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Antiviral Substances

Kenneth L. Rinehart, Lois S. Shield, and Martha Cohen-Parsons

1. INTRODUCTION

Even today, antiviral drugs are a rarity (Becker, 1976, 1983; Rothschild *et al.*, 1978; R. T. Walker *et al.*, 1979; Collier and Oxford, 1980; De Clercq and Walker, 1984; Mandell *et al.*, 1985; Rinehart, 1992): acyclovir is a notable success in reducing the severity of genital *herpes* infections, and newer analogues are under development; azidothymidine (AZT) is widely employed to extend the lifetime of AIDS sufferers, while other compounds with anti-AIDS potential are under investigation; vidarabine is approved for the treatment of idoxuridine- and acyclovir-resistant infections; ribavirin is an intranasal inhalant effective against respiratory syncitial virus (Stephen *et al.*, 1980); amantidine has been used for many years in treating some forms of influenza (Davies *et al.*, 1964). Even fewer antiviral agents were available in the 1970s when we began our systematic surveys designed to assess the bioactivity of marine organisms.

As the need to cope with human viruses becomes more pressing, interest in antiviral agents continues to mount (Munro *et al.*, 1987). The antiviral potential of extracts from red algae and clams was noted in a 1964 conference on antiviral substances sponsored by the New York Academy of Sciences and the National Institute of Allergy and Infectious Diseases (NIAID) (Hermann, 1965). Today,

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both the National Cancer Institute (NCI) and NIAID are involved in screening for anti-AIDS drugs, some of which are derived from marine sources (Kolberg, 1991). Moreover, research groups around the world regularly test marine organisms for antiviral activity, including cold- and deep-water species (Higa, 1986; Cross and Lewis, 1987; Munro *et al.*, 1989).

2. *MARINE-DERIVED ANTIVIRAL PROGRAM AT THE UNIVERSITY OF ILLINOIS*

The marine natural products chemistry program at the University of Illinois in Urbana was launched in earnest in 1974 with an 8-week expedition on board the *R/V Alpha Helix*, during which over 800 species of marine plants and animals found in Baja California waters were surveyed for antimicrobial activity (Hager *et al.*, 1976; Rinehart *et al.*, 1976; Shaw *et al.*, 1976). Subsequently some of the extracts were tested for antiviral activity with positive results. Antiviral activity and cytotoxicity were associated most frequently with tunicates (ascidians, sea squirts; phylum Chordata, subphylum Tunicata or Urochordata). Extracts of certain *Polyandrocarpa* and *Aplidium* species, for example, were modestly antiviral and cytotoxic; they yielded the polyandrocarpidines (Cheng and Rinehart, 1978; Rinehart *et al.*, 1983a) and aplidiasphingosine (Carter and Rinehart, 1978b), respectively. Although these compounds were of interest primarily for their antimicrobial and cytotoxic potentials, we were sufficiently impressed with the prospects for antiviral agents from marine sources that our screening protocol was expanded during a 5-week Caribbean expedition in 1978 to include a shipboard antiviral/cytotoxicity assay using herpes simplex virus type 1 (HSV-1) grown in monkey kidney (CV-1) cells (Rinehart *et al.*, 1981a). Such testing (Rinehart, 1988a) remains vital to an ongoing search for antiviral, antitumor, antimicrobial, and immunomodulatory metabolites in our marine collection of over 3000 samples from Florida, Texas, Maine, Washington, Alaska, Central America, the Bahamas, and the western Mediterranean (Rinehart, 1989; Rinehart *et al.*, 1990a,b).

3. *ANTIVIRAL ASSAYS*

Dependable antiviral assays must be the heart of any search for antiviral compounds in deciding which organisms to investigate, in following the isolation of the pure compounds, and in measuring the potency of the isolated compounds. Such assays may, of course, be *in vitro* or *in vivo*, but very few marine-derived compounds have been tested *in vivo*.

In our laboratory we have employed HSV-1, a DNA virus, as our primary *in*

vitro screen. This is a plaque assay in which CV-1 cells are infected with HSV-1, which proceeds to grow plaques or aggregates (Schroeder *et al.*, 1981) in cells. Reduction in plaque formation is deemed a positive assay. Similar plaque assays have been employed in determining antiviral activity against *Vesicular stomatitis* virus, an RNA virus, in CV-1 or baby hamster kidney (BHK) cells. Another RNA virus extensively employed for *in vitro* assays is the A59 corona virus in NCTC 1469 cells (Cross and Lewis, 1987). A variety of DNA and RNA viruses have been employed for secondary antiviral assays (Canonico *et al.*, 1982; Rinehart *et al.*, 1983b). For prediction of AIDS inhibition both the HIV virus itself and another retrovirus, a Visna (sheep) virus, have been employed (Frank *et al.*, 1987).

In an interesting spin-off, the antiviral assays also provide an assessment of cytotoxicity against normal (CV-1, BHK) cells and thus serve as a measure of potential for using the compounds as cytotoxic agents.

In the subsequent discussion we shall arbitrarily divide the antiviral compounds into those regarded as very active ($IC_{50} < 1$ μg/ml or well), active (IC_{50} 1–10 μg/ml or well), and modestly active ($IC_{50} > 10$ μg/ml or well) in *in vitro* assays. Unfortunately, many reports of "antiviral" activity do not include any quantitative measurements. Compounds lacking quantitation are, again arbitrarily, assigned to the modestly active group. In addition, only isolated compounds are included in the present review. Observations carried out on crude extracts, while providing useful stimuli, have often proved irreproducible and are omitted.

Following successful *in vitro* assays, *in vivo* studies must be carried out to measure the efficacy of an antiviral agent. Our most antiviral compounds, the didemnins and eudistomins, were tested successfully in a topical mouse vaginal herpes infection (HSV-2), which records prevention of death of the mice (Rinehart *et al.*, 1983b). Other *in vivo* herpes assays include rabbit eye herpes and herpes encephalitis assays. The latter is a difficult hurdle, since the drug must pass through the blood–brain barrier to be effective. Still other *in vivo* assays used with marine natural products include the A59 corona virus (Munro *et al.*, 1989) and Rift Valley fever virus (Canonico *et al.*, 1982). We note again that very few marine natural products have been tested *in vivo*, in part at least due to cost.

4. VERY ACTIVE ANTIVIRAL AGENTS

From the time of our earliest observations of marine antiviral activity (see above), we have become increasingly aware of the antiviral potential of substances derived from tunicates (Rinehart and Shield, 1983). That new emphasis was rewarded by the detection of strong antiviral activity in several species collected during the 1978 expedition (Rinehart *et al.*, 1981a). Our subsequent discovery of the didemnins and eudistomins was the beginning of a wide-ranging and continu-

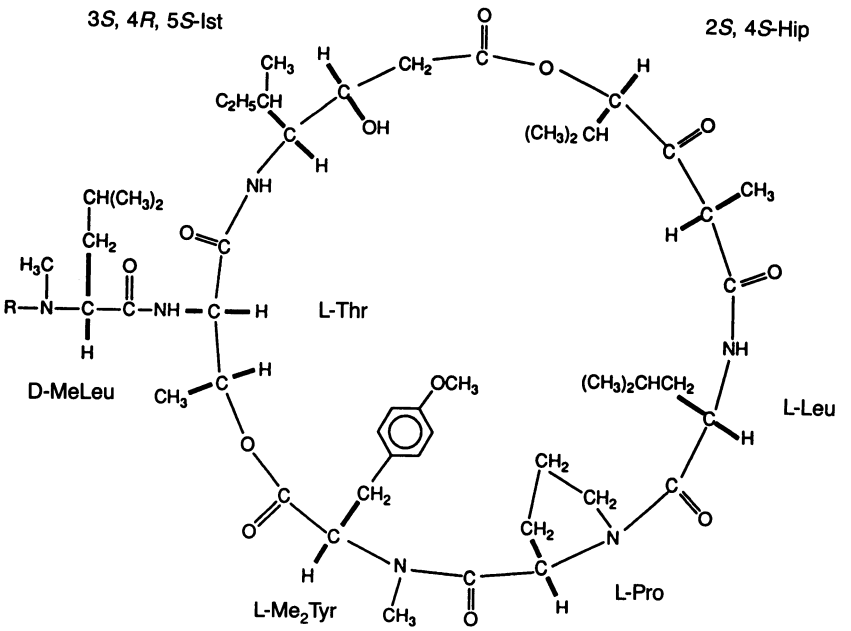
ing effort by chemists in this laboratory and others around the world, as well as by virologists, cancer researchers, taxonomists, and algologists. Structures have been assigned and confirmed by syntheses, *in vitro* and *in vivo* evaluations have been carried out, and analogues are currently being prepared for determining the relationship between structure and activity. At the same time, we continue to search for new, more potent members of the chemical families. The current status of our two furthest advanced projects—didemnins and eudistomins—will be reviewed first, with emphasis on their antiviral aspects, followed by other strongly antiviral compounds.

