[a] For brevity the arabic numerals 1 to 15 correspond to the following route sequences depicted in Figs. 1 and 2: 1 = ADFG; 2 = BDFG; 3 = ADHJKS; 4 = DBHJKS; 5 = ADHL; 6 = BDHL; 7 = ADFO; 8 = BDFO; 9 = ADFM; 10 = BDFM; 11 = C; 12 = ADFU; 13 = BDFU; 14 = ADHJKV; and 15 = DBHJKV. Processes 5 and 6 include products of route L and also subsequent routes Y and Z, and processes 9 and 10 include products of route M and also subsequent routes I and W. Note: Several routes actually represent two separate processes, as indicated in the legend to Fig. 2.

[b] For structures see Table 3.

[c] Side chain generated twice by this process.

[d] Number on left of slash represents the total number of alkyl radical oxidation processes producing the side chain and the number on the right is total number of alkyl radical β-scission processes producing the side chain.

isomerization of the alkoxy radical suggests a possible reason for the prevalence and relatively high concentrations of side chain (**XII**) (formed by 65 possible processes) and (**VIII**) (formed by 35 possible processes) in marine extracts. It is perhaps significant that C_{20} sterols, which did not cocur in any of the six marine organisms examined, also never occurred as products in the REACT simulation autoxidations, and that the C_{24} and C_{25} sterols which occurred at extremely low levels in the extracts also had only a few modes of production in the REACT analysis.

Sterol **X** found in *P. porosa* (Table 2) is not formed in the REACT simulation involving β scission of alkyl radicals, but is formed by REACT simulations involving alkyl radical oxidation. Also, sterol **VII**, which was found in each species of sponge, can only be generated by the alkyl radical β scission routes from marine sterol side chain *D* (Table 3), which is found in only a single sterol having very limited distributions and is not detected in any of the sponges. However, **VII** can be formed by 9 processes involving 7 reasonably abundant parent side chains by the alternate catalyzed alkyl radical oxidation process. The C_{21} sterol, **IX**, which is present at relatively high levels in *M. californica*, is only generated from one of the nine parent sterols present in *M. californica* by the alkyl radical β scission routes, and this parent sterol occurs at relatively minor levels (Table 2). The same side chain, **IX**, is generated from all nine *M. californica* major sterols by the catalyzed alkyl radical oxidation mechanism. Therefore, formation of unsaturated short side chain sterols from intermediate alkyl radicals is more readily explained by metal ion-catalyzed oxidations than by spontaneous β scission of alkyl radicals. The higher levels of saturated short side chain sterols relative to unsaturated short side chain sterols in the marine extracts (with the exception of *M. californica*) may reflect additional modes of alkyl radical reduction relative to oxidation or may reflect the availability of appropriate metal ions in suitable oxidation states for unsaturated short side chain production. Various marine organisms have been shown to contain many common and unusual metals which show wide variations in tissue concentration (*120*).

Comparison of Table 2 with Table 4 shows that autoxidation (Figs. 1 and 2) of the major sterols present in each species of marine invertebrate studied can, in principle, explain the general occurrences and relative concentrations of the short side chain sterol distributions. However, several unexplained observations exist e.g., the unexpectedly high concentrations of **V** and **IX** in *M. californica* and the absence of **VIII** and low relative concentration of **XII** in that organism as well as the apparently anomalous relative concentrations of saturated and unsaturated short side chains in the various organisms. Whether these unusual distributions indicate that certain short side chain sterols arise by some other autoxidative mechanism or have some entirely different origin is a matter awaiting experimental investigation, as is confirmation of the basic suggestion of an *in vivo* autoxidative origin of the saturated short side chain sterols.

SUMMARY AND CONCLUSIONS

Sterols with hydrocarbon side chains ranging from zero to six carbon atoms occur at minor concentrations in marine Coelenterata and Porifera. The side chains are apparently too short for the sterols to be functional membrane constituents.

