Invited Review

Progress in the Discovery of Biosynthetic Anticancer Drugs[1]

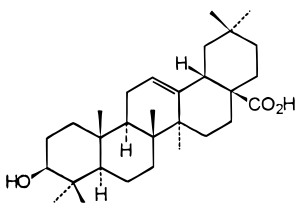
George R. Pettit

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287-2404

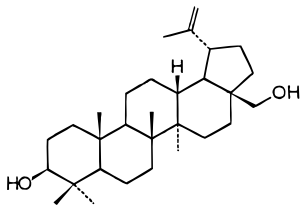
Received May 6, 1996

By September 1957 the U.S. National Cancer Institute (NCI) Cancer Chemotherapy National Service Center (CCNSC) was underway, and that fall Dr. J. L. Hartwell's recommendation[2] was being implemented[3] that five plant families prominent in the traditional treatment of cancer patients should be employed as sources of new anticancer drugs. That September, I began as an assistant professor of chemistry at the University of Maine and within 2 weeks began our still uninterrupted collaborative research program with the NCI directed at discovery of potentially useful anticancer drugs. The first objective of our CCNSC collaboration was to evaluate plant species from the family Labiatae, one of the five most promising families.[2,3]

Owing to the long delays in procurement of appropriate Labiatae species, exacerbated by the general insensitivity (to trace constituents in natural product extracts) and capricious nature of the NCI *in vivo* screen (e.g., sarcoma 180, adenocarcinoma 755) then in use,[4] progress was slow. Isolation of oleanolic acid (**1**) and related pentacyclic triterpenes from *Salvia* species (Labiatae)[5] was part of our related antineoplastic research at that time concerned with the tetracyclic triterpenes of shelf fungi[6] and the structures[7] of certain transformation products of betulin (**2**). We isolated betulin in quantity from the Maine white birch *Betula papyrifera*. Recently, the primary alcohol oxidation product, betulinic acid, was found to be uniquely effective against a line of human melanoma, and preclinical development is recommended.[8] Even oleanolic acid (**1**) has recently been proposed for development as an antiarthritic and antiinflamatory agent.[9]



1, Oleanolic acid



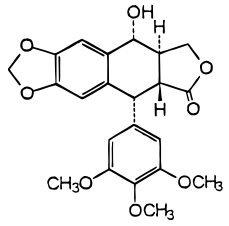
2, Betulin

The general plan envisioned in 1957 for attacking the cancer problem involved, in addition to the terrestrial plant and fungal antineoplastic constituents approach just noted, investigation of marine organisms, terrestrial arthropods, and amphibians and their associated microorganisms as new sources of presumed powerful and structurally unique anticancer drugs. The plan also included synthesis of modifications of steroid hormones in order to evaluate (NCI-CCNSC steroid screening program) their possible utility in the understanding of breast and prostate cancer, as well as structural modifications of the oxygenated aromatic system of podophyllotoxin (**3**)[2] and development of new synthetic methods for bonding the generally potent 2-haloethylamine alkylating unit to biologically active natural products such as steroid hormones, antibiotics, and peptides. Because my total financial resources for research in 1957 amounted to $500 and in 1958 some $3000, implementation of the overall strategy required considerable optimism, patience, and diligence. Indeed, these financial constraints caused postponement of the arthropod[10] and marine organism[11−14] research initiations until 1965−66. The marine microorganism research was begun during my expedition in the Gulf of Alaska on the then University of Alaska research ship *Alcona* in the fall of 1971. But this research area was quickly deferred owing to insufficient resources until 8 years ago.

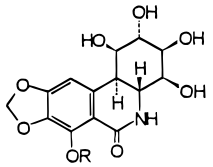
With my early graduate and postdoctoral students in the 1958−59 period, we were able to productively pursue structural modifications of podophyllotoxin[15,16] that more than 20 years later assisted in our discovery and development of pancratistatin (**4a**)[17] and the combretastatins (see **5**).[18,19] At the same time we began an extensive study aimed at degradation of lanosterol (**6**) by a 19-step reaction sequence to 14α-methylprogesterone (**7**),[20] in 20 steps to 14α-methyltestosterone (**8**),[21,22] and to other 14α-methyl steroids. On the presumption that certain steroidal bufadienolide constituents contained in the skin and venom glands of toads in the family Bufonidae might exhibit cancer cell growth inhibitory properties, we began (in 1958−59) a major effort to make such substances available by total synthesis for antineoplastic evaluation. At that time the other option involving the large-scale procurement of toads in the genus *Bufo* (long history of use in Chinese and Japanese medicine) and separation of the active constituents without an adequate bioassay system seemed even more daunting.

