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Secondary Metabolites from Marine Bryozoans. A review

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Secondary metabolites from marine bryozoans are reviewed. Two ctenosome bryozoans are dealt with, one, *Alcyonidium gelatinosum* containing a sulfoxonium ion acting as hapten in an allergic contact dermatitis and the other, *Zoobotryon verticillatum* yielding bromogramines. Five cheilostome bryozoans have given rise to the isolation of unique secondary natural products. *Bugula neritina* is the source of the antineoplastic bryostatins and *Bugula* purple while *Flustra foliacea* have yielded an array of bromoindole alkaloids and a brominated quinoline. *Chartella papyracea* also have bromoindole alkaloids while *Sessibugula translucens* have ecological active bipyrroles. A biological active xanthine derivative has been reported from *Phidolopora pacifica*. The structure and chemistry of these compounds are discussed as are their origin, function and biological activity.

Bryozoans (syn. Ectoprocta, Polyzoa) or moss animals are sedentary colonial filter-feeding organisms widely distributed throughout the world's marine and freshwater environments. There are about 4000 extant species and several times as many are known from the fossil record from the early Palaeozoic onwards. The bryozoan colony consists of a large number of tiny intercommunicating individuals, the zooids. The colony varies in size, shape and texture in the different species. Many bryozoans are common fouling organisms on marine facilities.[1]

Systematically phylum Bryozoa is divided into three classes, Phylactolaemata, the members of which are exclusively freshwater forms, Stenolaemata which are fossil except for some members of the order Cyclostomata, and Gymnolaemata being predominantly marine. Gymnolaemata encompass two orders Ctenostomata and Cheilostomata comprising 3000 or more living species.[1] A compilation of references to papers dealing with all aspects of bryozoan studies is published currently.[2]

Most accounts of secondary metabolites of bryozoans date back from before the introduction of modern separation and structure determination techniques. Consequently most of the earlier work needs reinvestigation. An example of a modern investigation is the identification of the apocarotenoid hopkinsiaxanthin from *Eurystomella bilabiata*.[3] Incidentally many ova and embryos in Cheilostomata and Ctenostomata are brightly colored due to carotenoids. Embryo color has been an useful taxonomic marker but the structures of the carotenoids are unknown.[4]

Apart from the above mentioned, extremely few accounts of the chemistry of bryozoans have appeared. As a result only very few secondary metabolites are known from this large

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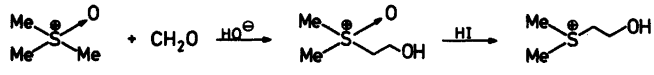
animal group and the ones isolated all originate from marine members of the two orders of Gymnolaemata.

The acceleration of chemical studies of bryozoans is nicely illustrated by the fact that two alkaloids were known from bryozoans in 1979,[5] nine in 1982,[6] and 15 in 1983.[7]

CTENOSTOMATA

Ctenostomata, being by far the smallest of the two orders, have two species, namely *Alcyonidium gelatinosum* (L.) and *Zoobotryon verticillatum* (Delle Chiaja, 1828) from which interesting although totally unrelated compounds have been isolated.

An eczematous allergic contact dermatitis named Dogger Bank itch caused by exposure to *A. gelatinosum* is a major occupational hazard to fishermen working in the North Sea area. Contact is established when handling the catch. The organism is so abundant in parts of the North Sea that the bryozoans alone may form a pile several feet across when the trawler nets are emptied.[1] Repeated exposure frequently provokes a sensitization, which may develop into hypersensitization. The dermatitis may evolve into a totally disabling state. Accordingly the disease was included in the Danish Workmen Compensation Act in



1 *2*

1939. The diagnosis was severely hampered prior to 1980, when the causative agent was isolated and identified.[8] The structure of the hapten was inferred from extensive analytical and spectroscopic studies as (2-hydroxyethyl)dimethylsulfoxonium ion (*1*). Reduction yielded the well known sulfocholine (*2*). This is the first example of a naturally occurring sulfoxonium compound, a class of substances only known from a few synthetic studies.[8]

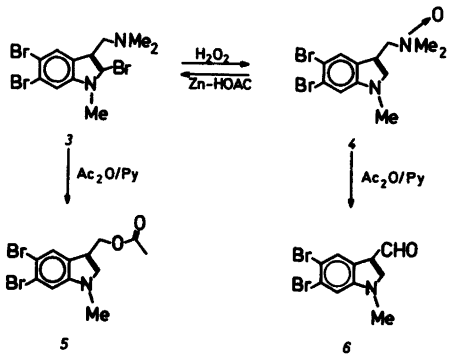
Since the yield of pure hapten is only about 5 ppm based on wet weight, a synthetic approach had to be developed. The preparation of this simple but unique natural product posed severe problems but was eventually accomplished by carefully controlled base catalyzed addition of formaldehyde to trimethylsulfoxonium chloride.[9] The chemical and medical aspects of these findings have been discussed.[10–12]

