Pure & Appl.Chem., Vol.54, No.10, pp.1919-1934, 1982.
Printed in Great Britain.

0033-4545/82/101919-16$03.00/0
Pergamon Press Ltd.
©1982 IUPAC

TOXINS, ANTICANCER AGENTS, AND TUMOR PROMOTERS FROM MARINE PROKARYOTES

Richard E. Moore

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

Abstract -- Marine prokaryotic organisms, in particular blue-green algae belonging to the Oscillatoriaceae, are potentially good sources of new antineoplastic agents. The dermatitis-producing substances in the blue-green alga Lyngbya majuscula, however, which exhibit marginal anticancer activities, are powerful tumor promoters. The potencies and modes of action of two of these tumor promoters, aplysiatoxin and lyngbyatoxin A, are essentially identical with the potency and mode of action of the well known tumor promoter from Croton oil, 12-O-tetradecanoylphorbol 13-acetate (TPA). Structure-activity relationships are presented for aplysiatoxin, lyngbyatoxin A, TPA, and related compounds. The absolute stereochemistries of the aplysiatoxin and lyngbyatoxin A are discussed.

INTRODUCTION

Cancer is the second leading cause of death in the United States and the Western World. Epidemiological studies of this insidious disease have now clearly shown that most human cancers are caused by environmental (exogenous) rather than endogenous factors. In recent years the emphasis of research on cancer has switched from studies on the treatment of cancer to studies on its cause and prevention. The rationale for this change is simple. If one could identify the causative exogenous factors, then presumably most human cancer could be eliminated either by reducing the human exposure or by developing protections for the human host against the responsible environmental agents (1,2).

As a result of this change the level of financial support for the development of new chemotherapeutic agents for the treatment of cancer has declined, whereas support for the identification of the causative carcinogens, mutagens, and cocarcinogens (tumor promoters) that are involved in the development of human cancer and the dietary constituents and other factors that protect the host by inhibiting or blocking the effects of the causative agents has increased. The present emphasis of research on cause and prevention is a reasonable one, since the control of this very complex disease will undoubtedly come only when the biochemistry of tumor formation is completely understood. Only then will it be possible to rationally develop new drugs that will not only specifically kill cancer cells (chemotherapeutic agents) but prevent their formation as well (chemopreventive agents), both in the early and late stages of tumor development.

One should not interpret the current research trend as a signal that searching for new drugs for the treatment of cancer using classical methodology is becoming a worthless endeavor. Quite the contrary, there are many leads that are worth pursuing. There is always the possibility that a new drug will be discovered that will cure a cancer without any undesirable side effects. Another reason why this search should continue is that new drugs will be discovered that will be invaluable pharmacological tools for studying and unravelling the complex biochemistry of the cancer cell rather than useful chemotherapeutic agents. As a result scientists will move a step closer to the day when their better understanding of the disease will permit a more scientific approach to finding new drugs for controlling cancer.

At this symposium I should like to focus on some of the leads that we have uncovered in the marine natural products area for finding new antineoplastic agents, to discuss the major difficulties that we have encountered in developing new drugs from these leads, to discuss the chemistry and significance of the compounds that we have isolated and identified, to show how these compounds are being used as tools for probing the mechanisms of chemical carcinogenesis, and to show how the compounds that we have isolated are being used to develop methods for searching for chemopreventive agents.

CYTOTOXICITY AND ANTINEOPLASTIC ACTIVITY

When the natural products chemist began to examine marine organisms in detail about two decades ago, it was not long before he realized that marine plants and animals contained an exciting array of structurally unusual organic compounds, frequently very different from those found in terrestrial plants and animals. Could more effective anticancer drugs be present among this vast assortment of new structures? The initial screening reports were encouraging, at least for extracts of certain marine animals (3) and plants (4). Actual progress in the isolation and identification of the active principles, however, has been extremely slow. Several new cytotoxins with novel structures have been isolated and identified, but to the author's knowledge none of these compounds have been shown yet to be effective in the treatment of human cancer (5,6,7,8,9,10,11).

There are many reasons for this sluggish progress. A major reason is undoubtedly linked with the total dependence of the investigator on a suitable bioassay to screen crude extracts and to guide him in the isolation and purification of an active drug from an extract. The majority of investigators have relied on outside testing, but our experience has shown that in-house testing is quicker, more reliable, less frustrating, and in the long run less expensive.

Mice are used exclusively in primary screens for antineoplastic activity. Since curative activity is a rare occurrence, a substance that prolongs the life of the diseased animal to a significant extent (50% or better) is considered to be active and worthy of further study. In our laboratory we are now using four systems, viz. Ehrlich ascites tumor, P-388 lymphocytic leukemia, Lewis lung carcinoma, and B16 melanoma. The Ehrlich ascites tumor assay was used extensively in the early 1960s by the National Cancer Institute; this is no longer used by NCI since the test is too variable, too long, and not selective enough. Interestingly we have found that the Ehrlich ascites system is fairly good screen if one uses complete cure as the sole criterion for activity.

Once it has been established by *in vivo* testing that an extract has antineoplastic activity, use of *in vivo* assays to monitor isolation and purification of the active principles is extremely tedious, slow, and expensive. Use of cell culture assays alleviate most of these problems. *In vitro* assays, however, only indicate cytotoxicity. Unfortunately there is no clear cut correlation between cytotoxicity and antineoplastic activity. Anticancer compounds are generally cytotoxic, but cytotoxins do not always display antineoplastic activity when tested *in vivo*.

Many researchers have become side-tracked with *in vitro* cytotoxicity data which have not been correlated with animal testing data. Consequently much effort can be spent on the isolation and identification of cytotoxic compounds that have no value as antineoplastic agents.

In our experience the only meaningful indicators of antineoplastic activity are the ones obtained from direct animal testing. Cytotoxicity assays in various cancer cell lines (e.g. KB, a human epidermoid carcinoma of the nasopharnx, and NIH 3T3; which are two systems that we are currently using) are useful for monitoring isolation and purification of the active compounds only after it has been established that the crude extract is active *in vivo*. After each purification step the cytotoxic fraction is checked for antineoplastic activity *in vivo*. Using these procedures we have been examining extracts of marine organisms for anticancer activity. So far we have found the largest incidence of activity in extracts of prokaryotic organisms and animals that have prokaryotes associated with them symbiotically.

Blue-Green Algae

For several years we have been interested in blue-green algae as sources of new anticancer agents. In our search we have found that extracts of blue-green algae collected in the field, especially those belonging to the Oscillatoriaceae, are frequently very active against P-388 lymphocytic mouse leukemia *in vivo*. Our attempts to isolate the active principles from most of these field specimens, however, have so far been largely unsuccessful. With the exception of a few species, e.g. *Lyngbya majuscula*, the vast majority of blue-green algae simply have not been available in sufficient quantities from field collections to insure isolation and characterization of the active principles. Even more frustrating, recollection attempts have generally failed as the algal blooms are sporadic in appearance and highly variable in production of drug. In late November, 1977, for example, Dennis Russell collected a marine *Phormidium* sp. at Pohakuloa on the island of Molokai; the methanolic extract of the cyanophyte showed a T/C (ratio of the average death time of treated mice to average death time of control mice x 100) value of 210 at a dose of 12.5 mg/kg in the P-388 lymphocytic leukemia test, with no signs of chronic toxicity. It also completely cured Ehrlich ascites (EA) tumor in mice at a dose of 10 mg/kg. Unfortunately Russell's *Phormidium* sp. was never found again. Species of *Phormidium* collected in May,

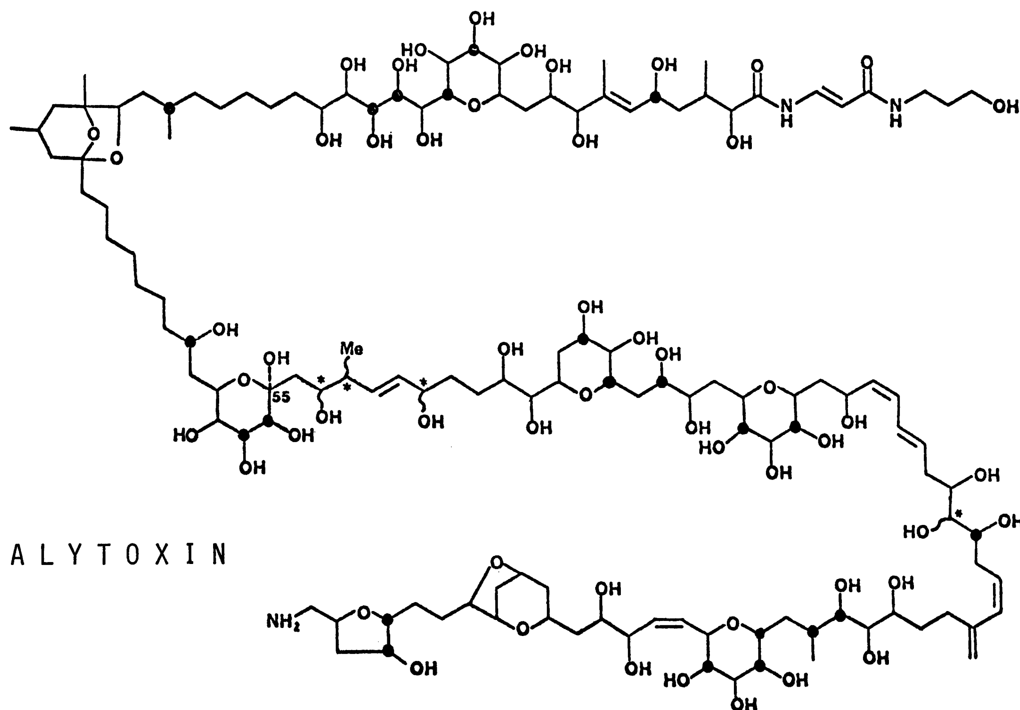
1978 and November, 1978 from the same area on Molokai proved to be inactive. Similarly in August, 1977, Jon Mynderse collected an Oscillatoria sp. from the reef in Arumizu Bay, Palau in the Western Caroline Islands; the crude aqueous ethanolic extract of this blue-green alga showed a T/C value of 205 at a dose of 5 mg/kg in the P-388 screen, again with no signs of chronic toxicity, and completely cured EA at 4 mg/kg. When this Oscillatoria sp. was recollected at this same site in Palau two years later, the extract showed no anticancer activity.

