Volatile Compounds from Marine Algae

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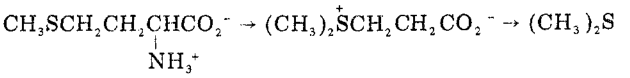
The fragrances of terrestrial plants have attracted the attention of man since antiquity. It is therefore not surprising that some of the earliest chemical investigations have been conducted on the odoriferous constituents. Organic chemists now immediately associate the odors of trees and shrubs with monoterpenes, of spices with phenols and their ethers, and of fruits and flowers with simple aliphatic esters.

The odors connected with marine plants are much less familar. Unlike the countless number of odoriferous terrestrial plants, relatively few marine plants possess odor. Prior to 1966, when we began our work on the odor of seaweed, only one volatile substance had been identified from wet, undecomposed seaweed and described in the literature. In 1935 Haas[1] had shown that dimethyl sulfide is spontaneously evolved from the red algae *Polysiphonia fastigiata* and *P. nigrescens* when the seaweeds are exposed to air. Bywood and Challenger[2] later found that dimethyl sulfide is similarly evolved from the green alga *Enteromorpha intestinalis*. Actually, the odor of dimethyl sulfide can be detected in many seaweeds immediately after crushing the plants. The formation of dimethyl sulfide results from an enzymatic decomposition of dimethyl-β-propiothetin,[3] a metabolite of methionine[4] that is fairly widespread in both benthic and planktonic marine plants.[5]

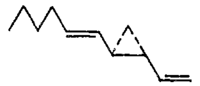
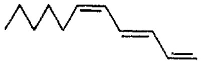
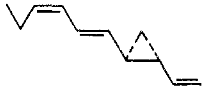
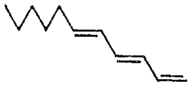
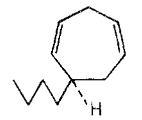
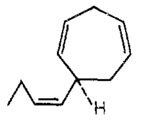
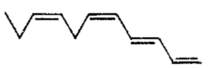
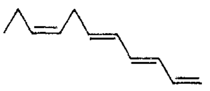


$$\underset{\displaystyle NH_3^+}{CH_3SCH_2CH_2CHCO_2^-} \rightarrow (CH_3)_2\overset{+}{S}CH_2CH_2CO_2^- \rightarrow (CH_3)_2S$$

The Odor of Brown Seaweed. From the steam distillate of dried *Dictyopteris divaricata*, a brown alga that grows on the coasts of Japan, Takaoka and Ando[6] obtained an oil with a "beach odor" which has been shown by Irie and co-workers to be a mixture of sesquiterpenes of the cadinene type.[7] A sesquiterpene, also of the cadinene type, has also been found to be the major hydrocarbon of *D. zonarioiodes* from Mexico.[8]

In striking contrast to the species of *Dictyopteris* that grow along the coasts of Japan and Lower California, the Hawaiian species *D. plagiogramma* or *D. australis* elaborates novel non-isoprenoid C_{11} compounds in the essential oil (Table I) instead of sesquiterpenes.[9] Each species grows abundantly on the sublittoral reef flats surrounding the Hawaiian Islands, and large amounts of the seaweed are deposited on the shores during the summer months by heavy surf. The odor of *Dictyopteris* can frequently be detected in the air around the beaches. Known as *limu lipoa* (seaweed gathered from the deep) in Hawaii, the freshly chopped seaweed is used as a condiment with raw fish and other foods.[10]

Table I
Odoriferous C_{11} Hydrocarbons of Hawaiian *Dictyopteris*

Cyclic compounds	$[\alpha]_D$, deg	Acyclic Polyenes
 dictyopterene A (1)	+72	 5
 dictyopterene B (2)	−43	 6
 dictyopterene C′ (3)	−12	 7  8
 dictyopterene D′ (4)	+75	 9  10

[a] Dictyopterenes C and D are (1*R*,2*S*)-*cis*-1-vinyl-2-(*trans*-1′-hexenyl)cyclopropane and (1*R*,2*S*)-*cis*-1-vinyl-2-(*trans*-1,*cis*-3-hexadienyl)cyclopropane, respectively, the proposed precursors of dictyopterenes C′ and D′.

Two interesting dialkenylcyclopropanes, dictyopterenes A and B, are the major constituents of the essential oil, and the related hydrocarbons, dictyopterenes C′ and D′, are minor components. Their

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(1) P. Haas, *Biochem. J.*, **29**, 298, 1297 (1935).

(2) R. Bywood and F. Challenger, *Biochem. J.*, **53**, xxvi (1953).

(3) F. Challenger and M. I. Simpson, *J. Chem. Soc.*, 1591 (1948).

(4) R. C. Greene, *J. Biol. Chem.*, **237**, 2251 (1962).

(5) (a) F. Challenger, R. Bywood, P. Thomas, and B. J. Hayward, *Arch. Biochem. Biophys.*, **69**, 514 (1957); (b) R. G. Ackman, C. S. Tocher, and J. McLachlan, *J. Fish. Res. Board Can.*, **23**, 357 (1966).

(6) M. Takaoka and Y. Ando, *J. Chem. Soc. Jpn., Pure Chem. Sect.*, **72**, 999 (1951).

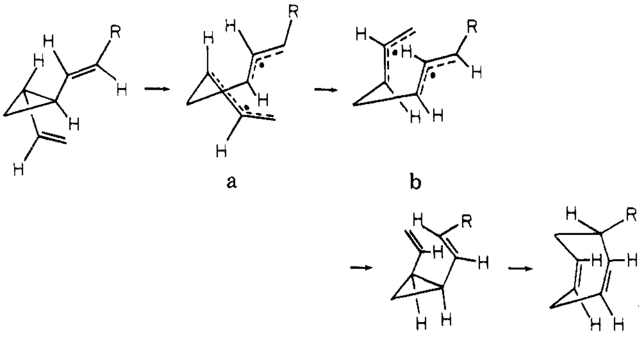
(7) (a) T. Irie, K. Yamamoto, and T. Masamune, *Bull. Chem. Soc. Jpn.*, **37**, 1053 (1964); (b) E. Kurosawa, M. Izawa, K. Yamamoto, T. Masamune, and T. Irie, *ibid.*, **39**, 2509 (1966).

(8) J. J. Sims, W. Fenical, R. M. Wing, and P. C. Radlick, *Phytochemistry*, **11**, 1161 (1972).