Eventually, we completed total synthesis of bufalin (**9**)[23−35] and all the major *Bufo* constituent bufadienolides including resibufogenin (**10**) and marinobufotoxin (**11**).[34,35] Although nearly all were found to exhibit a significant level of activity against the NCI KB nasopharynx carcinoma cell line,[3,4] marinobufagin (**12**) was found to provide a curative response level using the *in vivo* Ehrlich ascites system. While the Chinese *Bufo bufo gararizans* preparation Ch'an Su and the Japanese counterpart Sen So are still in common use in Asia, the western medical potential of the animal[36] and plant[37] bufadienolides require much further study. One of these, resibufogenin (**10**), is marketed in Japan as a

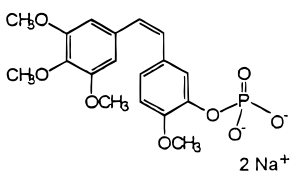
S0163-3864(96)00438-7 CCC: $12.00



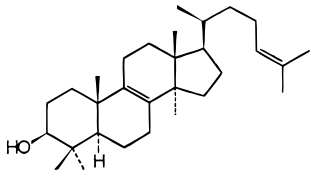
3, Podophyllotoxin



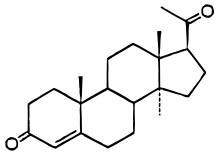
4a, Pancratistatin, R = H
b, Pancratistatin prodrug, R = $PO_3^{2-}.2Na^+$



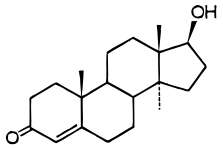
5, Combretastatin A-4 prodrug



6, Lanosterol

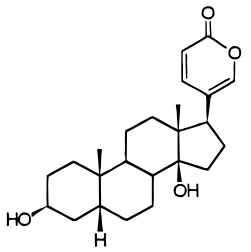


7, 14α-Methylprogesterone

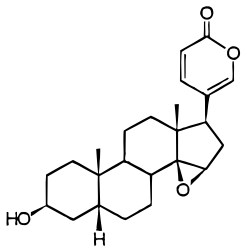


8, 14α-Methyltestosterone

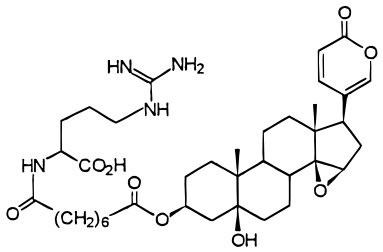
respiratory stimulant, and a bufalin (**9**)-type steroid is now believed to occur in human plasma and induce leukemia cell differentiation.[38]



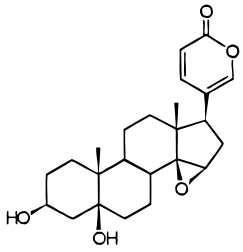
9, Bufalin



10, Resibufogenin

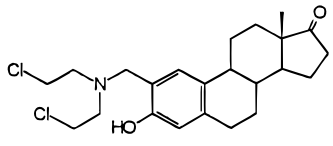


11, Marinobufotoxin

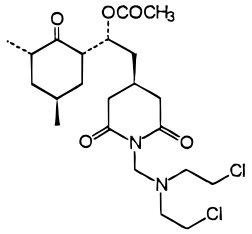


12, Marinobufagin

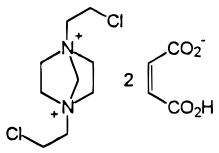
Our early (1959−65) explorations of 2-haloethylamine chemistry quickly led to a very potent series of antineoplastic agents[39] and quite significant improvements in the required synthetic methods.[40−43] Those advances allowed convenient syntheses of various steroid (**13**)[44] and antibiotic (**14**)[44] 2-haloethylamine derivatives. Most importantly, while investigating application of the Mannich reaction in 1959, we discovered a new heterocyclic ring system that became the precursor of our diazabicyclostatin maleate (**15**),[45−47] DABIS maleate, now being readied for phase II human cancer clinical trials.[48−50] DABIS maleate (**15**) was found to exhibit very strong (including curative levels) activity against a variety of NCI *in vivo* experimental cancer systems.[47] In phase I trials the MTD proved to be unusually high, and 750 mg/m^2 has been recommended for phase II clinical trials.[48] By 1965, NCI interest in developing 2-haloethylamines subsided and we accordingly began to phase out this area of research.