To circumvent these problems we began an intensive program in early 1981 to grow blue-green algae in the laboratory under carefully controlled conditions. We have not restricted ourselves to marine species as some freshwater and terrestrial cyanophytes readily adjust to marine environments and possibly might produce similar natural products to those found in marine varieties. Jon Mynderse, for example, has isolated an unusual antineoplastic agent, tolytoxin, from a terrestrial blue-green alga Tolypothrix conglutinata var. clorata found on Fanning Island (12); a closely related substance has recently been isolated from a marine organism (13).

In the past year Gregory Patterson has grown in culture over fifty strains of blue-green algae from various marine, aquatic, and soil sources. Eiichi and Shinobu Furusawa and Ted Norton in the University of Hawaii's Department of Pharmacology have tested the extracts of the cultured cyanophytes for cytotoxicity in vitro and for antineoplastic activity in vivo. One of the best activities is shown by the aqueous ethanolic extract of an Oscillatoria sp. that was collected from a freshwater pond in Manoa Valley, Oahu. T/C values of up to 150 were observed in the P-388 test and a T/C value of over 150 was obtained in a modified Lewis Lung carcinoma test (intraperitoneal injection of cancer cells rather than subcutaneous or intravenous administration) in which some mice were cured. Ten clones of this alga have been prepared by standard plating procedures. Only one of these clones have been examined. The major problem that confronts us at the moment is one of yield. The active compound is present in very small amount and we need to optimize conditions (pH, temperaure, light intensity and quality, aeration rate, time, organic additives) for production of drug. The chromatographic behavior of the active compound suggests that it has a molecular weight in the low thousands. Nothing else is known about this drug other than it is stable to heat and labile to both acid (pH 2) and base (pH 10).

Bacteria and Other Prokaryotes

The antineoplastic activity of many marine animals may be due to the presence of symbiotic prokaryotes. Palytoxin, for example, is an exceedingly poisonous, water-soluble substance that is found in coelenterates belonging to the genus Palythoa (14). Recently we have obtained evidence that palytoxin is produced by a bacterium, possibly a Vibrio sp., that is symbiotically associated with these coelenterates (15). Several clones of this bacterium were obtained from Hawaiian P. toxica, but only one clone produced a toxin that was chromatographically and pharmacologically identical with palytoxin. Only one subculture of this

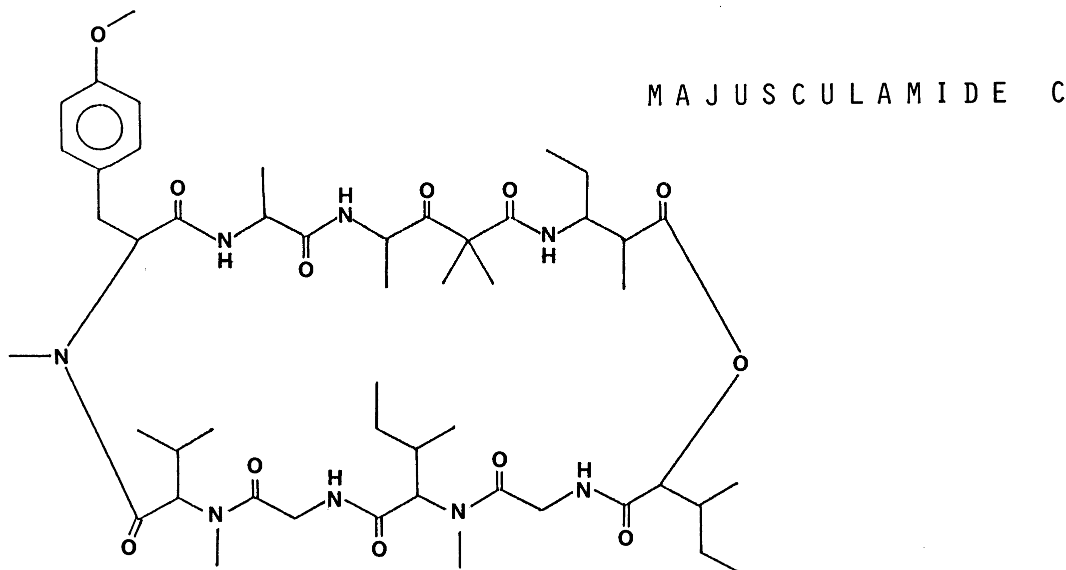


clone, however, displayed toxicity. From this behavior we conclude that certain requirements are needed to trigger toxin production in the bacterium outside of its animal host. These requirements are at present unknown.

Palytoxin is a powerful cytotoxin which shows some anticancer activity *in vivo*; for example, it completely cures Ehrlich ascites tumor in mice at doses as low as 1/10 the minimum lethal dose, but shows only marginal activity against P-388 leukemia (16). The absolute configurations of 60 of the 64 chiral centers of the major palytoxin from *P. toxica* have recently been determined; the remaining four asymmetric carbons, viz. C(57), C(58), C(61), and C(88), which are indicated by asterisks in the structural drawing are uncertain (17).

Didemnin B is a cyclic depsipeptide that the Rinehart group at the University of Illinois has isolated from a species of a Caribbean tunicate belonging to the genus *Tridemnum* (8). Didemnin B shows very good activity against P-388 leukemia and B16 melanoma in mice; the animal testing results have been encouraging enough that this compound has now reached clinical trial (18).

We were very interested in this discovery since Jon Mynderse had isolated a cytotoxic cyclic depsipeptide, majusculamide C, from the blue-green alga *Lyngbya majuscula*, which showed cell cycle activity that was similar to the mitosis blocker cytoclasin B (19). Majusculamide C, however, only showed 35% inhibition against X-5563 myeloma (0.5 mg/kg) and had marginal to nil activity against P-388 leukemia, 6C3HED lymphoma, and 755 carcinoma. A tentative structure has been proposed for majusculamide C by Daniel Carter (20); its mass spectrum indicates that a 2-[3-amido-2-methylpentoxy]-3-methylpentanoylglycyl-N-methyliso-leucyl-glycyl-N-methylvalyl-N,O-dimethyltyrosyl sequence is present, but the sequence of the remaining alanyl and alanylacetyl units is uncertain.



The didemnins could be metabolites of a *Prochloron* sp. that is associated symbiotically with all didemnid ascidians (21). This prokaryotic organism was originally thought to be a blue-green alga (22). *Prochloron*, however, belongs to a phylum (Prochlorophyta) that is different from the phyla that other prokaryotes, such as bacteria and blue-green algae, belong to. Gregory Patterson and Nancy Withers (23) have recently discovered that *Prochloron* is an algal mutant that requires tryptophan for growth outside of its host. The alga apparently obtains this essential amino acid in its symbiotic relationship with the ascidian. The *Prochloron* sp. in the didemnid ascidian *Diplosoma similis* from Kaneohe Bay, Oahu has been isolated and grown in culture. Testing of the extracts of the ascidian and the cultured prokaryote is planned.

ANTINEOPLASTIC ACTIVITY AND CHEMICAL CARCINOGENESIS

Most anticancer compounds that are used clinically for the treatment of human cancer are carcinogenic. Since there is generally a long lag time between carcinogen exposure and tumor formation, one wonders whether the drugs that are presently being used to treat and cure cancers are initiating new tumor cells that will emerge as different cancers 20-30 years from now.

In our work with anticancer agents from blue-green algae, we have isolated and identified representatives, e.g. debromoaplysiatoxin (24) and lyngbyatoxin A (25), of two classes of compounds which show activity against P-388 lymphocytic mouse leukemia. Unlike most antineoplastic agents, however, the aplysiatoxins and lyngbyatoxins do not appear to be carcinogenic, but rather cocarcinogenic (2,26,27). Cocarcinogens, which are generally called tumor promoters, accelerate the development of benign and malignant tumors from cells that have been exposed to carcinogens. The tumor promoters that are involved in the development of most human cancers have not been identified; in fact, only recently has it been recognized that such factors exist for cancers other than skin cancer. Many carcinogens can act as tumor promoters, but usually much higher concentrations of the carcinogen are required to accomplish this second stage.

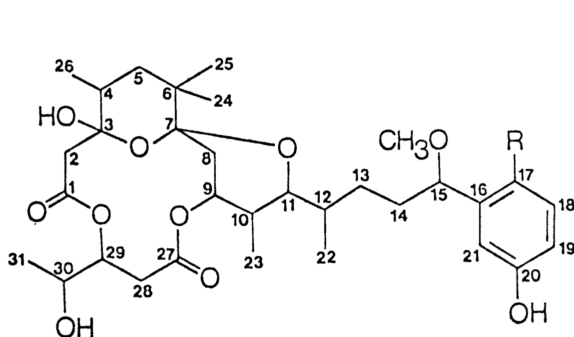
The blue-green algae that contain these tumor promoters may play a role in human stomach cancer, possibly among the Hawaiians who consume large amounts of seaweed and who have the highest incidence of gastrointestinal cancer in the world. The Hawaiians frequently eat seaweeds that contain carcinogenic and mutagenic halogen-containing compounds (e.g. *Asparagopsis taxiformis* (28) and *Laurencia nidifica* (29)). Since the tumor promoter-containing blue-green algae sometimes grow epiphytically on edible seaweeds (30), the possibility exists that the Hawaiians are obtaining in their seaweed diet alone all of the necessary exogenous ingredients for developing gastrointestinal cancer.