4.1. Didemnins

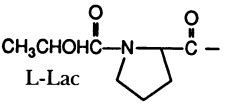
The didemnins (Rinehart *et al.*, 1987a; Rinehart, 1988b, and references therein) are a family of cyclic depsipeptides isolated from a *Tridemnum* species (family Didemnidae) that is found most often as a gray-green, flat, pancake-like coating on rocks or coral at depths to 120 feet. We first collected *Tridemnum* samples during our 1978 expedition in the waters off Belize, Honduras, Mexico, Colombia, and Panama. Independently, the bioactivity of such extracts attracted the attention of the NCI (Chun *et al.*, 1986). The structure elucidation of didemnins A, B, and C employed several types of mass spectrometry (Rinehart *et al.*, 1981b) and provided an early success with fast atom bombardment mass spectrometry when that then new technique became available. Modifications made in the course of our synthetic efforts and confirmed in other laboratories resulted in structures **1–9**, including two novel elements–2*S*,4*S*-hydroxyisovalerylpropionic acid (Hip) and (3*S*,4*R*,5*S*)-isostatine (Ist).

In shipboard testing, *Tridemnum* extracts inhibited HSV-1, with underlying cytotoxicity to the CV-1 cells. Both antitumor and antiviral activities were detected for the first didemnins isolated (Rinehart *et al.*, 1981a,c, 1983b; Canonico *et al.*, 1982), as well as for later members of the family, and, indeed, attention was soon focused on the anticancer potential of didemnin B and the ensuing clinical trials (Chun *et al.*, 1986). The didemnin family now includes many antitumor compounds from *T. solidum* (Rinehart *et al.*, 1990c; Sakai, 1991), including the even more potent dehydrodidemnin B from a Mediterranean tunicate, *Aplidium albicans* (Rinehart, 1990, 1992). Phase II clinical trials of didemnin B are expected to extend into 1993, with evaluation to follow. It was noted in early studies that **1** and **2** showed quantitatively altered bioactivities although they differed only in their side chain, suggesting that chemical modifications might lead to improved therapeutic potential.

After shipboard testing against HSV-1, the several samples of *Tridemnum* collected in 1978 were tested by Renis at the Upjohn Company, Kalamazoo, Michigan, against a battery of RNA (Coxsackie A21 virus, COE: equine rhino



(1) Didemnin A: R = H

(2) Didemnin B: R = 

(3) Didemnin C: R = L-Lac

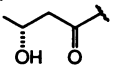
(4) Didemnin D: R = L-pGlu—$(L\text{-Gln})_3$—L-Lac—L-Pro—

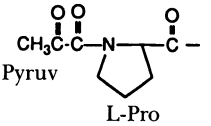
(5) Didemnin E: R = L-pGlu—$(L\text{-Gln})_2$—L-Lac—L-Pro—

(6) Didemnin G: R = CHO

(7) Didemnin X: R = Hydec—$(L\text{-Gln})_3$—L-Lac—L-Pro—

(8) Didemnin Y: R = Hydec—$(L\text{-Gln})_4$—L-Lac—L-Pro—

Hydec = 

(9) Dehydrodidemnin B: R = 

virus, ER; influenza virus, PR8; parainfluenza-3 virus, HA-1) and DNA (HSV-1; HSV-2; vaccinia, vacc) viruses. Later, individual didemnins (A, the major component; B, less abundant; and C, a trace component) from the initial extract were tested against the seven viruses. *In vitro*, didemnin B was 10–100 times as active as didemnin A. Didemnin B caused a >3 log reduction at 0.5 μg/ml in the growth of HSV-1 and HSV-2, while didemnin A caused a 1–2 log reduction at 5.0 μg/ml.

Topical application of didemnins A (at 1 mg/ml) and B (at 0.2 mg/ml) to mice inoculated intravaginally with HSV-2 produced improved survival rates and decreased virus titers. Neither didemnin A nor B was active against lethal Semliki Forest virus infections, and skin irritation was high for both compounds.

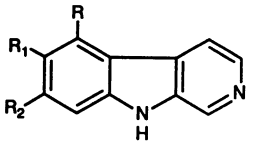
Didemnins A and B were tested by Canonico *et al.* (1982) against several virulent human pathogens for which neither treatment nor prevention is available–Rift Valley fever (RVF, Zagazig 501), Venezuelan equine encephalomyelitis (VEE, Trinidad donkey), yellow fever (YF, Asibi), and Pichinde arenavirus (PIC, AN3739), to give ID_{50} values of 0.04, 0.08, 0.08, and 0.22 μg/ml, respectively, for didemnin B and 1.37, 0.43, 0.4, and 2.9 μg/ml for didemnin A. Didemnin B was also effective in limiting the mortality of RVF-infected mice so that most lived for the duration of the study. Didemnin B is toxic, however; slightly higher doses proved lethal to mice. It is possible that, despite their low antiviral therapeutic indices, the didemnins could be modified or used in combination with other antiviral agents to combat such difficult disease.

4.2. Eudistomins

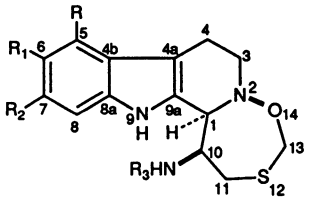
Extracts of *Eudistoma olivaceum* (family Polycitoridae), a shallow-water tunicate first collected during the Alpha Helix Caribbean Expedition in 1978, gave the strongest antiviral results in shipboard HSV-1 testing. The tunicate has since been recollected in Mexico, Belize, and Florida, usually by snorkeling or wading among mangrove roots, and 17 members of the eudistomin family have been isolated in this laboratory from toluene and chloroform extraction of the tunicate (J. Kobayashi *et al.*, 1984; Rinehart *et al.*, 1984, 1986, 1987b). The eudistomins we isolated showed a range of antiviral and antimicrobial activity and were characterized as a family of β-carbolines of four types–unsubstituted (**10–13**), pyrrolyl-substituted (**19**,**20**), pyrrolinyl-substituted (**21–25**), and tetrahydro-β-carbolines containing an oxathiazepine ring (**14–18**), a unique condensed ring system. Eudistomins G, H, I, and P were isolated and partially described simultaneously by the Cardellina group in Montana; they refined the separation process for those eudistomins and subsequently identified eudistomins R (**26**), S (**27**), and T (**28**) (Kinzer and Cardellina, 1987). Eudistomins D, E, H, and I have also been isolated from the Okinawan tunicate *Eudistoma glaucus*, together with related compounds such as eudistomidin B (**29**) (J. Kobayashi *et al.*, 1990).

From the compound ascidian *Ritterella sigillinoides*, Munro and Blunt and co-workers (Blunt *et al.*, 1987; Lake *et al.*, 1988a,b) isolated the trifluoroacetate salt of **17**. In the course of that work they revised the stereochemistry of the N–O bond of **17** (and **14**, **15**, and **18**), as confirmed later by x-ray analysis of the *p*-bromobenzoate derivative.

