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Marine animal and terrestrial plant anticancer constituents[a]

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Abstract - Substantial advances in improving human cancer treatment continue to require discovery and development of new and curative anticancer drugs. Our recent (1989-93) progress in discovery and development of new anticancer drugs derived from marine animal and terrestrial plant biosynthetic products has been reviewed. Special emphasis was placed upon the bryostatin (1,2), dolastatin (4-6), halichondrin (15), halistatin (16,17), spongistatin (18-20), cephalostatin (7-14) and pancratistatin (34) series of potentially useful anticancer drugs.

Real advances in improving human cancer treatment require discovery and development of new and curative anticancer drugs, and we are sharply focused on those objectives. To follow is a 1989-93 review of research progress aimed at discovery of structurally unique and promising anticancer drugs. The summary begins with a historical overview and then places major emphasis on our discoveries of the very important bryostatin (1, ref. 1), dolastatin (4,6, ref. 2), halichondrin (15, ref. 3), halistatin (16,17, ref. 4), spongistatin (18-20, ref. 5), cephalostatin (7-14, ref. 6) and pancratistatin (34, ref. 7) series of useful anticancer drugs.

The U. S. National Cancer Institute (NCI) research programs directed at discovery of new and clinically important animal, plant, and microorganism anticancer constituents were implemented in 1957 and have amply demonstrated that 2-4% of plant species and some 10% of marine animal species contain a great variety of antineoplastic and/or cytotoxic (ref. 8-18) constituents. The dramatic discoveries arising from the NCI research, such as taxol, (ref. 14,15) have stimulated considerable world-wide interest and initiation of analogous programs. Because of this vitally important NCI endeavor, new antineoplastic and/or cytotoxic biosynthetic products are being discovered at an increasing rate. The potential for discovering new animal, plant and microorganism biosynthetic products for treatment of human cancer is truly immense and offers great promise of many curative approaches to the cancer problem. Consider that the world's flora may number up to 800,000 and the more conspicuous terrestrial vegetation, the angiosperms, may number from 300,000 to some 500,000 (ref. 16). Furthermore, enormous numbers of marine animal (over 2,000,000) and microorganism species (ref. 17) are available for investigation. Even now, less than 10% of the higher plants and less than 0.5% of the marine animals (ref. 8-10) have received even a cursory effort to detect antineoplastic constituents. So the majority of important animal and plant cancer chemotherapeutic drugs still await discovery.

In 1965-66 we began the first systematic study of marine invertebrates and vertebrates as potential sources of new and potentially useful cancer chemotherapeutic drugs (ref. 18). By 1969 we found that 9-10% of marine animals yielded extracts with high and reproducible (confirmed active) antineoplastic activity in the NCI murine P388 *in vivo* lymphocytic leukemia screening system. From this evidence, it was abundantly clear that such natural products present an unusually good opportunity for discovering clinically useful anticancer drugs. To date we have isolated a large number of new cytotoxic and/or antineoplastic agents from marine animals.

Discovery (ref. 1) of the bryostatin series of marine Bryozoa constituents represents an especially important advance for the future and bryostatin 1 (1) has been undergoing

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Note a: Presented in part at the 19th IUPAC Symposium on the Chemistry of Natural Products, Karachi, Pakistan, January 16-20, 1994. Contribution 317 of "Antineoplastic Agents" and for part 316 refer to: C. Scheid, J. Prendiville, G. Jayson, D. Crowther, B. Fox, G.R. Pettit and P.L. Stern, *Cancer Research*, submitted.

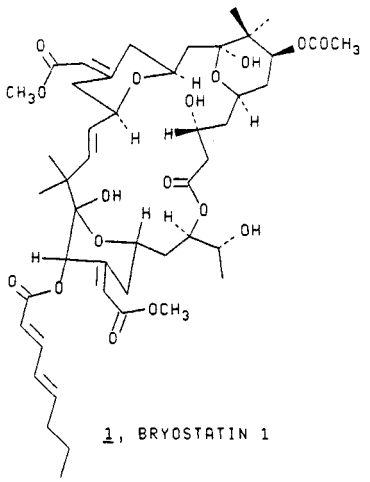
a very successful series of human Phase I clinical trials for over two years (ref. 19-21). The considerable therapeutic potential of bryostatin 1 (1) is based in part on its ability to influence protein kinase C which mediates one arm of a major signal transduction pathway involving lipophilic secondary messengers. We have been able to deduce (ref. 22) the absolute configuration of the bryostatins, convert bryostatin 2 (2) chemically to bryostatin 1 and related bryopyrans (ref. 23) to increase the supply of bryostatin 1, isolate and characterize the new bryostatins 14 and 15 (2, ref. 24), accomplish extensive structural modifications of bryostatin 2 (2, ref. 25), synthesize [26-^{3}H]-epi-bryostatin 4 for mechanistic studies (ref. 26,27) and discover the structurally novel neristatin 1 (3, ref. 28) a new cell growth inhibitory constituent of *Bugula neritina*. At the same time extensive biological and mechanistic studies of bryostatin 1 have been intensifying. These investigations have been yielding a wealth of useful and very exciting results for extending the clinical trials of bryostatin 1.