Anticancer Compounds from Oscillatoriaceae

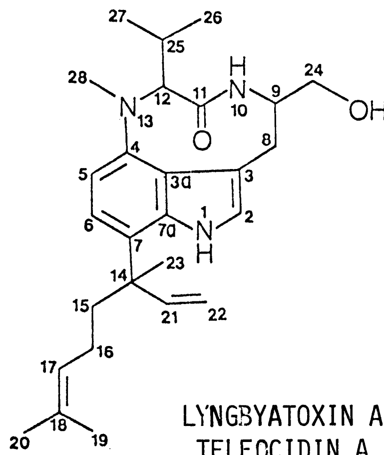
One of the most common and accessible marine cyanophytes in the subtropical and tropical oceans is *Lyngbya majuscula*. The first *L. majuscula* that we tested for antineoplastic activity was a deep-water variety found abundantly on many of the pinnacles of Enewetak Atoll in the Marshall Islands (24). The crude lipophilic extract of this blue-green alga consistently displayed activity in the P-388 screen at a T/C value of 140 (0.6 mg/kg dose). Using an *in vivo* bioassay to monitor the isolation of the drug, Jon Mynderse showed that the active principle was debromoaplysiatoxin, one of the toxic constituents that Kato and Scheuer had isolated from the digestive gland of the sea hare *Stylocheilus longicauda* (31). While collecting the *L. majuscula* at Enewetak, Mynderse found several *S. longicauda* feeding on the alga. This discovery provided clear evidence that debromoaplysiatoxin was being accumulated in the digestive tract of this gastropod mollusk through diet. Aplysiatoxin, a second major toxin that had been isolated from the Hawaiian sea hare, however, was not found in this deep water variety of *L. majuscula*.

When extracts of shallow water varieties of *L. majuscula* from Hawaii were examined, antineoplastic activity could generally be attributed to debromoaplysiatoxin and aplysiatoxin. The marginal activity of the shallow water *L. majuscula* on leeward Oahu, however, was shown to be due primarily to a different compound, lyngbyatoxin A (25).

Debromoaplysiatoxin was also identified as one of the compounds responsible for the antineoplastic activity of the lipophilic extract of a mixture of two blue-green algae, tentatively identified as *Schizothrix calcicola* and *Oscillatoria nigroviridis*, found on the seaward side of Enewetak Atoll (32). A second antineoplastic compound in this algal mixture, oscillatoxin A, was isolated and shown to be 31-nordebromoaplysiatoxin; it showed the same activity against P-388 leukemia *in vivo* as debromoaplysiatoxin. Small amounts of 17-bromooscillatoxin A, 17,19-dibromooscillatoxin A, and 19-bromoaplysiatoxin were also found in this algal mixture, but aplysiatoxin was not detected. The brominated toxins were not isolated in sufficient quantities for evaluation against P-388 *in vivo*.



APLYSIATOXIN, R = Br
DEBROMOAPLYSIATOXIN, R = H



LYNGBYATOXIN A
TELEOCIDIN A

Debromoaplysiatoxin and oscillatoxin A are fairly toxic substances. The minimum lethal dose of each compound in mice is roughly 0.2 mg/kg. Their best anticancer activities are observed only at the chronic toxicity levels and therefore do not appear to be potentially useful as anticancer drugs.

Seaweed Dermatitis

Lyngbya majuscula is the causative agent of a severe contact dermatitis that sometimes affects swimmers and bathers in Hawaii during the summer months. To date outbreaks of the dermatitis have only been observed on the windward side of the island of Oahu. Fortunately seaweed dermatitis is rare and large outbreaks occur several years apart. The most recent outbreak, where a total of 86 persons with symptoms were reported to the Hawaii Department of Health, occurred at the Kailua, Kalama, and Pilapu beaches on windward Oahu in August, 1980 (33). The symptoms of the dermatitis were a burning rash, generally involving the genital and/or perianal areas, which was followed by blister formation and deep desquamation. The dermatitis had resulted from the swimmer's contact with filaments of the blue-green alga, which had been broken loose from the ocean floor by the heavy surf and were floating freely in the water.

The 1980 outbreak provided our first opportunity to examine in detail a *L. majuscula* implicated directly with a major outbreak of the dermatitis. Giovanni Bartolini collected the free-floating cyanophyte and showed that debromoaplysiatoxin and aplysiatoxin were the major inflammatory and vesicatory constituents and the agents responsible for the dermatitis. This marked the first time that the bromine-containing aplysiatoxin had been isolated from *L. majuscula*. Jon Mynderse had only demonstrated the presence of debromoaplysiatoxin in the *L. majuscula* collected during an outbreak in Laie Bay on windward Oahu in September, 1976 (24).

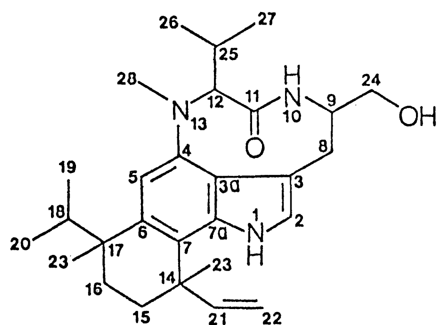
The *L. majuscula* on the leeward side of Oahu, which has never been implicated in an outbreak of seaweed dermatitis, also contains dermatitis-producing substances. The major inflammatory substance in a variety growing abundantly at Kahala Beach near Waikiki, Oahu for example, is lyngbyatoxin A (25), which is equipotent with the aplysiatoxins as an irritant. Surprisingly it is a compound which belongs to a complete different class of compounds. Lyngbyatoxin A is an indole alkaloid which is structurally related to teleocidin B, a potent inflammatory agent found in the soil fungus *Streptomyces mediocidicus* (34,35,36). Lyngbyatoxin A appears to be a minor constituent in the dermatitis-producing strains of *L. majuscula*. Murray Munro has examined *L. majuscula* from windward Oahu and Molokai and shown that lyngbyatoxin A is present.

S. mediocidicus is responsible for a contact dermatitis which affects workers in the antibiotic industry. Erthyromycin-producing strains of Streptomycetes sometimes become contaminated with teleocidin-producing strains and the workers that handle the contaminated fungi frequently contract severe rashes in the sensitive skin areas such as the genital and perianal regions.

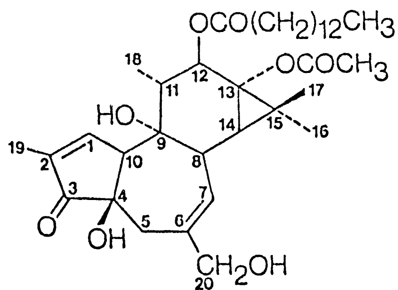
Structures of Teleocidins and Lyngbyatoxins

The teleocidins are the first representatives of this class of indole alkaloids and are so-named for their ichthyotoxicity. The telocidins have been isolated from the mycelia of several strains of Streptomyces (37,38). *S. mediocidicus* contains two dermatitis-producing components, viz. teleocidin A, which has a molecular weight of 437 daltons, and teleocidin B, which has a molecular weight of 451 daltons. Hirota Fujiki and Takashi Sugimura at the National Cancer Center Research Institute in Tokyo, Japan have recently found that teleocidin A is a mixture of lyngbyatoxin A and its C(14)-epimer. They have also discovered that teleocidin B is a mixture of the four possible C(14),C(17)-diastereomers. The structure of the 14*S**,17*S**-teleocidin B diastereomer has been previously solved by a X-ray crystallographic study of the corresponding 14*R**,17*S**-dihydroteleocidin B monobromoacetate derivative (35,36). Presumably the Hirata group had fortuitously crystallized this diastereomer, which is generally the major one, from a mixture of the four dihydroteleocidin B monobromoacetates.

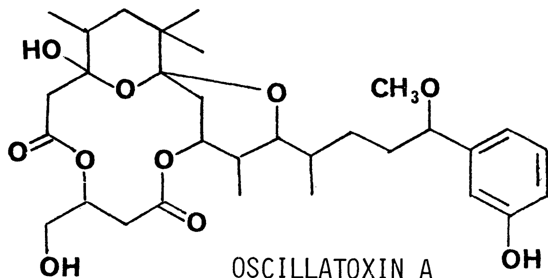
In our structural studies of lyngbyatoxin A (25), we found that the optical rotation of lyngbyatoxin A and the one reported in the literature for teleocidin B were both strongly levorotatory and that the circular dichroism curves of tetrahydrolyngbyatoxin A and a sample of dihydroteleocidin B obtained from the Hirata group were essentially the same. The dihydroteleocidin B that was used for the CD measurement and the teleocidin B that was used for the optical rotation determination were probably mixtures of the four C(14),-C(17)-diastereomers. Since the optical properties of lyngbyatoxin A and the teleocidin mixture and their catalytic hydrogenation products were essentially identical, the absolute stereochemistries of C(9) and C(12) for all of the compounds had to be the same. Unfortunately we misinterpreted the Hirata papers and inadvertently assigned the *L* configurations to the two amino acid units in the two toxins in our previous papers (12,25). In reexamining the Japanese papers we find that the absolute stereochemistry of