Access to the purified hapten has allowed the study of this type of allergy. It was shown that the reaction belongs to the delayed type cell-mediated hypersensitivity (type 4 reaction). Sensitizations of this type are irreversible and no prophylactic or therapeutic treatment is known.

At present the disease seems to be spreading. A thorough investigation of the incidence and mechanism of this severe occupational hazard is now possible.

The tropical cosmopolitan species, *Zoobotryon verticillatum* has yielded two simple bromogramine derived alkaloids namely 2,5,6-tribromo-*N*-methylgramine (*3*) and the corresponding side chain *N*-oxide (*4*).[13]

The alkaloids were present in 7.5×10^{-3} % (*3*) and 11.4×10^{-3} % (*4*) of dry weight, respectively. The structural elucidation was carried out spectroscopically. The structural assignments were confirmed by synthesis starting from gramine, which was *N*-methylated in 75 % yield and the resulting *N*-methylgramine brominated in 15 % yield giving the



2,5,6-tribromo derivative (*3*) as the major product, although in low yield. The bromogramine *3* gave a quantitative yield of *4* on hydrogen peroxide oxidation and conversely *4* could be reduced to *3*. Interestingly enough attempts to acetylate *3* afforded the primary acetate *5* and in the case of *4* the carbaldehyde *6*.

These findings are of interest in a taxonomical context since bromoindole alkaloids have been isolated from the totally unrelated *Flustra foliacea* (L.) of order Cheilostomata (*vide infra*).

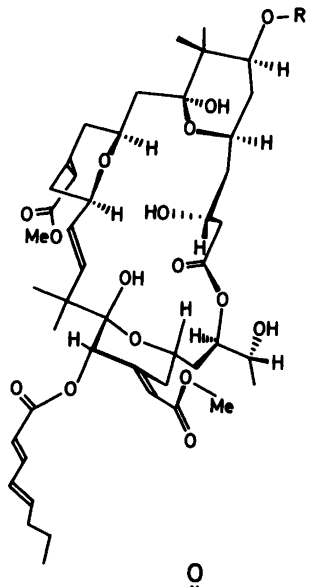
The *Zoobotryon* alkaloid *3* inhibits cell division in the fertilized sea urchin egg ($ED_{50} \cong 16$ μg/ml).

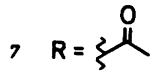
CHEILOSTOMATA

Cheilostomata are represented by the marine bryozoan *Bugula neritina* (L.). This species was formally described in the 10th edition of *Systema Naturae* in 1758, only three years after the appearance of *Natural History of the Corallines* by the London merchant John Ellis. The latter publication once and for all terminated more than 150 year's dispute and placed the corallines (bryozoans and coelenterates) in the animal kingdom.[1] The very cosmopolitan species *B. neritina* is of considerable economic importance being a common fouling organism on ships hulls and other marine facilities. Since many bryozoans are among the most resistant organisms to copper ions, a widely used anti-fouling component of marine coatings, fouling by these organisms constitutes a serious problem.

Early investigations of *B. neritina* deal with the structure of a purple pigment, believed to be the first recorded chemical study of bryozoan coloring matter.[14] This is not entirely true since a pigment, presumably chlorophyll, was reported as early as 1887 from *Flustra foliacea*, and, more important, *Bugula* purple was described at least as early as 1903 from *B. neritana* (presumably a misspelling of *B. neritina*).[15]

The pigment was described as adenochrome-like because the physical and chemical properties resemble those of adenochrome from the branchial heart of the common octopus, *Octopus vulgaris*. However, an absorption maximum was observed at 545 nm as compared to 505 nm for adenochrome.[14] A preliminary investigation on material collected around Okinawa indicated that the pigment contains sulfur.[16] The resemblance to adenochrome must then at best be superficial and the structure elucidation of this interesting pigment will have to await future studies.

