Phytochemistry, 1977, Vol. 16, pp. 367–374, Pergamon Press, Printed in England

HALOGEN CHEMISTRY OF THE RED ALGA *ASPARAGOPSIS*

OLIVER MCCONNELL and WILLIAM FENICAL

Institute of Marine Resources, Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A.

(*Revised received* 24 *September* 1976)

Key Word Index—*Asparagopsis taxiformis*; *A. armata*; Bonnemaisoniaceae; biological halogenation; naturally-occurring haloforms.

Abstract—Ethanol extraction of fresh *Asparagopsis taxiformis* and *A. armata*, followed by pentane partition, results in the isolation of a series of halomethanes, among them MeI, $CHCl_3$ and CCl_4. Under these extraction conditions, esterification readily occurs, allowing the isolation and identification of a series of polyhaloethyl acetates and acrylates. The recognition of acetone and polyhaloacetones in these extracts suggests biological halogenations occur which result in haloform reactions.

INTRODUCTION

Red algae (Rhodophyta) have been recognized since the late 1800's to be a rich source of the halogens, particularly bromine and iodine [1]. In recent years, halogen-containing organic compounds have been shown to be the major cellular forms that these elements assume [2]. To date, at least 10 families, representing 5 orders of red seaweeds (Nemaliales, Cryptonemiales, Gigartinales, Rhodymeniales, and Ceramiales), have been shown to be engaged in biological halogenation yielding a diverse array of organic compounds [2, 3]. Recent studies suggest that this process should also be considered for some seaweeds of the divisions Cyanophyta [4] and Chlorophyta [5]. In the majority of these cases, halogen-containing terpenes, acetylenes and phenols are produced. However, members of the family Bonnemaisoniaceae (Nemaliales) produce polyhalogenated compounds of quite different biosynthetic origin. The odorous alga *Bonnemaisonia hamifera*, for example, produces large amounts of 1,1,3,3-tetrabromo-2-heptanone and smaller amounts of dibromo, tribromo and bromoiodo-2-heptanones [6]. In an earlier communication, we described the isolation of a number of polyhalogenated acetones and butenones from *Asparagopsis taxiformis* (Delile) Trev. collected in the Gulf of California [7]. The results of preliminary studies of *A. taxiformis* from the Hawaiian Islands have also been described by Burreson *et al.* [8]. We wish to report here the results of more extensive chemical studies of the genus *Asparagopsis*, including both *A. taxiformis* from the Gulf of California and *A. armata* collected along the Spanish Mediterranean coast.

RESULTS AND DISCUSSION

The halogenated compounds from *Asparagopsis* possess a wide range of volatility and solubility and, hence, no single method of extraction and isolation can be considered entirely satisfactory. We have chosen two complementary methods which appear to capture the majority of the very volatile constituents, as well as those obtained only by investigation of the aqueous extract. In one method, lightly air-drying the samples at 25–30° followed by multi-solvent extraction and concentration yields the heavier, relatively non-volatile components formed in smaller amounts. To obtain the major, and most volatile, compounds, fresh algae were also preserved in 95% ethanol and the volatiles were directly extracted from the ethanol decant with purified pentane. This method results in the esterification of halogenated carboxylic acids which would otherwise be difficult to isolate. In both procedures, the components of *Asparagopsis* were readily separated and determined by GLC and MS. It should be noted that with all procedures elemental iodine was generated both spontaneously and during chromatographic treatments. In addition, even stored in the cold, certain fractions containing halogenated compounds decomposed, presumably by the action of liberated halogen acids. Hence, while we report here the structures of a number of substances detected in *Asparagopsis*, the relative amounts of these compounds may not accurately represent those present in the living plant. Also, and more importantly, the halogen acids and iodine liberated may be reacting with and altering the compounds initially present in the algae. Thus, since the production of artifacts is quite possible, we have not emphasized studies of the minor constituents not readily visible by GC–MS techniques (*ca* < 1%).

Air-drying procedures

The chloroform extract of air-dried *A. taxiformis* (Delile) Trev. collected near Isla Angel de la Guarda, Gulf of California, was chromatographed over deactivated silica gel, and all fractions eluted with benzene were ultimately combined. The benzene eluent was analyzed by GC–MS, which allowed the assignment of structure to eleven major halogen-containing compounds recognized as acetone and butenone derivatives [7] (Fig. 1). The spectral behavior of these metabolites confirmed their structures. Strong carbonyl bands were seen in the IR spectra between 1710 and 1760 cm^{-1}, reflecting the predicted shifts of $\nu_{C=O}$ by α-halogen substitution [9]. The mass spectra could easily be interpreted. Major fragments consisted of the halomethyl acylonium ions

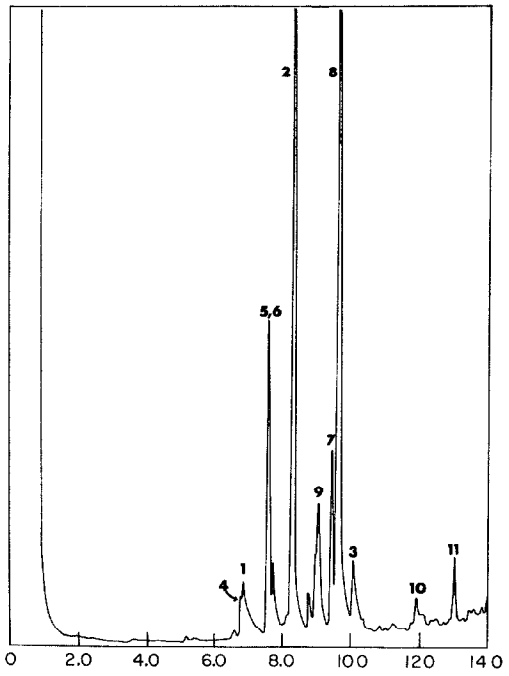
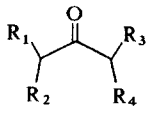
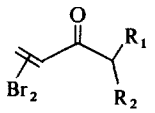


Fig. 1. *A. taxiformis*—dry extraction.





Cmpd	R_1	R_2	R_3	R_4
1	Br	H	Br	H
2	Br	Br	Br	H
3	Br	Br	Br	Br
4	Br	H	Cl	H
5	Cl	H	Br	Br
6	Cl	Br	Br	H
7	Cl	Br	Br	Br

Cmpd	R_1	R_2
8	Br	H
9	Cl	H
10	Cl	Br
11	Br	Br

and CR_3 [10] units from α-cleavage mechanisms and M^+-X fragments (see Experimental).