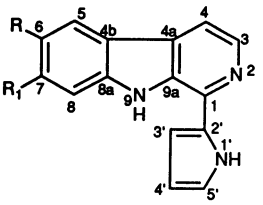
The *in vitro* antiviral potency of the eudistomins ranges from 5 to 500 ng/disk and follows the trend C, E, K, and L (oxathiazepino-tetrahydro-β-carbolines)



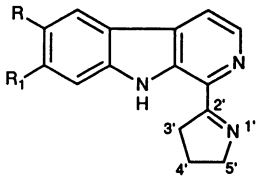
(**10**) Eudistomin D: R = Br, R_1 = OH, R_2=H
(**11**) Eudistomin J: R = H, R_1 = OH, R_2 = Br
(**12**) Eudistomin N: R = R_2 = H, R_1 = Br
(**13**) Eudistomin O: R = R_1 = H, R_2 = Br



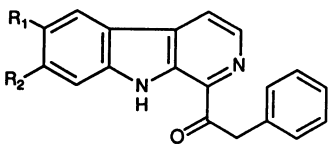
(**14**) Eudistomin C: R = R_3 = H, R_1 = OH, R_2 = Br
(**15**) Eudistomin E: R = Br, R_1 = OH, R_2 = R_3 = H
(**16**) Eudistomin F: R = H, R_1 = OH, R_2 = Br, R_3 = $C_2H_3O_2$
(**17**) Eudistomin K: R = R_1 = R_3 = H, R_2 = Br
(**18**) Eudistomin L: R = R_2 = R_3 = H, R_1 = Br



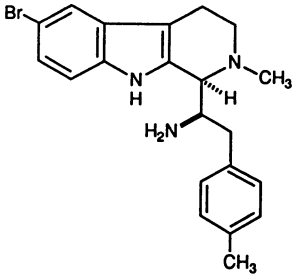
(**19**) Eudistomin A: R = OH, R_1 = Br
(**20**) Eudistomin M: R = OH, R_1 = H



(**21**) Eudistomin G: R = H, R_1 = Br
(**22**) Eudistomin H: R = Br, R_1 = H
(**23**) Eudistomin I: R = R_1 = H
(**24**) Eudistomin P: R = OH, R_1 = Br
(**25**) Eudistomin Q: R = OH, R_1 = H



(**26**) Eudistomin R: R_1 = H, R_2 = Br
(**27**) Eudistomin S: R_1 = Br, R_2 = H
(**28**) Eudistomin T: R_1 = R_2 = H



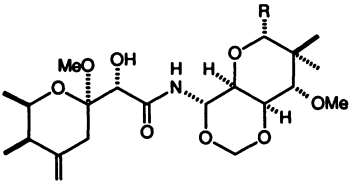
(**29**) Eudistomidin B

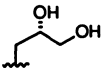
≫ H and P (1-pyrrolinyl-substituted) = D and N (+0) (1-unsubstituted) > A (1-pyrrolyl-substituted, no inhibition). The influence of Br and/or OH substituents on the β-carboline benzenoid ring follows the order E (5-Br, 6-OH) = C (6-OH, 7-Br) > L (6-Br) = K (7-Br), and P (6-OH, 7-Br) = H (6-Br) > G (7-Br) = Q (6-OH) = I (no substitution). Despite the challenge presented by the oxathiazepine ring, synthetic efforts in several laboratories around the world (Kirkup *et al.*, 1989;

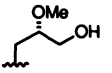
Nakagawa *et al.*, 1989; Still and Strautmanis, 1989; Hermkens *et al.*, 1990) have resulted in successful schemes for obtaining the most active eudistomins in amounts which should be sufficient for extensive *in vivo* testing.

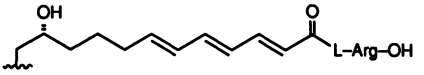
4.3. Mycalamides and Onnamide A

Perry *et al.* (1988) and Munro *et al.* (1988) reported the isolation and structure determination of mycalamide A (**30**) from a New Zealand sponge, a *Mycale* sp. (phylum Porifera). They found that a material consisting of 2% mycalamide A was effective against A59 coronavirus *in vivo* in mice at 0.2 μg/kg per day with 100% survival after 14 days. When pure mycalamide A was obtained,



(**30**) Mycalamide A: R = 

(**31**) Mycalamide B: R = 

(**32**) Onnamide A: R = 

it inhibited HSV-1 or polio virus type I at 5 ng/disk. The structure was assigned based on MS and NMR data, including HETCOR, COSY, long-range HETCOR, and difference NOE experiments, and by comparison with the known compound pederin, isolated from a terrestrial beetle. A related compound, onnamide A, was isolated from a Japanese sponge at about the same time (see the following).

Blunt *et al.* (1989) and Perry *et al.* (1990) reported further work on the mycalamides, including the antiviral and antitumor mycalamide B (**31**). Mycalamide B had greater antiviral activity and cytotoxicity than mycalamide A; *in vitro* antiviral testing showed a minimum dose of 1–2 ng/disk for B and 3.5–5.0 ng/disk for A. Neither has been tested *in vivo*.

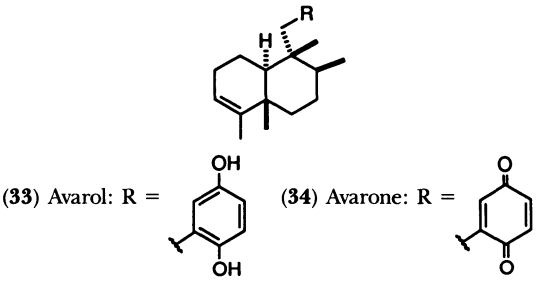
Onnamide A (**32**) was extracted from a *Theonella* sp. (phylum Porifera) collected off the coast of Okinawa (Sakemi *et al.*, 1988). Both the extract and the isolated compound were reported to have "potent activity" *in vitro* against HSV-1, VSV, and A59 coronavirus. Onnamide A was isolated in a procedure utilizing CCC and was assigned a structure based on UV, MS, and NMR data, including COSY, HETCOSY, and NOE difference experiments, and by analogy to pederin and mycalamide A (Sakemi *et al.*, 1988). Although details concerning the antiviral activity of **32** have not been published, its structural similarity to **30** and **31** suggests that the compound is probably quite active. In keeping with the antiviral,

antitumor, and antifungal activity, Higa *et al.* (1989) are pursuing the use of onnamide A and its derivatives as agricultural and medical fungicides and virucides.

Mycalamides A and B, pederin, and onnamide A are protein synthesis inhibitors with commonalities in structure. Although the active solution conformation for this group of compounds is unknown, a correlation between substructure and bioactivity has been suggested (Perry *et al.*, 1990). However, due to the high variability in antiviral potency between these related compounds, it will probably be necessary to investigate a wide range of derivatives before any such correlation can be established (Perry *et al.*, 1990).

4.4. Avarol and Avarone

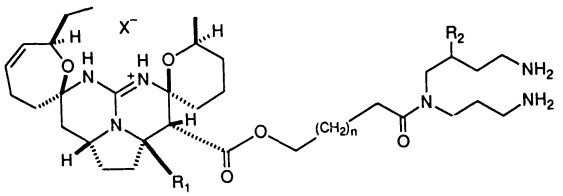
Avarol (**33**) and avarone (**34**) were recently reported to inhibit human immunodeficiency virus at doses of 0.1–1 μg/ml *in vitro* and thus are of potential use in treatment of AIDS (Sarin *et al.*, 1987). Extracted from the sponge *Disidea avara* (phylum Porifera), the compounds were identified by IR and NMR spectra



as sesquiterpenes attached to a quinone or hydroquinone unit and are related to a number of other compounds, including aureol, zonarol, chromazonarol, panicein, kamalonen, spongiaquinone, and ilimaquinone (Minale *et al.*, 1974). Avarol and avarone are of particular interest in the development of clinical application because of their high therapeutic indices and ability to cross the blood–brain barrier (Sarin *et al.*, 1987).

4.5. Ptilomycalin A and Crambescidins

Kashman *et al.* (1989a) reported the isolation of ptilomycalin A (**35**) from the Caribbean sponge *Ptilocaulis spiculifer* and a Red Sea sponge, a *Hemimycale* sp. (phylum Porifera). Activity against HSV was observed at a concentration of 0.2 μg/ml (Kashman *et al.*, 1989a). In addition to the high antiviral activity, the compound exhibited antitumor and antifungal activity. Its polycyclic guanidine structure was assigned based on UV, IR, MS, and NMR spectroscopy, including



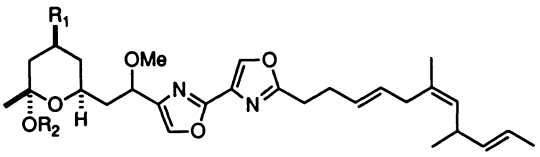
(**35**) Ptilomycalin A: $R_1 = R_2 = H$, n = 13
(**36**) Crambescidin 816: $R_1 = R_2 = OH$, n = 13
(**37**) Crambescidin 830: $R_1 = R_2 = OH$, n = 14
(**38**) Crambescidin 844: $R_1 = R_2 = OH$, n = 15
(**39**) Crambescidin 800: $R_1 = H$, $R_2 = OH$, n = 13

COLOC, NOESY, ROESY, HMBC, and HOHAHA data. The discovery of **35** revealed a new class of alkaloids linked to spermidine via an ω-hydroxy acid.