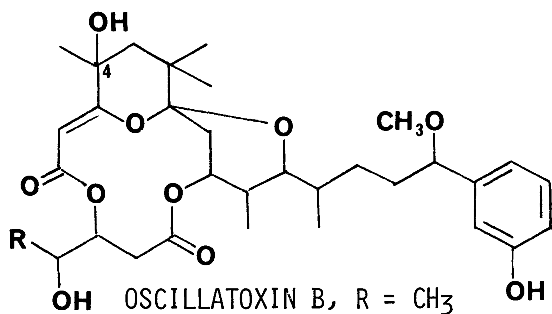
TELEOCIDIN B



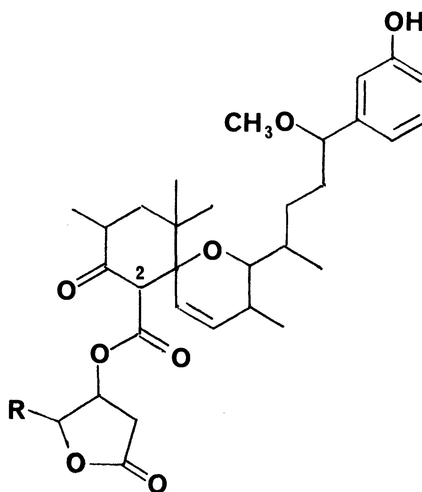
12-O-TETRADECANOYLPHORBOL-13-ACETATE
TPA



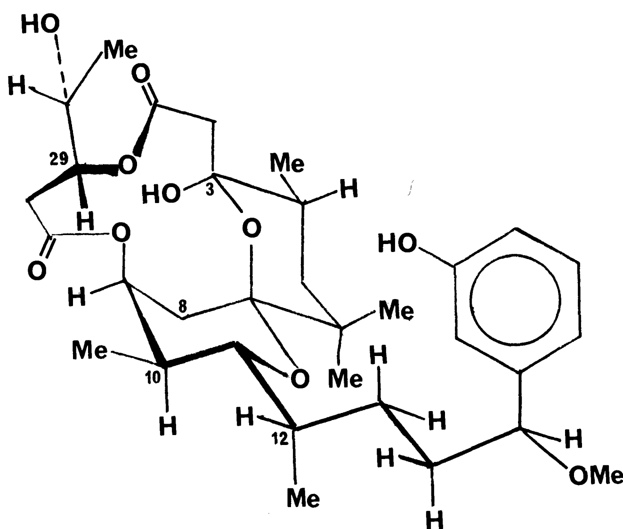
OSCILLATOXIN A



OSCILLATOXIN B, R = CH_3
31-NOROSCILLATOXIN B, R = H



OSCILLATOXIN D, R = H
30-METHYLOSCILLATOXIN D, R = CH_3



Absolute stereochemistry of debromoaplysiatoxin

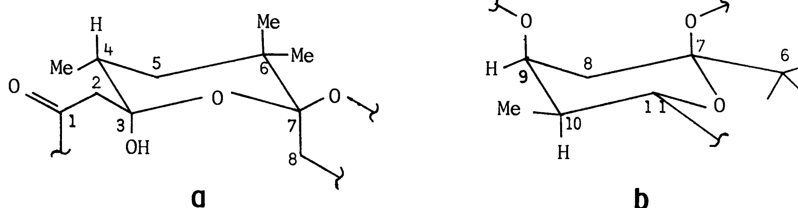
14R*,17S*-dihydroteleocidin B monobromoacetate was not determined; the X-ray data was not refined enough to permit a choice. Evidence presented below suggests that the two amino acid residues might be D and we are presently accumulating sufficient lyngbyatoxin A to solve this problem by chemical degradation.

Only one C(14)-epimer of lyngbyatoxin A is present in L. majuscula. At this writing we do not know what the relative stereochemistry of lyngbyatoxin A's C(14) is with respect to the two chiral centers in the lactam ring. At times we have thought that lyngbyatoxin A might be a mixture of two structural isomers (12). Variable temperature proton NMR studies, however, now clearly show that this doubling is due to two slowly interconverting conformers as we had originally proposed in our first paper on lyngbyatoxin A (25). Interestingly, Hirota Fujiki and Takashi Sugimura have found that the proton NMR signals of the two teleocidin A epimers, one of which is identical to lyngbyatoxin A, and the four teleocidin B diastereomers are all doubled, presumably due to two slowly interconverting conformers for each compound.

Murray Munro has found that L. majuscula contains a trace amount of a substance having a molecular weight of 451 daltons. Preliminary examination of a dermonecrotic-active fraction containing this compound indicates that it is not a teleocidin B. The mass spectrum of the fraction shows a peak at m/z 451 for the molecular ion, but none of the fragmentation peaks that are seen in the mass spectra of the four teleocidin B diastereomers are present. There is a peak at m/z 368 which appears to be a fragmentation peak of the 451 compound. The 368 peak, which may correspond to loss of C_6H_{11} from a linalyl side chain (cleavage of the C(14)-C(15) bond) in the 451 compound, suggests that the additional CH_2 is on the indole or lactam ring.

Structure of Aplysiatoxins and Oscillatoxins

The gross structure of aplysiatoxin (AT) was solved by Kato and Scheuer in the early 1970s (31). On the basis of its chemical reactivity and spectral properties, Kato and Scheuer were able to propose the relative stereochemistry of the two oxane rings A and B as shown in drawings a and b (39). Rings A and B were assumed to have the preferred chair conforma-

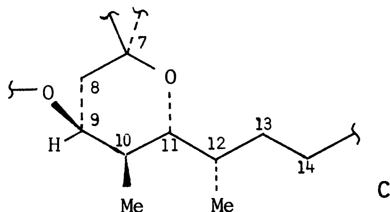


tions. Since AT was extremely unstable to acid and readily lost water to form anhydroaplysiatoxin (AAT), the hemiketal OH on C-3 and the hydrogen of C-4 of ring A had to be trans diaxial to each other. The methyl group on C-4 was therefore equatorial. The proton NMR spectrum of aplysiatoxin supported these assignments as W-coupling was observed between the hydroxyl proton of C-3 and the hydrogen of C-4 and the C-4 proton signal showed trans and gauche couplings to the hydrogens on C-5 (12). Since dehydration of AT to AAT caused an appreciable change in chemical shift for the C-9 proton, Kato and Scheuer felt that C-8 had to be attached axially to C-7. For ring B the NMR data indicated that the lactone oxygen was connected axially to C-9 and that the methyl on C-10 and the side chain on C-11 were in equatorial positions. In the construction of a Dreiding model, the bislactone system could only be closed if the oxygen of ring A was attached axially to ring B. No stereochemical assignments of C-12, C-15, C-29, and C-30 were made. Debromoaplysiatoxin (DAT) had the same relative stereochemistry.

In our work on the toxins of L. majuscula, Jon Mynderse was able to crystallize DAT (24). Unfortunately it has not been possible to solve its structure by X-ray crystallography and so far we have not been able to crystallize AT or to prepare any crystalline derivatives containing bromine for X-ray analysis.

Ronald Woodard and John Craig at the University of California Medical Center have been able to determine the absolute configuration of C-15 in DAT as S by comparing its circular dichroism spectra with those of appropriate model compounds. They have also shown that oscillatoxin A (OT-A) has a circular dichroism curve that is identical with that of DAT. The absolute stereochemistries of DAT and OT-A are therefore identical. Adrian Blackman has recently degraded ADAT by acid hydrolysis to dextrorotatory cis-3-hydroxy-4-methyl-γ-butyrolactone, which exhibits a positive CD curve. He has also degraded anhydrooscillatoxin A (AOT-A) to dextrorotatory 3-hydroxy-γ-butyrolactone which also exhibits a positive CD curve; the optical properties are opposite to those of the S(-)-lactone synthesized from L-malic acid (40). The absolute configuration of C-29 in both DAT and OT-A is therefore assigned as R from the positive CD curves (41) of the two lactone degradation products.

We have now confirmed the relative stereochemistry shown in a and b with extensive NOE studies on debromoaplysiatoxin 20,30-diacetate. Irradiation of the equatorial methyl group on C-6 shows a positive NOE on the axial C-8 hydrogen, but no NOE effect on the equatorial C-8 proton. Irradiation of the axial methyl group on C-6, on the other hand, produces no NOE effect on either of the C-8 protons. When the equatorial C-8 proton is irradiated, an appreciable positive NOE is observed for the proton of the axial hydroxyl group on C-3. These three NOE experiments prove that C-8 is attached axially to ring A at C-7. The most informative NOEs are seen when the equatorial methyl group on C-10 is irradiated; signal enhancements are seen for the protons on C-9, C-11, C-12, and C-29 (Fig. 1). The NOE effect on the C-12 proton indicates that the methyl group on C-10 and the hydrogen on C-12 are eclipsed in the preferred conformation of this molecule in solution. The NOE on C-29 establishes the relative stereochemistry of thedioxyvalerate portion of the molecule with respect to the rest of the molecule; note in the figure that one of the protons on C-28 may also be experiencing a slight NOE. The relative stereochemistry of the chiral centers in the oxane rings and the two asymmetric carbon atoms in the dioxyvalerate unit is therefore 3S,4R,7S,9S,10R,11R,29R,30R. Since C-29 has been shown already to be R, the absolute configurations of these eight asymmetric carbons are as indicated. In the preferred conformation of this molecule in solution, the C(12)-C(13)-C(14)-C(15)···· side chain is fully extended and coplanar with C(11)-C(10)-C(9) in ring B as shown in c. This means that



the absolute stereochemistry of C-12 is S. The C-12 assignment is supported by a NOE experiment whereby irradiation of the methyl group on C-12 produces appreciable NOEs on the C-10 hydrogen and one of the hydrogens on C-14, possible only if the side chain is fully extended with the carbons of ring B so that the methyl group can eclipse both of these protons.