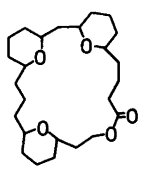


7 R = 

9 R = H

Recently *B. neritina* has attracted considerable interest due to the exciting discovery of metabolites with exceptionally high levels of antineoplastic activity. The first of these to be reported, bryostatin 1 (*7*), in a dose level of 10–70 μg/(kg/injection dose) shows 52–96 % life extension in the murine P388 lymphocytic leukemia system, ED_{50} of 0.89 μg/ml against P388 *in vitro* cell line,[18] 34–51 % life extension at 37.5–150 μg/kg in L1210 lymphocytic leukemia, 40–48 % life extension at 5–20 μg/kg in M5076 ovarian carcinoma, and 20–65 % curative effect in the tumor regression model at 20–40 μg/kg.[19] The structure elucidation of bryostatin 1 (*7*) was a result of spectroscopic and crystallographic studies.

The relative stereochemistry of bryostatin 1 is depicted in 7, which also shows the most likely absolute configuration. Bryostatin 1 is based on an unprecedented 26-membered ring system – the bryopyran system, *8*. In the crystalline state bryostatin 1 exhibits a large oxygen-rich cavity suggesting that the molecule may have cation binding capabilities comparable to the polyether antibiotics.



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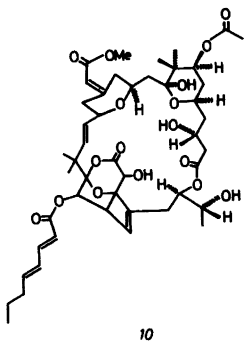

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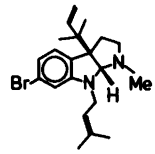

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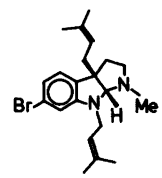

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The bryozoan contains a further sixteen exceptionally active macrolides. Spectroscopic studies of bryostatin 2 (*9*) revealed this compound to be the deacetyl analogue of bryostatin 1 (*7*).[20] In the PS system (The National Cancer Institute's murine P-388 lymphocytic leukemia system) bryostatin 2 exhibits 60 % increase in life-span at 30 μg/kg, a remarkably high activity. At the same dose a life extension of 63 % was observed in the latter system for bryostatin 3 (*10*). The structure was inferred from spectroscopic studies.[20]

The yield of bryostatin 1 (*7*) is unpublished but bryostatin 2 (*9*) and bryostatin 3 (*10*) were isolated from 500 kg wet bryozoans in 6.2×10^{-5} and 1.6×10^{-5} % respectively (the yields are given as 6.2×10^{-7} and 1.6×10^{-7} % but this must be a misprint, since the actual material isolated was 314.5 mg[20] and 81.5 mg[19] from 500 kg wet weight). The biogenesis is unknown but it has been suggested that the bryopyran ring system is acetate derived with the gem-dimethyl groups at C-8 and C-18 originating from methionine.[20]

Another cheilostome bryozoan, namely *Flustra foliacea* (L.), has been studied. The lemon-like odor characteristic of this organism was shown to be due to a mixture mainly of geraniol with *cis*- and *trans*-citral and small amounts of citronellol and nerol.[21] This mixture was tested for antifouling activity under field conditions and found to display activity comparable with commercially available additives.[22] At this point the investigations were discontinued due to the appearance of a patent[23] covering the commercial exploitation of these compounds.

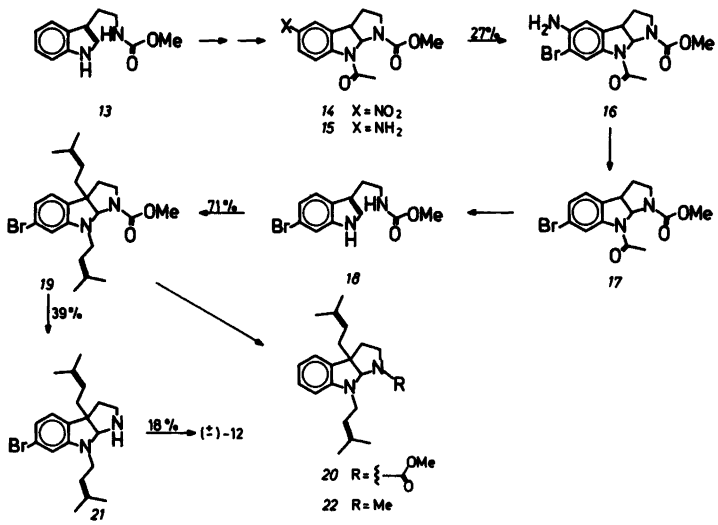
The older parts of the fronds of *F. foliacea* are known to exhibit antibiotic activity.[24] This activity has not yet been fully investigated but bioactive metabolites have emerged. As a result of spectroscopic studies two indole alkaloids, flustramine A and flustramine B, were identified as 3a,8a-*cis*-1-methyl-3a-(2-methyl-3-buten-2-yl)-6-bromo-8-(3-methyl-2-buten-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*11*) and 3a,8a-*cis*-1-methyl-3a,8-bis(3-methyl-2-butenyl)-6-bromo-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*12*).[25,26] These two alkaloids exhibit muscle relaxant activity *in vivo* as well as *in vitro* affecting both skeletal and smooth muscle.