Biosynthesis of these sterols by terpene cyclizations would require the unlikely and unprecedented existence of an entire series of nonsqualene, nonpolyprenoid precursors. A biological degradative origin must also be considered unlikely since products of such processes lead to highly functionalized side chains. Formation of nonfunctional sterols by dealkylation mechanisms is also untenable, and therefore an autoxidative origin for these compounds was suggested. However, careful studies with *M. californica* demonstrated that these compounds occur in the tissue of that organism and are not artifacts produced by sample handling or laboratory work-up. These findings suggested an *in vivo* autoxidative or dietary origin for the short side chain sterols.

It was noted that hydrocarbon production in mammals has been correlated with *in vivo* autoxidation of unsaturated fatty acids (*109*, *110*). We suggested that similar reactions could give rise to short side chain sterols from the *in vivo* autoxidation of membrane sterols. In this regard, factors unique to the marine environment which would promote *in vivo* or environmental autoxidation processes were discussed.

In vivo autoxidation is implicated in the normal aging processes of organisms (*121*) and in various pathological conditions (*76*). It should be mentioned that autoxidation processes may be more important in the aging processes of invertebrates than in mammals, since biological antioxidants have been shown to increase significantly the life spans of certain invertebrates (*122*), but have no effect on the life spans of mammals (*123*). A considerable quantity of evidence (*124*) supporting *in vivo* autoxidative damage to lipid membranes does exist. The *in vivo* autoxidation of cholesterol in the B-ring has been implicated in various hemolytic diseases in humans (*125*). Autoxidative attack on the cholesterol side chain is expected to be minimal relative to B-ring attack, since cholesterol has no side chain unsaturations which would facilitate side chain autoxidative attack (*126*). However, it is important to note that marine invertebrates often contain a large percentage of sterols with unsaturated side chains. Finally, it is reasonable to suggest that accumulation of autoxidation products may be more prevalent in marine invertebrates than in mammals, since many marine organisms are known to store excretion products (*127*).

As a prelude to further experimental studies, we attempted to evaluate the possible autoxidative origins of the short side chain sterols by generating the autoxidation products of all known marine sterol side chains using expected autoxidation mechanisms. We employed the REACT computer program in those evaluations because of the large number of reactants, products, and processes involved. Results of the REACT analysis lend support to the suggested direct autoxidative origin for the marine short side chain sterols. Further experiments are now in progress in this laboratory to help clarify the origin of the marine short side chain sterols. If these sterols are shown to arise by *in vivo* or environmental autoxidation in the oceans, it will have important implications in marine biochemistry and ecology.