Very recently (Jares-Erijman *et al.*, 1991) a series of compounds related to **35** was isolated from the Mediterranean sponge *Crambe crambe* (phylum Porifera). The structures of these new compounds, the crambescidins (**36–39**), were assigned based on FABMS/MS, HRFABMS, and a series of NMR studies including HMBC. Compounds **36–39** differ from **35** by the presence of a hydroxyspermidine unit and from one another in the chain length of the long-chain hydroxy acid and in the presence or absence of a hydroxyl group in the guanidine-containing heterocyclic system. All of the crambescidins show activity against HSV-1 at 1.25 μg/ml and exhibit 98% inhibition of L1210 cell growth at 0.1 μg/ml.

4.6. Hennoxazoles

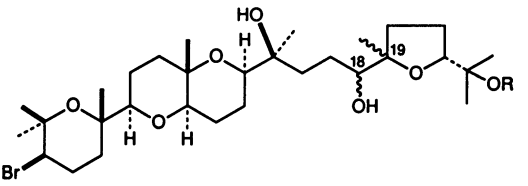
Hennoxazoles A–D (**40–43**) were isolated from a sponge, a *Polyfibrospongia* sp. (phylum Porifera), collected on the island of Miyako in Okinawa (Ichiba *et al.*, 1991). The names derive from the presence of two oxazole units in the molecules, which otherwise appear to be formed from a polyketide-amino acid biosynthetic pathway. In addition to displaying analgesic activity, hennoxazole A, the major component (0.01% of wet weight), showed strong activity against HSV-1 (IC_{50} = 0.6 μg/ml).



(**40**) Hennoxazole A: $R_1 = OH$, $R_2 = CH_3$
(**41**) Hennoxazole B: $R_1 = OH$, $R_2 = CH_2CH_3$
(**42**) Hennoxazole C: $R_1 = OH$, $R_2 = CH_2CH_2CH_2CH_3$
(**43**) Hennoxazole D: $R_1 = H$, $R_2 = CH_3$

4.7. Thyrsiferol and Related Triterpenes

Blunt *et al.* (1978) isolated thyrsiferol (**44**) from the red alga *Laurencia thyrsifera* (phylum Rhodophyta) collected in New Zealand. Although no biological activity was observed at that time, its structure was assigned as a squalene-derived triterpene tetracyclic ether. Years later, Gonzalez *et al.* (1984) isolated a



(**44**) Thyrsiferol: 18*S*, 19*R*; R = H
(**45**) Thyrsiferol acetate: 18*S*, 19*R*; R = Ac
(**46**) Venustatriol: 18*R*, 19*S*; R = H

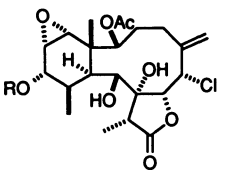
number of inactive compounds from *L. pinnatifida*, some of which were terpenoids related to thyrsiferol (especially dehydrothyrsiferol and thyrsiferol monoacetate). By contrast, Suzuki *et al.* (1985) reported that a crude extract of *L. obtusa* was strongly cytotoxic to P388 cells (ED_{50} = 0.18 μg/ml), and they isolated thyrsiferol, thyrsiferol acetate, and teurilene from the extract. Structures for these compounds were assigned based on NMR, IR, and x-ray data.

In subsequent studies, thyrsiferol-23 acetate (**45**) and a related compound, venustatriol (**46**), proved to be strongly antiviral when isolated from an extract of an Okinawan sample of *L. venusta* which showed "significant activity" against VSV and HSV-1 (Sakemi *et al.*, 1986). Based on MS, NMR, and x-ray data, venustatriol was found to be a stereoisomer of thyrsiferol. Still later, all three compounds that had been isolated by Sakemi *et al.* (1986), viz. thyrsiferol, thyrsiferol-23 acetate, and venustatriol, showed *in vitro* activity against VSV and HSV-1 (Higa *et al.*, 1988a), with efficacies reported for all three compounds at levels of 0.1–0.5 μg/well (Rinehart, 1992). Some accompanying cytotoxicity has also been observed as well as slight activity against A59 corona virus without concurrent cytotoxicity (Rinehart, 1992). Suzuki *et al.* (1987) isolated additional compounds of this type from *L. obtusa* collected in Japan. Included were five new compounds with some cytotoxicity against P388 *in vitro*–15(28)-anhydrothyrsiferol diacetate, 15-anhydrothyrsiferol diacetate; magireols A, B, and C. Their structures were established on the basis of IR, NMR, and HR mass spectra compared to known compounds of this type as well as chemical derivatization. Antiviral activity has not yet been reported for the additional compounds.

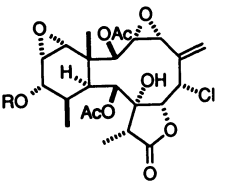
The cytotoxicity and antiviral activity reported for thyrsiferol-related compounds stimulated interest in their synthesis. As a result, thyrsiferol was partially synthesized by Hashimoto *et al.* (1987) and total syntheses of (+)-thyrsiferol (Hashimoto *et al.*, 1988) and (+)-venustatriol (Hashimoto *et al.*, 1988; Corey and Ha, 1988) were subsequently achieved.

4.8. Solenolides and Briantheins

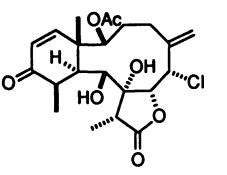
Groweiss *et al.* (1988) reported the isolation of solenolides A–F (**47–52**) from a *Solenopodium* sp. (phylum Coelenterata), a newly identified Indopacific gorgonian collected at Palau. The diterpenoid lactone structures of **47–52** were assigned from NMR data, including NOESY and NOE difference experiments; UV data; and chemical derivatization. These diterpenoid compounds represent a



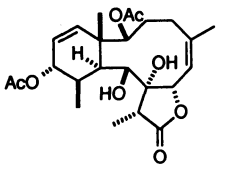
(**47**) Solenolide A: R = $C_5H_{11}CO$
(**48**) Solenolide B: R = Ac



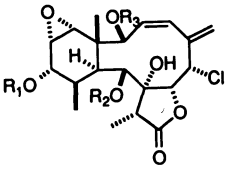
(**49**) Solenolide C: R = H
(**50**) Solenolide D: R = Ac



(**51**) Solenolide E



(**52**) Solenolide F



(**53**) Briantheín V: $R_1 = R_3 = COCH_2CH_2CH_3$, R_2 = Ac
(**54**) Briantheín X: $R_1 = R_2$ = Ac, R_3 = H
(**55**) Briantheín Y: $R_1 = R_2$ = Ac, $R_3 = COCH_2CH_2CH_3$
(**56**) Briantheín Z: $R_1 = R_2 = R_3$ = Ac

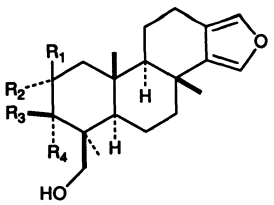
variation on the class of briarein marine products previously found to have biomedical potential. Three of the five new solenolide compounds exhibited antiviral activity, the most notable of which were the inhibitions of rhinovirus by solenolides A (IC_{50} = 0.39 μg/ml) and E (IC_{50} = 12.5 μg/ml). Additional findings included activities against HSV (solenolides A and E), polio III (solenolide A), Ann Arbor (solenolides A, D, and E), Maryland (solenolide A), and Semliki Forest viruses (solenolide D). Solenolides A, D, E, and F also exhibited anti-inflammatory activity. The solenolides are closely related to the briantheins (see below), which have also been reported to be antiviral.