(9) (a) R. E. Moore, J. A. Pettus, Jr., and M. S. Doty, *Tetrahedron Lett.*, 4787 (1968); (b) J. A. Pettus, Jr., and R. E. Moore, *Chem. Commun.*, 1093 (1970); (c) J. A. Pettus, Jr., and R. E. Moore, *J. Am. Chem. Soc.*, **93**, 3087 (1971); (d) R. E. Moore, J. Mistysyn, and J. A. Pettus, Jr., *J. Chem. Soc., Chem. Commun.*, 326 (1972); (e) R. E. Moore, J. A. Pettus, Jr., and J. Mistysyn, *J. Org. Chem.*, **39**, 2201 (1974).

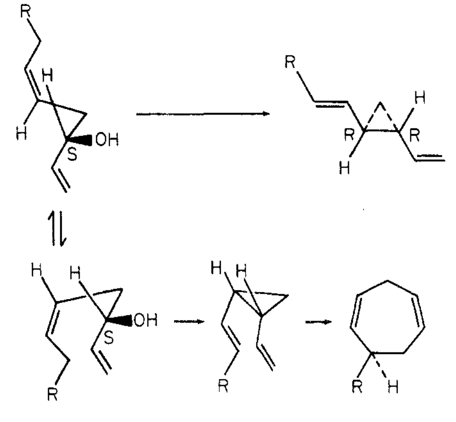
(10) I. A. Abbott and E. H. Williamson, "Limu: An Ethnobotanical Study of Some Edible Hawaiian Seaweeds", Pacific Tropical Botanical Garden, Lawai, Kauai, Hawaii, 1974.

structures were deduced from spectral and chemical data[9e] and verified by synthesis.[11] Lemieux oxidation of either **1** or **2** gave (+)-*trans*-cyclopropanedicarboxylic acid, establishing the absolute configuration of the cyclopropane ring as *R,R*. Both **1** and **2** underwent stereoselective, thermally induced Cope rearrangements[9c] to 1,4-cycloheptadienes, and the products were identical, except for optical properties, with natural **3** and **4**. To rationalize the stereoselectivity in the isomerization of **1** or **2**, the allyl system in the intermediate diradial a[12] must rotate faster[13] than the bulkier heptenyl (R = *n*-butyl) or heptadienyl (R = *cis*-1-butenyl) group to give the conformer b, which then recyclizes to the *cis*-1,2-dialkenylcyclopropane[14] and rearranges by a conventional Cope rearrangement to the cycloheptadiene. The absolute configurations of the cycloheptadienes were secured by converting them to the known butylsuccinic acids.

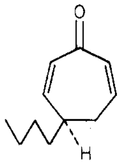


Several acyclic undecapolyenes, viz. three 1,3,5-undecatrienes (**5**–**7**), four 2,4,6-undecatrienes, of which only one (**8**) has been fully characterized, and two 1,3,5,8-undecatetraenes (**9**, **10**), are found with the dictyopterenes in the essential oil in moderate to trace amounts.[9e] The compositions of the 1,3,5-undecatriene, 2,4,6-undecatriene, and 1,3,5,8-undecatetraene mixtures are essentially the same as the photostationary-state mixtures resulting from the *p*-benzoquinone photosensitized equilibrations of the trienes and tetraene in the presence of azulene as a quencher. For example, **5** and **6** are the predominant isomers of 1,3,5-undecatriene in both the essential oil and photostationary state mixture. From proton NMR evidence all four natural 2,4,6-undecatrienes are trans-Δ^4 isomers.[9e] The same mixture of 2,4,6-undecatrienes is formed when 1,*cis*-3,*cis*-5-undecatriene[15] is thermally isomerized and the resulting *cis*-2,*cis*-4,*trans*-6-undecatriene is photoisomerized in the presence of *p*-benzoquinone and azulene.

Scheme I
Proposed Biogenesis of the Dictyopterenes from (3*S*)-1,*cis*-5-Undecadien-3-ol (R = *n*-butyl) and (3*S*)-1,*cis*-5,*cis*-8-Undecatrien-3-ol (R = *cis*-1-butenyl)



Mass spectral analysis of the oil indicates that small amounts of compounds having the molecular compositions $C_{11}H_{14}O$ and $C_{11}H_{16}O$ are also present. Two odoriferous, dextrorotatory dihydrotropones related to dictyopterene C′ have been isolated, and their structures have been deduced to be **11** and **12**.[16] Selenium dioxide oxidation of **3** produced **11**, indicating that C-6 in **11** has the *R* configuration. Catalytic hydrogenation of **12** led to a 3-butylcycloheptanone, which exhibited a CD curve similar to that of (–)-(3*S*)-methylcycloheptanone, showing that C-6 in **12** is also *R*.[17]



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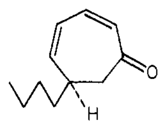

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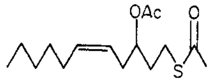
Less volatile organosulfur compounds such as **13** and **14** (absolute configurations undetermined) have been isolated from the chloroform–methanol extract of the dried alga.[9d,18] None of the sulfur compounds, however, exhibits a penetrating odor like the hydrocarbons and ketones. All of the compounds possess a C_{11} unit attached to sulfur with an oxygen substituent at C-3 and are obviously related to the hydrocarbons.



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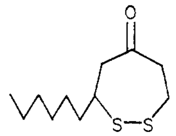

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The most attractive precursors to both the hydrocarbons and sulfur compounds appear to be 1-undecen-3-ols.[19] 1-Alken-3-ols are ubiquitous[20] minor constituents of animal and plant lipids and are me-

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(11) (a) G. Ohloff and W. Pickenhagen, *Helv. Chim. Acta*, **52**, 880 (1969); (b) K. C. Das and B. Weinstein, *Tetrahedron Lett.*, 3459 (1969); (c) A. W. Burgstahler and C. M. Groginsky, *Trans. Kans. Acad. Sci.*, **72**, 486 (1969); (d) B. Weinstein, A. Ali, and D. Sarantakis, *Chem. Commun.*, 940 (1971); (e) W. E. Billups, W. Y. Chow, and J. H. Cross, *J. Chem. Soc., Chem. Commun.*, 252 (1974).

(12) The preferential ring opening to intermediate a is suggested from circular dichroism and nuclear magnetic resonance studies of **1** and **2** at elevated temperatures: S.-Y. C. Lee, J. C. Craig, P. A. Kollman, R. E. Moore, J. A. Pettus, Jr., and S. Rothenberg, submitted for publication. The upper energy state conformation of a *trans*-dialkenylcyclopropane is gauche (dihedral angle <90°) and not s-cis as needed for formation of diradicals with *cis*-alkenyl radical groups. Cyclopentenes are not formed in the Cope rearrangements of **1** and **2** as the intermediate diradical has structure a.

(13) Internal rotational preference is shown in the thermal enantiomerization and diastereoisomerization of *cis*- and *trans*-1-cyano-2-isopropenylcyclopropane: W. von E. Doering and K. Sachdev, *J. Am. Chem. Soc.*, **96**, 1168 (1974).