Flustramine A and B caused 50 % inhibition in the amplitude of the contraction of electrically stimulated rat diaphragms at a dose level of 59 and 63 μg/ml. The effects were not antagonized by synstigmine. The electrically induced twitches of the isolated guinea-pig ileum was 50 % inhibited by concentrations of 69 and 71 μg/ml of flustramine A and B respectively. The non-stimulated ileum was not affected but the histamine evoked twitches were reduced. In the latter test the crude extract exhibited stronger activity (50 % inhibition at 45 μg/ml) than pure *11* and *12* meaning presumably that some of the minor alkaloids have pronounced activity. In mice the minimum lethal dose of crude extract is 500 mg/kg with symptoms of respiration paralysis.[27]

The crude petroleum ether extract of *F. foliacea* strongly inhibits plaque formation (>90 %) for influenza virus (WSN strain) in a concentration of 100 μg/ml.[28]

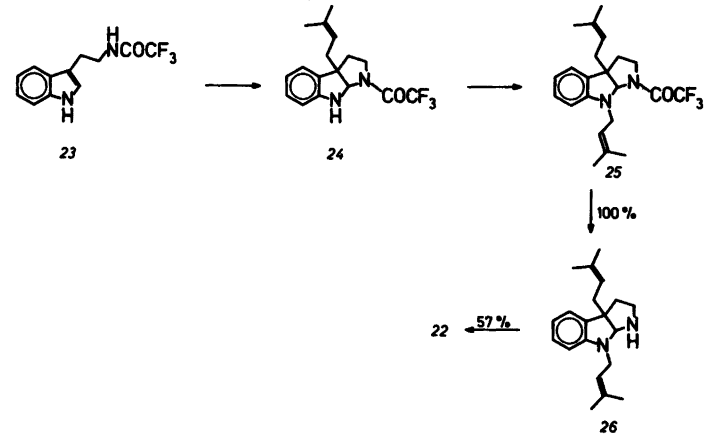
The structure elucidation of *11* and *12* was carried out on samples isolated from bryozoans collected in the North Sea. Flustramine A and B were present in equal amounts and the combined yield was 0.07 % of dry weight. The pharmacological studies were based on material collected at the northern Swedish west-coast at a depth of 10 m. The latter material gave almost twice as high yield of the two alkaloids (combined 0.16 %). Whether the difference in yields is due to seasonal or geographical variations or some as yet unknown factor has not been investigated.

A synthesis of (±)-flustramine B (*12*) has been carried out (Scheme 1).[29] Starting from N_b-methoxycarbonyltryptamine (*13*) the 5-nitropyrroloindole *14* was produced. Catalytic

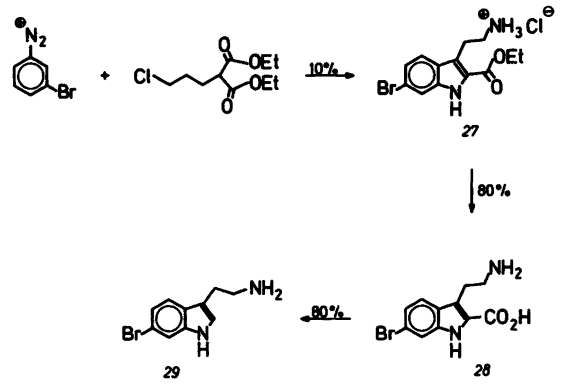


Scheme 1.

reduction of *14* gave the corresponding 5-amino derivative *15*, which could be brominated with NBS in dimethylformamide to give *16* in 27 % yield. Deamination of *16* was affected by reaction with excess isoamylnitrite to give *17*. The synthesis of *17* from *14* could be carried out in an overall yield of 60 % without purification of the intermediates. Acid catalyzed ring opening of *17* gave the 6-bromotryptamine *18* in excellent yield. Excess dimethylallyl bromide afforded 71 % diprenylated pyrroloindole *19* as an oil. Under comparable conditions *13* gave 75 % yield of *20*. Base catalyzed hydrolysis of *19* gave *21* in 39 % yield. On methylation with methyl iodide at room temperature *21* gave an 18 % yield of (±)-flustramine B (*12*). Debromoflustramine B (*22*) was isolated in excellent yield by lithium aluminium hydride reduction of *19*. Another synthetic approach to (±)-debromoflustramine B (*22*) has appeared (Scheme 2).[30] Treatment of N_b-trifluoroacetyltryptamine



Scheme 2.