The briarein and asbestinin series of diterpenes consists of highly oxidized compounds isolated from gorgonian coral (*Briareum asbestinum* and *B. polyanthes*; phylum Coelenterata) found in Caribbean and Bermudan waters (Stierle *et al.*, 1980; Grode *et al.*, 1983a). These compounds are very closely related chemically to the solenolides but are much less active as antiviral agents. Among the briareins, briantheins V, Y, and Z inhibited A59 mouse corona virus *in vitro* at 50, 400, and 80 μg/ml, respectively (Coval *et al.*, 1988). Brianthein Z, first

isolated by Grode *et al.* (1983a), inhibited HSV-1 at 80 μg/ml. Briantheins Z and V displayed *in vitro* cytotoxicity toward P388 (Coval *et al.*, 1988), and brianthein Y has been noted for its insecticidal potential (Grode *et al.*, 1983b). In light of certain taxonomic discrepancies (for example, *B. polyanthes* had also been known as *Ammothea polyanthes*, *Erythropodium polyanthes*, and *B. asbestinum*), compounds V, Y, and Z were of interest as potential chemotaxonomic markers. The brianthein structures were assigned by x-ray analysis of V (**53**) (Coval *et al.*, 1988) and X–Z (**54–56**) (Grode *et al.*, 1983b).

4.9. Spongiadiol and Related Compounds

Kazlauskas *et al.* (1979) isolated eight tetracyclic furanoditerpenes from an Australian sponge of the genus *Spongia* (phylum Porifera) collected from the Great Barrier Reef. The compounds were originally given one of three trivial classifications, spongiadiol [3α,19-dihydroxyspongia-13(16),14-dien-2-one], spongiatriol [3α,17,19-trihydroxyspongia-13(16),14-dien-2-one], and epispongiadiol [3β,19-dihydroxyspongia-13(16),14-dien-2-one]. Structures were determined on the basis of NMR, x-ray analysis of one of the compounds, and CD data. Earlier, degraded C-21 terpenes of this general form had been isolated from a *Spongia* sp. (Cimino *et al.*, 1974; Kazlauskas *et al.*, 1976), as had the diterpene spongi-12-en-16-one (Kazlauskas *et al.*, 1976), but no bioactivities were reported. Later, Kohmoto *et al.* (1987) reported the isolation from a deep-water Caribbean *Spongia* sp. of spongiadiol (**57**), epispongiadiol (**58**), and the new isospongiadiol [2α,19-dihydroxyspongia-13(16),14-dien-3-one] (**59**). In their study, spongiadiol was



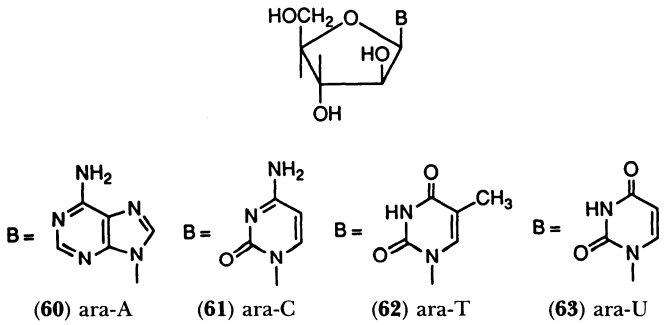
(**57**) Spongiadiol: $R_1 + R_2 = O$, $R_3 = H$, $R_4 = OH$
(**58**) Epispongiadiol: $R_1 + R_2 = O$, $R_3 = OH$, $R_4 = H$
(**59**) Isospongiadiol: $R_1 = H$, $R_2 = OH$, $R_3 + R_4 = O$

isolated as 0.13% of the frozen weight, epispongiadiol as 0.87%, and isospongiadiol as 0.2% in an isolation process that involved CCC, and structures were assigned based on IR, MS, and NMR data, including COSY and NOE experiments. Kohmoto *et al.* (1987) reported both antiviral activity and cytotoxicity for all three spongiols. *In vitro* assays against HSV-1 revealed a spectrum of activities ranging from the very active spongiadiol (IC_{50} = 0.25 μg/ml) to the modestly active epispongiadiol (IC_{50} = 12.5 μg/ml), with isospongiadiol exhibit-

ing intermediate activity (IC_{50} = 2.0 μg/ml). In additional reports of the antitumor and antiviral activities of these three furanoditerpenoids, spongiadiol and isospongiadiol gave 100% inhibition of HSV-1 plaque formation at 20 and 0.5 μg/(6-mm disk), and epispongiadiol gave partial inhibition at 12.5 μg/disk (Kohmoto *et al.*, 1988).

4.10. Ara-A

A family of potent antiviral and antitumor compounds including two presently in clinical use as antiviral or antitumor agents (i.e., ara-A, 9-β-D-arabinofuranosyladenine, **60**; ara-C, 1-β-D-arabinosylcytosine, **61**) is related to the arabinosides isolated in the early 1950s from the marine sponge *Cryptotethia crypta* (Bergmann and Feeney, 1950, 1951). Bergmann first collected *C. crypta* in



(**60**) ara-A (**61**) ara-C (**62**) ara-T (**63**) ara-U

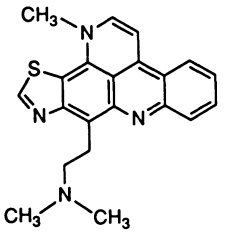
1945, and in the next few years he reported the presence of spongothymidine (ara-T, 1-β-D-arabinofuranosylthymidine, **62**), spongouridine (ara-U, 1-β-D-arabinofuranosyluracil, **63**), and spongosine (1-β-D-ribofuranosyl-2-methoxyadenine) [reviewed by Cohen (1966)]. Cimino *et al.* (1984) identified ara-U, as well as ara-A and the 3′-*O*-acetyl derivative of ara-A, in the 1-butanol extract of the gorgonian *Eunicella cavolini* (phylum Coelenterata) on the basis of UV, IR, NMR, and MS data, and by comparison with authentic samples. This was the first discovery of ara-A in a natural marine source, although it had been synthesized as one of many bioactive variations on the naturally occurring spongouridine.

Early *in vitro* studies showed the antiviral activity of the arabinosides to vary depending upon whether the challenge was against HSV-1 or -2. Using rabbit kidney and human skin fibroblast cultures, De Clercq *et al.* (1977) reported MICs (minimum inhibitory concentration) as low as 0.02 and 1 μg/ml for ara-C and ara-A, respectively, against HSV-1; and 200 and 10 μg/ml, respectively, against HSV-2. Significant *in vitro* activity has also been observed for a number of xylofuranonucleosides against three DNA viruses (HSV-1, HSV-2, vaccinia) and one RNA virus (rhinovirus-9) (Gosselin *et al.*, 1986).

5. ACTIVE ANTIVIRAL AGENTS

5.1. Dercitin

A fused pentacyclic aromatic alkaloid, dercitin (**64**), was isolated from a *Dercitus* sp. (sponge; phylum Porifera) by Gunawardana *et al.* (1988). It was observed to be a violet pigment having antitumor, antiviral, and immunomodulatory activity *in vitro* and antitumor activity *in vivo*. Dercitin was obtained in a yield of 0.69% of the wet weight of the sponge, and its structure was assigned as

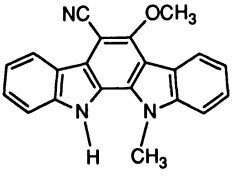


(**64**)

N,*N*,1-trimethyl-1*H*-pyrido[4,3,2-*mn*]thiazolo[5,4-b]acridine-9-ethanamine on the basis of UV, MS, and NMR data, including COSY, NOE, HETCOSY, COLOC, and INADEQUATE experiments. The presence of the fused thiazole unit was thought to be unique to this pentacyclic aromatic alkaloid. Its cytotoxicity and antiviral activity were reported as 10, +++ at 5 μg/well against HSV-1 and 0, +++ at 1 μg/well against A59 murine corona virus. Further study showed that the antitumor activity of dercitin was associated with its intercalation into nucleic acids (Burres *et al.*, 1989). Other bioactive compounds isolated from sponges of the family Pachastrellidae have been found to contain the same pyrido[4,3,2-*mn*]-acridine skeleton as **64** (Gunawardana *et al.*, 1989).

5.2. Indolocarbazole

Knübel *et al.* (1990) extracted a bioactive blue-green alga, *Nostoc sphaericum* (phylum Cyanophyta), from an Oahu mud sample. From the cultured Hawaiian alga they isolated indolo[2,3A]carbazole compounds and found the major component, 6-cyano-5-methoxy-12-methylindolo[2,3A]carbazole (**65**), to

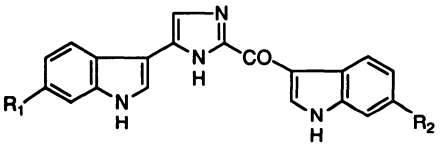


(**65**)

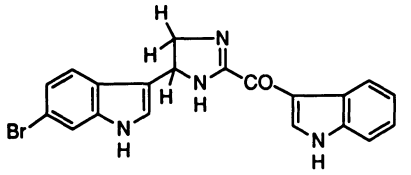
be responsible for most of the antiviral activity and cytotoxicity. The virus titer in mink lung cells infected with HSV-2 was reduced 95% at ca. 1 μg/ml, but some virus remained before the cytotoxic MIC was reached at 100 μg/ml. Similar activity was observed for the 12-demethyl analogue. The major compound was obtained in a 0.22% dry weight yield, and its structure was assigned on the basis of UV, MS, and NMR, including COSY, HMQC, HMBC, and NOE experiments. Although this *Nostoc* species is not, strictly speaking, a marine blue-green alga, other cyanobacteria are found in the ocean.