(14) C. Ullenius, P. W. Ford, and J. E. Baldwin, *J. Am. Chem. Soc.*, **94**, 5910 (1972); J. E. Baldwin and C. Ullenius, *ibid.*, **96**, 1542 (1974).

(15) F. Näf, R. Decorzant, W. Thommen, B. Willhalm, and G. Ohloff, *Helv. Chim. Acta*, **58**, 1016 (1975).

(16) R. E. Moore and G. Yost, *J. Chem. Soc., Chem. Commun.*, 937 (1973).

(17) G. Yost, M.S. Thesis, University of Hawaii, 1974.

(18) (a) P. Roller, K. Au, and R. E. Moore, *Chem. Commun.*, 503 (1971); (b) R. E. Moore, *ibid.*, 1168 (1971); (c) A. E. Asato and R. E. Moore, *Tetrahedron Lett.*, 4941 (1973).

(19) R. E. Moore, *Lloydia*, **39**, 181 (1976).

(20) (a) W. Stark and D. A. Forss, *J. Dairy Res.*, **31**, 253 (1964); (b) W. Stark, J. F. Smith, and D. A. Forss, *ibid.*, **34**, 123 (1967).

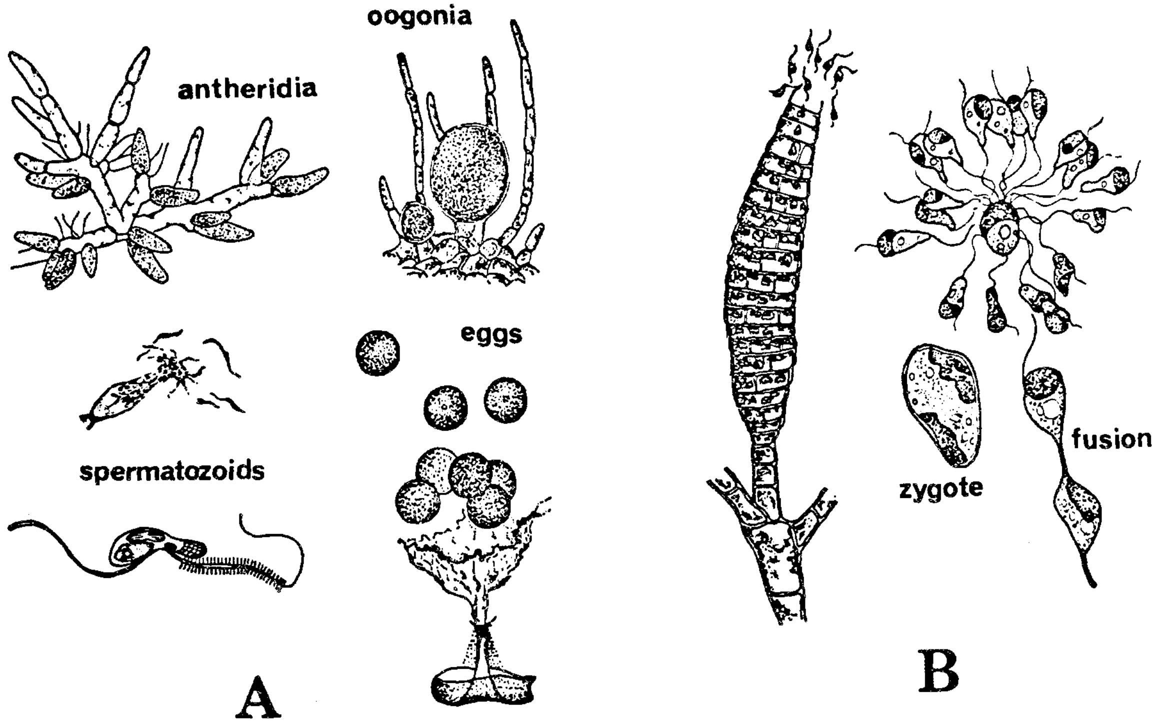

Figure 1. Sexual reproduction in (A) *Fucus serratus* and (B) *Ectocarpus siliculosus* (copyright 1972, Westdeutscher Verlag GmbH; reproduced, with permission, from *Rheinisch-Westfael. Akad. Wiss.*, **No. 217** (1972)).

tabolites of fatty acids. 1-Undecen-3-ols have not been detected in *Dictyopteris* and other seaweeds as yet. (3*S*)-*cis*-1,5-Octadien-3-ol (**15**), however, is a minor component of the essential oil of the red seaweed *Chondrococcus hornemanni*,[21] but C_8 hydrocarbons related to the C_{11} hydrocarbons of *Dictyopteris* are not associated with it. If the hypothetical 1-undecen-3-ols have the *S* configuration at C-3, then concomitant dehydration and cyclization of (3*S*)-*cis*-1,5-undecadien-3-ol leads to either **1** or (1*R*,2*S*)-*cis*-1-vinyl-2-(*trans*-1′-hexenyl)cyclopropane (dictyopterene C), the latter rearranging spontaneously at biotic temperatures[11a] to **3** (Scheme I). Similarly, (3*S*)-1,-*cis*-5,*cis*-8-undecatriene-3-ol leads to either **2** or **4** via (1*R*,2*S*)-*cis*-1-vinyl-2-(*trans*-1,*cis*-3-hexadienyl)cyclopropane (dictyopterene D).

The Role of Volatile Hydrocarbons in the Sexual Reproduction of Brown Seaweeds. In the late 1940's Cook and his collaborators[22] discovered that motile spermatozoa are attracted to mature eggs of *Fucus serratus* by a volatile substance secreted by the eggs. The same phenomenon was observed for *F. vesiculosus*. The chemotactic principle could be readily expelled from an aqueous extract of the eggs by a stream of inert gas and was completely recovered when the carrier gas was passed through a strongly cooled trap containing a small amount of frozen seawater. Interestingly, the active substance from *F. serratus* not only attracted the sperm of *F. serratus* but also attracted those of *F. vesiculosus* and *F. spiralis*. The nonspecificity of the pheromone indicated that the same chemotactic agent was produced by the closely related Fucales. The mechanism of this very striking phenomenon was concluded to be purely physical, as the chemotactic response was sensitive only to a gradient concentration of the pheromone. Chemotactic activity could be induced by a large number of simple organic compounds. Of the hydrocarbons that were tested, *n*-hexane corresponded closest in biological and physical behavior with the natural pheromone. An attempt to identify the chemotactic principle by mass spectrometry gave inconclusive results, and work was discontinued.