To confirm the structures of the haloacetones **1–7**, acetone and chloroacetone were each brominated according to the method of Rappe [11]. With each precursor, complex mixtures were produced which contained major amounts of **1–7**. GC–MS analysis of these standards proved them to be identical with the natural products. The halobutenones were assigned, based upon analogous mass spectral fragmentation patterns, carbonyl bands in the region of 1670 cm^{-1}, and the existence of singlet olefin signals in the NMR spectra at ~7·55 ppm. While we prefer to consider the vinyl-substituted (C_3–C_4) bromines *trans*-vicinal, based upon predicted chemical shifts for the lone olefin proton, these substituents appear to be prone to facile isomerizations (see Experimental). Halogenated acetones and butenones have also been found in *A. taxiformis* from Hawaii [8].

Analysis of the CH_2Cl_2 extracts of lightly air-dried *A. armata* Harvey collected near La Escala, Spain gave similar results; however, **11** was the only halobutenone found, but in very small amounts and only from Si gel chromatography. Since this alga was not dried as rigorously, volatile constituents were also observed, with retention times less than 4 min. (The nature of the volatile components will be discussed in the next section.) In addition to the haloacetones **2–7**, found first in *A. taxiformis*, a group of highly chlorinated acetones **12–21** was observed. Column chromatography of this sample provided two additional iodine-containing acetone isomers, **20** and **21**, which are obscured by more abundant components in Fig. 2. Also present in the crude extract but not observed from column chromatography were mono- and dibromoacetaldehyde **22–23**, which were identical with standards.

Fresh extractions

Air-drying *Asparagopsis* prior to extraction procedures results in the loss of a great deal of volatile chemical constituents. Freshly collected plants have pungent odors

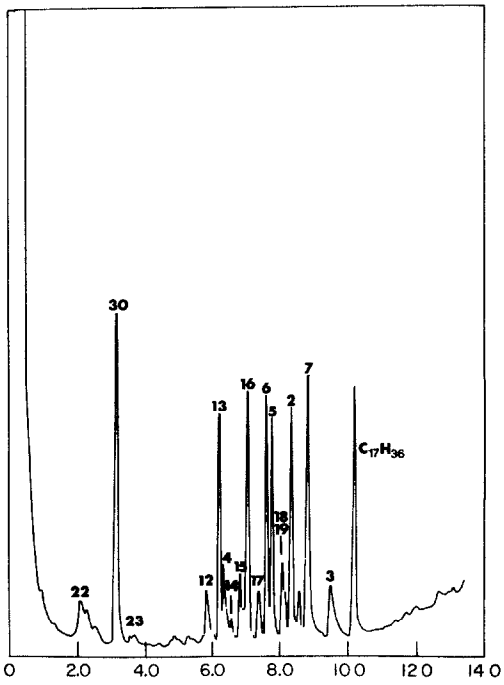
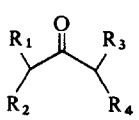


Fig. 2. *A. armata*—dry extraction.

Cmpd	R_1	R_2	R_3	R_4
12	H	Cl	H	Cl
13	H	Cl	Cl	Cl
14	Cl	Cl	Cl	Cl
15	Cl	Cl	H	Br
16	Cl	Br	H	Cl
17	Cl	Cl	Cl	Br
18	Cl	Cl	Br	Br
19	Cl	Br	Cl	Br
20	H	Cl	H	I
21	Br	Br	H	I



which rapidly disappear after collection. To avoid the loss of these substances, fresh plants of *A. armata* were placed in 95% ethanol immediately after collection. The ethanol solution was next extracted directly with purified pentane. The pentane extract was investigated after careful removal of solvent by fractional distillation. The major components of fresh *A. armata* are not those which are visible in the dry extraction but a series of halomethanes and esters of halocarboxylic acids. The extremely volatile halomethane components were determined by capillary column electron-capture GLC, while those less volatile than pentane were analyzed by GC–MS. Nine halomethanes, **24–32**, were detected by these combined methods and identified, for the most part, using standards. Four of these metabolites, **29–32**, have been also found in Hawaiian *A. taxiformis* [8].

Cmpd	*Halomethane*	
24	MeI*	
25	CH_2ClI	* detection by ECGC methods
26	$CHCl_3$*	
27	CCl_4*	
28	$CHBrCl_2$*	
29	$CHBr_2Cl$	
30	$CHBr_3$	
31	CBr_4	
32	$CHBr_2I$	

It is important to point out our confirmation of MeI, $CHCl_3$, CCl_4, and CBr_4 as natural products in *Asparagopsis*. Lovelock has recently reported that MeI concentrations were greatly increased near beds of the brown seaweed *Laminaria digitata* [12]. However, the algal source for this halocarbon was not confirmed. Also, the constancy of atmospheric CCl_4 concentrations have indicated a ubiquitous natural source [13]. While CCl_4 and MeI are indeed produced by *A. armata*, and while plants of this genus are distributed world-wide, the ability of these species to provide environmentally significant amounts of these halocarbons must be questioned.

Figure 3 outlines the GC–MS results obtained by ethanol–pentane extraction of *A. armata*. In addition to the halomethanes (compounds of less than 2 min retention time were not analyzed by MS), five ethyl esters were discovered, which are assigned as halogen-containing derivatives of acetic and acrylic acids.