5.3. Topsentins

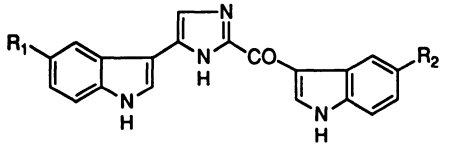
The bioactivity (P388, HSV-1) of the genus *Spongosorites* (phylum Porifera) was found in our laboratory to be associated with the bis(indolyl)imidazoles shown here–topsentin (**66**), bromotopsentin (**67**), and isotopsentin (**68**) (Tsujii *et al.*, 1988; Gunasekera *et al.*, 1989), whose structures were assigned based on HREIMS and NMR. Synthetic work undertaken to confirm the structure assignments and to study structure–activity relationships afforded the family of com-



(**66**) Topsentin: R_1 = H, R_2 = OH
(**67**) Bromotopsentin: R_1 = Br, R_2 = OH
(**68**) Isotopsentin: R_1 = OH, R_2 = H
(**69**) Hydroxytopsentin: R_1 = R_2 = OH
(**70**) Deoxytopsentin: R_1 = R_2 = H



(**71**) 4,5-dihydro-6″-deoxybromotopsentin

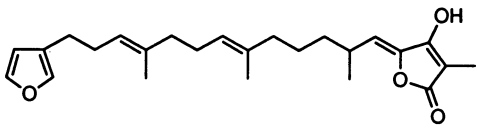


(**72**) Neotopsentin: R_1 = H, R_2 = OH
(**73**) Neoisotopsentin: R_1 = OH, R_2 = H
(**74**) Neohydroxytopsentin: R_1 = R_2 = OH

pounds **69–74**. The most active antiviral compound, topsentin, inhibited A59 corona virus at 2 μg/disk and HSV-1 at 50 μg/disk in tissue culture assays.

5.4. Variabilin

Faulkner (1973) reported the isolation of several furanosesterterpenes from a sponge indigenous to New Zealand and belonging to the genus *Ircinia* (phylum Porifera). Among them was variabilin (**75**), an antimicrobial agent (vs. *Staphylococcus aureus*) accounting for 0.2% of the dry weight of the sponge. Its structure



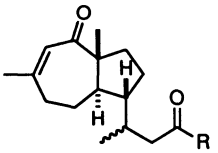
(**75**)

was assigned on the basis of UV, IR, and NMR data and comparisons with other tetronic acids reported earlier, such as ircinins 1 and 2 and fasciculatin. Due to its ubiquitous presence in the genus *Ircinia*, variabilin served as a valuable taxonomic marker and was later used as a chemotaxonomic marker to facilitate the study of sponges of the order Dictyoceratida (Perry *et al.*, 1987). Variabilin proved to be a major component in extracts of six *Ircinia*, three *Psammocinia*, and one *Sarcotragus* samples.

Four new furanosesterterpene tetronic acids were identified by Barrow *et al.* (1988b). In the course of that work, crude *Ircinia* extracts displayed *in vitro* antiviral activity against VSV-1 and polio virus type I in BSC (green monkey kidney) host cells. Some cytotoxicity at 2 μg/disk was also observed. Although variabilin purportedly showed varying antiviral behavior, an additional study found the compound to be cytotoxic but not antiviral (Barrow *et al.*, 1988a). Nevertheless, the stereochemistry of variabilin, the major bioactive component in a *Sarcotragus* sample, was completed and three new terpenes of the same general type were reported. Later, Barrow *et al.* (1989) studied the decomposition products of variabilin and obtained some of its bioactive yet stable analogues. The derivatives were more stable in the presence of light and air than was variabilin, but there remained the problem of any useful antiviral effect (either *in vitro* or *in vivo*) being overshadowed by cytotoxicity. In the group of compounds examined, the activity observed at 2–20 μg/disk depended not on the presence of a furan or a tetronic acid unit, but on the presence of such terminal groups as hydroxyl or carboxyl.

5.5. Reiswigins

Kashman *et al.* (1987) reported the isolation of reiswigins A (**76**) and B (**77**), bioactive terpenes from the sponge *Epipolasis reiswigi* (phylum Porifera). Their

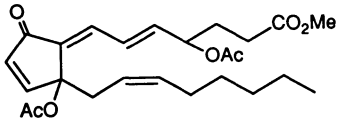


(**76**) Reiswigin A: R = $CH_2CH(CH_3)_2$
(**77**) Reiswigin B: R = $-CH=C(CH_3)_2$

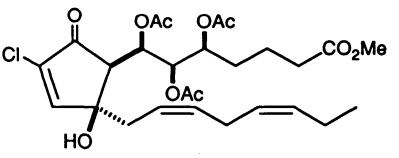
structures were assigned on the basis of UV, IR, MS, and NMR data, including COSY, INADEQUATE, NOESY, and NOE experiments. Both compounds inhibited HSV-1 completely at 2 μg and A59 virus partially at 20 μg (++), and reiswigin A completely inhibited VSV at 2 μg without accompanying cytotoxicity. Antiviral activity was reported for a series of six related diterpenes, including **76** and **77** (Kashman *et al.*, 1989b).

5.6. *Prostaglandins*

Activity against both RNA and DNA viruses has been recorded for a number of prostaglandins (Santoro *et al.*, 1980; Ankel *et al.*, 1985), some of which occur in the marine environment among the soft corals. This observation of antiviral activity has been extended to the more unusual clavulone II (**78**), a prostanoid isolated from the soft coral *Clavularia viridis* (phylum Cnidaria) and identified



(**78**) Clavulone II



(**79**) Punaglandin-1

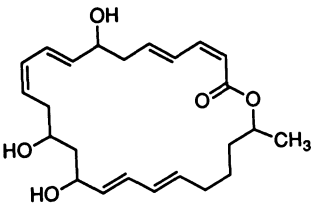
by UV, IR, and NMR data (Kikuchi *et al.*, 1982; M. Kobayashi *et al.*, 1982). Clavulone II was found to be the most active prostanoid in tests conducted against VSV (IC_{50} ca. 2 μg/ml) and encephalomyocarditis (EMC) (Bader *et al.*, 1991).

Punaglandins (halogenated eicosanoids, e.g., **79**) are unusual prostaglandins obtained from the octacoral *Telesto riisei*. Although the original descriptions of the natural products did not report antiviral activity, subsequent patent applications (Noyori *et al.*, 1987a,b) indicated that some punaglandin derivatives were antiviral agents.

5.7. *Macrolactin A*

Gustafson *et al.* (1989) reported the isolation of macrolactins A–F from the culture broth of a deep-sea bacterium that could not be classified taxonomically.

Structures were established on the basis of UV, IR, MS, and NMR data, including COSY, HETCOR, and COLOC experiments. The compounds were found to be 24-membered ring lactones and their glucose β-pyranoside analogues and included the open-chain macrolactinic and isomacrolactinic acids. Macrolactin A (**80**) showed some activity against *Bacillus subtilis* and *S. aureus* as well as B16-F10 murine melanoma cells *in vitro*. Against HSV-1 (strain LL) and HSV-2 (strain



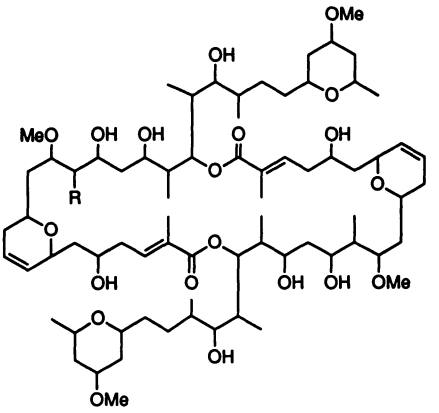
(**80**)

G), the IC_{50} was 5.0 and 8.3 μg/ml, respectively. Although no cytotoxicity data were provided, Gustafson *et al.* (1989) indicated that the potential therapeutic index fell in the range 10–100. In ongoing tests conducted by the NCI, a concentration of 10 μg/ml of macrolactin A gave maximum protection against human HIV replication (Gustafson *et al.*, 1989).