Some 20 years later Müller and Jaenicke[23] reisolated the volatile attractant from the eggs and oogonia of *F. serratus* and purified it by gas chromatography. Spectral and chemical examination of the resulting odoriferous oil showed that it was an isomer of 1,3,5-octatriene which they named fucoserratene. From biogenetic considerations fucoserratene was predicted to be 1,*trans*-3,*cis*-5-octatriene (**16**),[24] and this was later proven by synthesis.[25] Compared with *n*-hexane, the sperm attractant had enormous chemotactic activity.

Earlier, Müller had studied chemotaxis in another brown seaweed *Ectocarpus siliculosus*.[26] Noting that the female gametes possessed odor whereas the isomorphic male gametes did not, he collected an odoriferous oil from female cultures and tested it for chemotactic activity. When Vaseline droplets impregnated with the pheromone were placed in the vicinity of active spermatozoids, these simulated eggs became surrounded by halos of male gametes. A collaborative effort with Jaenicke resulted in the first successful isolation and characterization of a sperm attractant from a marine alga.[27] The pheromone, ectocarpene (**4**), was a C_{11}

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(21) F. X. Woolard, B. J. Burreson, and R. E. Moore, *J. Chem. Soc., Chem. Commun.*, 486 (1975).

(22) (a) A. H. Cook, J. A. Elvidge, and I. Heilbron, *Proc. R. Soc. London, Ser. B*, **135**, 293 (1948); (b) A. H. Cook, J. A. Elvidge, and R. Bentley, *ibid.*, **138**, 97 (1951).

(23) D. G. Müller and L. Jaenicke, *FEBS Lett.*, **30**, 137 (1973).

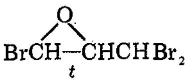
(24) L. Jaenicke, D. G. Müller, and R. E. Moore, *J. Am. Chem. Soc.*, **96**, 3324 (1974).

(25) L. Jaenicke and K. Seferiadis, *Chem. Ber.*, **108**, 225 (1975).

(26) D. G. Müller, *Planta*, **81**, 160 (1968).

(27) (a) D. G. Müller, L. Jaenicke, M. Donike, and T. Akintobi, *Science*, **171**, 815 (1971); (b) L. Jaenicke, T. Akintobi, and D. G. Muller, *Angew. Chem., Int. Ed. Engl.*, **10**, 492 (1971).

Table II
Volatile Constituents of Hawaiian *Asparagopsis taxiformis*[a]

Type of Compound	Structure	Type of Compound	Structure
Haloforms	$CHBr_3$	Halogenated 2-acetoxypropanes	$BrCH_2CH(OAc)CHBr_2$
	$CHBr_2I$		$Br_2CHCH(OAc)CHBr_2$
	$CHBrI_2$		
	CHI_3		
	$CHBr_2Cl$	Halogenated 1,2-epoxypropanes	 $BrCH\text{—}CHCHBr_2$ (O bridge; *t*)
	$CHBrClI$		
Dihalomethanes	CH_2Br_2	1,1,3,3-Tetrahalopropenes	$Br_2C{=}CHCHBr_2$
	CH_2BrI		$Br_2C{=}CHCHBrCl$
	CH_2I_2		$Br_2C{=}CHCHCl_2$
Carbon tetrahalides	CBr_4		$BrIC{=}CHCHBr_2$
Carbonyl dihalides	COI_2	3,3-Dihaloacroleins	$Br_2C{=}CHCHO$
2-Haloethanols	ICH_2CH_2OH	Halogenated butenones	$Br_2C{=}CHCOCH_3$[b]
1,2-Dihaloethanes	$BrCH_2CH_2I$		$Br_2C{=}CHCOCH_2Br$[b]
Halogenated acetaldehydes	Br_2CHCHO		$Br_2C{=}CHCOCH_2I$[b]
Halogenated acetones	CH_3COCH_2Br		$Br_2C{=}CHCOCHBr_2$
	CH_3COCH_2I		$Br_2C{=}CHCOCHBrCl$[b]
	$CH_3COCHBr_2$		$BrClC{=}CHCOCHBr_2$[b]
	$BrCH_2COCH_2Br$		$Cl_2C{=}CHCOCHBr_2$[b]
	$BrCH_2COCH_2I$		$BrClC{=}CHCOCHBrCl$[b]
	CH_3COCBr_3		
	CH_3COCBr_2Cl		
	$BrCH_2COCHBr_2$		
	$ICH_2COCHBr_2$		
	$Br_2CHCOCHBr_2$		
	$Cl_3CCOCCl_3$		

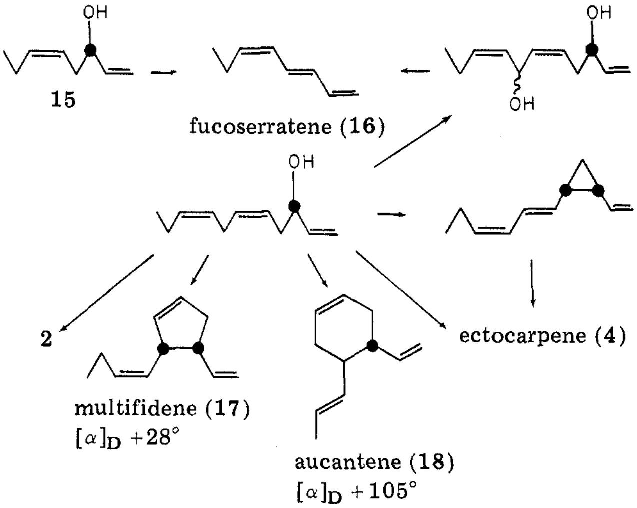
[a] Collected from the reef between Diamond Head and Waikiki, Oahu. [b] The placement of the two olefinic halogens on C-4 is tentative.

hydrocarbon that proved to be identical in all respects, including optical properties, with the dictyopterene D′ that my group had isolated from Hawaiian *Dictyopteris*.[9c]

The studies of chemotaxis in *F. serratus* and *E. siliculosus* represented studies of two completely different fertilization processes. For the dioecious seaweed *F. serratus*, fertilization was oogamous: motile, biflagellated spermatozoids released by the antheridia of male plants fused with nonmotile, spherically shaped eggs released from the oogonia of female plants (Figure 1A). A C_8 hydrocarbon was produced by the eggs to attract the sperm to them. In the dioecious *E. siliculosus*, however, fertilization was isogamous and involved the fusion of motile, isomorphic male and female gametes (zoogametes) (Figure 1B). In this second case male gametes were attracted to female gametes by a C_{11} hydrocarbon excreted by the females.