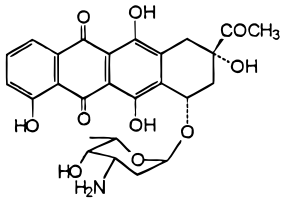
13, Estrone derivative



14, Cycloheximide derivative



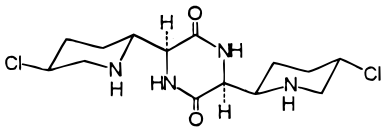
15, DABIS maleate



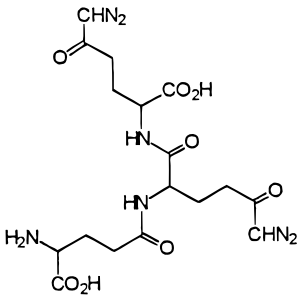
16, Carminomycin

Our initial research with anticancer antibiotics such as actidione (cycloheximide, see **14**) was productively extended during the 1970's and included the preparation, crystallization, and X-ray crystal structure determination of carminomycin (**16**),[51] employed for cancer treatment in Russia and elsewhere (including clinical trials against breast cancer in the United States). That was followed by our X-ray crystal structure elucidation of the South African *Streptomyces griseoluteus* constituent 593A (**17**, NSC-135758).[52] Initial NCI clinical trials against certain human lymphomas were encouraging, but clinical supplies proved to be a problem. In keeping with nature's virtuosity in synthesizing a phenomenal variety of anticancer substances, 593A appears to be the first naturally occurring prototype of the 2-haloethylamines to be isolated. Obviously, this is one of the very small number of occurrences where synthetic structures preceded the biosynthetic lead. Another example of this precedence of synthesis involves the diazoketone groups of the anticancer antibiotic azotomycin (**18**), of which we completed the first total synthesis[53] as well as a new synthesis of DON (**19**).[54] At the time both anticancer antibiotics were in preclinical and clinical development. Over the past 8 years we have been pursuing the isolation and structural characterization of cancer cell growth inhibitory substances from marine and terrestrial microorganisms, and results of these endeavors will be reported in the future.

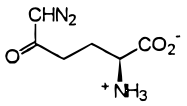
Now returning to 1965, this proved to be an especially important year. It included the move to expanded laboratory facilities at Arizona State University, which allowed initiation of the marine organism[11−14] and arthropod[10] approaches to new types of antineoplastic substances, as well as my appointment as an advisor to the NCI for anticancer drug discovery and development. Because of the latter responsibilities, I decided for ethical reasons not to compete for any of the then current NCI natural products (plants and microorgan-



17, NSC-135758 (593A)



18, Azotomycin

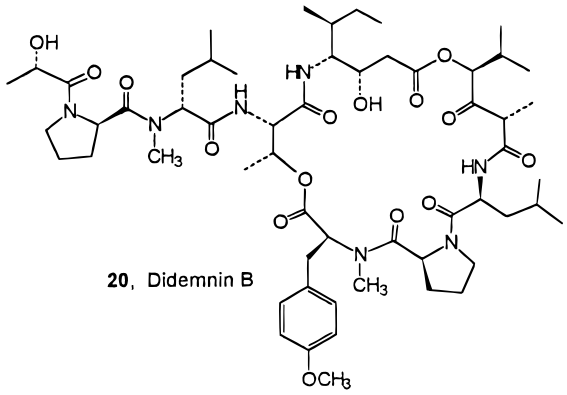


19, Don

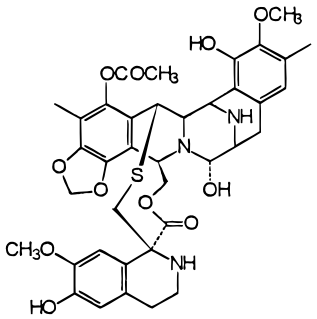
isms) research contracts or to be a recipient of plant leads (and later animal leads) from any of the NCI natural products procurement contracts.[3,4] With those self-imposed limitations, it seemed necessary, except for a brief period in 1979−81 (with plants), to increase our own field collections of plants from Mexico to Alaska and Western Canada and later worldwide. The same constraints were employed, albeit worldwide from the start, with field collections of marine organisms and arthropods. While the consequences of these ethical decisions continue to prove (in respect to field biology) very challenging, they did leave me free to better serve the NCI and recommend new NCI research contract initiatives such as the first marine animal research (including procurement) contract (with Professor A. Weinheimer) then (1970) at the University of Oklahoma. That NCI contract led to Weinheimer's discovery[55] of didemnin B[56] (**20**) and the ecteinascidin leads that were developed by Rinehardt.[57] The clinical trials of didemnin B are ongoing, and the ecteinascidin 729 (**21**) clinical trials will soon begin.

Another important consequence of 1965 was the NCI development of the *in vivo* murine P388 lymphocytic leukemia based on its ability to select a majority of the then (and now) anticancer drugs active against human cancer. Very importantly, the P388 leukemia, unlike the NCI L1210 leukemia,[3,4] proved sensitive to trace antineoplastic components in complex natural product extracts. Application of that bioassay system caused a positive real change in our ability to isolate arthropod, marine animal, and plant antineoplastic constituents. Some of our earliest successes came with our Arizona and Oregon plant leads.

The first real advance was with a 1967 collection (Oregon) of *Helenium autumnale* (Compositae) where helenalin (**22**),[58,59] not previously known to be antineoplastic, proved to be the most active (P388, T/C 220 at 3 mg/kg) constituent accompanied by some previously unknown components such as autumnolide.[60] Although not further developed at the time, that situation is changing. Recently, helenalin (**22**) as the glutaric acid ester has been found to have potent topoisomerase II activity.[61] Parallel investigations employing a 1966 collection of *Baileya multiradiata* (Compositae) led to other active (P388) and new pseudoguaianolide lactones exemplified by multigilin (**23**, P388 T/C 164 at 12.5 mg/kg) and multistatin (**24**, P388 T/C 131 at 32 mg/kg).[62,63]