Bryostatin 1 has been found to cause differentiation of B-chronic lymphocytic leukemia in an unprecedented fashion, (ref. 29) and be capable of converting leukemia cells *in vitro* to those typical of hairy cell leukemia which is curable (ref. 30). Successful extension of these experiments to the clinic may result in the first really curative technique for human chronic lymphocytic leukemia. The potential for treating chronic myelogenous leukemia patients is also very promising (ref. 31,32). Bryostatin 1 was found capable of inducing macrophage-like differentiation in maturing CML cells (ref. 32). Most importantly, bryostatin 1 was dramatically effective against cells taken from patients in the CML blast phase. Against a line of acute lymphoblastic leukemia, bryostatin 1 was found capable of inducing further differentiation along the B-cell lineage (ref. 33,34). In general, bryostatin 1 was found to be a B-cell differentiating agent with a potential therapeutic role in treatment of such lymphomas (ref. 35,36). Indeed, the early potential of bryostatin 1 with its immunomodulatory and antineoplastic properties as a biological response modifier is already being realized in clinical trials. Interestingly, bryostatin 1 has been found to potentiate ARA-C apoptosis or programmed cell death, and this combination looks very promising for clinical evaluation (ref. 37,38,39,40). Another facet of the activity of bryostatin 1 against lymphomas involves its ability to convert a high-grade lymphoma cell line to an intermediate grade, again offering clinical potential (ref. 41). The majority of the studies just reviewed were conducted with fresh tissue from human cancer patients.

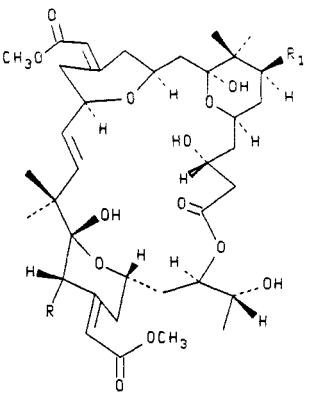
Among the many animal or *in vitro* experiments conducted with the bryostatins, the potential of one has already been realized in the clinic (ref. 19), namely the ability of bryostatin 1 to reverse murine B16 melanoma pulmonary metastases (ref. 42). Another nice advance that will eventually be tested in the clinic involves its use in adoptive immunotherapy (ref. 43). With an intradermal murine tumor model, the adoptive transfer of bryostatin 1-stimulated DLN cells induced regression of established liver and pulmonary metastases resulting in curative responses (ref. 43). In other murine experiments, bryostatin 1 has proved to be dramatically (1 μg/mouse) effective against lethal whole body irradiation producing a 70% survival rate that increased to about 80% using coadministered GM-CSF (ref. 44). Another 19 research publications involving more fundamental research with bryostatin 1 in various cell systems will now be just cited (ref. 45-63). In short, the bryostatin research has been very productive and stimulated much world-wide interest. From all appearances, bryostatin 1 and eventually perhaps other members in the series will become successful anticancer drugs, in addition to their already well established role as unique biochemical probes.

Dolastatin 10 (4) continues to be a high priority clinical development objective by the NCI and European collaborators. Our challenging isolation and structural elucidation research with shell-less mollusks such as *Dolabella auricularia* has continued and resulted in discovery of the strong cell growth inhibitor dolastatin 14 (5) (ref. 64) along with further chemical and antineoplastic studies of dolastatins 10-15 (ref. 65). In order to provide sufficient quantities of dolastatins 10 (4) and 15 (6) for clinical development, an intense research effort was devoted to these vital total synthetic objectives, (ref. 66-70) and include our new and more stereoselective total synthesis of dolastatin 10. We have also improved our initial total synthesis of dolastatin 15 (ref. 71). At the same time, an extensive series of structure/activity investigations using total synthetic approaches have been underway and reference 72 provides an illustration. *Dolabella a.* continues to yield new antineoplastic substances along with the first example of the nickel chelate, tunichlorin, occurring in a mollusc (ref. 73).