Müller then turned his attention to *Cutleria multifida* and a third type of fertilization process. In this dioecious alga fertilization was anisogamous: macroscopic, flagellated female gametes were fertilized by microscopic, flagellated male gametes. Again Müller observed that the females attracted the males with an odoriferous substance. Similar methodology was used to collect and purify the male-attracting oil of *Cutleria*, which consisted of three C_{11} hydrocarbons, only one of which was biologically active. From spectral and biogenetic arguments the sperm attractant multifidene was concluded to be a 3-(*cis*-1-butenyl)-4-vinylcyclopentene.[24] Synthesis has now shown that multifidene is the cis compound **17**.[28] The two biologically inactive components of the male-attracting oil were also present in the essential oil of the male and asexual plants. One of these hydrocarbons was **4**, whereas the other one, aucantene, was identified as *trans*-4-(*trans*-1-propenyl)-5-vinylcyclohexene (**18**).[24] The structure of aucantene has also been confirmed by synthesis[29] (see Scheme II).

Scheme II
Proposed Biogenesis of Sperm Attractants and Related Hydrocarbons in Brown Algae from (3*S*)-1,*cis*-4-Alkadien-3-ols



Discovery of Haloforms in the Bonnemaisoniaceae. *Asparagopsis taxiformis* is an edible red seaweed that is highly favored in Hawaii for its strong aroma and flavor. Known locally as *limu kohu* (the supreme seaweed),[10] the alga is a dioecious, gametophytic plant that alternates in its life cycle with a heteromorphic sporophyte, *Falkenbergia rufanolosa*. *A. taxiformis* is rich in iodine, but free molecular iodine is not present in the live plants. Its essential oil is composed of mainly bromine- and iodine-containing haloforms[30] and small to trace amounts of many other

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(28) L. Jaenicke and W. Boland, *Justus Liebigs Ann. Chem.*, 1135 (1976).

(29) F.-J. Marner and L. Jaenicke, *Chem. Ber.*, **108**, 2202 (1975).

(30) B. J. Burreson, R. E. Moore, and P. Roller, *Tetrahedron Lett.*, 473 (1975).

Table III
Halogenated Acids, Alcohols, and Amides in Extracts of Dried Hawaiian *Asparagopsis taxiformis*

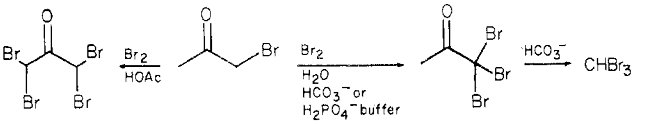
CH_2Cl_2 Extract[a]	H_2O Extract[b]
1,1-Dihalo-2-propanols	Haloacetic acids
1,3-Dihalo-2-propanols	Dihaloacetic acids
1,1,3-Trihalo-2-propanols	Monohaloacrylic acids
1,1,1,3-Tetrahalo-2-propanols	3,3-Dihaloacrylic acids
1,1,3,3-Tetrahalo-2-propanols	Trihaloacrylic acids
1,4,4-Trihalobut-3-en-2-ols	4,4-Dihalo-2-hydroxybut-3-enoic acids
1,1,4,4-Tetrahalobut-3-en-2-ols	
1,1,1,4,4-Pentahalobut-3-en-2-ols	
2,2-Dihaloacetamides	

[a] $Br_2CHCH(OH)CH_2Br$, $Br_2CHCH(OH)CH_2Cl$, $Br_2CHCH(OH)CHBr_2$, $Br_2C{=}CHCH(OH)CHBr_2$, $Br_2CHCONH_2$, $BrICHCONH_2$, and $I_2CHCONH_2$ are the major constituents. [b] Br_2CHCO_2H, $BrICHCO_2H$, I_2CHCO_2H, and $Br_2C{=}CHCO_2H$ are the major constituents.

halogenated compounds (Table II).[31,32] Related nonvolatile compounds are present in the methylene chloride[33] and aqueous extracts[34] of the lyophilized seaweed (Table III). Interestingly, none of these halogenated compounds exist in the asexual *F. rufanolosa.*

The haloforms are apparently formed by the classical haloform reaction of methyl or carboxymethyl ketones. Monohaloacetones, 1,1-dihaloacetones, and 1,1,1-trihaloacetones, which are intermediates in the conversion of acetone or acetoacetic acid to haloforms, are all found in the essential oil. Small amounts of 1,2-dihaloacetones, 1,1,2-trihaloacetones, 1,1,2,2-tetrahaloacetones, and even a hexahaloacetone are also present. Introduction of the halogens into the acetones is undoubtedly enzymatic,[35,36] but the degradation of 1,1,1-trihaloacetones into haloforms may not be. On standing in aqueous sodium bicarbonate solution, 1,1,1-trihaloacetones such as 1,1,1-tribromoacetone and 1,1-dibromo-1-chloroacetone decompose slowly into haloforms and acetic acid.[31]

In the nonenzymatic bromination of acetone, the introduction of the first bromine is slow. Further bromination of bromoacetone in aqueous solution, however, is rapid and leads to 1,1,1-tribromoacetone. In glacial acetic acid, on the other hand, bromination of bromoacetone leads slowly to 1,1,3,3-tetrabromoacetone (**19**), as the reaction proceeds through an enol intermediate rather than through the enolate ion.



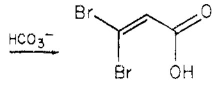
19

Further bromination of **19** occurs only in aqueous solution where it is brominated to pentabromoacetone, which decomposes into bromoform and dibromoacetic acid. Pentabromoacetone has not been detected in Hawaiian *Asparagopsis*, but dibromoacetic acid is a constituent of the aqueous extract of the seaweed.[31]

Most interesting is the presence of bromochloroiodomethane in *Asparagopsis.* The amount of this haloform is extremely small, and we have been unable to concentrate a sufficient quantity of this material to determine its optical properties. In the gas chromatograph it is very poorly resolved from the enormous amount of bromoform, the major constituent of the essential oil. All of the iodine-containing haloforms rapidly liberate iodine on exposure to air and light. This sensitivity suggests that the dihalomethanes, 1-bromo-2-iodoethane, and carbonyl diiodide may be artifacts from photooxidation of iodoform, bromodiiodomethane, and dibromoiodomethane. The carbon tetrabromide, however, is probably formed by further bromination of bromoform in the seaweed.