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REFERENCES

1. F. J. SCHMITZ, "Marine Natural Products" (P. J. Scheuer, Ed.), Vol. 1, pp. 241–297. Academic Press, New York, 1978.
2. L. J. GOAD, "Biochemical and Biophysical Perspectives in Marine Biology" (D. C. Malins and J. R. Sargent, Eds.), Vol. 3, pp. 213–318. Academic Press, London, 1976.
3. J. T. BAKER AND V. MURPHY, "Handbook of Marine Science. Compounds from Marine Organisms," Vol. 1, CRC Press, Cleveland, Ohio, 1976.
4. C. FRANCISCO, G. COMBAUT, J. TESTE, AND B. F. MAUME, *Biochim. Biophys. Acta* **487**, 115 (1977).
5. M. KOBAYASHI AND H. MITSUHASHI, *Steroids* **26**, 605 (1975).
6. S. POPOV, R. M. K. CARLSON, A. WEGMANN, AND C. DJERASSI, *Tetrahedron Lett.* 3491 (1976).
7. R. L. HALE, J. LECLERQ, B. TURSCH, C. DJERASSI, R. A. J. GROSS, A. J. WEINHEIMER, K. C. GUPTA, AND P. J. SCHEUER, *J. Amer. Chem. Soc.* **92**, 2179 (1970).
8. N. C. LING, R. L. HALE, AND C. DJERASSI, *J. Amer. Chem. Soc.* **92**, 5281 (1970).
9. Y. M. SHEIKH, C. DJERASSI, AND B. TURSCH, *Chem. Comm.*, 217 (1971).
10. S. POPOV, R. M. K. CARLSON, A. WEGMANN, AND C. DJERASSI, *Steroids* **28**, 699 (1976).
11. T. TAMURA, B. TRUSCOTT, AND D. R. IDLER, *J. Fish. Res. Bd. Canad.* **21**, 1519 (1964).
12. D. R. IDLER, P. M. WISEMAN, AND L. M. SAFE, *Steroids* **16**, 451 (1970).
13. T. R. ERDMANN AND R. H. THOMSON, *Tetrahedron* **28**, 5163 (1972).
14. M. KOBAYASHI, R. TSURU, K. TODO, AND H. MITSUHASHI, *Tetrahedron* **29**, 1193, (1973).
15. D. R. IDLER, M. W. KHALIL, J. E. GILBERT, AND C. J. W. BROOKS, *Steroids* **27**, 155 (1976).
16. C. DELSETH, L. TOLELA, P. J. SCHEUER, R. J. WELLS, AND C. DJERASSI, *Helv. Chim. Acta*, submitted for publication.
17. M. DE ROSA, L. MINALE, AND G. SODANO, *Comp. Biochem. Physiol.* **46B**, 823 (1973).
18. D. R. IDLER AND P. WISEMAN, *J. Fish. Res. Bd. Canad.* **29**, 385 (1972).
19. S. YASUDA, *Comp. Biochem. Physiol.* **50B**, 399 (1975).
20. S. YASUDA, *J. Sci. Hiroshima Univ., Ser. A.* **39**, 131 (1975).
21. R. GAGOSIAN, *Limnol. Oceanogr.* **21**, 702 (1976).
22. W. R. NES, "Advances in Lipid Research" (R. Paoletti and D. Kritchevsky, Eds.), Vol. 15. Academic Press, New York, 1977.
23. M. FRYBERG, A. C. OEHLSCHLAGER, AND A. M. UNRAU, *Chem. Commun.*, 1194 (1971).
24. C. DJERASSI, R. M. K. CARLSON, S. POPOV, AND T. H. VARKONY, "Marine Natural Products Chemistry" (D. J. Faulkner and W. H. Fenical, Eds.), pp. 111–123. Plenum, New York, 1977.
25. J. A. BALLANTINE, K. WILLIAMS, AND B. A. BURKE, *Tetrahedron Lett.*, 1547 (1977).
26. H. D. HIGGS AND D. J. FAULKNER, *Steroids* **30**, 379 (1977).
27. J. F. KINGSTON, B. GREGORY, AND A. G. FALLIS, *Tetrahedron Lett.*, 4261 (1977).
28. A. KANAZAWA AND S. TESHIMA, *Bull. Japan. Soc. Sci. Fish.* **37**, 675 (1971).
29. K. BLOCH, "Reflections on Biochemistry" (A. Kornberg, B. L. Horecker, L. Cornudella, and J. Oro, Eds.), pp. 143–150. Pergamon, New York, 1976.
30. W. R. NES, *Lipids* **9**, 596 (1974).
31. B. DE KRUYFF, "Lipids" (R. Paloetti, G. Porcellati, and G. Jacini, Eds.), Vol. 1, pp. 169–179. Raven, New York, 1976.
32. R. A. DEMEL AND B. DE KRUYFF, *Biochim. Biophys. Acta* **457**, 109 (1976).
33. C. GREEN, "Biochemistry of Lipids" (T. W. Goodwin, Ed.), Vol. 14, pp. 101–152. University Park Press, Baltimore, Md., 1977.
34. E. HEFTMANN, "Steroid Biochemistry," Chap. 12, Academic Press, New York, 1970.
35. K. E. SUCKLING AND G. S. BOYD, *Biochim. Biophys. Acta* **436**, 295 (1976).
36. R. W. STEVENS AND G. GREEN, *FEBS Lett.* **27**, 145 (1972).