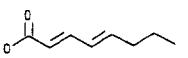
A large number of biological experiments are being conducted with dolastatins 10 and 15.

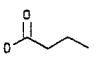
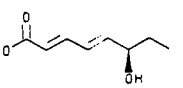


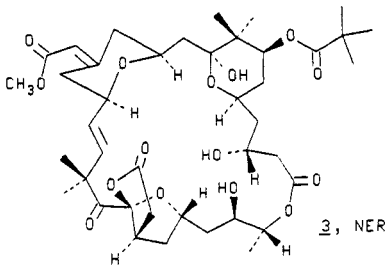
1, BRYOSTATIN 1



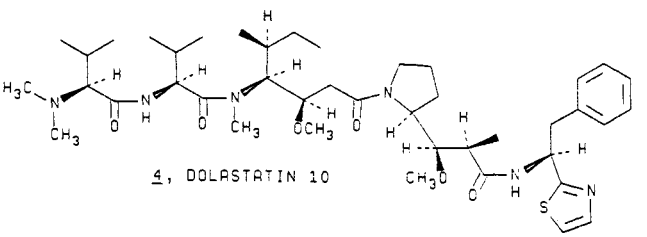
R	R_1	
B	OH	BRYOSTATIN 2
D	C	BRYOSTATIN 4
A	C	BRYOSTATIN 5
A	D	BRYOSTATIN 6
A	A	BRYOSTATIN 7
D	D	BRYOSTATIN 8
D	A	BRYOSTATIN 9
H	C	BRYOSTATIN 10
H	A	BRYOSTATIN 11
B	D	BRYOSTATIN 12
H	D	BRYOSTATIN 13
OH	C	BRYOSTATIN 14
E	A	BRYOSTATIN 15