Ethyl 2,2-dibromoacetate (**33**) and ethyl *E*-2,3-dibromoacrylate (**34**) were the major ester components, while the haloesters **35–37** were clearly present in lower amounts. As in the case of the haloacetones, the MS and IR properties of **33–37** were very useful in the structure assignments. Ester bands ($\nu_{C=O}$) were observed at 1720–1755 cm^{-1}. The mass spectral fragmentation patterns clearly indicated ethyl esters. Ethyl 2,2-dibromoacetate (**33**) exhibited a small molecular ion cluster at *m/e* 244, 246, 248 (rel. int. 1:2:1) and relatively intense fragment ions at M^+-45 (C_2H_5O) and M^+-73 ($C_3H_5O_2$). Both of these α-cleavage fragments readily lost Br to give the corresponding monobromo fragment ions. Ethyl *E*-2,3-dibromoacrylate (**34**) exhibits a stronger molecular ion cluster at *m/e* 256, 258, 260 (rel. int. 1:2:1) and was characterized by a strong M^+-28 (C_2H_4) fragment ion as well as intense M^+-45 and M^+-73 fragment ions. In both spectra, the base peaks resulted from α-cleavage yielding M^+-73 and loss of Br. Compounds **35–37** exhibited

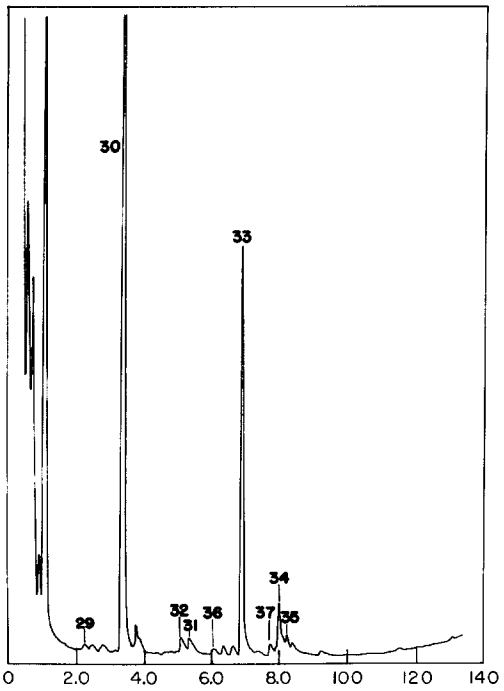
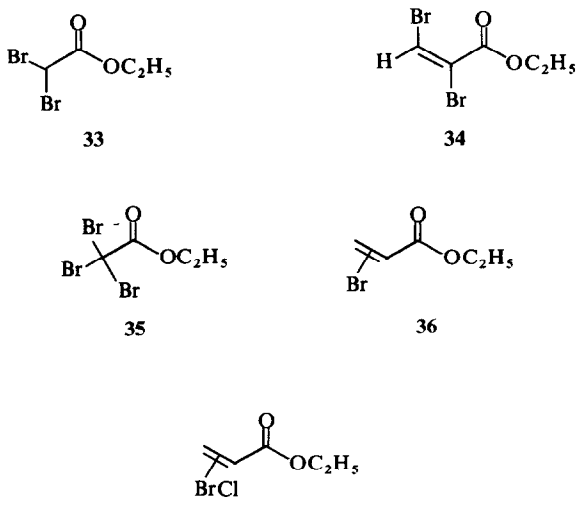


Fig. 3. *A. armata*—fresh extraction.



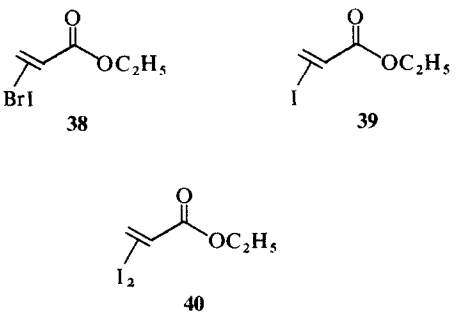
similar fragmentation patterns. To confirm the structures of the haloacrylates, **34** was synthesized. Ethyl propiolate was obtained by Jones oxidation of propargyl alcohol, followed by esterification with 95% ethanol and sulfuric acid. Addition of Br_2 in CCl_4 to the ester gave good yields of both *E* and *Z* ethyl 2,3-dibromoacrylate. Both synthetic products had identical mass spectra with that of the natural product. The natural product was assigned as the *E*-isomer by its PMR characteristics. Each synthetic isomer was represented by a singlet olefin band in the PMR spectrum at 7·04 and 8·20 ppm (taken as a mixture with polymer impurities). The natural product (**34**) corresponded to the higher field band, with a singlet measured

at 6·97 ppm (taken as a mixture with esterified fatty acids). $LiAlH_4$ reduction of naturally-occurring **34** gave *E*-2,3-dibromoallyl alcohol in high yield. The alcohol exhibited an olefin singlet at 6·27 ppm and therefore must be C-3 substituted. Since the chemical shifts for the lone olefin proton of both the ester (6.97) and the alcohol (6.27) are in accord with calculated data for *E* isomers [14], we feel confident in assigning this stereochemistry to **34**.

The fresh extraction methods outlined above were also applied to a number of samples of *A. taxiformis*. This mild isolation procedure allows for an accurate appraisal of the halogen-containing metabolites present. Three samples of *A. taxiformis* are compared here, collected from three locales encompassing a 400-mile range within the Gulf of California. In each case, the algae were handled in an identical fashion, and all GC–MS comparisons were made under identical conditions.

When samples of *A. taxiformis* collected near Isla Angel de la Guarda (29° 35′ NL) were extracted fresh with ethanol, rather than after preliminary drying, only haloforms and small amounts of ethyl esters were observed (Fig. 4). A new acrylate ester, **38**, could be easily recognized as ethyl bromoiodoacrylate from its MS fragmentations, which showed M^+-I and fragments analogous to **34**, **36**, and **37**. Column chromatography, followed by GC–MS of various column fractions, showed that ethyl monoiodoacrylate (**39**) and ethyl diiodoacrylate (**40**) are present in the algae but are obscured in Fig. 4. A major non-halogenated metabolite which was readily resolved was ethyl dodecenoate, $C_{14}H_{26}O_2$, **41**.

A collection of *A. taxiformis* from Isla Carmen (25° 59′ NL) gave interesting and comparable results (Fig. 5). All the halogen-containing compounds in the Isla Angel de la Guarda collection were recognized in this collection, with no new halogen-containing substances. The major non-halogen metabolite, **42**, was confirmed as



mesityl oxide (4-methyl-3-pentene-2-one) by mass spectral comparison with a commercial sample.

A. taxiformis collected at Cabo San Lucas (22° 80′ NL) gave somewhat different results (Fig. 6). Bromoform (**30**) and dibromochloromethane (**29**) were abundant as generally observed, but the major esters present were ethyl dibromo- and tribromoacetate, **33** and **35**. Smaller amounts of ethyl *E*-3,4-dibromoacrylate (**34**) and an as yet unrecognized ester, ethyl 2-bromo-2-iodoacetate (**43**) were observed.