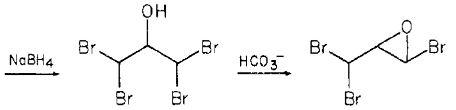
The 3,3-dihaloacrylic acids in *Asparagopsis* may be formed from either the haloform reaction of 4,4-dihalobutenones or from the Favorskii rearrangement of 1,1,3,3-tetrahaloacetones. When **19** is allowed to stand in aqueous sodium bicarbonate solution, it is converted cleanly into 3,3-dibromoacrylic acid.[34]

19



The halogenated 2-propanols appear to be the precursors of the halogenated propenes and epoxypropanes. In nonenzymatic studies we have been unable to dehydrate 1,1,3,3-tetrabromopropan-2-ol (**20**) or to convert 2-acetoxy-1,1,3,3-tetrabromopropane to 1,1,3,3-tetrabromopropene under mild conditions. In aqueous bicarbonate solution, however, **20** readily cyclizes to *trans*-1,3,3-tribromoepoxypropane (**21**).[34]

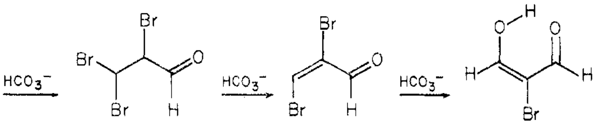
19



20 21

There is a possibility that **21** is involved in the enzymatic biogenesis of 3,3-dibromoacrolein. Under nonenzymatic conditions, however, **21** slowly rearranges in aqueous bicarbonate and eliminates hydrogen bromide to form (*E*)-2,3-dibromoacrolein, a labile compound that is converted into the remarkably stable bromomalonodialdehyde on further standing in bicarbonate solution. Even though **21** is a constituent of the essential oil, neither 2,3-dibromoacrolein nor bromomalonodialdehyde can be detected in the algal extracts.[34]

21



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(31) B. J. Burreson, R. E. Moore, and P. P. Roller, *J. Agric. Food Chem.*, **24**, 856 (1976).

(32) While our work was in progress the isolation of several bromine- and chlorine-containing acetones and butenones from *A. taxiformis* collected in the Gulf of California was reported: W. Fenical, *Tetrahedron Lett.*, 4463 (1974).

(33) F. X. Woolard, R. E. Moore, and P. P. Roller, *Tetrahedron*, **32**, 2843 (1976).

(34) Presented by R. E. Moore, F. X. Woolard, and P. P. Roller, 172nd National Meeting of the American Chemical Society, San Francisco, Calif., August 1976, Abstract ORGN-083.

(35) For studies of enzymatic halogenation see: J. R. Beckwith, R. Clark, and L. P. Hager, *J. Biol. Chem.*, **238**, 3086 (1963); A. Taurog and E. M. Howells, *ibid.*, **241**, 1329 (1966); J. A. Thomas, D. R. Morris, and L. P. Hager, *ibid.*, **245**, 3129 (1970), and references therein.

(36) Chloroperoxidase from the mold *Caldariomyces fumago* catalyzes the peroxidative chlorination of β-ketadipic acid to δ-chlorolevulinic acid and carbon dioxide: P. D. Shaw and L. P. Hager, *J. Biol. Chem.*, **236**, 1626 (1961).

3,3-Dihaloacroleins may serve as precursors to the 4,4-dihalobut-3-en-2-ols. A number of substrates such as acetate, malonate, haloacetate, dihaloacetate, and even haloform and methylene halide could provide the additional carbon atom.[33,37] The 4,4-dihalobutenones, on the other hand, are probably formed by the Claisen condensation of a 3,3-dihaloacrylate with a one-carbon donor.

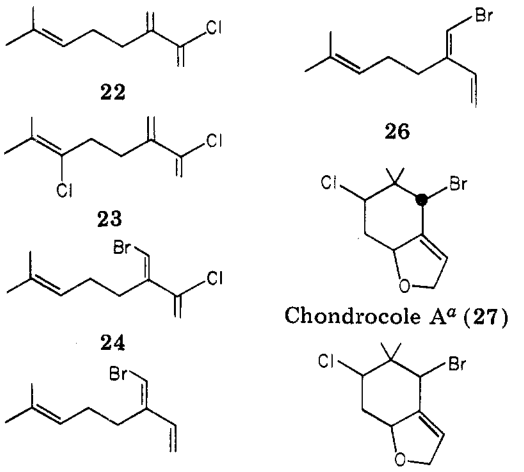
The presence of haloforms and other halogenated compounds in *A. taxiformis* suggests that this seaweed might be potentially dangerous to eat. In Hawaii the uncooked seaweed is frequently eaten with meats and fish. Since the odor and flavor are quite strong, however, relatively small quantities of the *limu* are added to foods. To the author's knowledge there has never been a case of illness attributed to the ingestion of *limu kohu*. Many of the compounds in *Asparagopsis* appear to be carcinogenic, and an epidemiological study might provide some valuable information. Although extensive data have been accumulated on the carcinogenicity of $CHCl_3$, CCl_4, and other chlorine-containing compounds, relatively little is known about the bromine and iodine analogues.

Odoriferous halogenated compounds have also been isolated from other Bonnemaisoniaceae. Rinehart et al. have identified several bromine-containing 2-heptanones, primarily 1,1,3,3-tetrabromo-2-heptanone, and also 1-iodo-3,3-dibromo-2-heptanone, in the essential oil of *Bonnemaisonia hamifera*,[38] and very recently the research group at the Roche Institute of Marine Pharmacology in Sidney has found 3,4,4-tribromobutenone, 1,1,2-tribromooct-1-en-3-one, and six closely related compounds in *Ptilonia australasica*.[39]

The Origin of Halocarbons in the Atmosphere and Water. Chloroform and carbon tetrachloride are constituents of the chlorocarbon complement of the upper atmosphere[40] and have been identified as trace contaminants of chlorine-treated drinking water.[41] It is quite possible that the $CHCl_3$ and CCl_4 in both the air and purified water have algal origins and arise by reactions analogous to those in *Asparagopsis* for $CHBr_3$ and CBr_4. To date we have not determined whether or not $CHCl_3$ and CCl_4 are produced by Hawaiian *A. taxiformis*. The presence of hexachloroacetone in the essential oil certainly suggests that they are. The Fenical and Goldberg groups, however, have recently shown that $CHCl_3$ and CCl_4, as well as bromodichloromethane, are constituents of *A. armata* from the Mediterranean.[42]

Methyl chloride, the dominant chlorocarbon in the atmosphere[43] and a possible regulator of the stratospheric ozone density, appears to have a marine origin and is not the result of direct industrial pollution.[44] In the sea its proposed precursor, methyl iodide, reacts nucleophilically with chloride ion and is converted into the more volatile methyl chloride, which then escapes into the air.[45] Methyl iodide is found in all ocean waters and may be a common extracellular metabolite of marine algae. The water in the vicinity of seaweeds such as the iodine-rich kelp *Laminaria digitata* contains a thousand times more methyl iodide than the open oceans, but its actual existence in this seaweed has not been proven. Methyl iodide, however, is produced by *Asparagopsis armata*.[42]

Table IV
Volatile Halogenated Monoterpenes from Hawaiian *Chondrococcus hornemanni*



22, 23, 24, 25, 26, Chondrocole A[a] (27), Chondrocole B[a] (28)

[a] The optical rotation and absolute configuration has not been determined.