2, A,  B,  C, 

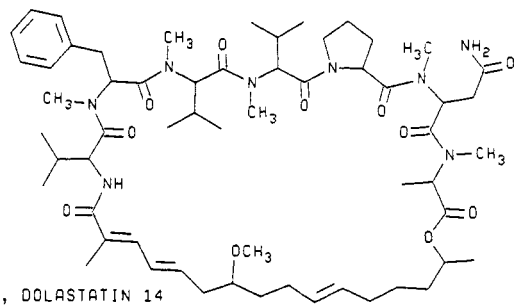
D,  E, 



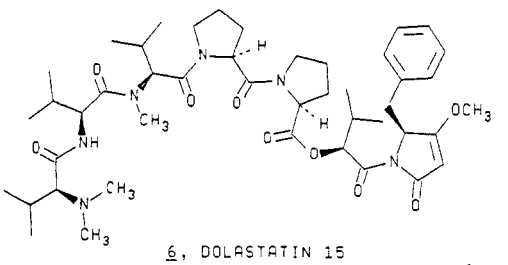
3, NERISTATIN 1



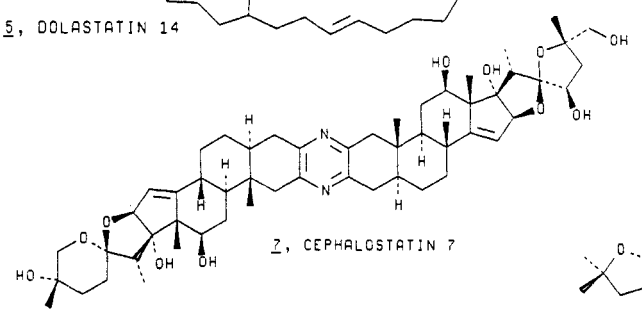
4, DOLASTATIN 10



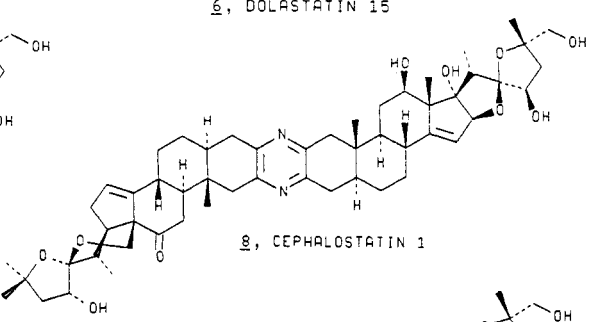
5, DOLASTATIN 14



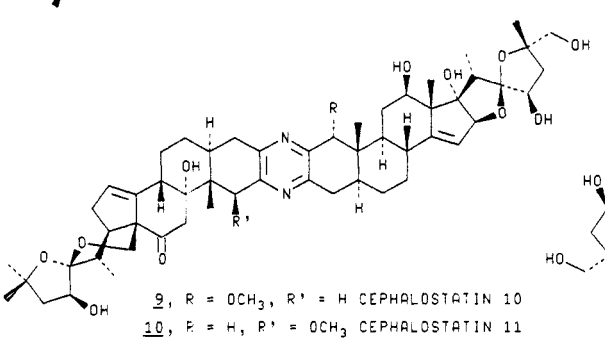
6, DOLASTATIN 15



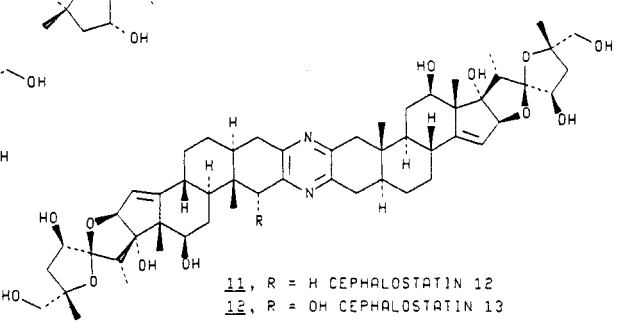
7, CEPHALOSTATIN 7



8, CEPHALOSTATIN 1



9, R = OCH_3, R' = H CEPHALOSTATIN 10
10, R = H, R' = OCH_3 CEPHALOSTATIN 11



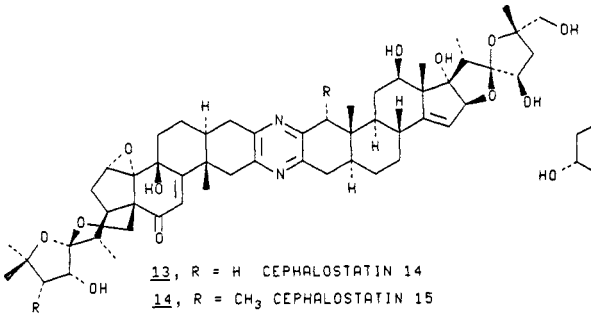
11, R = H CEPHALOSTATIN 12
12, R = OH CEPHALOSTATIN 13

Among these studies, dolastatins 10 and 15 were found to inhibit proliferation of human leukemia cell lines at concentrations lower than those that inhibited the growth of normal cells (ref. 74) and to inhibit the proliferation of several myeloid types from patients with acute myeloid leukemia (ref. 75). The discovery that dolastatin 10 (4) was an extraordinarily effective (appears to be the most potent) inhibitor of microtubule assembly, tubulin-dependent GTP hydrolysis and the binding of vincristine to tubulin has stimulated a series of very productive research directions concerned with structure/activity (ref. 76,77) relationships and mechanistic considerations (ref. 75,78,79). Indeed, this area has been quite productive and shows promise of leading to a much clearer molecular model for understanding the interaction of tubulin with certain strongly active anticancer drugs (ref. 78,80). By contrast, the inhibition of glutamate-induced polymerization of tubulin by dolastatin 15 (6) was 23 μM as compared to 1.2 μM for dolastatin 10 (4) and 1.5 μM for vinblastine (ref. 79). But, dolastatin 15 proved to be a very potent antimitotic with a profile of human cancer cell line inhibition differing from that of dolastatin 10 (ref. 79). The human cancer cell line selectivity of dolastatin 10 offers much promise. Indeed, there is every indication (ref. 81,82) from the preclinical studies that the clinical trials of dolastatins 10 and 15, due to begin in 1994, should be successful.