6. *MODESTLY ACTIVE ANTIVIRAL AGENTS*

6.1. *Misakinolide A and Bistheonellides*

Misakinolide A was first isolated from a *Theonella* sp. (phylum Porifera) collected in Okinawa (Sakai *et al.*, 1986). *In vitro* antiviral and antifungal activities were reported. On the basis of MS and NMR data, including COSY, Sakai *et al.* (1986) assigned a 20-membered macrolide structure similar to that of swinholide A, a known antifungal compound isolated from a sponge of the same genus (Carmely and Kashman, 1985). Comparisons with swinholide A led to the assignment of a monomeric structure as found in the scytophycins from the blue-green alga *Scytonema pseudohofmani*. Upon further study, Kato *et al.* (1987) revised the structure of misakinolide A from a monomeric to a dimeric macrolide and concluded that the dimeric structure was identical to that of bistheonellide A (**81**), newly isolated from a *Theonella* sp. The structure of bistheonellide B (**82**), a related compound, was also assigned. These are the first reports of dimeric macrolides having a 40-membered ring (Kato *et al.*, 1987). The dimeric structure determination included FABMS data and was confirmed by chemical degradation. Kato *et al.* (1987) also reported that bistheonellides A and B inhibited starfish (*Asterina pectinifera*) embryo development, a finding suggestive of *in vivo*

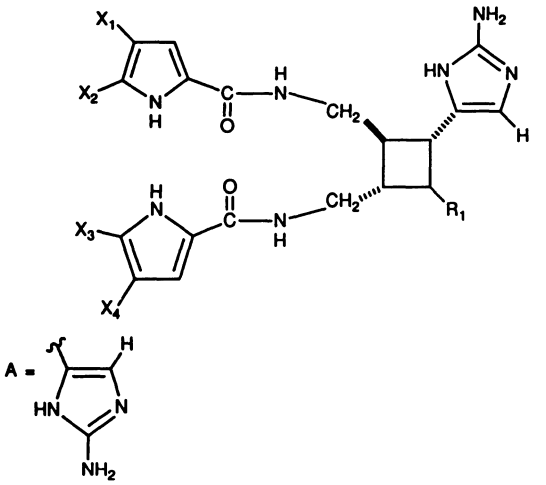


(**81**) Bistheonellide A: R = Me
(**82**) Bistheonellide B: R = H

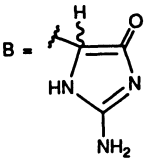
cytotoxicity. In addition, Higa *et al.* (1988b) recorded antitumor, antiviral, and antifungal activity for misakinolide A (bistheonellide A), citing activity against HSV-1 and VSV in CV-1 cells at 8 μg/0.5 ml.

6.2. Sceptrins and Ageliferins

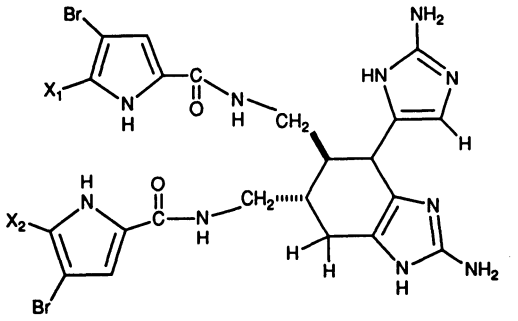
Extracts of the Caribbean sponge *Agelas conifera* (phylum Porifera) yielded the diacetate salts of the series of bromopyrroles shown here (**83–87**, **88–90**)



		X_1	X_2	X_3	X_4	R_1
(**83**)	Sceptrin:	Br	H	H	H	A
(**84**)	Debromosceptrin:	Br	H	H	Br	A
(**85**)	Dibromosceptrin	Br	Br	Br	Br	A



		X_1	X_2	X_3	X_4	R_1
(**86**)	Debromooxysceptrin:	H	H	H	Br	B
(**87**)	Oxysceptrin:	Br	H	H	Br	B

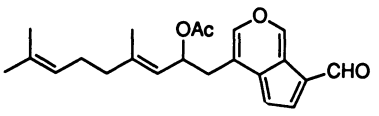


(**88**) Ageliferin: $X_1 = X_2 = H$
(**89**) Bromoageliferin: $X_1 = Br$, $X_2 = H$
(**90**) Dibromoageliferin: $X_1 = X_2 = Br$

(Rinehart, 1988c, Keifer *et al.*, 1991). Based on spectroscopic comparisons to the known sceptrin (R. P. Walker *et al.*, 1981), as well as on FABMS and NMR data, the structures assigned included the oxysceptrins and ageliferins. The latter compounds have sceptrinlike formulas with less symmetrical structures. Compounds of the sceptrin and ageliferin groups are active against HSV-1 at 20 μg/disk and VSV at 100 μg/disk, while the oxysceptrins are less active (Keifer *et al.*, 1991).

6.3. Halitunal

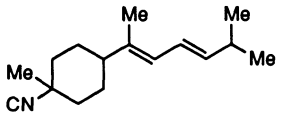
Halitunal (**91**), a diterpene isolated from the green alga *Halimeda tuna* (phylum Chlorophyta) by Koehn *et al.* (1991), was collected near Chub Point in the Bahamas, and constituted 0.01% of the wet weight of the alga. The molecular formula was assigned mainly from NMR spectroscopic measurements and required extensive use of HMBC correlations. Halitunal showed ca. 50% inhibition of viral replication of A59 murine corona virus in NCTC 1469 mouse liver cells at a dose of 20 μg per test well.



(**91**)

6.4. Sesquiterpenoid Isocyanide

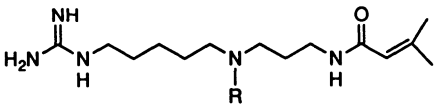
Wright *et al.* (1988) have reported the antitumor, antiviral, and antifungal activities of a sesquiterpenoid isocyanide (**92**) isolated from the marine sponge *Bubaris* (phylum Porifera). At 20 μg/0.5 ml, the A59 coronavirus in mouse liver cells was partially inhibited, indicating that the sesquiterpenoid compound is only weakly virucidal.



(**92**)

6.5. Acarnidines and Polyandrocarpidines

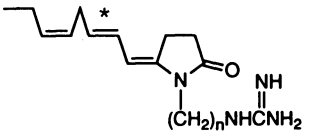
Acarnidines 1a–1c (**93–95**) were isolated from *Acarnus erithacus* (de Laubenfels), a sponge (phylum Porifera), and were among the antiviral substances identified from our collections in the Gulf of California (Carter and Rinehart, 1978a). The homospermidine skeleton common to these three guanidino compounds was assigned based on GC/MS data, and the compounds were distinguished from one another by their fatty acid constituents. In addition to



(**93**) Acarnidine 1a: R = $CO(CH_2)_{10}CH_3$
(**94**) Acarnidine 1b: R = $CO(CH_2)_3CH{=}CH(CH_2)_5CH_3$ (*Z*)
(**95**) Acarnidine 1c: R = $COC_{13}H_{21}$

some antibacterial activity, we observed activity against HSV-1 at 100 μg/disk. However, Munro *et al.* (1987) reported a lack of activity against "a range of DNA and RNA viruses" despite observations of cytotoxicity and antibacterial activity.

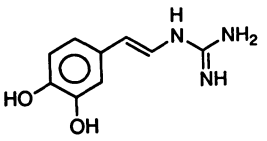
We obtained a mixture of the homologues, polyandrocarpidines I and II, from an extract of a *Polyandrocarpa* sp. (tunicate, phylum Chordata) collected in Baja California (Cheng and Rinehart, 1978). The mixture displayed antibacterial activity and was cytotoxic to CV-1 cells at 200 μg/well. We also observed slight antiviral activity against HSV-1. From studies utilizing NMR data, Carté and Faulkner (1982) found that each homologue was a mixture of γ-methylene-γ-lactam isomers (**96**, **97**; **98**, **99**) in varying proportions and the structure assignment was confirmed by synthesis of derivatives (Rinehart *et al.*, 1983a).