Scheme 3.

(*23*) with γ,γ-dimethylallylbromide gave after purifiifaction of the reaction mixture 5.6 % of *24*, 4.8 % *25* and 28 % unreacted *23*. Sodium borohydride reduction of *25* gave a quantitative yield of *26* which could be methylated by reaction with formaldehyde and sodium cyanoborohydride in 57 % yield. The analogous reactions with N_b-acetyltryptamine and N_b-methoxycarbonyltryptamine (*13*) were abandoned due to difficulties in removing the protecting groups. The latter publication records the ^{13}C NMR and UV spectroscopic details of the derivatives treated.

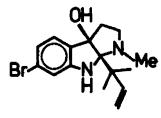
Obviously 6-bromotryptamine is a key starting material in the synthesis of these alkaloids. The preparation of 6-bromotryptamine from 3-bromoaniline in an overall yield of 6.4 % has been reported (Scheme 3).[31] Japp-Klingemann reaction of diethyl 3-chloropropylmalonate with 3-bromophenyldiazonium ion prepared by diazotation of 3-bromoaniline obtained by reduction of 3-bromonitrobenzene gave a 10 % yield of indole *27*. Saponification of *27* produced *28* in 80 % yield, the same yield as obtained in the acid catalyzed decarboxylation of *28* to give 6-bromotryptamine (*29*). The spectroscopic properties (^1H NMR, ^{13}C NMR, and UV) are reported for *27, 28,* and *29*. Another route to *29* is *via* the reaction of 6-bromogramine, prepared from 6-bromoindole with nitromethane followed by catalytic reduction of the 6-bromo-3-(2-nitroethyl)indole.[32] 6-Bromotryptamine (*29*) in mixture with 4-bromotryptamine was obtained from the reaction of 2-methoxy-*N*-benzoylpyrrolidine with 3-bromophenylhydrazine followed by saponification.[32]

Flustramine C (3×10^{-4} % of dry weight), flustraminol A (6×10^{-4} % of dry weight), and flustraminol B (8×10^{-5} % of dry weight) all originate from the same organism and all possess the physostigmine skeleton of *11* and *12* but represent a two-equivalents higher oxidation state than these. The structures were determined by spectroscopic studies[33] as 1-methyl-3a-(2-methyl-3-buten-2-yl)-6-bromo-1,2,3,3a-tetrahydropyrrolo[2,3-*b*]indole (*30*), 1-methyl-3a-hydroxy-6-bromo-8a-(2-methyl-3-buten-2-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*31*), and 1-methyl-3a-hydroxy-6-bromo-8-(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*32*), respectively. Flustramine C could be reduced (lithium aluminum hydride) to debromo-8,8a-dihydroflustramine C (*33*) in 87.5 % yield. Interestingly enough, 8,8a-dihydroflustramine C (*34*) was recently isolated from a sample of *F. foliacea* collected in Minas Basin, Nova Scotia.[34] The yield was about 3×10^{-3} % of wet weight which is presumably equal to about 0.03–0.05 % of dry weight – a figure very much comparable to the amount found for flustramine A and B from specimens collected

around Scandinavia. The structure was identitifed as 1-methyl-3a-(2-methyl-3-buten-2-yl)-6-bromo-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*34*) as a result of spectroscopic studies. Dihydroflustramine C showed strong activity against *Bacillus subtilis*. The Scandinavian specimens also showed mass spectrometric evidence for the presence of *34*, however, the concentration must have been lower in this material.[35] Flustramide A (*35*), isolated in about 5×10^{-3} % yield, is formally related to flustramine A (*11*) by oxidation of the methylene group at position 2 forming a γ-lactam.[36] Spectroscopic studies defined the structure as 3a,8a-cis-1-methyl-2-oxo-3a-(2-methyl-3-buten-2-yl)-6-bromo-8-(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole (*35*).