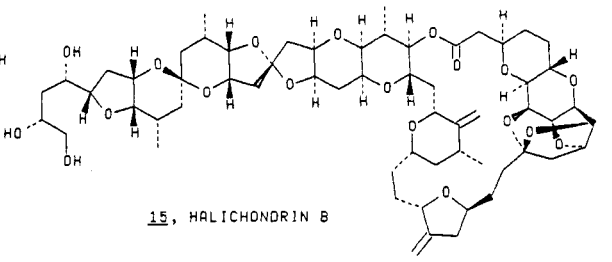
We discovered cephalostatins 1-9 (ref. 6) as constituents of the African marine worm *Cephalodiscus gilchristi*. Cephalostatin 7 (7), especially, displayed remarkable potency with TGI values to $>10^{-10}$ μg/ml against a number of human cancer cell lines such as those derived from non-small cell lung cancer, small cell lung cancer, renal, brain and leukemias (ref. 6). Those dramatic results combined with the need for preclinical supplies of cephalostatins 1 (8) and 7 (7) necessitated a 450 kg recollection of this tiny (less than 5 mm) Indian Ocean (Southeast Africa) worm. Because of the substantial (and challenging) research effort here, we discovered new cephalostatins 10 (9) and 11 (10, ref. 83), cephalostatins 12 (11) and 13 (12, ref. 84) and cephalostatins 14 (13) and 15 (14, ref. 85). As usual in this series, the structural determinations were difficult but the extraordinary and selective effects against various human cancer cell lines in the NCI panel have been very rewarding. Presently, several of the newer cephalostatins appear to compete quite favorably with cephalostatins 1 (8) and 7 (7) in terms of cancer cell growth inhibition. We have detected the presence of several more new cephalostatins that will extend the series and provide additional useful structure/activity relationship information. The new cephalostatins occur in yields of about 10^{-8}% and give every evidence of being exceptionally potent. The preliminary activity against brain cancer xenografts provides a good basis for the difficult research in progress here.

The preclinical studies of halichondrin B (15, ref. 86) continue to yield splendid results. We independently (the Uemura Japanese group was first to publish) discovered (ref. 3) halichondrin B (15) in a Western Caroline Island marine sponge and our investigations have continued at a rapid pace. The urgent need to meet clinical supplies has led us to discover four new sources of halichondrin B in diverse sponge types in geographically widely separated areas of the Western Pacific Ocean and Western Indian Ocean (ref. 3,5,87). Those investigations constitute successful completion of difficult research ranging from the field expeditions to devising new techniques for the more rapid isolation of this trace constituent. Meanwhile, we succeeded in discovering halistatin 1 (16, ref. 4) and the related halistatin 2 (17, ref. 87). Both were found to be exceptionally potent antineoplastic constituents of two different marine sponges we located in The Republic of Comoros. Against the NCI human cancer cell line panel, the negative $\log_{10}$ GI_{50} values range to over nine and represent an excellent selection of human cancer types. Briefly stated, the halistatins offer considerable promise for improving future human cancer treatment.

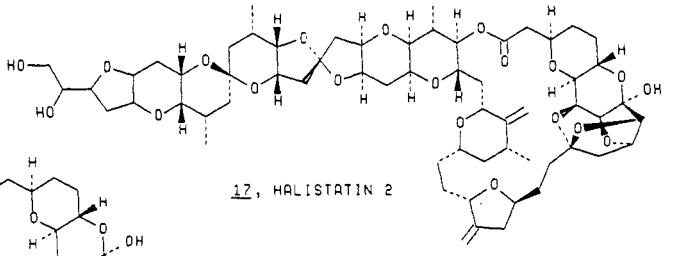
From current information, it's clear spongistatin 1 (18) we discovered in a *Spongia sp.* will become a very important clinical candidate. Fortunately, the new and improved isolation and high field 2D NMR procedures we developed for solving the halistatin research problems allowed us to accelerate structural assignments for spongistatin 1 (18, ref. 5), spongistatins 2 (19a) and 3 (19b, ref. 88) and spongistatins 4 (19c) and 5 (20, ref. 89). A brief outline of the latter (ref. 89) discoveries and remarkable human cancer cell line results provides a useful illustration. In 1973 we began a 20 year investigation of antineoplastic constituents in the bright red marine sponge *Spirastrella spinispirulifera* collected off the south coast of Africa. Increasingly larger (to 360 kg) recollections of this sponge and chemical/biological research over the period to 1980 proved inadequate but did provide by 1982 the first submilligram amounts of spongistatins. By that time all effort was focused on a 2,409 kg sponge



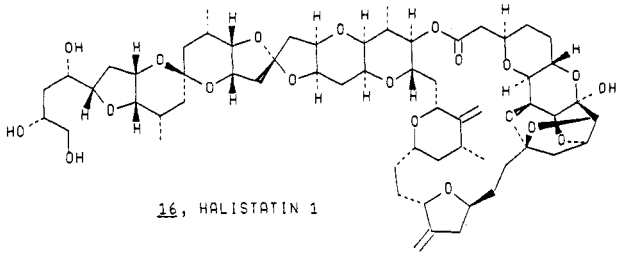
13, R = H CEPHALOSTATIN 14
14, R = CH_3 CEPHALOSTATIN 15