The samples compared above by GC–MS (Figs 4–6) represent the variation in halogen chemistry throughout the distributional range of *A. taxiformis* in the Gulf of California. While some comparisons can be made, not surprisingly the greatest variation exists between the collections in the extreme north and south. We interpret these data to suggest that environmental features influence the biosynthesis of halogen-containing metabolites in *Asparagopsis*.

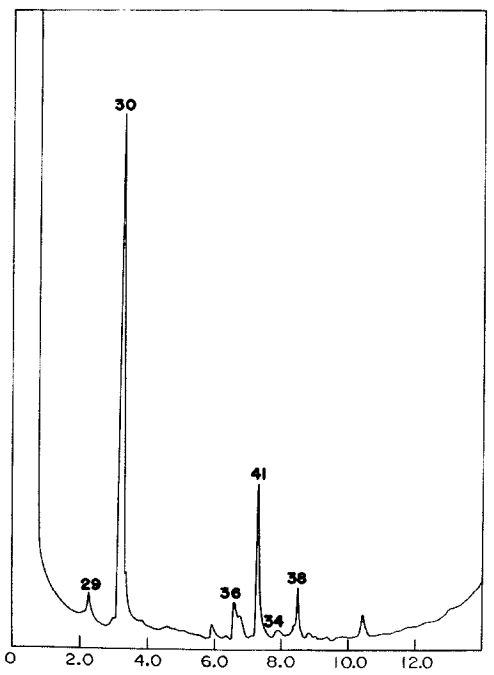


Fig. 4. *A. taxiformis*—Isla Angel de la Guarda.

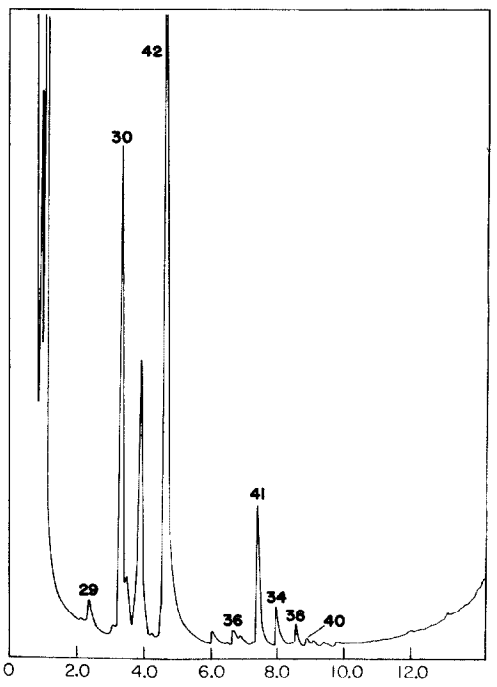


Fig. 5. *A. taxiformis*—Isla Carmen.

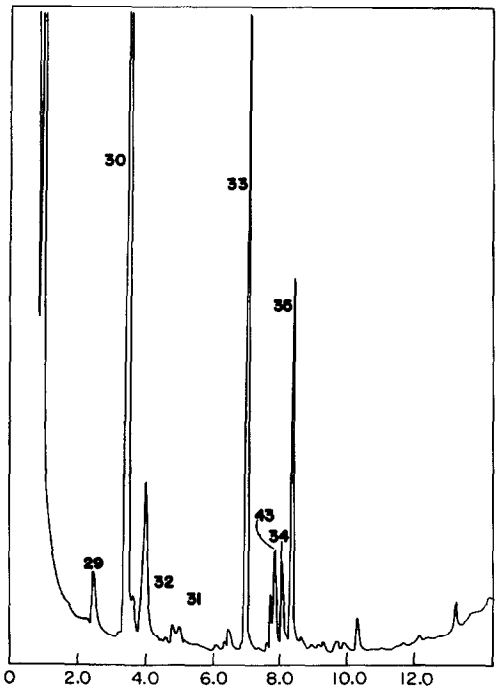


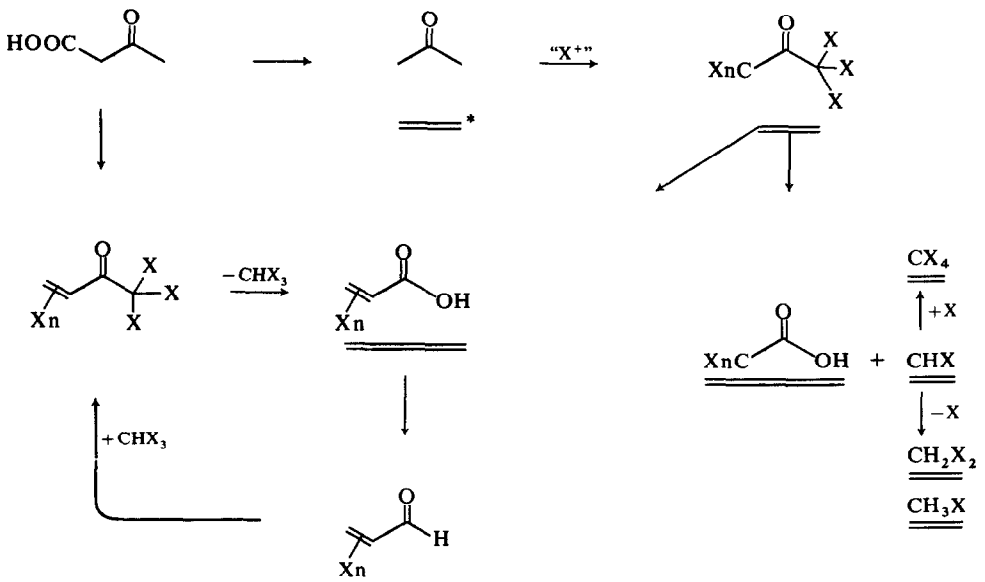
Fig. 6. *A. taxiformis*—Cabo San Lucas.

Formation of ethyl esters

The use of ethanol as an extraction solvent was a fortuitous choice with respect to the recognition of halogenated carboxylic acids as major metabolic products in *Asparagopsis*. Extracting fresh plants with chloroform or trapping volatiles by cold finger condensation yields only haloforms, acetones, and minor products of a variety of structure types. If methanol is utilized, the corresponding methyl esters are produced. In this way, the analogous methyl esters of **33**, **34**, **35**, and **43** were obtained from extracts analogous to those depicted in Fig. 6. One possible method of producing esters not involving esterification reactions is the solvolysis of 1,1,1-trihaloketones in alcohol, which could produce esters and haloforms. To discount this possibility, we treated 1,1,3,3,3-pentabromoacetone with ethanol and traces of HBr for 12 hr. GC–MC analysis showed that ethyl esters were not produced. On the other hand, treatment of bromoacetic acid under these conditions resulted in quantitative esterification.