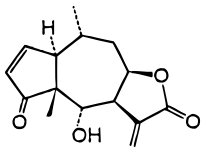


20, Didemnin B

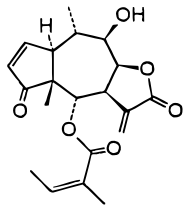


21, Ecteinascidin 729

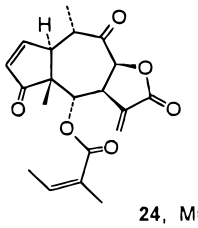
In this period we also completed a practical synthesis of the plant quinone lapachol then in clinical development.[64]



22, Helenalin

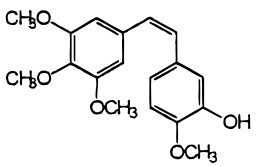


23, Multigilin

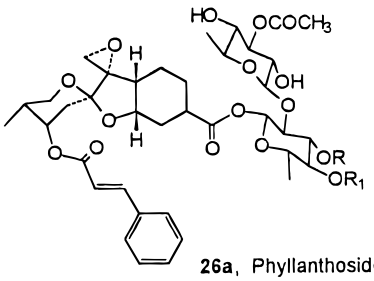


24, Multistatin

In the 1970's, we achieved better success with terrestrial plants, especially with *Combretum caffrum* (Combretaceae), an African willow tree that has a history of being used by the Zulu for various purposes. One of those was as a charm to ward off enemies. (In retrospect, how appropriate when one considers the cancer problem!) From that tree we were able to isolate (about 1 mg from 70 kg of the plant) combretastatin A-4 (**25**) among roughly 20 other active substances.[19] Combretastatin A-4 has proved to be a powerful inhibitor of tubulin assembly and a variety of human cancer cell lines.[19] Recently, we have converted it to a prodrug (**5**), which was realized by a fairly classic phosphate ester procedure.[18] The product proved to be a substance about as soluble as sucrose in water, whereas the original phenol sodium, potassium, and other salts dissociate so fast in water that they are useless in terms



25, Combretastatin A-4

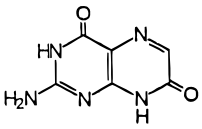


26a, Phyllanthoside, R = $COCH_3$, R_1 = H
b, Phyllanthostatin 1, R = H, R_1 = $COCH_3$

of improving the solubility. Recently, combretastatin A-4 was used against tissue from 47 ovarian cancer patients (tissue taken at the University of Arizona), and this substance turned out to be very active against about 27 of those specimens. We've also found recently that the phosphate prodrug (**5**) is very active *in vivo* and causes tumors to hemorrhage. On the basis of the promising antiangiogenesis and other antineoplastic effects, clinical development of combretastatin A-4 prodrug (**5**) is underway.

One of the plant families we began investigating in parallel with the Combretaceae was the Amaryllidaceae. Hippocrates, about 200 B.C., used extracts from plants of the genus *Narcissus* for treating breast cancer patients. The most active substance located so far in this plant family is pancratistatin[17] (**4a**, from *Pancratium littoralis*, later reidentified as *Hymenocallis littoralis*). We were able to determine the structure by X-ray crystallography and more recently have converted it to a phosphate prodrug (**4b**). This time, synthesis of the phenol phosphate proved to be quite challenging, and eventually we resorted to starting with a trivalent phosphite and oxidizing later in the synthesis.[17] The prodrug (**4b**) again proved to have a very nice spectrum of activity and is in preclinical development. In addition to important antineoplastic activity, pancratistatin[17] also has an unusual spectrum of antiviral activity. So far, it is the first substance to cure Japanese encephalitis in an animal model.[65,66] Another interesting series of plant-derived clinical candidates (**26a,b**) was obtained from the Costa Rican tree *Phyllanthus acuminatus* (Euphorbiaceae).[67] Both phyllanthoside (**26a**) and phyllanthostatin 1 (**26b**) intraconvert in aqueous ethanol through an ortho acid rearrangement[68] of the ester group to give an equilibrium mixture. Phyllanthoside has completed phase I human trials, and we hope that the phase II trials will be successful.

For reasons noted above, it became possible in 1965−66 to begin a broadly based evaluation of the arthropoda classes Insecta, Arachnida, Crustacea, and Myriapoda for potential antineoplastic constituents, and this represented the first such directed investigation of the arthropods.[10] Over the next 4 years we isolated antineoplastic substances that ranged from simple purines and isoxanthopterin (**27**, from Asian butterflies)[69] to an approximately 100-unit protein from the legs of the female Asian beetle *Allomyrina dichotomus*.[70] By 1972, the need for increasingly larger scale (500 000+ members of each species) recollections of insects and the consequent drain of meager resources combined with the generally more promising antineoplastic activity shown by our then rapidly increasing marine animal extract leads led to a deferral of this important research thrust. Meanwhile, for obvious reasons, the decision to follow the marine organisms, especially the marine invertebrates, has continued to consume a major portion of our resources. In retrospect, the 1972 decision has, given the difficult circumstances, proven to be quite correct. The marine invertebrates have been shown by us and others to be a most remarkable source of antineoplastic substances and anticancer drugs with unprecedented structural types.[71−73]