In most cases the 3a,8a-*cis* junction of the hexahydropyrrolo[2,3-*b*]indole system was inferred from ^{1}H-[^{1}H] Nuclear Overhauser Enhancement-difference (NOE)-difference spectroscopic studies, *e.g.* in the case of flustramine A (*11*) and flustramide A (*35*) the enhancements of the proton attached to C-8a was 3.1 and 10.5 %, respectively, on irradiation at the frequency corresponding to the signals from the C-15 and C-16 methyl groups. The same technique also served to assign the position of the bromine atom unambiguously since the assignments of the signals originating from the aromatic protons could be determined from the enhancement data. Thus in the NOE experiment mentioned above enhancements of 3.6 and 2.1 % respectively were found for the protons at C-4 allowing an assignment of the aromatic signals and thereby defining the site of the bromine substituent.

Structure elucidation by spectroscopic methods served to identify still two alkaloids from *F. foliacea*. One of them, 6-bromo-N_b-methyl-N_b-formyltryptamine (*36*) was present in about 2×10^{-3} % of dry weight.[36] According to NMR analysis *36* exists in solution as a



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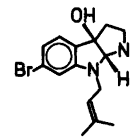

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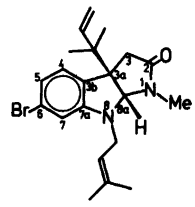
mixture of two rotational isomers with *Z* and *E* configuration around the carbon-nitrogen bond of the formamide function. The situation is further complicated by the formation of intramolecularly associated forms giving rise to duplication of lines in the NMR spectra. The existence of *Z,E*-isomers was demonstrated also in N_b-methyl-N_b-formyltryptamine prepared for comparison. In the other alkaloid, flustrabromine, N_b-methyl-N_b-formyl-6-bromo-2-(2-methyl-3-buten-2-yl)tryptamine (*37*, 6.3×10^{-3} % of dry weight)[37] the predominant rotamer was found to be the *E*-isomer. Also in this case does the existence of intramolecularly associated forms reveal itself from a duplication of the resonances of both the *E* and *Z* isomers. The proximity of the formamide group and the aromatic system was



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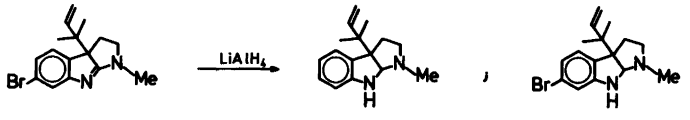
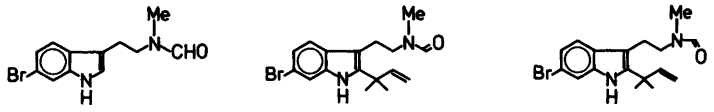
demonstrated by NOE difference spectroscopic measurements. Presumably this type of intramolecular association is of general occurrence in N_b-formyl-N_b-methyltryptamine derivatives since it was demonstrated in N_b-formyl-N_b-methyltryptamine, 1-(3-methyl-2-butenyl)-N_b-formyl-N_b-methyltryptamine, and 2-(3-methyl-2-butenyl)-N_b-formyl-N_b-methyltryptamine as well.[37]



30 33 34

The first naturally occurring bromoquinoline, 7-bromo-4-(2-ethoxyethyl)quinoline (*38*), was identified by spectroscopic methods.[38] The 2-ethoxyethyl substituent could be an artifact since the natural product was in contact with ethanol during the isolation procedure. If the ethoxy group was introduced during isolation it must have replaced a very reactive group.



36 E Z

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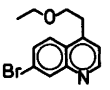

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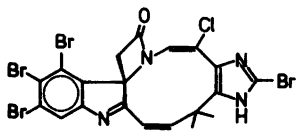
The absolute configurations of the *Flustra* alkaloids *11*, *12*, *30*, *31*, *32*, *34* and *35* are unknown, consequently none of the formulas are intended to depict absolute stereochemistry. Presumably *31* and *32* have the same relative stereochemistry as *11*, *12*, *34* and *35*, *i.e.* 3a,8a-*cis*.