Lyngbya majuscula and other DAT-containing species of Oscillatoriaceae elaborate several non-toxic compounds that are related to DAT. These compounds, referred to as oscillatoxins, were first isolated by Jon Mynderse from the Schizothrix calcicola and Oscillatoria nigroviridis mixture from Enewetak Atoll. Two of the compounds, oscillatoxin B (OT-B) and 31-noroscillatoxin B (31-norOT-B), have molecular weights that are two mass units lower than those of DAT and OT-A, respectively. Two structures, which are presented elsewhere (12), have been considered for both OT-B and 31-norOT-B. New data indicate that OT-B and

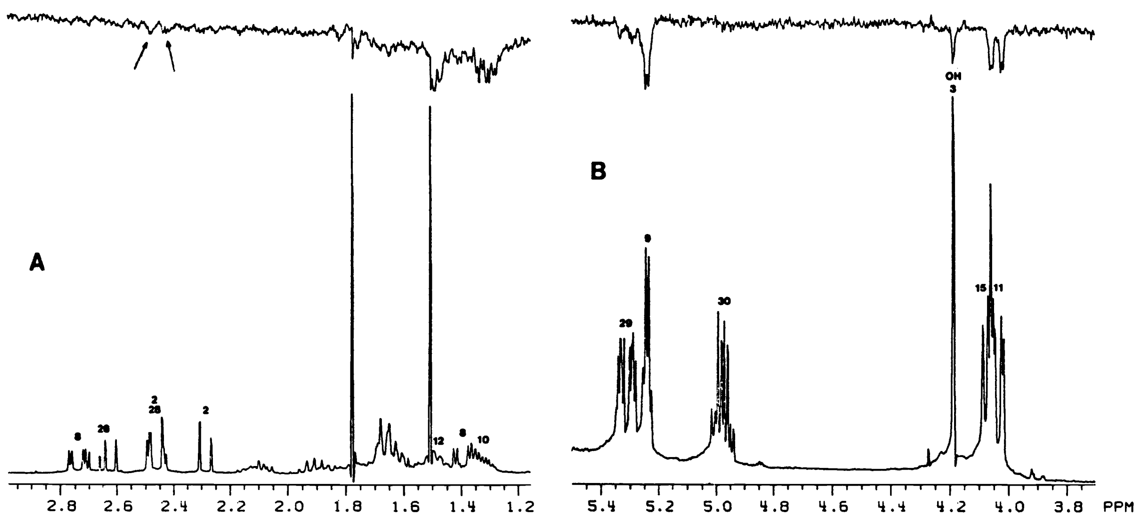


Fig. 1. 300 MHz ^{1}H NMR spectrum of debromoaplysiatoxin 20,30-diacetate in benzene-d_6. Lower traces in A and B represent 2.98-1.15 ppm and 5.50-3.70 ppm sections of the normal spectrum. Upper traces in A and B are comparable sections of the NOE difference spectrum resulting from irradiation of the methyl group on C-10 (δ 0.659). Note that significant NOEs are shown by protons on C-12, C-10, C-29, C-9, and C-11 and also by the OH group on C-3 (NOE transfer from proton on C-9). There also appears to be a very small NOE shown by one of the protons on C-28 (indicated in upper trace of A by arrows).

31-norOT-B are 4-hydroxy-2,3-anhydrodebromoaplysiatoxin and 4-hydroxy-2,3-anhydrooscillatoxin A, respectively, where the Δ^2-double bond in both compounds is Z. Interestingly Adrian Blackman has isolated OT-B and the C-4 epimer of OT-B from the deep water Lyngbya majuscula in Enewetak. Another compound, oscillatoxin C, appears to be a stereoisomer of DAT; unfortunately, it has only been found once in trace amount in the Schizothrix-Oscillatoria mixture. Oscillatoxin D is a minor constituent of the Schizothrix-Oscillatoria mixture (12). Its structure has been secured from spectral data. The naturally-occurring compound is apparently the thermodynamically less stable C-2 epimer since it isomerizes gradually to 2-epioscillatoxin D. 30-Methyloscillatoxin D is a minor constituent in deep water L. majuscula.

Anhydroaplysiatoxin, anhydrodebromoaplysiatoxin, and anhydrooscillatoxin A are naturally occurring and generally accompany AT, DAT,and OT-A in the alga. These non-toxic anhydro compounds are not artifacts generated during the isolation. Extreme caution must be exercised during the isolation of AT, DAT and OT-A; exposure of these toxins to traces of acid in solvents and on chromatographic columns (even reverse-phase systems) results in rapid and complete dehydration to the corresponding anhydro compounds.

Inflammation and Tumor Promotion

Until recently there was only a single class of chemical substances known, the phorbol esters, that were capable of inducing tumor promotion at nanomolar concentrations, i.e. levels at which hormonal activity is observed. The most potent tumor promoter of these diterpenoid esters is 12-O-tetradecanoylphorbol-13-acetate (TPA), a highly inflammatory agent found in Croton oil. Detailed studies of the tumor promoting activity of TPA by many investigators has strongly suggested that inflammation is an important event in the development of a tumor after carcinogen exposure.

About three years ago Hirota Fujiki and Takashi Sugimura began an intensive search to find other inflammatory agents that could act as tumor promoters. They screened over 270 substances and found that 43 of these were skin irritants. Of these 43 only two compounds, teleocidin B and its catalytic hydrogenation product, dihydroteleocidin B, proved to be tumor promoters. Both compounds were found to be equipotent with TPA, acting at nanomolar concentrations. At about the time that the tumor promoting activity of the teleocidins was discovered, our work on lyngbyatoxin A appeared in print. The Sugimura group immediately recognized the similarity of lyngbyatoxin A and teleocidin B, not only in structure but in bioactivity, and requested samples of lyngbyatoxin A and its catalytic hydrogenation product, tetrahydrolyngbyatoxin A, from us to test in their systems. Needless to say, both compounds were found to be powerful tumor promoters, essentially identical in potency with the teleocidins. Samples of aplysiatoxin and debromoaplysiatoxin, which we knew were just as inflammatory as lyngbyatoxin A, were also submitted to the Sugimura group for testing. Aplysiatoxin was found to be a potent tumor promoter, comparable in potency with TPA, teleocidin B, and lyngbyatoxin A. To everyone's surprise, however, debromoaplysiatoxin was found to be a much weaker tumor promoter, about a hundred times less effective.

Tumor Promotion and Skin Cancer

Over two hundred years ago the British surgeon Sir Percivall Pott recognized that cancer of the scrotum, a common disease among chimney sweeps, was associated with their occupational exposure to soot (42). This clever observation marked the first time that a human cancer had been linked to an environmental agent. It is now well established that most of the causative agents in the soot are certain polycylic aromatic hydrocarbons such as benzo[a]pyrene. These compounds represent major potential public health hazards even today. In the early 1970s it was estimated that about 1300 tons of benzo[a]pyrene was being emitted into the air of the United States annually (43).

Chemically induced skin cancer proceeds in two major stages. This has been known for at least four decades and was first observed in mouse skin (44). The first stage, initiation, is accomplished when the skin is exposed to a single small dose of a carcinogen (initiator). This one time contact with the carcinogen, however, is insufficient for visible tumors to be produced over the life-span of the animal. Tumors will only develop when the skin is repeatedly exposed a cocarcinogen (tumor promoter) in the second stage, promotion. Generally a carcinogen can act as both initiator and promoter at high doses. Tumor promoters alone are not carcinogenic. It is only after initiation has occurred that tumor promoters can cause cancer.

In mice, for example, tumors can be produced by a single application of a large dose of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) to the skin. When a very small dose (100 μg) of DMBA is applied to the skin, no tumors are produced during the remaining lifetime of the mouse, as long as the animal does not come into prolonged contact with a tumor promoter. If 2.5 μg applications of a tumor promoter such as lyngbyatoxin A, teleocidin B, aplysiatoxin, or 12-O-tetradecanoylphorbol-13-acetate (TBA) is begun one week after exposure to DMBA and continued two times a week for a period of 30 weeks, however, skin tumors are formed in all animals treated in this manner.

In the case of TPA it has been shown that skin tumors will develop even if a year has elapsed between the application of the initiator and the promoter. Once application of the tumor promoter has commenced, it must be continued at frequent intervals for tumors to develop. If the application schedule is interrupted or changed so that there are longer intervals between applications, tumors are not produced. Tumor promotion, therefore, is a reversible process.

Initiation leads to a permanent change in the genetic machinery of the cell, presumably by irreversible interactions of the metabolized carcinogen with the DNA. Although enzymatic mechanisms for removing DNA defects exist in murine and human cells, the repair processes appear to be very slow. For this reason, tumors can be produced even when a long period has elapsed between initiation and exposure to the promoter.

The promotion stage is an exceedingly complex one whereby the initiated cell is transformed either into the cell of a benign tumor or into the cell of a malignant cancer. Although TPA forms more tumors in mouse skin than teleocidin and dihydroteleocidin, most of the TBA-induced tumors are benign tumors (papillomas). Teleocidin and dihydroteleocidin, by contrast, form more malignant tumors (squamous cell carcinomas) (26,45). Similar evaluations of lyngbyatoxin A and aplysiatoxin are in progress at the National Cancer Center Research Institute in Japan.

Recently it has been demonstrated that the promotion stage of chemical carcinogenesis is itself a multistage process, consisting of at least two well established stages (46,47) each of which can be accomplished by a certain chemical agent. Skin tumors can be produced in DMBA treated mice, for example, by a single application of TPA to carry out the first stage of promotion, followed by repeated application of a nonpromoting, mitogenic irritant to effect the second stage.

The chemistry by which DMBA reacts with the DNA of the cell in the initiation stage is known in exquisite detail. In the presence of the cytochrome P-450 monooxygenase system, which requires O_2 and NADPH as cofactors, the DMBA is converted to several metabolites. The active metabolite is a diolepoxide which has a structure that is analogous to that of 7β(R),8α(S)-dihydroxy-9α(S),10α(R)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, the active metabolite of benzo[a]pyrene (43). Apparently the carcinogenic potency of a polycyclic aromatic hydrocarbon depends on how good a substrate it is for the cytochrome P-450 monooxygenase system; benzo[e]pyrene, for example, is essentially non-carcinogenic because it is a poor substrate for P-450. The diolepoxide of DMBA is a pure initiator and has no tumor promoting activity; it reacts preferentially with the N-2 position of guanine in DNA, leading to an adduct in which the 2-amino group of guanine has opened the epoxide ring at position 10 in a trans addition reaction (48,49). Interestingly polycyclic aromatic hydrocarbon carcinogens are taken up into mitochondrial DNA almost two orders of magnitude greater than into nuclear DNA (1). The functional significance of this preferential attack is not clear, but this could cause disturbances in energy metabolism and perturbations in mitochondrial ion flux, in particular intracellular calcium ion homeostatis, leading to alternations in growth control.