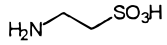


27, Isoxanthopterin

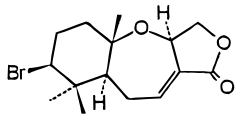
As mentioned earlier, we were not able to begin investigating marine animals until 1965−66 (8 years after the start of our research group) and that delay was strictly due to financial and facility reasons. Historically, the concept began to grow during my graduate school period. It arose from my own experience growing up about a mile from the Atlantic Ocean. I had never seen a marine invertebrate with cancer, and that made me wonder whether these organisms have protective agents that we could adopt to human use. From 1966, we were able to begin in earnest, and in that period, again thanks to the collaboration of my colleagues at the NCI, we began to explore specimens from various worldwide ocean areas. This was the first effort ever to systematically look at marine organisms as new sources of anticancer drugs, and within 3 years we had the compelling positive evidence. By 1968[3,11] we had ample evidence that at least 10% of such organisms would yield extracts with antineoplastic activity. The corollary expectation was that if we were able to isolate and characterize the antineoplastic substances they would probably reveal structures we organic chemists would have never thought of as being useful for treating cancer patients. Fortunately, that hope has been realized.

Because of the extent and complexity of our research over the past 30 years directed at discovery of marine organism anticancer constituents, the remainder of this progress overview will be devoted to five of the currently most promising preclinical and clinical series of anticancer drugs: the cephalostatins, halichondrin/halistatins, spongistatins, dolastatins, and bryostatins. The exception that follows is a brief look at the rather inauspicious beginning of the isolation/structure studies late in the 1960's. By utilization of the P388-leukemia-guided bioassay, the first marine invertebrate constituents were isolated from Florida and Taiwan mollusca species and proved to be the marginally active (P388, T/C 123−131 at 100 mg/kg) amino acid taurine (**28**).[74] Fortunately, that near-nonevent was soon followed by the P388-bioassay-directed isolation from the hammerhead shark (*Sphyrna lewini*)[75] and green sea urchin (*Strongylocentrotus droebachiensis*)[76] of antineoplastic

(P388 *in vivo*) glycoproteins and by the isolation and structural elucidation (by X-ray crystallography) of the P388 cell growth inhibitor aplysistatin (**29**) from the Australian sea hare *Aplysia angasi*.[77] Meanwhile, in the 1968−73 period a series of most challenging, albeit very productive, marine invertebrate antineoplastic constituent leads were undertaken, and progress with five of these to the present will complete this review.



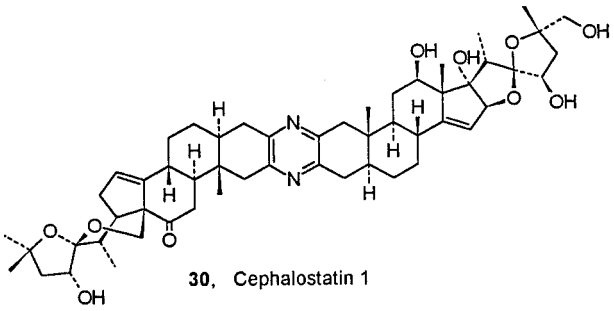
28, Taurine



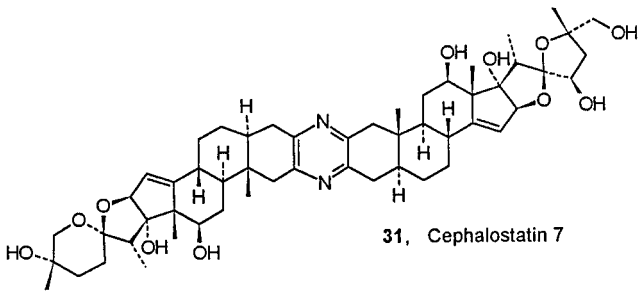
29, Aplysistatin

Generally, when diving, you find marine worms very difficult to collect owing to a lightning-fast retraction mechanism, and the result is a hole in the coral or sand as the organism quickly recedes. The South African marine worm we became very interested in, namely, *Cephalodiscus gilchristi*, is usually only a couple of millimeters long. However, it actually comes out of its tube and swims outside for feeding operations. We began working on this species in 1972 and continued to the present; recently we investigated a half-ton recollection. Some 10 years ago, we isolated the first member of a new series of very potent cancer cell growth inhibitory compounds, the cephalostatins.[78−80] The genus *Cephalodiscus* is rare among marine worms, and there are only a few species. Cephalostatins 1 (**30**)[78] and 7 (**31**)[79] have been selected for clinical development. Both have remarkable activity with a somewhat different overall spectrum. Against the P388 lymphocytic leukemia and the NCI human cancer cell line panel, the ED_{50} values drop to 10^{-9} μg/mL when cephalostatin 1 is used. Recently, we isolated cephalostatins 16 and 17 (**32**).[80] This very impressive series of substances is still not complete. A Japanese group has recently begun to isolate parallel compounds from a tunicate,[81] so it seems these compounds occur more widely than we originally believed.