The marine bryozoan *Chartella papyracea* (Ellis and Solander) is closely related to *Flustra foliacea*, since both are members of the family Flustridae. The former species has yielded a series of unique indole alkaloids. The structure of the first compound, chartelline



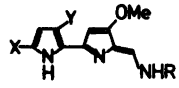
39

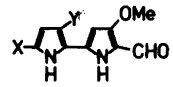
A (*39*), isolated in about 0.07 % yield of dry weight, was solved by X-ray diffraction methods.[39] The central aza-2,4,7-decatriene ring system is *condensed* with a bromoimidazole ring, a tribromoindole ring system, and a β-lactam ring. In the crystal the 10-membered ring

assumes a boat-like conformation where the imidazole ring and the indole derived moiety are nearly coplanar. Except for the imidazole group and the aromatic benzene ring all other double bonds are nonconjugated due to the geometry of the framework. The β-lactam ring is nearly perpendicular on the plane of the indole system. Biogenetically chartelline A (*39*) seems to be derived from tryptamine, histidine, and an isoprene unit. The absolute stereochemistry of the chiral center of the spirolinkage was determined as *S*.

Another member of Flustridae, *Securiflustra securifrons* (Pallas) has yielded alkaloids of as yet undetermined structure.[6,7]

A series of bipyrroles, tambjamine A (*40*), B (*41*), C (*42*), and D (*43*), has been isolated and identified from the green cheilostome bryozoan *Sessibugula translucens* Osburn 1950.[40] The structure elucidation was carried out by chemical and spectroscopic comparisons of the enamines (*30–43*) and the corresponding aldehydes and their *N*-methylated derivatives. The total yield of tambjamines and the artifacts (*44–46*) was about 0.45 % of dry weight.



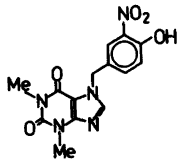


40 X = H, Y = H, R = H
41 X = Br, Y = H, R = H
42 X = H, Y = H, R = Bu^i
43 X = H, Y = Br, R = Bu^i

44 X = H, Y = H
45 X = Br, Y = H
46 X = H, Y = Br

Since the bipyrroles turn green on standing it is suspected that the green color of the bryozoan originates from dimers of these compounds. The enamines *40* and *41* inhibited cell division at 1 μg/ml in the fertilized sea urchin egg assay and showed moderate antimicrobial activity at 50 μg/disc in the disc assay method against *Eschericia coli, Staphylococcus aureus, Bacillus subtilis,* and *Vibrio anguillarum.* The isobutylamines *42* and *43* inhibited cell division at 1 μg/ml and showed antimicrobial activity at 5 μg/disc against *Candida albicans, B. subtilis, S. aureus* and *V. anguillarum* and mild activity at 50 μg/disc against *E. coli.*

The tambjamines seem to be involved in complex ecological relationships since they are present in two small nembrothid nudibranchs, *Tambje eliora* (Marcus and Marcus, 1967) and *Tambje abdere* Farmer 1978, undoubtedly originating from their diet *S. translucens.* The smaller nudibranchs are preyed upon by the large carnivorous nudibranch *Roboastra tigris* Farmer 1978 which in turn contains the tambjamines originating from its prey. *T. abdere* when attacked, produced a yellow mucus that often caused *R. tigris* to break off its attack while *T. eliora* attempted to escape without apparent use of defensive secretions.



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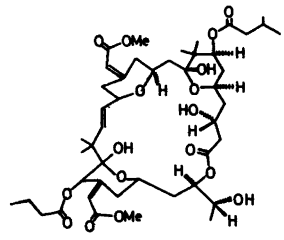

48

Accordingly *R. tigris* seems to prefer to eat *T. eliora*. *R. tigris* is capable of following the tambjamine containing slime trail let down by the *Tambje* species. Undoubtedly the tambjamines are defensive secretions used to deter potential predators.

The "lacey bryozoan", *Phidolopora pacifica,* has yielded phidolopin (*47*) identified by X-ray structural analysis of the *p*-bromophenacyl derivative.[41] Phidolopin (*47*) exhibits *in vitro* antifungal activity against *Pythiam ultimum, Rhizoctonia solani* and *Helminthosporium sativum* with a minimum inhibitory concentration of 70 μg/6 mm disc and antialgal activity against the pennate diatom *Cylindrotheca fusiformis*. Like caffeine, theophylline, and theobromine, phidolopin is based on the xanthine nucleus and in addition has the relatively rare nitro functionality.