Odoriferous Monoterpenes from Red Algae. Halogenated monoterpenes were first discovered in the digestive gland of the sea hare *Aplysia californica*.[46] Suspected to be of dietary origin, these compounds were eventually traced to species of *Plocamium* that are ingested by the mollusk.[47] Since then a fascinating array of novel compounds have been isolated from this genus of red seaweeds.[48] Most of the monoterpenes from *Plocamium*, however, are not very volatile and possess only faint odors.

Chondrococcus hornemanni, a red seaweed that grows in the subtropical and tropical Pacific Ocean in regions of heavy surf, has a strong turpentine-like odor that is manifested on crushing the plants. When inspected under a microscope, numerous oil droplets or possibly specialized cells can be detected throughout the thalli. These secondary structures are ruptured on freezing the seaweed, and the characteristic odor of the plant is liberated. Lyophilization of the seaweed removes the odoriferous constituent along with water, and the essential oil is recovered simply by extracting the condensate with methylene chloride. The essential oil

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(37) H. Taguchi, H. Yamamoto, and H. Nozaki, *J. Am. Chem. Soc.*, **96**, 3020 (1974).

(38) J. F. Siuda, G. R. Van Blaricom, P. D. Shaw, R. D. Johnson, R. H. White, L. P. Hager, and R. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **97**, 937 (1975).

(39) Paper presented by R. Kazlauskas, P. T. Murphy, R. Quinn, and R. J. Wells at the 10th International Symposium on Chemistry of Natural Products, IUPAC, Dunedin, New Zealand, August 1976.

(40) J. E. Lovelock, R. J. Maggs, and R. J. Wade, *Nature (London)*, **241**, 194 (1973).

(41) *Chem. Eng. News*, **54** (16), 7 (1976).

(42) O. McConnell and W. Fenical, private communication; C.-W. Su and E. D. Goldberg, private communication.

(43) E. P. Grimsrud and R. A. Rasmussen, *Atmos. Environ.*, **9**, 1010, 1014 (1975).

(44) J. E. Lovelock, *Nature (London)*, **256**, 193 (1975).

(45) The residence time of methyl iodide in seawater is comparable to its rate of reaction with chloride ion: O. C. Zafiriou, *J. Mar. Res.*, **33**, 75 (1975).

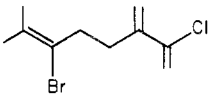
(46) (a) D. J. Faulkner, M. O. Stallard, J. Fayos, and J. Clardy, *J. Am. Chem. Soc.*, **95**, 3413 (1973); (b) D. J. Faulkner and M. O. Stallard, *Tetrahedron Lett.*, 1171 (1973).

(47) M. O. Stallard and D. J. Faulkner, *Comp. Biochem. Physiol. B*, **49**, 25 (1974).

(48) (a) J. S. Mynderse, D. J. Faulkner, J. Finer, and J. Clardy, *Tetrahedron Lett.*, 2175 (1975); (b) J. S. Mynderse and D. J. Faulkner, *Tetrahedron*, **31**, 1963 (1975); (c) P. Crews and E. Kho, *J. Org. Chem.*, **40**, 2568 (1975).

of subtropical *C. hornemanni* is composed of mostly myrcenes that are mono- and disubstituted with bromine and/or chlorine.[49] Usually bromine is found on the C-3 methylene and chlorine is attached to the C-2 and C-6 carbons of 7-methyl-3-methylene-1,6-octadiene (myrcene). For example, in a variety found at Black Point, Oahu, **22** and **23** are the most abundant volatile compounds, whereas in another variety collected at the Halona Blowhole, Oahu, **24** and **25** are the major constituents of the essential oil (Table IV). In addition to the myrcenes, the essential oil of the latter alga also contains a large amount of a cyclic monoterpene, chondrocole A, a dimethylhexahydrobenzofuran having the structure **27**. Chondrocole A is only a minor volatile constituent of the alga from Black Point, but accompanies the isomeric chondrocole B (**28**) in that seaweed.[49b] The chlorine and bromine of chondrocoles A and B are in positions analogous with the placement of a chlorine on C-6 and a bromine on the C-3 methylene of myrcene.

Chondrococcus also produces halogenated myrcenes with bromines at C-2 and C-6 and a chlorine on the C-3 methylene. Although we have not encountered any of these compounds in the essential oils of Hawaiian *Chondrococcus*, the ethereal extract of the lyophilized seaweed from Black Point curiously contains a 6-bromomyrcene, **29**, along with a related cyclic compound, chondrocole C.[50] Ichikawa et al. have also reported that **31** and **32** are minor constituents of *C. hornemanni* from the Amami Island coasts of Japan.[49a]



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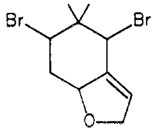

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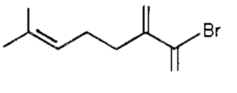

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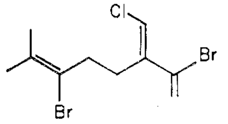
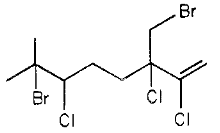


32

Several halogenated dihydro- and tetrahydromyrcenes are present in the ethereal extracts of the dried seaweeds. The *Chondrococcus* from Black Point elaborates mainly **33**, with smaller amounts of three closely related compounds. All four compounds display a Markownikoff addition of BrCl to the C-3 methylene, and two of these show that BrCl has been added to an anti-Markownikoff manner to the Δ^6 double bond. None of the major polyhydromyrcenes have BrCl added to the vinyl group; however, a related cyclic compound, **34**, has been found with this feature.[50] The variety from the Halona Blowhole, on the other hand, elaborates **35** and **36** as the major di- and tetrahydromyrenes.