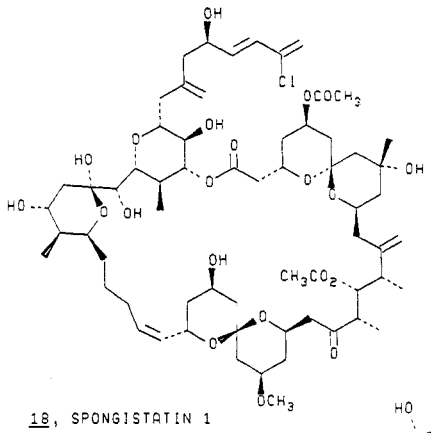
15, HALICHONDRIN B



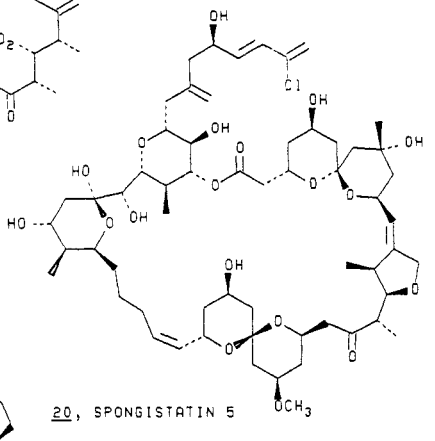
17, HALISTATIN 2



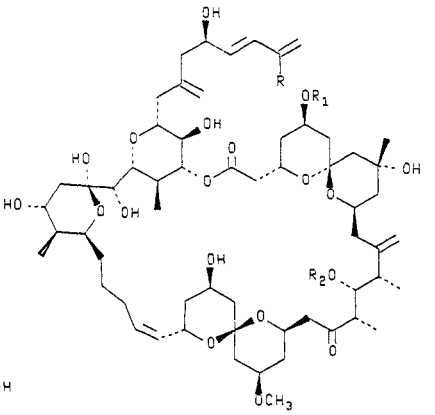
16, HALISTATIN 1



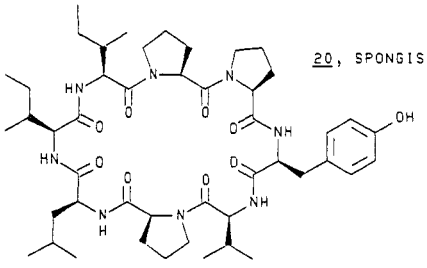
18, SPONGISTATIN 1



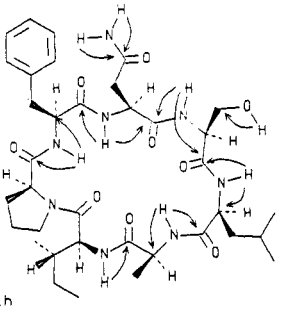
20, SPONGISTATIN 5



19a, R = H, R_1 = R_2 = $COCH_3$
SPONGISTATIN 2
b, R = Cl, R_1 = H, R_2 = $COCH_3$
SPONGISTATIN 3
c, R = Cl, R_1 = $COCH_3$, R_2 = H
SPONGISTATIN 4



21, HYMENISTATIN 1



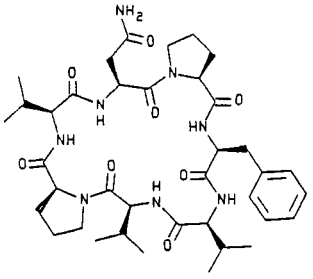
22, STYLOSTATIN 1 with
HMBC correlations

recollection that recently led us to 10.7 mg (4.4×10^{-7}% yield) of spongistatin 4 (19c) and a comparable amount of spongistatin 5 (20). Evaluation of spongistatins 4 and 5 against the NCI human cancer panel (60 cell lines) gave dramatic results. Comparative testing of spongistatins 2 (19a), 4 (19c) and 5 (20) exhibited an overall potency comparable to spongistatin 1 (18) (panel mean GI_{50} 10^{-10}M). These three compounds proved to be among the most potent of all substances tested to date in the NCI panel. Interestingly, several of the human breast cancer cell lines recently incorporated into the NCI panel were among the most sensitive (e.g., GI_{50} 10^{-11}-10^{-12}M). Furthermore, results of pattern-recognition analyses revealed that the highly distinctive mean-graph "fingerprint" (pattern of relative cellular sensitivity) produced in common by spongistatins 1 (18) and 5 (20) is closely correlated to the important class of microtubule-interactive antimitotics. Among the major objectives for continued research with the spongistatins will be completing an X-ray crystal structure determination to establish the stereochemistry at each asymmetric center and the absolute configuration.