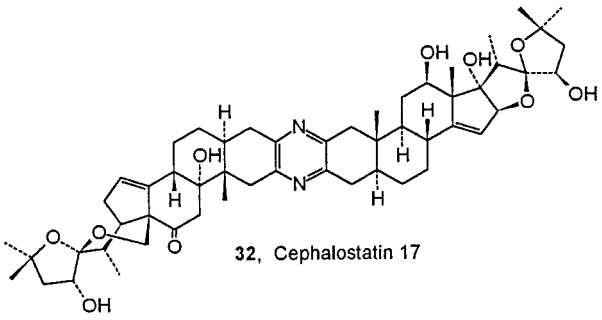
A 1973 collection of the South African marine sponge *Spirastrella spinispirulifera*[82] gave extracts that would double the life span of animals given the P388 lymphocytic leukemia: a very important lead. Once we started the actual separations, we found that some of these fractions would give a curative level of activity. While a very exciting lead at that time, it is still so today. From 1973 to 1979 we completed several scale-up operations and extensive separations aimed at the active constituents. Before we could isolate the active components on each attack, we ran out of material. Each time we found less than 1 μg and needed to overcome that serious problem. From 1979 to 1980, we went to the next scale-up entailing about 3 tons of the red sponge. Then we had the problem of trying to reduce the biomass so that we could work with the active fractions on a laboratory scale. At one point in this endeavor it was necessary to use HPLC columns that were nearly 3 m tall and 15 cm in diameter. That really helped with the initial concentration. By 1981, thanks to efforts on that scale, we isolated the first anticancer substance from the red sponge.[82] We obtained about 800 μg but were unable to solve the structure in 1981 using early 400 MHz NMR equipment with which it was still necessary to decouple



30, Cephalostatin 1



31, Cephalostatin 7



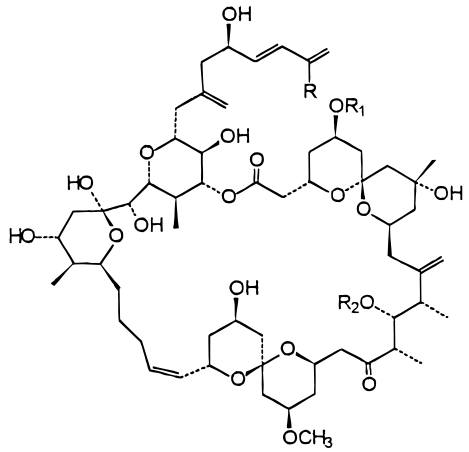
32, Cephalostatin 17

every complex signal by hand. At that time the instrumentation was really not up to the challenge of such a complex structure with only 800 μg (now spongistatin 4) available.[82] Before proceeding further, we need to review another endeavor that led to the final solution of the red sponge anticancer components' structural challenges.

The Republic of Maldives is about 1500 miles long comprising some 1200 islands: a very interesting place with a great variety of marine fauna. In 1986, we collected a black sponge initially identified as a *Spongia* sp. but recently reidentified as a *Hyrtios* sp. We found very quickly that it had potential anticancer constituents. In this case, the initial activity was very modest and we didn't think it was a high-priority lead for over 1 year. Then the activity of key fractions markedly increased. We returned to the Maldives in 1988 for a 400-kg wet weight recollection. After launching a determined effort to separate the anticancer substances, we found some of the most active constituents to be the red sponge spongistatin series that we had been working with from 1973.

We finally isolated the first of the Maldive spongistatins, albeit only a few milligrams, but by 1988 that was enough to solve the structure problems. The first of the Maldives series was spongistatin 1 (**33a**)[83] with 63 carbon atoms in a macrocycle, 21 oxygen atoms, and a chlorine atom. The structure required our solving it three times, in three different solvents, over the course of 1 year. Then we finally felt confident the structure was solved, although it had posed a lot of difficulties with the NMR interpretations even 6 years ago. Now we know spongistatin 1 (**33a**) is a 32-membered mac-

rocyclic lactone with a chlorine atom and 23 chiral centers. Currently the chiral centers are still a challenge. Although we obtained 10 mg of spongistatin 1 from the original 400 kg of sponge, we're still trying to grow crystals for the X-ray analysis.

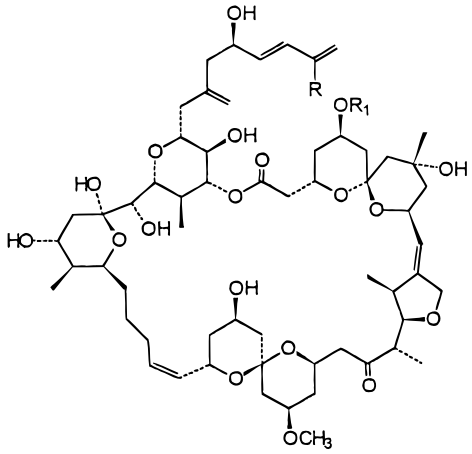


33a, Spongistatin 1, R = Cl, $R_1 = R_2 = COCH_3$
b, Spongistatin 2, R = H, $R_1 = R_2 = COCH_3$
c, Spongistatin 3, R = Cl, R_1 = H, $R_2 = COCH_3$
d, Spongistatin 4, R = Cl, $R_1 = COCH_3$, R_2 = H
e, Spongistatin 6, R = H, $R_1 = COCH_3$, R_2 = H