37. C. Litchfield, A. J. Greenberg, G. Noto, and R. W. Morales, *Lipids* **11**, 567 (1976).
38. C. Delseth, R. M. K. Carlson, C. Djerassi, T. R. Erdman, and P. J. Scheuer, *Helv. Chim. Acta* **61**, 1470 (1978).
39. T. R. Erdman and P. J. Scheuer, *Lloydia* **38**, 359 (1975).
40. E. G. Bligh and W. J. Dyer, *Canad. J. Biochem. Physiol.* **37**, 911 (1959).
41. A. Windaus, *Chem. Ber.* **42**, 238 (1909).
42. R. Schoenheimer and H. Dan, *Z. Physiol. Chem.* **215**, 59 (1933).
43. S. Popov, R. M. K. Carlson, and C. Djerassi, in preparation.
44. A. M. Krubiner, N. Gottfried, and E. P. Oliveto, *J. Org. Chem.* **34**, 3502 (1967).
45. A. Tait, *Biochem. J.* **128**, 467 (1972).
46. J. E. van Lier and L. L. Smith, *J. Org. Chem.*.**36**, 1007 (1971).
47. J. E. van Lier and L. L. Smith, *J. Org. Chem.* **35**, 2627 (1970).
48. H. de Vries and H. J. Backer, *Rec. Trav. Chim.* **69**, 759 (1950).
49. W. G. Salmond and M. C. Sobala, *Tetrahedron Lett.*, 1695 (1977).
50. J. P. Dusza and W. Bergmann, *J. Org. Chem.* **25**, 79 (1960).
51. H. McKennis Jr., *J. Biol. Chem.* **172**, 313 (1948).
52. J. P. Schmit, M. Piraux, and J. F. Pilette, *J. Org. Chem.* **40**, 1586 (1975).
53. C. Djerassi, *Pure Appl. Chem.* **21**, 205 (1970).
54. H. Budzikiewicz, "Biochemical Applications of Mass Spectrometry" (G. Waller, Ed.), pp. 251–289. Wiley–Interscience, New York, 1972.
55. Z. V. Zaretskii, "Mass Spectrometry of Steroids," Israel Universities Press, Jerusalem, 1976.
56. S. G. Wyllie, B. A. Amos, and L. Tokes, *J. Org. Chem.* **42**, 725 (1977).
57. L. G. Partridge and C. Djerassi, *J. Org. Chem.* **42**, 2799 (1977).
58. G. Patterson, *Anal. Chem.* **43**, 1165 (1971).
59. B. A. Knights, "Modern Methods of Steroid Analysis" (E. Heftmann, Ed.), Chap. 5. Academic Press, New York, 1973.
60. T. Itoh, T. Tamura, T. Lida, and T. Matsumoto, *Steroids* **23**, 681 (1973).
61. T. Itoh, T. Tamura, T. Lida, and T. Matsumoto, *Steroids* **26**, 93 (1975).
62. T. Itoh, T. Tamura, T. Lida, and T. Matsumoto, *Steroids* **27**, 729 (1975).
63. H. Budzikiewicz and W. Ockels, *Tetrahedron* **32**, 143 (1976).
64. C. K. A. Martin, "Advances in Applied Microbiology" (D. Perlman, Ed.), Vol. 22. Academic Press, New York, 1977.
65. J. E. van Lier, J. Rousseau, R. Langlois, and G. J. Fisher, *Biochim. Biophys. Acta* **487**, 395 (1977).
66. C. A. Riely, G. Cohen, and M. Lieberman, *Science* **183**, 208 (1974).
67. E. E. Dumelin and A. L. Tappel, *Lipids* **12**, 894 (1977).
68. J. E. van Lier and L. L. Smith, *Steroids* **15**, 485 (1970).
69. J. E. van Lier and L. L. Smith, *J. Org. Chem.* **35**, 2627 (1970).
70. J. E. van Lier, A. L. Da Costa, and L. L. Smith, *Chem. Phys. Lipids* **14**, 327 (1975).
71. L. L. Smith, W. S. Matthews, J. C. Price, R. C. Bachmann, and B. Reynolds, *J. Chromatogr.* **27**, 187 (1967).
72. A. L. J. Beckwith, *Proc. Chem. Soc.*, 194 (1958).
73. S. Bergstrom and B. Samuelson, "Autoxidation and Antioxidants" (W. O. Lundberg, Ed.), Vol. 1, pp. 233–248. Interscience, New York, 1961.
74. M. J. Kulig and L. L. Smith, *J. Org. Chem.* **38**, 3639 (1973).
75. L. L. Smith, J. I. Teng, M. J. Kulig, and F. L. Hill, *J. Org. Chem.* **38**, 1763 (1973).
76. G. L. Plaa and H. Witschi, *Annu. Rev. Pharmacol.* **16**, 125 (1976).
77. W. A. Pryor, "Free Radicals in Biology" (W. A. Pryor, Ed.), Vol. 1, pp. 1–43. Academic Press, New York, 1976.
78. P. Buchner, "Endosymbiosis of Animals with Plant Microorganisms," Interscience, New York, 1965.
79. D. C. Smith, "Symbiosis of Algae with Invertebrates," Oxford Univ. Press, London, 1973.
80. J. W. Kanwisher and S. A. Wainwright, *Biol. Bull.* **133**, 378 (1967).
81. T. Wyatt, "Ecology of the Seas" (D. H. Cushing and J. J. Walsh, Eds.). W. B. Saunders, Philadelphia, Pa., 1976.