In our more recent investigations of marine Porifera antineoplastic constituents, we discovered the murine P388 lymphocytic leukemia cell growth inhibitor and cyclooctapeptide hymenistatin 1 (21) which represented the first such combination of source, structural type, and biological activity (ref. 90). Isolation and characterization of such a seemingly unexceptional cyclic peptide with cell growth inhibitory properties provides a new insight into structure requirements for antineoplastic activity (hymenistatin 1 also inhibits growth of the P388 system *in vivo*). That advance allowed us to concentrate on discovery of new marine animal cyclic peptides with antineoplastic activity. The novel substances we are presently investigating are providing considerable insights into structural requirements for antineoplastic activity among sponge cyclic peptide constituents. Illustrative is our isolation and structural elucidation of stylostatin 1 (22) from the Papua New Guinea marine sponge *Stylotella aurantium* (ref. 91). Those successes have been extended to discovery of axinastatins 1-4 (23-26) from *Axinella* species of marine sponges ranging from Palau in the Western Pacific to Republic of Comoros in the Indian Ocean (ref. 3,92,93). In a series of parallel and successful investigations of marine sponge antineoplastic constituents from the genus *Phakellia* ranging from the Federated States of Micronesia in the Western Pacific to Republic of Comoros, isolation and structural determination has led to phakellistatins 1-3 (27-29, ref. 94,95,96). All of these very interesting and potentially useful new marine animal cyclic peptides have been found to exhibit moderate to strong cancer cell growth inhibition ranging from GI_{50} 0.1 to 0.001 μg/ml. The structures were all assigned on the basis of high field (400 and 500 MHz) 2D-NMR, high resolution MS/MS tandem type mass spectrometry, X-ray crystallography and chiral gas chromatographic analyses of hydrolysis products.

Another productive advance with marine porifera antineoplastic constituents was discovery of cribrostatins 1 and 2 (30,31) in a blue sponge we uncovered in the Republic of Maldives (ref. 97). Cribrostatin 1 has shown very selective activity against all of the nine human melanoma cell lines comprising the NCI panel. Human cancer xenograft evaluations are now in progress. Presently we are engaged in determining the structures of five more, albeit complex, antineoplastic constituents of this unusual blue sponge. Other marine animal constituent endeavors have led to the isolation and structure of axinohydantoin (32, ref. 98) and related pyrrologuanidines from a Western Pacific *Axinella*, toxins from a Gulf of California *Geodia* black sponge (ref. 99) and a new type of sterol from a *Xestospongia* species (ref. 100).

Exploratory investigations of new plant species for antineoplastic constituents has remained an important direction. Extension of our phyllanthostatin 1 (33)-phyllanthoside lead (for the first Phase I clinical trial refer to ref. 101) has resulted in devising a simpler method for isolation of phyllanthoside and discovery of a new member of this strongly antineoplastic series, namely, phyllanthostatin 6 (ref. 101). A major research effort has been devoted to advancing our clinical candidate pancratistatin (34). By utilization of plant tissue culture techniques (ref. 102), we have been able to propagate one bulb of *Hymenocallis littoralis* (formerly *Pancratium littoralis*) with subsequent planting in our greenhouses and open fields to the present some 60,000 bulbs that will form a principle source of the clinical supply. Simultaneously, we have been conducting a world-wide evaluation of *Hymenocallis* species and evaluating their pancratistatin content (ref. 7). That led to discovery of a new antineoplastic constituent found to be 7-deoxy-*trans*-dihydronarciclasine (35). While investigating a related *Amaryllidaceae* family member *Zephyranthes candida*, we uncovered a closely related antineoplastic constituent *trans*-dihydronarciclasine (36, ref. 103). Through a combination of isolation and synthetic structural modifications, a number of



23, AXINASTATIN 1

Cyclo-(Pro-Val-Asn-Pro-Phe-Val-Leu)

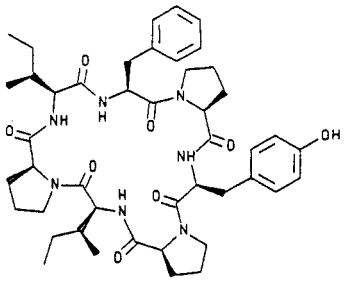
24, AXINASTATIN 2

Cyclo-(Pro-Val-Asn-Pro-Phe-Ile-Val)

25, AXINASTATIN 3

Cyclo-(Pro-Leu-Thr-Pro-Leu-Trp-Val)