Biogenesis in Asparagopsis

On the basis of these studies of the two major cosmopolitan *Asparagopsis* species, and considering the results of *A. taxiformis* studies from Hawaii [8], we conclude that biological haloform reactions produce extracellular haloforms, probably as environmental adaptations. With the exception of our first appraisal of *Asparagopsis* chemistry [7], halobutenones have not been recognized as significant components in our extracts, whereas haloacetones are ubiquitous in varying concentration in all collections studied. There are many possible origins for the compounds which we have observed. Logically, acetone or its precursor, acetoacetate, appears to undergo halogenation reactions analogous to those of chloroperoxidase [15]. The variety of haloacetone isomers discovered indicates that enzymatic halogenation occurs with bromine, chlorine and iodine, in that order of significance of incorporation. 1,1,1-Trihaloacetone derivatives either chemically or enzymatically react with base to yield haloforms and the corresponding acetic acids. We do not consider butenones to be the only or even the most probable source for the haloacrylic acids which are formed.



Possible routes for the production of halogenated metabolites in *Asparagopsis*. * Double underlining indicates major metabolites isolated in this study.

Rather, base rearrangements of haloacetones (Favorsky Reaction) would yield the observed acrylic acids and would also explain the prevalence of the terminal olefin in these C_3 acids. Precedence is found for facile Favorsky-type reactions with chloroacetones [16]. Halobutenones could be produced by at least two methods, from acetoacetate via a multistep reduction, dehydration and halogenation, or via a base-catalyzed condensation of haloacrylaldehydes with halomethanes. A logical precursor for the latter reaction, dibromoacrolein, was observed in low concentrations in the Hawaiian *A. taxiformis*. If butenones were produced from acetoacetate, small amounts of metabolic intermediates resulting from reduction, dehydration and halogenation should be observed. Neither esters of acetoacetate nor intermediates in this synthesis have been observed in ethanol or multisolvent extraction procedures.

Free acetone exists in appreciable amounts in the ethanol extracts of *Asparagopsis*. Instead of pentane partition, the aqueous ethanol extract of Isla de la Guarda *A. taxiformis* was investigated by fractional distillation. The forerunner to ethanol contained a sharp singlet at 2·1 ppm in its PMR spectrum and reacted readily with 2,4-dinitrophenyl hydrazine. The hydrazone obtained was identical in all respects to a standard sample of acetone 2,4-DNP. From the decant of 250 ml ethanol used to extract 300 g wet algae, 50 mg of DNP were obtained. An equivalent amount of extraction solvent, when analyzed by identical methods, did not generate measurable amounts of acetone 2,4-DNP. In the extracts of the Isla Carmen collection, mesityl oxide (**42**) was observed as a major metabolite (see Fig. 5). The formation of this product substantiates the existence of free acetone and also suggests the existence of *in vivo* base catalyzed reactions.

While the aforementioned array of halogen-containing products is probably obtained from acetate biosynthesis, it is not clear whether or not they are of catabolic origin, as are some recognized methyl ketones [17]. From the dried collection of *A. armata*, we isolated and esterified the fatty acid fractions obtained by column chromatography. β-Oxidation precursors (β-ketoesters) were not observed, and the mixture of esters obtained contained a normal distribution of plant acids. Also, no methyl ketones larger than acetone were found. Thus, we conclude that acetone and its halogen metabolism products are anabolic products of acetate metabolism.

Biological signficance

The extracts of our collections of *A. taxiformis*, as well as those from Caribbean collections [18], show considerable antibacterial activity. Extracts of *A. armata* have also been reported to be antibacterial [19]. It can be assumed, then, that the major halogenated metabolites, halomethanes, haloacetones and haloacetates, and acrylates are responsible for this toxicity. The general toxicity of low-molecular-weight halogen-containing compounds appears to be derived from their efficiencies as alkylating agents. Haloacetones, for example, are well-known enzyme inhibitors which are capable of cross-linking serine and histidine residues in various proteins [20]. Bromo- and iodoacetic acids were also shown to effectively alkylate proteins at numerous amino acid sites [20], and some halomethanes are strong biocides. Hence, it would be highly advantageous for these metabolites to be both produced and stored in isolation. Curiously, specialized cells exist in this genus which have been termed vesicular cells or 'Blasenzellen' by early European investigators [21]. These specialized cells are known to exude substances which result in a positive starch–iodine test, and it was proposed that the cells liberated the molecular halogens. We suggest that the lipid metabolites reported here, particularly the halomethanes and acetones, are responsible for these observations. Since these compounds are generally unstable, they diffuse from the vesicular cells and ultimately decompose thermally and photochemically on the plant exterior, yielding low levels of elemental halogens. A study of the gross chemical nature of the vesicular cells by X-ray fluorescence–electron microprobe methods has confirmed the high halogen content of these unusual cells [22].

The biological importance of the chemical activities of *Asparagopsis* appears related to environmental adaptation. *Asparagopsis* species generally occur in subtropical to tropical waters and are abundant in areas of extremely high algal predation.

EXPERIMENTAL

MS were recorded on the LKB 9000 and Hewlett–Packard 5930A mass spectrometer systems. Gas chromatograms (FID) and GC–MS were obtained by temp. programming; 30° to 250° at 16°/min with 2 min delay using a 2 m × 2 mm glass column packed with 3% SP-2401 and using He as the carrier gas at 60 ml/min. Figures 1–6 were obtained using these conditions.

Dry extraction. A. taxiformis (Delile) Trev and *A. armata* were air-dried at ambient temps, ground in a Wiley mill and Soxhlet extracted with $CHCl_3$ and CH_2Cl_2, respectively. The solvent was then carefully removed *in vacuo*.

Fresh extraction. Fresh algae were immediately stored in 95% EtOH and kept cold. EtOH soln was decanted and extracted with pentane, purified by treatment with aq. H_2SO_4 and $KMnO_4$ followed by distn at 36°. The pentane extract was reduced in volume by fractional distn.