82. D. L. Fox, *Ann. N.Y. Acad. Sci.* **90**, 617 (1960).
83. C. A. Hetzel, T. Chang, and E. Buhle, "Identification of Some Sterols in a Gorgonian Coral," Abstr. 22nd Annual Conference on Mass Spectrometry and Allied Topics, Philadelphia, pp. 206–209, 1974.
84. D. R. Kearns, *Chem. Rev.* **71**, 395 (1971).
85. C. S. Foote, *Acc. Chem. Res.* **1**, 104 (1968).
86. K. Gollnick, "Advances in Photochemistry" (W. A. Noyes, Jr., G. S. Hammond, and J. N. Pitts, Jr., Eds.). Interscience, New York, 1968.
87. R. G. Zepp, N. L. Wolfe, G. L. Baughman, and R. C. Hollis, *Nature* (*London*) **267**, 421 (1977).
88. F. R. Mayo, *Acc. Chem. Res.* **1**, 193 (1968).
89. A. Fish, "Organic Peroxides" (D. Swern, Ed.), pp. 141–198. Wiley–Interscience, New York, 1970.
90. E. G. E. Hawkins, "Organic Chemistry" (W. A. Waters, Ed.), Series 2, Vol. 1, pp. 169–210. Butterworths, Boston, 1975.
91. E. G. E. Hawkins, "Organic Chemistry" (W. A. Waters, Ed.), Series 1, Vol. 10, pp. 95–138. Butterworths, Boston, 1973.
92. J. A. Howard, "Free Radicals" (J. K. Kochi, Ed.), Vol. 2, pp. 3–62. Wiley–Interscience, New York, 1973.
93. T. H. Varkony, D. H. Smith, and C. Djerassi, *Tetrahedron* **34**, 841 (1978).
94. T. H. Varkony, R. E. Carhart, and D. H. Smith, "Computer-Assisted Organic Synthesis" (W. T. Wipke, Ed.). Pp. 188–216. American Chemical Society, Washington, D.C. (1977).
95. R. E. Carhart, D. H. Smith, H. Brown, and C. Djerassi, *J. Amer. Chem. Soc.* **97**, 5755 (1975).
96. P. A. Voogt, *Neth. J. Zool.* **26**, 84 (1976).
97. D. R. Idler and P. Wiseman, *Int. J. Biochem.* **2**, 516 (1971).
98. D. R. Idler and P. Wiseman, *Comp. Biochem. Physiol.* **38**, 581 (1971).
99. S. Teshima, A. Kanazawa, and T. Ando, *Comp. Biochem. Physiol.* **47B**, 507 (1974).
100. R. M. K. Carlson, Ph.D. Thesis, Stanford University (1977).
101. E. Y. Dawson, "Marine Botany," Holt, Rinehart, & Winston, New York, 1966.
102. B. Tursch, *Pure Appl. Chem.* **48**, 1 (1976).
103. H. E. De La Mare, J. K. Kochi and F. F. Rust, *J. Amer. Chem. Soc.* **85**, 1437 (1963).
104. G. Sosnovsky and D. J. Rawlinson, "Organic Peroxides" (D. Swern, Ed.), Vol. 2, pp. 153–268. Wiley–Interscience, New York, 1970.