26, AXINASTATIN 4



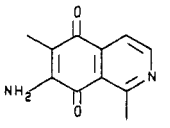
27, PHAKELLISTATIN 1

Cyclo-(Pro-Tyr-Pro-Phe-Ile-Ile)

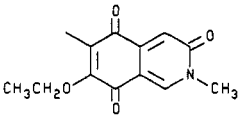
28, PHAKELLISTATIN 2

Cyclo-(Pro-Phe-Pro-Thr-Leu-*trans*-photo-Trp)

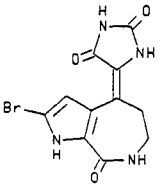
29, PHAKELLISTATIN 3



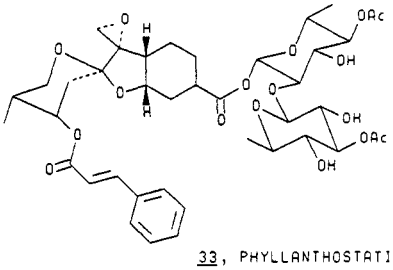
30, CRIBROSTATIN 1



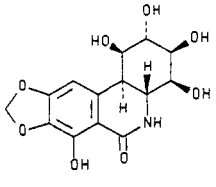
31, CRIBROSTATIN 2



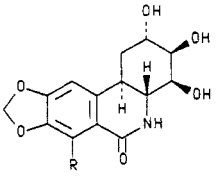
32, AXINOHYDANTOIN



33, PHYLLANTHOSTATIN 1

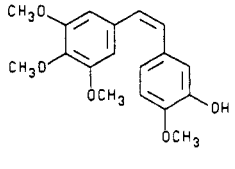


34, PANCRATISTATIN

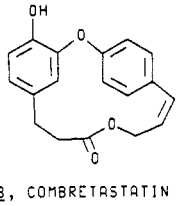


35, R = H 7-DEOXY-*trans*-DIHYDRONARCICLASINE

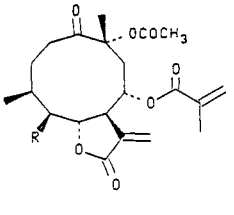
36, R = OH *trans*-DIHYDRONARCICLASINE



37, COMBRETASTATIN A-4

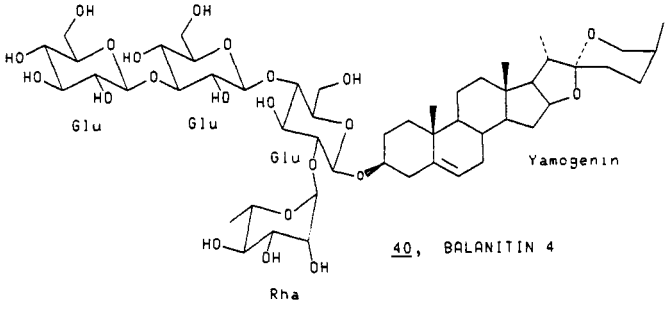


38, COMBRETASTATIN D-2



39a, R = OH LYCHNOSTATIN 1

b, R = H LYCHNOSTATIN 2



40, BALANITIN 4

derivatives were prepared for further antineoplastic and antiviral evaluations (ref. 104). These latter experiments led to a dramatic result: pancratistatin was found to be the first substance to cure Japanese encephalitis in an experimental animal (ref. 104). Other research has been focused on devising an efficient total synthesis of pancratistatin. Among the many isolation, synthetic and antineoplastic evaluations (ref. 105) we have been pursuing to advance the combretastatin A-4 (37) lead has been successful synthesis of a water soluble pro-drug and the isolation and structure determination of combretastatin D-2 (38, ref. 106). The surprisingly strong and selective activity of combretastatin A-4 against unusually difficult human cancer cell types continues to be very impressive (ref. 105).

In the general area of terrestrial plant cell growth inhibitory and/or antineoplastic constituents, discoveries as diverse as lychnostatins 1 and 2 (39, ref. 107) and the new balanitin 4 (40, ref. 108) were made along with cytostatic acteosides (ref. 109) and aceratioside (ref. 110). The balanitin series was further extended by isolation and characterization of balanitins 5-7 (ref. 108). As usual, structure determinations were nicely completed employing a series of high resolution 2D-NMR, high resolution mass spectrometry and X-ray crystallographic techniques (ref. 111,112).

In summary, the foundation is now available for a rapid acceleration in the discovery of new animal, plant and microorganism derived anticancer and antiviral drugs. That vitally necessary objective will continue to guide our future research endeavors.

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