The structural elucidation of spongistatin 1 (**33a**) quickly led to structural assignments for spongistatin 2 (**33b**)[84] followed by spongistatin 3 (**33c**).[84] Both were found to involve some variations in the acetylation pattern of the parent macrocyclic lactone. We discovered spongistatin 4 (**33d**),[82] as noted above, in 1981 in the African red sponge. Once we solved the structures of spongistatins 1−3, we were able to elucidate the structure of spongistatin 4 and continue in the same fashion with the other red sponge constituents (very small amounts). At present, we have determined the structures up to spongistatin 9 (**34d**). With the red sponge components, there is a departure in the structure at number 5 (**34a**) due to an additional tetrahydrofuran ring. Spongistatins 5 (**34a**),[82] 7 (**34b**),[85] 8 (**34c**),[86] and 9 (**34d**)[86] all carry that ring system. However, the cancer cell growth inhibitory activity of spongistatin 5 was not diminished compared to that of spongistatin 1. Furthermore, spongistatin 6 (**33e**)[85] from the red sponge represents a return to the original spongipyran structure minus the tetrahydrofuran ring.

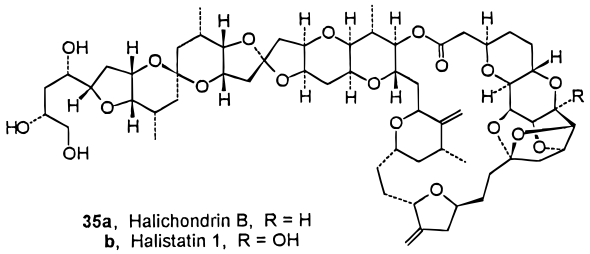
The profile of spongistatin 1 against the NCI human cancer cell line panel is probably the best to date in the NCI's evaluation programs. For example, with small cell lung cancer, the amount of spongistatin 1 required for 50% growth inhibition is 10^{-10} M, and that activity continues in lines from the colon cancer, renal cancer, ovarian cancer, and breast cancer sets. The latter are the most strongly inhibited, at roughly 10^{-12} M. Even more exciting are the current *in vivo* results. Spongistatin 1, used against the OVCAR-3 xenograph at 25 μg/kg, led to better than 70% long term survivors. In terms of mechanism of action, spongistatin 1 inhibits mitosis and microtubule assembly.[87,88] Both the anticancer *in vivo* and mechanistic studies are in progress, while spongistatin 1 is being readied for preclinical development.

Now we'll turn to another very important research area beginning with a series of sponges from the western Pacific. One of the best of these was an

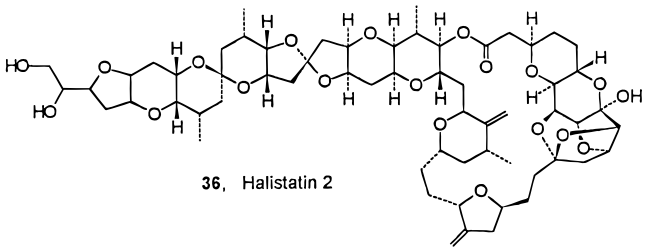


34a, Spongistatin 5, R = Cl, R_1 = H
b, Spongistatin 7, R = H, R_1 = H
c, Spongistatin 8, R = H, $R_1 = COCH_3$
d, Spongistatin 9, R = Cl, $R_1 = COCH_3$

Axinella species we collected (1979) in the Western Caroline Islands in the Palau group and another from the Republic of Comoros off east Africa. With the sponge from Palau, we were proceeding ahead rapidly when, unfortunately, for reasons beyond control of any of us, the NCI's natural-products-based anticancer drug discovery research suddenly came to an end in October 1981. All of us contributing to that program were set back several years. As a result, we didn't recover to a point where we could isolate key anticancer constituents from the Palau sponge until 1983−84. Then we isolated the component now known as halichondrin B (**35a**).[89] However, a Japanese group very capably led by Uemura published the structure (**35a**) just as we were completing this important discovery and assisting the NCI with initial antineoplastic evaluations. Halichondrin B (**35a**) is now in preclinical development in the NCI and looks excellent. That comment also applies to other powerful anticancer substances in our series such as halistatins 1 (**35b**)[90] and 2 (**36**).[91]



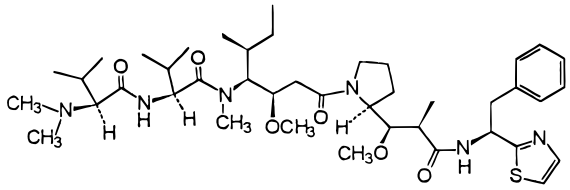
35a, Halichondrin B, R = H
b, Halistatin 1, R = OH



36, Halistatin 2

Next, we'll review the exceptionally important dolastatin leads. In 1972, specimens of *Dolabella auricularia* were collected off the island of Mauritius in the Indian Ocean. Like the red sponge, when we got this sea hare to our laboratories and began the evaluation, we found it would more than double the lifespan of