105. S. W. Benson, "Oxidation of Organic Compounds" (R. F. Gould, Ed.), Vol. 2, Amer. Chem. Soc., Washington, D.C., 1968.
106. D. M. Brown and A. Fish, *Proc. R. Soc. Ser. A.* **308**, 547 (1969).
107. R. J. Horvat, W. G. Lane, H. Ng, and A. D. Shepherd, *Nature* (*London*) **203**, 524 (1964).
108. C. D. Evans, G. R. List, A. Dolev, D. G. McConnell, and R. L. Hoffman, *Lipids* **2**, 432 (1967).
109. C. J. Dillard, E. E. Dumelin, and A. L. Tappel, *Lipids* **12**, 109 (1976).
110. D. G. Hafeman and W. G. Hoekstra, *J. Nutr.* **107**, 666 (1977).
111. L. Hotez, J. W. Dailey, G. W. Geelhoed, and J. L. Gainer, *Experientia* **33**, 1424 (1977).
112. S. W. Benson, "Thermochemical Kinetics," Wiley, New York, 1968.
113. P. J. O'Brien, *Canad. J. Biochem.* **47**, 485 (1969).
114. G. A. Russell, "Free Radicals" (J. K. Kochi, Ed.), Vol. 1, pp. 275–332. Wiley, New York, 1973.
115. B. Acott and A. L. J. Beckwith, *Austral. J. Chem.* **17**, 1342 (1964).
116. J. D. B. MacDougall, S. Biswas, and R. P. Cook, *Brit. J. Exp. Pathol.* **46**, 549 (1965).
117. H. W. Chen, A. A. Kandutsch, and C. Waymouth, *Nature* (*London*) **251**, 420 (1974).
118. R. H. Hesse, "Advances in Free-Radical Chemistry" (G. H. Williams, Ed.), Vol. 2, pp. 83–138. Academic Press, New York, 1969.
119. C. Walling, "Free Radicals in Solution," pp. 179–238. Wiley, New York, 1957.
120. A. P. Vinogradov, "The Elementary Chemical Composition of Marine Organisms," Sears Foundation, New Haven, Conn., 1953.
121. C. J. Dillard and A. L. Tappel, *Lipids* **6**, 715 (1971).
122. L. Epstein and D. Gershon, *Mech. Ageing Develop.* **1**, 257 (1972).
123. D. Harman, *J. Gerontol.* **23**, 476 (1968).
124. J. F. Mead, "Free Radicals in Biology" (W. A. Pryor, Ed.), Vol. 1, pp. 51–68. Academic Press, New York, 1976.

125. C. S. Foote, "Free Radicals in Biology" (W. A. Pryor, Ed.), Vol. 2, pp. 112–133. Academic Press, New York, 1976.
126. M. J. Thompson, J. N. Kaplanis, and H. E. Vroman, *Steroids* **5**, 551 (1965).
127. J. A. C. Nicol, "The Biology of Marine Animals," Interscience, New York, 1969.
128. E. Ayanoglu, C. Djerassi, T. R. Erdman, and P. J. Scheuer, *Steroids* **31**, 815 (1978).