Detection of I_2. I_2 was present and was produced in nearly all extracts containing halogenated compounds as evidenced by the rapid formation of a pinkish color. It was identified in the distd solvent from Si gel chromatography of both dried algae extracts by UV-$\lambda_{max}^{C_6H_6}$: 500.

A. taxiformis (*Delile*) *Trev.—dry extraction.* A collection of algae was made in the spring, 1974, near Isla Angel de la Guarda in the Gulf of California [23]. A conc $CHCl_3$ extract was chromatographed on Si gel. Fractions eluted with C_6H_6 (0.08% dry wt) were combined to yield compounds **1–11** by CG–MS analysis. The mass spectral details of these compounds were reported in an earlier communication [7].

Synthesis of haloacetones [11]. Me_2CO and chloroacetone were separately brominated by addition of 4 equivs Br_2 to a well-stirred and ice-cooled soln of ketone mixed with 48% HBr (100 ml/mol ketone). Mixtures were obtained and by CG–MS, the halogenated ketones produced were identical to the natural products, **1–7**.

Halobutenones. Bromination of but-3-yn-2-one in CCl_4 gives *Z*-3,4-dibromobut-3-en-2-one as the predominant isomer, but chlorination of methyl propiolate in CCl_4 gives a *ca* 2·3 ratio of *Z*- to *E*-methyl 2,3-dichloroacrylate [24]. Chlorination in the presence of UV light does not change this ratio, but treatment of *E*-3,4-dibromobut-3-en-2-one with heat or LiBr in HOAc gives a facile isomerization to the *Z*-isomer [8]. The dibromobutenone equilibrium thus appears to favor the *Z*-isomer, while the dihalo-acrylate shows a more equal isomer distribution (see later details regarding ethyl 2,3-dibromoacrylate).

A. armata—*dry extraction.* A collection of this alga was made on June 30, 1975 at La Escala, Spain [23] (along the Mediterranean coast). The CH_2Cl_2 extract of 2.25 kg ground algae yielded

16 g. To obtain Fig. 2, a portion of this extract was passed quickly over Si gel with 100% Et_2O. Another portion of the crude extract (13.0 g) was also chromatographed on Si gel (5.5 × 100 cm). Halogenated methanes were eluted with 100% petrol. Increasing the solvent polarity to 10% C_6H_6 in petrol began eluting the halogenated acetones. At 100% C_6H_6, all of the halogenated acetones and tetrabromobutenone (**11**) were eluted. Combined haloacetone fractions yielded *ca* 6 g. Low resolution mass spectral data of the additional halogenated acetones not found in *A. taxiformis* yielded the following (50 eV); **12**—126[M^+] (10, Cl_2), 77 (100, Cl), 49 (49, 75); **13**—160[M^+] (1, Cl_3), 83 (21, Cl_2), 77 (100, Cl), 49 (43, Cl); **14**—194[M^+] (2, Cl_4), 111 (21, Cl_2), 83 (100, Cl_2); **15**—204[M^+] (3, $BrCl_2$), 121 (100, Br), 93 (32, Br), 83 (25, Cl_2); **16**—204[M^+] (3, $BrCl_2$, 127 (14, BrCl), 77 (100, Cl), 49 (29, Cl); **17**—238[M^+] (5, $BrCl_3$), 155 (5,4 BrCl), 127 (100, BrCl), 111 (18, Cl_2), 83 (63, Cl_2); **18**—282[M^+] (3, Br_2Cl_2), 199 (75, Br_2), 171 (88, Br_2), 120 (100, Br), 111 (13, Cl_2), 83 (50, Cl_2); **19**—282[M^+] (3, Br_2Cl_2), 155 (50, BrCl), 127 (75, BrCl), 120 (100, Br); **20**—218[M^+] (7, ClI), 169 (43, I), 141 (21, I), 77 (100, Cl), 49 (14, Cl); **21**—340[M^+] (2, Br_2I), 171 (18, Br_2), 169 (100, I), 141 (27, I), 127 (36, I). *Haloacetaldehyde.* **22**—122[M^+] (26, Br), 94 (100, Br), 93 (22, Br), 79 (20, Br); **23**—200[M^+] (2, Br_2), 172 (20, Br_2), 171 (2, Br_2), 120 (4, Br), 93 (100, Br), 79 (26, Br). The mass spectra of these two haloacetaldehydes were identical to GC–MS of a mixture of synthetic compounds obtained by treating acetaldehyde, neat, with two equivalents of Br_2.

A. armata—*fresh extraction.* The EtOH decant (50 ml) of a sample of fresh algae was extracted 5 × 50 ml with purified pentane. The pentane was very carefully reduced in vol. by distn to *ca* 20 ml. This solution was then examined both by EC gas chromatography and GC–MS.

(a) *EC analysis—halomethanes.* The EC data were obtained using a 6.1 m × 3.0 mm stainless steel column packed with 16.5% Si oil DC-550. The carrier gas (He) flow was 81 ml/min and the column was run at 60°. Compounds detected exclusively by EC–GC methods were MeI (**24**), $CHCl_3$ (**26**), CCl_4 (**27**), and $CHBrCl_2$ (**28**). These compounds were identified by comparing the retention time with that of standards.

(b) *GC–MS analysis—halomethanes.* Under conditions previously described, CH_2ClI (**25**), $CHBr_2Cl$ (**29**), $CHBr_3$ (**30**), CBr_4 (**31**), and $CHBr_2I$ (**32**) were identified in this extract by GC–MS. Compounds **30** and **31** were identical to commercial samples. The low resolution mass spectral data yielded the following (50 eV); **25**—176[M^+] (29, ClI), 141 (19, I), 127 (40, I), 49 (100, Cl); **29**—206[M^+] (2, Br_2Cl), 127 (100, BrCl), 91 (17, Br), 79 (30, Br); **30**—250[M^+] (5, Br_3), 171 (100, Br_2), 158 (5, Br_2), 91 (20, Br), 79 (20, Br); **31**—328[M^+] (< 1, Br_4), 249 (100, Br_3), 171 (28, Br_2), 91 (22, Br), 79 (28, Br); **32**—298[M^+] (17, Br_2I), 219 (10, BrI), 171 (100, Br_2), 127 (50, I).