(**96**) Polyandrocarpidine A: n = 5; * *cis* isomer
(**97**) Polyandrocarpidine B: n = 5; * *trans* isomer
(**98**) Polyandrocarpidine C: n = 4; * *cis* isomer
(**99**) Polyandrocarpidine D: n = 4; * *trans* isomer

6.6. Tubastrine

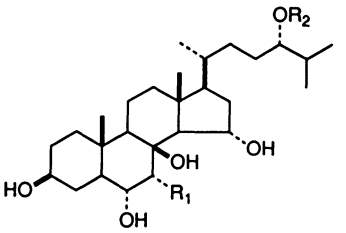
Sakai and Higa (1987) isolated tubastrine (**100**), a guanidino styrene compound obtained from the Okinawan coral *Tubastrea aurea* (phylum Coelenterata). For the extract they reported mild activity against HSV-1 and VSV. The structure was assigned as β-(aminoiminomethyl)-amino-3,4-dihydroxystyrene on the basis of spectroscopic data and chemical derivatization. An additional report claimed that tubastrine completely inhibits VSV and HSV-1 in CV-1 cells at 200 μg/0.5 ml (Higa and Sakai, 1988).



(**100**)

6.7. Saponins

The steroidal glycoside saponins obtained from a variety of starfish exhibit a wide array of activities of biological importance. Shimizu (1971) provided an early example of saponin antiviral activity when he discovered that an extract of the common Atlantic starfish *Asterias forbesi* (phylum Echinodermata) had activity against influenza virus in chick embryos. The purified active components were also obtained from *Acanthaster planci* and *Asterias pectinifera* and found to be polyhydroxylated steroidal glycosides, i.e., asterosaponins (Shimizu, 1971). More recently, Andersson *et al.* (1989) assayed 18 compounds derived from nine starfish and two brittle-stars and identified two polyhydroxylated steroidal glycoside saponins (crossasterosides B and D, **101** and **102**) showing more than a 25% reduction of SHV-1 (Suid herpes virus) plaque formation in porcine kidney-15 cells. The two compounds also showed moderate or weak cytotoxicity and activity against *S. aureus*.



(**101**) Crossasteroside B: R_1 = H, R_2 = 4-O-Me-Xyl$^{1\rightarrow2}$3-O-Me-Xyl-
(**102**) Crossasteroside D: R_1 = OH, R_2 = Xyl$^{1\rightarrow2}$3-O-Me-Xyl-

6.8. BDS-1

Citing unpublished data, Driscoll *et al.* (1989a) reported the presence of antiviral activity in the antihypertensive protein, BDS-I (**103**). They isolated BDS-I from the sea anemone *Anemonia sulcata* (phylum Coelenterata) and determined its three-dimensional solution structure using NMR techniques, including

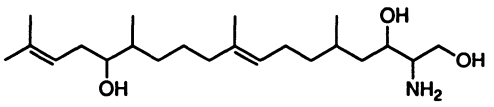
1 5 10 15 20 25 30 35 40
AAPCFCSGKPGRGDLWI$^{L}_{F}$RGTCPGGYGYTSNCYKWPNICCYPH

(**103**)

NOESY, DQF-COSY, HOHAHA, and E-COSY (Driscoll *et al.*, 1989b). BDS-I showed neither the cardiotoxicity nor the neurotoxicity usually associated with sea anemone peptides, but at an unreported concentration it protected mouse liver cells "completely" from the mouse hepatitis virus strain MSV-A59. BDS-I is an approximately 1:1 mixture of the isoproteins (Leu^{18})- and (Phe^{18})-BDS-I.

6.9. Aplidiasphingosine

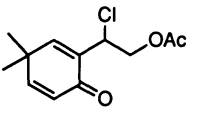
Our collection of tunicates from the Gulf of California included an *Aplidium* species (phylum Chordata) from which we isolated aplidiasphingosine (**104**) (Carter and Rinehart, 1978b). The terpenoid structure, identified as a derivative of sphingosine, was assigned on the basis of IR and NMR data. Broad-spectrum antibacterial activity as well as antifungal, antitumor, and modest antiviral activity (HSV-1) were observed.



(**104**)

6.10. Cyclohexadienone

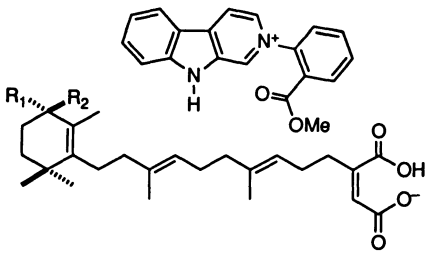
Extraction of the red alga *Desmia hornemanni* (phylum Rhodophyta) yielded a series of octodene-type halogenated cyclohexadienones (monoterpenes) identified by IR, MS, and NMR spectral data (Higa, 1985; Higa *et al.*, 1985; Snader and Higa, 1986a). Along with some of their derivatives, the compounds were tested against L1210, HSV-1, and VSV; of these the acetate shown (**105**) was reported to have "potent antiviral activity" against HSV-1 and VSV.



(**105**)

6.11. Reticulatines

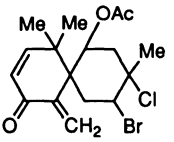
Reticulatines A and B (**106** and **107**) were isolated from the Fijian sponge *Fascaplysinopsis reticulata* (phylum Porifera) and were found to be closely related to the known fascaplysins, isolated from the same species (Jiménez *et al.*, 1991). Positive and negative ion FABMS played an important role in assigning the structures of these β-carbolinium salts. The cationic structure was an unusual feature for the molecules, and both **106** and **107** were said to show "potency" in antiviral assays, although no data were provided.



(**106**) Reticulatine A: $R_1 + R_2 = O$
(**107**) Reticulatine B: $R_1 = R_2 = H$

6.12. Chamigrene Derivatives

Snader and Higa (1986b) obtained chamigrene derivatives (e.g., **108**) from the sea hare *Aplysia dactylomela* (phylum Mollusca). Although no data were provided, *in vitro* HSV-1 and VSV inhibitions were claimed.



(108)

6.13. Polysaccharides

Carrageenan is a cell-wall polysaccharide constructed from galactose with varying amounts of sulfate substituents and is isolated in large quantity from red algae (phylum Rhodophyta). Samples collected in Senegal, including *Hypnea musciformis*, *Anatheca montagnei*, *Agardhiella tenera*, and *Euchema cottonii*, inhibited the activity of yellow fever virus by up to 25.8% (Ferrer-Di Martino *et al.*, 1985). A carrageenan sample obtained commercially (Sigma Chem. Co.) by Gonzalez *et al.* (1987) inhibited HSV-1 cell growth in HeLa cells without becoming cytotoxic when concentrations were maintained as high as 200 μg/ml. Their studies indicated that the time course of HSV-1 infection is a critical factor in determining the success of carrageenan treatment. Neushul (1991) observed activity against HIV in water-soluble substances extracted from *Schizymenia californica* and reported that a component of the extract, carrageenan, inhibited reverse transcriptase. The use of marine-derived polysaccharides in the treatment of retroviruses had previously been proposed (Muto *et al.*, 1988).

7. CONCLUSIONS

From the foregoing discussion of antiviral substances found in marine extracts, two general observations stand out. First, antiviral activity is by no means limited to any one class of chemical compounds any more than it is to any one phylum of marine species. Peptides, heterocycles, and terpenes all contribute compounds with confirmed antiviral activity. From this it follows that a number of different mechanisms of action will be found for this disparate collection of compounds. Very little is known about these mechanisms, but studies of modes of action of the compounds should provide an active area of investigation in years to come.

The second generality is less optimistic. Only in very few cases have any of the compounds discussed above been tested *in vivo*, an obvious prerequisite for any attempt to introduce an antiviral agent into the clinic. Moreover, where *in vivo* activity has been measured, the specificities and margins of safety have been relatively narrow. Thus, toxicity seems likely to be a serious problem with most marine-derived drugs as with most other antiviral agents. The one clinically useful

compound at present, ara-A, originally resulted from a structure–activity relationship study of arabinosyl nucleosides but was subsequently found in nature.

Although one can envision cases where these antiviral compounds could be used in life-threatening situations, in the main we are still a long way from introducing any marine natural products as marketable antiviral agents. A potential area for introduction of a marine-derived drug would be in treatment of AIDS and perhaps efforts should be increased in this direction.

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