The biochemistry of tumor promotion is not well understood. The key steps in the mechanism(s) by which an initiated cell is transformed into a tumor cell are not known. The promotion process appears to begin when a promoter such as TPA, teleocidin, lyngbyatoxin A, or aplysiatoxin binds to a receptor on the plasma membrane of the cell (50,51,52), frequently referred to as the phorboid receptor. Associated with this phorboid receptor binding are a number of biologic responses, for example, inhibition of binding of epidermal growth factor (EGF), a hormone-like protein which stimulates several events in cell division (53), to its receptor (50) and stimulation of choline and arachidonic acid release from cellular phospholipids (50), resulting in increased prostaglandin synthesis as one consequence (54).

The binding potency of a promoter to the phorboid receptor and the degree to which a promoter inhibits EGF binding to its receptor appear to correlate with the promoter's strength as a tumor promoter _in vivo_. Interestingly debromoaplysiatoxin binds much weaker to the phorboid receptor and is a weaker inhibitor of binding of EGF to its receptor (50). TPA, the teleocidins, lyngbyatoxin A, and aplysiatoxin have all been shown to be strong tumor promoters _in vivo_; debromoaplysiatoxin, however, is a very weak tumor promoter. Curiously debromoaplysiatoxin stimulates the release of choline and arachidonic acid from cellular phospholipids to the same degree as teleocidin, lyngbyatoxin A, and aplysiatoxin. These results strongly suggest that induction of the phospholipid metabolism is mediated by a receptor that is different from the phorboid receptor.

At first it was thought that the phorboid and EGF receptors might be one and the same as tumor promoters and EGF show some similar biologic effects (53). EGF, however, does not inhibit phorboid receptor binding. Inhibition of binding of EGF to its receptor is an indirect consequence of binding of a tumor promoter to the phorboid receptor and not to binding of EGF and tumor promoters to the same receptor site (55,56). Apparently the binding of the tumor promoter to the phorboid receptor causes alternations in the membrane;

as a result, EGF is no longer able to recognize its receptor.

The normal function of EGF is to initiate and maintain a complex network of biochemical and morphological events leading to cell growth and multiplication. When a cell undergoes division in the normal situation, one of the daughter cells replaces the parent (stem) cell whereas the other daughter cell is programmed to perform a special function (differentiation). In the case of the skin cell, the daughter cell that is destined to differentiate does so by becoming keratin. The switches that control the cell's capability to proliferate or differentiate are on the surface of the cell membrane. When cellular contact is broken, for example by a wound, the cellular biochemistry is reorganized to allow proliferation and a transient inhibition of terminal differentiation. When the tissue is restored so that cellular contact is reestablished at the healed wound site, proliferation ceases and terminal differentiation proceeds once again. The phorboid receptor system has been proposed to play a role in inhibiting terminal differentiation and stimulating cell growth during wound healing (1). This would necessitate the generation of an endogenous factor on wounding that would bind to the phorboid receptor to shut off the differentiation process and stimulate stem cell growth. Recently evidence has been obtained that such factors exist (56). Stimulation of the phorboid receptor by a tumor promoter, however, might result in inhibition of terminal differentiation and preferential growth stimulation of aberrant stem cells formed during initiation.

The EGF receptor appears to be a glycoprotein on the plasma membrane and has an estimated molecular weight of about 100,000 daltons (53). EGF, which has a molecular weight of 6045 daltons, binds with its receptor and the resulting EGF-receptor complex is then internalized in the cell. Nothing, however, is known about the phorboid receptor.

The ability of tumor promoters to inhibit terminal differentiation may be central to their ability to develop tumors from initiated cells (1). TPA, teleocidin, lyngbyatoxin A, and aplysiatoxin are potent inhibitors of terminal differentiation in a variety of cell systems; for example, these compounds inhibit induced differentiation (hemoglobin synthesis) in Friend erythroblastic leukemia cells (26,27), inhibit induced melanogenesis in B16 melanoma cells (57), and inhibit induced myogenesis in human myoblasts (57). TPA, teleocidin, lyngbyatoxin A, and aplysiatoxin are also potent inducers of differentiation in other cell systems; for example, these compounds induce differentiation of human promyelocytic leukemia cells (HL-60) into macrophage-like cells, characterized by induction of cell adhesion and increased release of lysozyme (58,59). The weak tumor promoter, debromoaplysiatoxin, is a much weaker inhibitor and inducer of terminal differentiation in all of the above cell systems.

Inflammation and hyperplasia are necessary events for tumor promotion. Evidence for this intimate connection comes from the fact that tumor development in mouse skin is inhibited by pretreatment with indomethacin, an aspirin-like drug, prior to application of the tumor promoter (60). The causative agent of the inflammation and hyperplasia appears to be prostaglandin E_2, which is produced from the arachidonic acid released on deacylation of membrane phospholipids by the tumor promoter (54,61). Prostaglandin synthesis is blocked by the cyclooxygenase inhibitor indomethacin and other aspirin-like drugs (62,63). In mouse skin inflammation and hyperplasia are inhibited when indomethacin is applied before the tumor promoter (47). This inhibition is reversed if prostaglandin E_2 is applied simultaneously with the tumor promoter. Inflammation and hyperplasia induced by tumor promoters are also inhibited by corticosteroidal drugs, such as dexamethasone, which blocks the formation of arachidonic acid from phospholipids (62,63). The inhibition of inflammation and hyperplasia, and also tumor development, by indomethacin and corticosteroids is only effective, however, when the drug is applied prior to the tumor promoter.

The role of prostaglandin E_2 in tumor promotion is not clear. It is suspected that prostaglandin-induced inflammation is necessary to attract leukocytes to the inflammatory site by chemotaxis (64). After arrival of polymorphonuclear leukocytes (PMNLs) into the inflammed area, lipoxygenase products (65), such as (5S,12R)-dihydroxy-6,8,10,14-icosatetraenoic acid (leukotriene B), might then become more important as chemotactic factors in attracting more leukocytes to the area. Leukotriene production may be stimulated in PMNLs by tumor promoters; in support of this proposal, divalent cation ionophore A23187, which produces some biologic effects that are similar to tumor promoters (61), stimulates the production of leukotriene B from human PMNLs (67). To date, however, the importance, if any, of the leukotrienes (68,69,70) in tumor promotion has not been determined. It will be interesting to test whether lipoxygenase blockers (71,72) inhibit tumor promotion.

Generally a burst of oxygen consumption accompanies the activation of phagocytic cells during the inflammatory process, resulting in the production of superoxide anion radical $O_2^{\bullet-}$) and hydrogen peroxide. The function of these active oxygen species is to kill microbes, but these potent oxidants are also potentially genotoxic (73). Superoxide anion radical production by human PMNLs is stimulated by TPA, teleocidin B, and the second stage promoter mezerein (74,75,76). Active oxygen species generated during the activation of phagocytic cells could act on the DNA of the initiated cell to cause expression of the

tumor phenotype, perhaps by causing rearrangements of the genetic material. Protease inhibitors, vitamin A derivatives, and dexamethasone block superoxide formation by tumor promoter-stimulated phagocytes (74,77). Protease inhibitors (78,79) and vitamin A derivatives such as 13-cis-retinoic acid (60) inhibit tumorigenesis in mouse skin.

Tumor promoters express in normal cells the phenotype of the tumor cell. For example, increased ornithine decarboxylase (ODC) activity, an activity which is characteristic of cells of fast-growing neoplasms, is observed in normal cells that have been treated with a tumor promoter. When a tumor promoter is applied topically to mouse skin, increased ODC activity is noted almost immediately, reaching a maximum about four hours after application. ODC activity is inhibited if 13-cis-retinoic acid is applied to the skin prior to the tumor promoter. TPA, the teleocidins, lyngbyatoxin, aplysiatoxin, and even debromoaplysiatoxin show the same amount of activity (26,27), indicating that this activity is associated with the second stage of tumor promotion. The significance of increased ODC activity, however, is unclear.

What happens in tumor promotion from this point on is uncertain. This discussion has presented only some of the current concepts in chemical carcinogenesis. It is obvious that the dermatitis-producing toxins of *Lyngbya majuscula* are playing and will continue to play an important role in determining the mechanisms of tumor promotion.

Structure-Activity Relationships of Tumor Promoters

Three classes of chemical compounds are now known to act as tumor promoters at nanomolar concentrations, viz. diterpenoid esters (12-O-tetradecanoylphorbol-13-acetate), indole alkaloids (teleocidin B and lyngbyatoxin A), and phenolic bislactones (aplysiatoxin). Even though these three groups of compounds have totally different structures, all three of them act essentially the same way in several biologic systems. TPA, the teleocidins and lyngbyatoxin A, and aplysiatoxin bind to the same phorboid receptor and produce the same biologic responses to this binding; in addition, these compounds show similar potencies as tumor promoters *in vivo*. Interestingly all three classes display antileukemic activities (24,25,76,80). Their similar bioactivities, in particular the same binding behavior to the phorboid receptor, suggest that the compounds in these three distinct classes have certain structural features in common.