(c) *GC–MS analysis—ethyl esters.* Low resolution mass spectral data (50 eV): **33**—244[M^+] (< 1, Br_2), 199 (20, Br_2), 172 (93, Br_2), 171 (93, Br_2), 120 (80, Br), 93 (100, Br), 79 (33, Br); **34**—256[M^+] (4, Br_2), 228 (21, Br_2), 211 (64, Br_2), 183 (18, Br_2), 133 (21, Br), 104 (100, Br); **35**—322[M^+] (≪ 1, Br_3), 277 (< 1, Br_3), 249 (26, Br_3), 199 (16, Br_2), 198 (8, Br_2), 171 (15, Br_2), 170 (42, Br_2), 119 (83, Br), 107 (63, Br), 91 (100, Br); **36**—178[M^+] (1, Br), 150 (18, Br), 133 (100, Br), 105 (41, Br); **37**—212[M^+] (1, BrCl), 184 (15, BrCl), 177 (60, BrCl).

Synthesis of ethyl 2,3-dibromoacrylate, **34**. To 0.75 g of propargyl alcohol in 25 ml Me_2CO which was cooled to 0° was added dropwise with stirring Jones reagent until the soln remained slightly orange-brown. After stirring an additional hr at room temp., the reaction mixture was worked up. NMR (60 MHz, $CDCl_3$): δ 3·18 (1H, *s*), δ 10·2 (1H, *s*). To 0·44 g of the acid was added 1.3 ml 95% EtOH and 0.13 g H_2SO_4 (conc). The mixture was stirred for 20 hr at room temp. and 2 hr at 60°. NMR (60 MHz, CCl_4): δ1.3 (3H, *t*), δ 2.8 (1H, *s*), δ 4.3 (2H, *g*). Stoichiometric bromination using Br_2 with 0.04 g of ethyl propiolate in 5 ml CCl_4 at room temperature for 5 min gave, after work-up, a 3:2 mixture of *Z*- to *E*-ethyl 2,3-dibromoacrylate, and bromination of the same amount in 35 ml CCl_4 at 0° for 30 min gave a 1:1 mixture. GC–MS of this mixture of dibromo-acrylates showed that both products had identical mass spectra with that of the natural product. NMR (60 MHz, CCl_4): δ 1.31 (3H, *t*), δ 4.3 (2H, *q*), *Z*-δ 8.20 (1H, *s*), *E*-δ 7.04 (1H, *s*).

LAH Reduction of **34**. Two different Si gel column chromatography fractions of *A. taxiformis* from Isla Angel de la Guarda stored in 95% EtOH were reduced in Et_2O with LAH at room temp. under N_2. These fractions contained only ethyl 2,3-dibromoacrylate and differing amounts of esterified fatty acids. The IR of both reaction product mixtures showed the complete disappearance of the broad carbonyl band centered at 1740 cm^{-1} and the appearance of a strong broad hydroxyl absorbance centered at 3150 cm^{-1}. In the first case the PMR singlet of ethyl 2,3-dibromoacrylate shifted from δ 6.97 to a singlet at δ 6.27 in 2,3-dibromoallyl alcohol and in the second case the singlet shifted from δ 6.96 to δ 6.33. These chemical shifts and the changes of chemical shifts to higher field are consistent with the predicted values of ethyl *E*-2,3-dibromoacrylate being reduced to *E*-2,3-dibromoallyl alcohol [14].

A. taxiformis—*Isla Angel de la Guarda—fresh extraction.* The fresh algae stored in 95% EtOH was worked up by the procedure described previously. This extract revealed the presence of ethyl bromoiodoacrylate, a previously undescribed compound. GC–MS gave the following (50 eV): **38**—304[M^+] (4, BrI), 276 (10, BrI), 259 (20, BrI), 231 (4, BrI), 153 (74, I), 127 (100, I). Instead of decanting the 95% EtOH, a large portion of this alga was homogenized using a Waring blender and heated to reflux for 2 hr. The filtrate was reduced in volume *in vacuo* and chromatographed on Si gel. Fractions eluted with 5% C_6H_6 in petrol to 100% C_6H_6 yielded varying amounts of halogenated ethyl acrylates and esterified fatty acids. The new halogenated ethyl acrylates were recognized, iodo-, **39**, and ethyl diiodoacrylate, **40**. GC–MS yielded the following (50 eV): **39**—226[M^+] (27, I), 198 (37, I), 181 (93, I), 153 (53, I), 127 (100, I); **40**—352[M^+] (6, I_2), 324 (2, I_2), 307 (4, I_2), 153 (87, I), 127 (100, I). The following two collections were made in April, 1975 and April, 1976, respectively.

A. taxiformis—*Isla Carmen—fresh extraction.* The pentane partition of this fresh 95% EtOH extract gave a significant amount of mesityl oxide, **42**, which was identical to a commercial sample by GC–MS (50 eV): **42**—98[M^+] (15.6), 83 (48.4), 55 (100.0), 43 (70.3), 39 (54.7).

A. taxiformis—*Cabo San Lucas—fresh extraction.* The pentane partition resulted in a new compound, ethyl 2-bromo-2-iodoacetate, **43**. GC–MS gave the following (50 eV): 292[M^+] (33, BrI), 247 (11, BrI), 219 (40, BrI), 165 (49, Br), 127 (100, I), 120 (49, Br), 109 (55, Br), 92 (76, Br).

Solvolysis of 1,1,3,3,3-pentabromoacetone in EtOH. A collection of *A. taxiformis* was collected in April, 1976 at Isla San Jose in the Gulf of California and stored fresh in $CHCl_3$. Algae were homogenized and heated at 50° for 1 hr in $CHCl_3$. The filtrate was carefully concd *in vacuo* and passed quickly over Si gel with 100% Et_2O. By GC–MS, this extract was comprised of 1,1,3,3-tetrabromoacetone, **3**, 1,1,1,3-tetrabromoacetone, **44**, 1,1,3,3,3-pentabromoacetone, **45**, 1,1,3-tribromoacetone, **2**, and bromoform, **30**, in order of abundance. 1 g of this extract was treated with 60 ml 95% EtOH, both with and without 1 ml of 48% HBr, for 12 hr at room temp. At the end of this time there was very little relative change in the abundance of these compounds as evidenced by GC (FID) and GC–MS. During this same time period, 0.139 g bromoacetic acid, obtained by hydrolyzing bromoacetyl bromide, was quantitatively esterified by treatment with 0.1 ml 48% HBr and 30 ml 95% EtOH at room temp.