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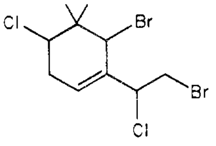

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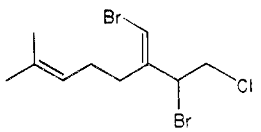

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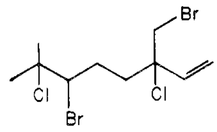
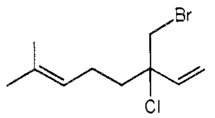


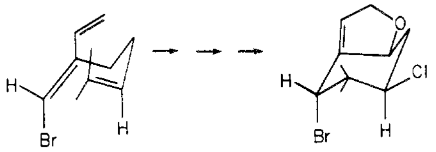
36

In **36** BrCl has added in a Markownikoff fashion to the Δ^6 double bond but in **35** has added anti-Markownikoff to the vinyl group.[50] Interestingly *C. hornemanni* from tropical Sri Lanka (Ceylon) does not produce halogenated myrcenes. In the extract of the seaweed, however, the major halogenated compound is **37**.[51]



37

Biogenetically the halogenated myrcenes are probably formed by the enzymatic addition of BrCl to myrcene (**38**), a minor constituent of the essential oil,[49a] followed by a dehydrohalogenation (e.g., **38** → **37** → **25** or **26**). In the nonenzymatic addition of halogen to myrcene, the first equivalent adds almost exclusively to the Δ^6 double bond, but monohalomyrcenes substituted at C-6 have not been found in *Chondrococcus*. Chondrocole A may be formed from **25**. Similarly chondrocoles B and C could arise from **26**.[49b]



25 **27**

Future Directions. Actually, only a few benthic marine algae have been examined in any detail for volatile compounds. The isolation of **15** from *Chondrococcus hornemanni* suggests that 1-undecen-3-ols and C_8 hydrocarbons related to the dictyopterenes may eventually be found in seaweeds. The isolation of numerous chlorine-, bromine-, and iodine-containing compounds from *Asparagopsis* moves one to wonder whether organofluorine compounds might exist in some marine plants. Ethylene, an important regulator in the growth and development of terrestrial plants,[52] has not been demonstrated in marine plants but may be produced concomitantly with dimethyl sulfide. The sulfur odor of seaweeds may not always be due to dimethyl sulfide. Some seaweeds are rich sources of sulfur and sulfur-containing lipids; Wratten and Faulkner,[53] for example, have recently identified lenthionine (**39**) and other cyclic polysulfides and their oxidation products in the odoriferous red alga *Chondria californica*. A few species of *Laurencia*, a genus of red seaweeds that has been a very rich source of interesting halogenated C_{15} compounds,[54] have a faint odor attributed mostly to

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(49) (a) N. Ichikawa, Y. Naya, and S. Enomoto, *Chem. Lett.*, 1333 (1974); (b) B. J. Burreson, F. X. Woolard, and R. E. Moore, *Tetrahedron Lett.*, 2155 (1975).

(50) B. J. Burreson, F. X. Woolard, and R. E. Moore, *Chem. Lett.*, 1111 (1975).

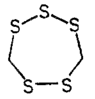
(51) F. X. Woodlard, R. E. Moore, M. Mahendran, A. Sivapalan, *Phytochemistry*, 15, 1069 (1976).

(52) F. B. Abeles, "Ethylene in Plant Biology", Academic Press, New York, N.Y., 1973.

(53) S. J. Wratten and D. J. Faulkner, *J. Org. Chem.*, 41, 2465 (1976).

(54) For a review see: W. Fenical, *J. Phycol.*, 11, 245 (1975).

unhalogenated sesquiterpenoids.[55] In a study of Hawaiian *L. nidifica*, Erickson and her collaborators have described the isolation and structure elucidation of an interesting sesquiterpenoid alcohol, **40**,[56] a possible link to dactyloxene B (**41**), isolated from the sea hare *Aplysia dactylomela*.[57] Several species of benthic algae are currently being investigated in my laboratory. In one case we have found that *p*-(methoxymethyl)phenol (**42**) is responsible for the foul odor of the red alga *Martensia fragilis*.[58]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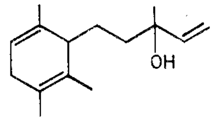

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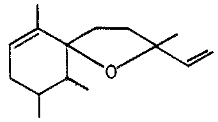

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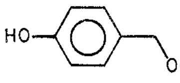

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It is a great pleasure to acknowledge the efforts of my collaborators, especially B. J. Burreson, J. A. Pettus, Jr., P. P. Roller, and F. X. Woolard, whose work is discussed here. I am very grateful to the National Science Foundation and to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(55) J. Cardellina, B. J. Burreson, P. P. Roller, and R. E. Moore, unpublished work.

(56) H. H. Sun, S. M. Waraszkiewicz, and K. L. Erickson, *Tetrahedron Lett.*, 585 (1976).

(57) F. G. Schmitz and F. J. McDonald, *Tetrahedron Lett.*, 2541 (1974).

(58) J. Battaile and R. E. Moore, unpublished work.

Spin-Labeled Nucleic Acids

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Numerous spectroscopic techniques have been used to study the relationships which exist between the structure and the function of nucleic acid molecules. Some of the significant structure–function problems in the macromolecular nucleic acid field are: (a) helix–coil transition, (b) unfolding of the tertiary structure by temperature or chemical agents, (c) binding of metal ions such as magnesium, (d) interaction and intercalation of drugs, causing perturbations of the native structure, (e) recognition site on the transfer RNA (tRNA) by the corresponding aminoacyl-synthetase, and (f) interaction of specific proteins with RNA in the ribosomes.

Certain aspects of these problems have been illuminated by spin-labeling studies.

Nitroxide spin-labels are stable synthetic organic free radicals that can interact either covalently or noncovalently with biological macromolecules of interest. Much of the organic chemistry of nitroxides is presented elsewhere.[1-7] Spin-labels can provide information concerning structure and conformational changes because they are particularly sensitive to molecular motion and orientation, as well as to electric and magnetic environments. All these features can be found in biological systems, and they can be detected and monitored by using an electron paramagnetic resonance (EPR) spectrometer.

The spin-label technique or method has been applied to a variety of biological systems, but polynucleotides have received little attention despite publication of an initial study in 1967.[8] Compared to other biosystems, nucleic acids present a particular challenge because they contain essentially only four types of monomers or bases (G, C, A, U, and/or T), which most of the time are either stacked or hydrogen-bonded to one another. Thus, specific spin-labeling of a particular site or base becomes almost impossible!

Because of this difficulty, most specific labeling studies of nucleic acids have concerned transfer RNAs, in which some rare bases are selectively reactive toward certain spin-label reagents. This Account treats this topic in depth and gives less exhaustive attention to investigations of DNA and RNA molecules. Spin-labeled mononucleotides were reviewed recently by Gaffney[9] and are not covered here. Numerous articles or books treat the general topic of biological applications of spin-labeling.[10-19]

Hermann Dugas received his B.Sc. (1964) from the Université de Montréal and his Ph.D. (1967) in organic synthesis from the University of New Brunswick. After 1 year of postdoctoral work at New Brunswick and 2 years at the National Research Council of Canada in Ottawa, he joined the staff of the Département de Chimie of the Université de Montréal in 1970. Professor Dugas's research interests relate to the study of structure and function of biological systems using NMR and EPR techniques.

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