ORIGIN AND FUNCTION

At present there is a growing realization that aquatic chemical ecology is much more diverse and complex than anticipated from a superficial survey of the area. Until now ecological investigations have been severely hampered by the lack of chemical information. As this is now accumulating with increasing momentum the foundation for rational studies is emerging. In the case of bryozoans there are, however, questions of a biological nature which must be studied before serious conclutions can be drawn regarding the origin and function of the secondary metabolites encountered. First of all, many bryozoans have adopted an epiphytic or epizoic way of life. The selection of substratum by the bryozoan larvae seems no affair of chance. It has been demonstrated that the larvae actively search for a suitable substratum to settle and metamorphose.[1] Undoubtedly, the search is often guided by chemicals released by the host as in the example of settling of larvae induced by extracts of *Fucus serratus*.[42] Whether the bryozoan colony, once established, has any exchange of metabolites with the host, is unknown. The same holds true for the epizoic flora and fauna colonizing bryozoans. An even more intriguing and important question concerns the presence and role of endosymbionts. Certainly some bryozoans, as for example *F. foliacea* and *S. securifrons* harbour, at least incidentally, a green alga, *Epicladia flustra* Reinke.[6] The monoterpenes isolated from *F. foliacea* might conceivably originate with the green algal symbiont, however, it is most unlikely that the indole alkaloids should be connected with the metabolism of the latter. Only once has an indole alkaloid (caulerpin) been isolated from green algae of genus *Caulerpa,*[6] and in this case the indole alkaloid may be an artifact. If some of the compounds discussed here are actually derived from endosymbionts it is much more likely that these are moulds or bacteria, as these organisms are known to have the biosynthetic ability to produce compounds structurally related to the ones treated here.[6] In this connection it is very interesting that some bryozoans were found to have sac-like organs – the funicular bodies – with cultures of non-pathogenic species specific bacteria.[43–45] Furthermore, in three out of seven species investigated the larvae of the bryozoans consistently carried rod-shaped bacteria in the pallial sinus indicating a mechanism for transferring these species specific symbionts to the new colony.[46] These associations were observed in all larvae independent of geographic location, year, or season. The species associated with bacteria are among the most frequently encountered fouling bryozoans and are often the first to appear on new surfaces. In the case of *B. neritina,* bacteria-containing bodies have not been reported, but bacteria, anatomically similar to those of the larvae are found in the lumen of the funicular system.[47] If the symbionts are actually responsible for the synthesis of the bryostatins these observations might account for the rather low yields reported. The only known natural product even distantly related to the bryostatins is the

cyclic ionophore aplasmomycin from *Streptomyces griseus*.[48,49] It has been suggested that the tambjamines of *S. translucens* may arise from prodigiosin synthesized by the red marine bacteria of genus *Benechea*.[40]

Whatever are the sources of the bryozoan compounds treated here; dietary, epibiont, symbiont, or true bryozoan metabolites, the biogenetic pathways responsible for their appearance are at present totally obscure. Before conclusive experiments can be performed in this area more knowledge concerning the role of the symbionts must be made available. Only the surface of the problem of marine symbiotic relationships has been scratched as evidenced by a recent report of the occurrence of mycoplasmalike organisms in the larvae and adults of the marine bryozoan *Watersipora cucullata*.[50]

Even ignoring the problem of the origin of these compounds their ecological roles are far from clear, although one relationship has been established in the case of the tambjamines.[40] For all the metabolites, however, their varied but pronounced biological activities point to important functions in the struggle for survival of the bryozoans. Undoubtedly chemical investigations of bryozoan ecology would be very rewarding, since many biological hypotheses and observations, lending themselves to interpretation in chemical terms, exist. For example, does the nudibranch *Polycera atra* (sorcerer's nudibranch) feeding on *B. neritina* concentrate and use the bryostatins? Does the nudibranch *Crimora papillata* feeding on *F. foliacea* and *C. papyracea* use the alkaloids for defensive purposes? What is the nature of the chemicals inducing larvae to settle preferentially on one specific alga?

As demonstrated above phylum Bryozoa is a promising target for natural products research. Much needs to be done and it is hoped that the glimpses of this fascinating field unravelled so far should stimulate future research of the ecological role of the metabolites known and the ones to be reported in the future.

ADDENDUM

Since the conclusion of this review, an important addition to the bryostatins, namely bryostatin 4 (*48*), has appeared.[51] Bryostatin 4 was isolated in a yield of 8.9×10^{-5} % of wet weight from material collected from the Gulf of Mexico (USA), Gulf of California (Mexico), and Gulf of Sagami (Japan). The structure was inferred from hydrolysis experiments and spectroscopic studies, in particular solution phase secondary ion mass spectra. Bryostatin 4 (*48*) exhibits pronounced activity, ED_{50}, $10^{-3}-10^{-4}$ μg/ml in PS cell line and 62 % increase in life extension at 46 μg/kg. Because of the isolation of *48* from the diverse geographical areas bryostatin 4 presumably is not of dietary origin. Debromo-8,8a-dihydroflustramine C has been synthetisized.[52]

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