Alan Jeffrey, I. Bernard Weinstein, and others at Columbia University have recently compared the structures of TPA, dihydroteleocidin, and aplysiatoxin using computer graphic analysis (50). From evidence in the literature (81,82,83) that an unsaturated keto group at C-3, a primary allylic hydroxyl at C-20, a non-methylated tertiary hydroxyl at C-4, and a hydrophobic ester at C-12 are needed for maximum activity in TPA, the Columbia group has compared the 3-dimensional structures of dihydroteleocidin B and TPA to see if there are any groups in the two molecules that are superimposable. They find that the best fit is obtained when the two amino acid units of the dihydroteleocidin molecule are *D*, not *L* (25). In their model, the C-11 carbonyl, N-13, N-1, and the OH on C-24 in dihydroteleocidin occupy very similar positons in space with the C-3 carbonyl, the OH at C-4, the OH at C-9, and the OH on C-24 in TPA, respectively; the monoterpenoidal portion of dihydroteleocidin and the C_{14} ester group of TPA, which are needed for effective binding of these tumor promoters to the hydrophobic regions of the phorboid receptor, are also in similar positions.

The activities of the teleocidins and lyngbyatoxin A are not changed by hydrogenation of the monoterpenoidal portion. In fact the activity does not depend at all whether the monoterpenoidal portion of the molecule is cyclic or acyclic, saturated or unsaturated, C_{10} or C_{11}. Lipophilic character in this region of the indole alkaloid is undoubtedly necessary for non-specific binding to the hydrophorbic region of the phorboid receptor. The indole alkaloid without its monoterpenoid moiety may exhibit activities similar to those of phorbol which has no tumor-promoting activity. Proof of this is needed. The intact lactam ring is needed for maximum toxicity, as is a free OH on C-24 (38); these two functionalities are probably also needed for maximum tumor promoting activity.

The absolute stereochemistry of aplysiatoxin was not known at the time a computer graphics analysis of various possible stereoisomers was made by the Columbia group (50). A fit was obtained, but the absolute stereochemistry of that model, in which the C-1 carbonyl, OH on C-3, ether oxygen of ring B, and the OH on C-30 of aplysiatoxin were correlated with the C-3 carbonyl, the OH at C-4, the OH at C-9, and the OH on C-24 in TPA, respectively, has an absolute stereochemistry that is opposite to what we have determined from degradative and spectral studies. Now that the absolute stereochemistry of aplysiatoxin has been solved, it should be possible to compare its 3-dimensional structure with TPA and dihydroteleocidin with much more reliability and confidence.

To aid in this comparison we have begun activity studies of derivatives of aplysiatoxin. It is already known that the OH on C-3 is necessary for activity. Anhydroaplysiatoxin is non-toxic and inactive in all of the test screens for tumor promoting activity. Aplysiatoxin 30-acetate has also been found to be non-toxic (84), but it has not been tested for

tumor promoter activity yet. Toxicity is also lost when the phenolic OH is methylated. It is not clear at all why the bromine on C-17 markedly enhances the potency of this compound as a tumor promoter. Recent studies by Hirota Fujiki and Takashi Sugimura indicate that 19-bromoaplysiatoxin shows less ODC activity in mouse skin than aplysiatoxin and that 19,21-dibromoaplysiatoxin exhibits no ODC activity, suggesting that 19-bromoaplysiatoxin will probably be a weaker tumor promoter _in vivo_ and 19,21-dibromoaplysiatoxin a nonpromoter.

CHEMICAL CARCINOGENESIS AND CHEMOPREVENTION

Since initiation requires only a single exposure to a carcinogen, there is not much hope that this stage of chemical carcinogenesis can be completely eliminated or avoided. There are just too many carcinogens in the environment. Realistically the goal should be one where there is a marked reduction in human exposure to carcinogens. Since tumor promotion requires prolonged contact with the causative agent for cancer to occur, however, there is a real possibility that this stage can be effectively controlled and even eliminated for most human cancers by limiting exposure to tumor promoters and by using chemopreventive agents to protect the human host. Some of these protective substances, like the vitamins, are dietary constituents (85).

There is already evidence (85) that tumorigenesis is inhibited by vitamin A derivatives, protease inhibitors (86), non-steroidal and steroidal antinflammatory agents, antioxidants (87), certain trace elements, and other compounds (88,89). Others will be found as the secrets of tumor promotion are uncovered. It is envisioned that someday chemopreventives will be used as supplements to diet much in the same way than vitamins are used today.

The promotion stage, at least of some cancers, appears to commence when a tumor promoter interacts with the phorboid receptor. One wonders whether chemopreventives could be developed that would inhibit tumor promoters from binding to the phorboid receptor. It would be interesting, therefore, to examine the cells of sea hares to find out why tumor promoters fail to elicit the biologic responses noted in murine (90) and human cells. Are inhibitors of phorboid receptor binding present in the digestive gland of the sea hare or is the lipid character (91) of the cell membrane sufficiently different to allow binding of an endogenous growth factor but not an exogenous tumor promoter.

Certain fish are also resistant to the tumor promoters of _Lyngbya majuscula_. The rabbit-fish _Siganus fuscescens_, for example, has been observed to feed on sea grass entangled with _L. majuscula_ with no apparent ill effects (92). Human intoxication in the Ryukyus Islands from rabbitfish may result from eating the viscera of the fish that has accumulated _L. majuscula_ toxins. This suggests that the tumor promoters of _L. majuscula_ might be found in the digestive tract and viscera of other fish, thereby contributing to gastrointestinal cancer in Japan.

In the next few years studies on tumor promotion and chemoprevention will accelerate. These preliminary studies that I have just briefly touched on here at this symposium indicate that these will be truly exciting years.

Acknowledgment -- The author gratefully acknowledges the invaluable contributions of several collaborators to this study: Adrian Blackman (currently on sabbatical leave from the University of Tasmansia), Jon Mynderse, Gregory Patterson, Daniel Carter, John Cardellina, Franz-Josef Marner, Murray Munro, Giovanni Bartolini, Midori Kashiwagi, Eiichi and Shinobu Furusawa, and Ted Norton at the University of Hawaii; Hirota Fujiki and Takashi Sugimura at the National Cancer Institute Research Cancer, Tokyo, Japan; Ronald Woodard and J. Cymerman Craig at the University of California, San Francisco; and I Bernard Weinstein, Alan Jeffrey, and others at Columbia University. Research in the author's laboratory was generously supported by PHS grant CA12623, awarded by the National Cancer Institute, Department of Health and Human Services and in part by grants CHE79-25416 and CHE81-00240 from the National Science Foundation.

REFERENCES

1. I.B. Weinstein, in _Mechanisms of Chemical Carcinogenesis_, C. Harris and P. Cerutti, eds., in press.
2. T. Sugimura, _Cancer_ _49_, 1970-1984 (1982).
3. G.R. Pettit, J.F. Day, J.L. Hartwell, and H.B. Wood, _Nature_ _227_, 962-963 (1970).
4. M. Kashiwagi, J.S. Mynderse, R.E. Moore, and T.R. Norton, _J. Pharm. Sci._ _69_, 735-738 (1980).