Characterization of Me_2CO in A. taxiformis. 250 ml of decanted 95% EtOH soln containing *A. taxiformis* from Isla de la Guarda was fractionally distd (in thoroughly-dried glassware) to yield a forerun of 50 ml with bp 71–76°. This forerun was treated with 2,4-dinitrophenylhydrazine and the resultant solid mixture yielding 50 mg of dimethyl-2,4-dinitrophenyl-hydrazone after PLC (Si gel, 1500 μm, petrol–CH_2Cl_2 (1:1)). This material was identical by NMR and IR to an authentic 2,4-DNP derivative of Me_2CO. An equivalent amount of Rossville (Gold Shield) 95% EtOH was treated identically but no hydrazones were formed.

Attempt to locate β-ketoacids and methyl ketones in A. armata—

dry extraction. The dry extract of *A. armata* was investigated further by Si gel column chromatography after the halomethanes, haloacetones and tetrabromobutenone had been eluted to find β-ketoacids and methyl ketones. Fractions eluted with 1% Et_2O in C_6H_6 to 50% Et_2O in C_6H_6 were examined because they contained similar TLC R_f values when compared to fatty acid standards such as caproic and palmitic acid. No methyl ketones were observed by NMR in these or any previously eluted fractions. Portions of these fractions were combined to yield 0.1 g. This mixture was esterified by adding 0.7 ml H_2SO_4 (conc) and 2 ml MeOH and heating at 60° for 30 min. After work-up, a mixture of methyl esters was purified by PLC (Si gel, 1500 μm, Et_2O–C_6H_6 (1:1) $R_f \geqslant 0.4$). NMR (60 MHz, CCl_4): δ 3.65, *s*. This mixture was invetigated by GC (FID) and GC–MS. (Conditions: same column as used previously, run at 160° (iso) with He flow = 60 ml/min). By comparison with standard fatty acid methyl ester N.I.H. mixtures E (caprylate, caproate, laurate, myristate, palmitate) and F (myristate, palmitate, stearate, arachidate, behenate, lignocerate) from Applied Science Labs, Inc., the naturally occurring mixture contained the following fatty acid methyl esters: palmitate (100%); myristate (25%), stearate (14%), and laurate (5%). The odd-numbered fatty acids, C-17 (12%) and C-15 (2%) were present, but in typically smaller quantities. Interestingly, only saturated fatty acid esters were observed.

Acknowledgements—We thank Dr. Chih-Wu Su for analysis of volatile halomethanes by EC–GC. We thank the National Science Foundation for supporting this work under grant DES75-03824 and by provision of ship funds for support of R/V DOLPHIN in the Gulf of California. Our use of the mass spectral and NMR facilities of the Chemistry Department, UC-San Diego, supported under an NIH grant (RR-708) is gratefully acknowledged. A major portion of this research was sponsored by NOAA, Office of Sea Grant, Department of Commerce, under grant 04-3-158-22. The U.S. Government is authorized to produce and distribute reprints for governmental purposes, notwithstanding any copyright notation that may appear hereon.

REFERENCES

1. Robertson, D. (1896) *Trans. Nat. Hist. Soc. Glasgow* **41**, 172.
2. Fenical, W. (1975) *J. Phycol.* **11**, 245, and references therein.
3. Two recent reports extend the list of halogenating red algae to two new genera. See Fenical, W., Finer, J. and Clardy J. (1976) *Tetrahedron Letters* 731, and Crews, P., Ng, P., Kho-Wiseman, E. and Pace, C. (1976) *Phytochemistry* **15**, 1707.
4. Pedersen, M. and DaSilva, E. J. (1973) *Planta* **115**, 83.
5. Högberg, H.-E., Thomson, R. H. and King, T. J. (1976) *Chem. Commun.* 1696.
6. Siuda, J. F., Van Blaricom, G. R., Shaw, P. D., Johnson, R. D., White, R. H., Hager, L. P. and Rinehart, K. L., Jr. (1975) *J. Am. Chem. Soc.* **97**, 937.
7. Fenical, W. (1974) *Tetrahedron Letters* 4463.
8. Burreson, B. J., Moore, R. E. and Roller, P. (1975) *Tetrahedron Letters* 473.
9. Nakanishi, K. (1962) *Infrared Absorption Spectroscopy*, p. 42. Holden-Day, San Francisco.
10. McLafferty, F. W. (1962) *Interpretation of Mass Spectra.* W. A. Benjamin, New York.
11. Rappe, C. (1963) *Arkiv. Kemi* **21**, 503.
12. Lovelock, J. E., Maggs, R. J. and Wade, R. J. (1973) *Nature* **241**, 194. Also see Lovelock, J. E. (1975) *Nature* **256**, 193.
13. Lovelock, J. E. (1974) *Nature* **252**, 292.
14. Pasto, D. J. and Johnson, C. R. (1969) *Organic Structure Determination.* Prentice-Hall, Englewood Cliffs, N.J.
15. Morris, D. R. and Hager, L. P. (1966) *J. Biol. Chem.* **241**, 1763.
16. Wagner, W. M., Kloosterziel, H. and Bickel, A. F. (1961) *Rec. Trav. Chim.* **81**, 933.
17. Dartey, C. K. and Kinsella, J. E. (1973) *J. Agr. Food Chem.* **21**, 721. and references cited therein.
18. Burkholder, P. R., Burkholder, L. M. and Almodovar, L. R. (1960) *Bot. Marina* **2**, 149. Also see Starr, T. J., Kajima, M. and Piferrer, M. (1966) *Texas Rep. Biol. Med.* **24**, 208.
19. Hornsey, I. S. and Hyde, D. (1974) *Br. Phycol. J.* **9**, 353.
20. Freedman, R. B. (1961) *Quart, Revs* **25**, 431.
21. Kylin, H. (1915) *Arch. Bot.* **14**, 1.
22. Wolk, C. P. (1968) *Planta* **78**, 371.
23. Voucher specimens of *A. taxiformis* and *A. armata* have been deposited in the National Herbarium, Smithsonian Institution, Washington, D.C.
24. Kurtz, A. N., Billups, W. E., Greenlee, R. B., Hamil, H. F. and Pace, W. T. (1965) *J. Org. Chem.* **30**, 3141.