5. G.R. Pettit, Y. Kamano, P. Brown, D. Gust, M. Inoue, and C. L. Herald, J. Am. Chem. Soc. 104, 905-907 (1982).
6. K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D. VanEngen, J. Clardy, Y. Gopichand, and F.J. Schmitz, J. Am. Chem. Soc. 103, 2469-2471 (1981).
7. F.J. Schmitz, R.S. Prasad, Y. Gopichand, M.B. Hossain, D. van der Helm, and P. Schmidt, J. Am. Chem. Soc. 103, 2467-2469 (1981).
8. K.L. Rinehart, Jr., J.B. Gloer, J.C. Cook, Jr., S.A. Mizsak, and T.A. Scahill, J. Am. Chem. Soc. 103, 1857-1859 (1981).
9. G.T. Carter and R.L. Rinehart, Jr., J. Am. Chem. Soc., 100, 7441-7442 (1978).
10. M.T. Cheng and K.L. Rinehart, Jr., J. Am. Chem. Soc. 100, 7409-7411 (1978).
11. G.R. Pettit, C.L. Herald, M.S. Allen, R.B. Von Dreele, L.D. Vanell, J.P.Y. Kao, and W. Blake, J. Am. Chem. Soc. 99, 262-263 (1977).
12. R.E. Moore, in Marine Natural Products: Chemical and Biological Perspectives, P.J. Scheuer, ed., Vol. 4, Academic Press, New York, 1981, pp. 1-52.
13. J.A. Roesener and P.J. Scheuer, unpublished results.
14. R.E. Moore and G. Bartolini, J. Am. Chem. Soc., 103, 2491-2494 (1981).
15. R.E. Moore, P. Helfrich, and G.M.L. Patterson, Oceanus 25(2), 54-63 (1982).
16. R.J. Quinn, M. Kashiwagi, R.E. Moore, and T.R. Norton, J. Pharm. Sci. 63, 257-260 (1974).
17. R.E. Moore, G. Bartolini, J. Barchi, A.A. Bothner-By, J. Dadok, and J. Ford, J. Am. Chem. Soc. 104, 3776-3779 (1982).
18. K.L. Rinehart, Jr., J.B. Gloer, R.G. Hughes, Jr., H.E. Renis, J.P. McGovern, E.B. Swynenberg, D.A. Stringfellow, S.L. Kuentzel, and L.H. Li, Science, 212, 933-935 (1981).
19. F. Kelly and J. Sambrook, Nature New Biol. 242, 217-219 (1973); W.F. Wright and L. Hayflick, Cell Tissue Kinet. 5, 351-364 (1972).
20. D.C. Carter, M.S. Thesis, University of Hawaii, 1980.
21. R.A. Lewin, submitted for publication.
22. R.A. Lewin and N.W. Withers, Nature 256, 735-737 (1975).
23. G.M.L. Patterson and N.W. Withers, Science, in press.
24. J.S. Mynderse, R.E. Moore, M. Kashiwagi, and T.R. Norton, Science 196, 538-540 (1977).
25. J.H. Cardellina II, F.J. Marner, and R.E. Moore, Science 204, 193-195 (1979).
26. H. Fujiki, M. Mori, M. Nakayasu, M. Terada, T. Sugimura, and R.E. Moore, Proc. Natl. Acad. Sci. USA 78, 3872-3876 (1981).
27. H. Fujiki, M. Suganuma, M. Nakayasu, H. Hoshino, R.E. Moore, and T. Sugimura, Gann 73, 497-499 (1982).
28. B.J. Burreson, R.E. Moore, and P.P. Roller, J. Agric. Food Chem. 24, 856-861 (1976).
29. S.M. Waraszkiewicz, H.H. Sun, K.L. Erickson, J. Finer, and J. Clardy, J. Org. Chem. 43, 3194-3204 (1978).
30. R.E. Moore, in The Water Environment: Algal Toxins and Health, W.W. Carmichael, ed., Plenum, New York, 1981, pp. 15-23.
31. Y. Kato and P.J. Scheuer, Pure Appl. Chem. 41, 1-14 (1975).
32. J.S. Mynderse and R.E. Moore, J. Org. Chem. 43, 2301-2303 (1978).
33. M. Serdula, G. Bartolini, R.E. Moore, N. Wiebenga, and J. Gooch, Hawaii Med. J., in press.
34. N. Sakabe, H. Harada, Y. Hirata, Y. Tomiie, and I. Nitta, Tetrahedron Lett., 2523-2525 (1966).
35. H. Nakata, H. Harada, and Y. Hirata, Tetrahedron Lett. 2515-2522 (1966).
36. H. Harada, N. Sakabe, Y. Hirata, Y. Tomiie, and I. Nitta, Bull. Chem. Soc. Japan 39, 1773-1775 (1966).
37. M. Takashima, H. Sakai, and K. Arima, Agr. Biol. Chem. 26, 660-668 (1962).
38. M. Takashima, H. Sakai, R. Mori, and K.Arima, Agr. Biol. Chem. 26, 669-678 (1962).
39. Y. Kato and P.J. Scheuer, Pure Appl. Chem. 48, 29-33 (1976).
40. K. Mori, T. Takigawa, and T. Matsuo, Tetrahedron 35, 933-940 (1979).
41. A.F. Beecham, Tetrahedron Lett., 2355-2360, 3591-3594 (1968).
42. A. Haddow, Perspect. Biol. Med. 17, 543 (1974).
43. I.B. Weinstein, A.M. Jeffrey, K.W. Jennette, S.H. Blobstein, R.G. Harvey, C. Harris, H. Autrup, H. Kasai, and K. Nakanishi, Science 193, 592-595 (1976).
44. I. Berenblum, Cancer Res. 1, 807-814 (1941).
45. H. Fujiki, M. Suganuma, N. Matsukura, T. Sugimura, and S. Takayama, submitted for publication.
46. T.J. Slaga, S.M. Fischer, K. Nelson, and G.L. Gleason, Proc. Natl. Acad. Sci. USA 77, 3659-3663 (1980).
47. G. Fürstenberger, D.L. Berry, B. Sorg, and F. Marks, Proc. Natl. Acad. Sci USA 78, 7722-7726 (1981).
48. A.M. Jeffrey, I.B. Weinstein, K.W. Jennette, K. Grzeskowiak, K. Nakanishi, and R.G. Harvey, H. Autrup, and C. Harris, Nature 269, 348-350 (1977).
49. K. Nakanishi, H. Kasai, H. Cho, R.G. Harvey, A.M. Jeffrey, K.W. Jennette, and I.B. Weinstein, J. Am. Chem. Soc. 99, 258-260 (1977).
50. A.D. Horowitz, H. Fujiki, I.B. Weinstein, A. Jeffrey, E. Okin, R.E. Moore, and T. Sugimura, submitted for publication.
51. R. Schmidt, B. Sorg, A. Marston, H. Fujiki, T. Sugimura, R.E. Moore, and E. Hecker, submitted for publication.

52. K. Umezawa, I.B. Weinstein, A. Horowitz, H. Fujiki, T. Matsushima, and T. Sugimura, Nature 290, 411-413 (1981).
53. G. Carpenter and S. Cohen, Annu. Rev. Biochem. 48, 193-216 (1979).
54. H. Sakamoto, M. Terada, H. Fujiki, M. Mori, M. Nakayasu, T. Sugimura, and I.B. Weinstein, Biochem. Biophys. Res. Commun. 102, 100-107 (1981).
55. L.S. Lee and I.B. Weinstein, Carcinogenesis 1, 669-679 (1980).
56. A.D. Horowitz, E. Greenebaum, and I.B. Weinstein, Proc. Natl. Acad. Sci USA 78, 2315-2319 (1981).
57. P.B. Fisher, A.F. Miranda, R.A. Mufson, L. S. Weinstein, H. Fujiki, and T. Sugimura, and I.B. Weinstein, submitted for publication.
58. M. Nakayasu, H. Fujiki, M. Mori, T. Sugimura, and R.E. Moore, Cancer Letters 12, 271-277 (1981).
59. E. Huberman, G.R. Braslawsky, M. Callaham, and H. Fujiki, Carcinogenesis, 3, 111-114 (1982).
60. A.K. Verma and R.K. Boutwell, in Yearbook of Science and Technology, McGraw-Hill, New York, 1980, pp. 298-300.
61. F. Marks, G. Fürstenberger, and E. Kownatski, Cancer Res. 41, 696-702 (1981).
62. T.K. Schaaf, Annu. Rep. Med. Chem., 12, 182-190 (1977).
63. F.A. Kuehl, Jr. and R.W. Egan, Science 210, 978-984 (1980).
64. E.L. Becker and H.J. Showell, Annu. Rep. Med. Chem. 15, 224-232 (1980).
65. D.M. Bailey and L. W. Chakrin, Annu. Rep. Med. Chem. 16, 213-227 (1981).
66. P. Borgeat and B. Samuelsson, Proc. Natl. Acad. Sci. USA, 76, 3213-3217 (1979).
67. P. Borgeat and B. Samuellsson, Proc. Natl. Acad. Sci. USA 76, 2148-2152 (1979).
68. R.C. Murphy, S. Hammarström, and B. Samuelsson, Proc. Natl. Acad. Sci. 76, 4275-4279 (1979).
69. E.J. Corey, D.A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson, and S. Hammarström, J. Am. Chem. Soc. 102, 1436-1439 (1980).
70. D.-E. Sok, C.-O. Han, W.-R. Shieh, B.-N. Zhou, and C.J. Sih, Biochem. Biophys. Res. Commun. 104, 1363 (1982).
71. E.J. Corey and H. Park, J. Am. Chem. Soc. 104, 1750-1752 (1982).
72. E.J. Corey and J.E. Munroe, J. Am. Chem. Soc 104, 1752-1754 (1982).
73. H.C. Birnboim, Science 215, 1247-1249 (1982).
74. B.D. Goldstein, G. Witz, M. Amoruso, D.S. Stone, and W. Troll, Cancer Letters 11, 257-262 (1981).
75. W. Troll, G. Witz, B. Goldstein, D. Stone, and T. Sugimura, in Carcinogenesis: Cocarcinogenesis and Biological Effects of Tumor Promoters, E. Hecker, N.E. Fusenig, W. Kunz, F. Marks, and H.W. Thielmann, eds., Vol. 7, Raven Press, New York, 1982, pp. 593-597.
76. S.M. Kupchan and R.L. Baxter, Science 187, 652-653 (1975).
77. B.D. Goldstein, G. Witz, M. Amoruso, and W. Troll, Biochem. Biophys. Res. Commun. 88, 854-860 (1979).
78. M. Hozumi, M. Ogawa, T. Sugimura, T. Takeuchi, and H. Umezawa, Cancer Res. 32, 1725-1728 (1972).
79. W. Troll, A. Klassen, and A. Janoff, Science 169, 1211-1213 (1971).
80. S.M. Kupchan, I. Uchids, A.R. Branfmann, R.G. Daily, and B.Y. Fei, Science 191, 571-572 (1976).
81. E. Hecker, in Carcinogenesis: Mechanisms of Tumor Promotion and Cocarcinogenesis, T.J. Slaga, A. Sivak, and R.K. Boutwell, eds., Vol. 2, Raven Press, New York, 1978, pp. 11-48.
82. B.L. Van Duuren, S.S. Tseng, A. Segal, A.C. Smith, S. Melchionne, and I. Seidmann, Cancer Res. 39, 2644-2646 (1979).
83. H. Yamasaki, I.B. Weinstein, and B.L. Van Duuren, Carcinogenesis 2, 537-543 (1981).
84. Y. Kato, Ph.D. Dissertation, University of Hawaii, 1973.
85. Diet, Nutrition, and Cancer, National Research Council, National Academy Press, Washington, D.C., 1982.
86. W. Troll, R. Wiesner, C.J. Shellabarger, S. Holtzman, and J.P. Stone, Carcinogenesis 1, 469-472 (1980).
87. L.W. Wattenberg, J. Natl. Cancer Inst. 46, 1425-1430 (1972).
88. S. Belman and W. Troll, Cancer Res. 34, 3446-3455 (1974).
89. M. Froscio, G.R. Guy, and A.W. Murray, Biochem. Biophys. Res. Commun. 98, 829-835 (1981).
90. M. Hergenhahn and E. Hecker, Carcinogenesis 2, 1277-1281 (1981).
91. P.B. Fisher, U. Cogan, A.D. Horowitz, D. Schachter, and I.B. Weinstein, Biochem. Biophys. Res. Commun., 100, 370-376 (1981).
92. Y. Hashimoto, H. Kamiya, K. Yamazato, and K. Nozawa, in Animal, Plant, and Microbial Toxins, A. Ohsaka, K. Hayashi, and Y. Sawai, eds., Vol. 1, Plenum, New York, 1976, pp. 333-338.