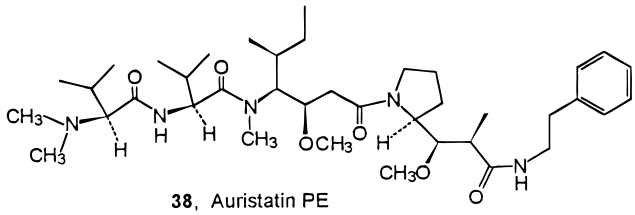
animals with the P388 leukemia. Again, this was a very high priority lead that we pursued very intensively and with which we encountered a very similar sequence of events. We never seemed to collect enough of the sea hare to solve the problem during the 1970's. Over the next 10 years to 1982, we had to recollect a number of times in Mauritius, and it wasn't until we obtained an almost 2-ton collection that we were finally able to solve the problem. The separation was extremely challenging. The simplest way we ever found to isolate the key substance, dolastatin 10 (**37**),[92] involved about 20 000 fractions and some 23 separate chromatographic steps using various techniques. We first isolated dolastatin 10 (**37**)[92] in 1984, and it took about 1 year to solve the structure problem with that first milligram. Crystallization failed, so we had to solve the structure employing high-field NMR, high-resolution mass spectrometry, and finally total synthesis.



37, Dolastatin 10

Dolastatin 10 (**37**)[92] was found to be a peptide in which, except for valine, the other four amino acid components, *N*,*N*-dimethylvaline, dolaisoleuine, dolaproine, and dolaphenine (derived from phenylalanine), were all unprecedented—one of nature's marvelous secrets revealed. The antineoplastic activity of dolastatin 10, the most active of the dolastatin series, was such that we knew it was going to clinical development. For example, at 11 μg/kg it afforded an 80% cure rate against the *in vivo* B16 melanoma. In general, dolastatin 10 has yet to meet an experimental cancer it couldn't attack.[93] The next problem was the nine chiral centers in that novel peptide of which we didn't even know the chirality of one. Furthermore, to prepare dolastatin 10 for eventual clinical trials, we would have needed about 700 tons of the sea hare. For ecological and many other reasons, that was not an option. Dolastatin 10 had to be synthesized, and that required determination of the chirality. So we started the total syntheses and then relied on our knowledge of the high-field NMR characteristics of the components to direct the total synthetic approaches. Each of the total syntheses we completed required about 28 steps; they were not easy at the beginning and it took 15 total syntheses.[94−96] However, that was better than the theoretical 512! Subsequently, we have been preparing dolastatin 10 by total synthesis and have recently completed another 13-g total synthetic scale-up of dolastatin 10 to meet clinical supply requirements.

The dolastatin 10 mechanism of action is complex and seems focused on inhibiting tubulin assembly.[97−99] It is a noncompetitive inhibitor of vincristine and is much more active than vincristine. Dolastatin 10 inhibits microtubule assembly at 1.2 μmol. In that respect it is the most potent such inhibitor known. With spongistatin 1 (**33a**), interaction (5-μmol region) with tubulin seems to be only part of the mechanism, and this spongipyran is a noncompetitive inhibitor of both vincristine and dolastatin 10. With regard to combretastatin A-4 (**25**), it is a noncompetitive inhibitor of colchicine and is almost as active as dolastatin 10 in terms of inhibiting tubulin assembly. In general, these are a most interesting series of compounds. Dolastatin 10 (**37**) entered phase I human clinical trials in November 1995 through the NCI. The first human trials began at the Mayo Clinic in Rochester and at the University of Texas M. D. Anderson unit in Houston. We hope that, as those trials are expanded, dolastatin 10 will prove to be a very useful anticancer drug. Only expert clinical research and time will provide the answer. Meanwhile, one of a large number of dolastatin 10 structural modifications we synthesized, designated auristatin PE (**38**),[97] has been entered into seven phase I cancer clinical trials in Japan.

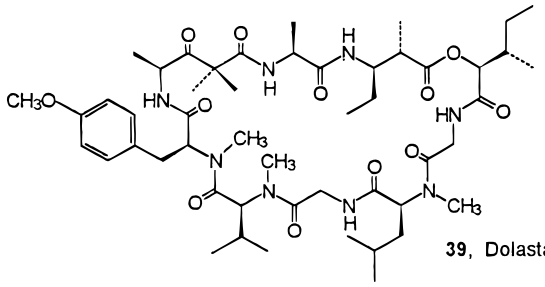


38, Auristatin PE

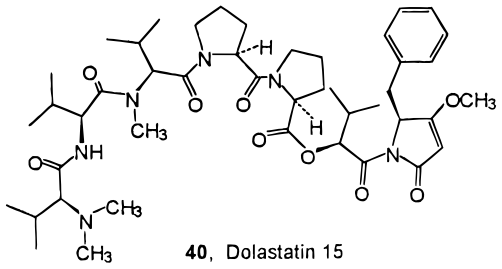
Because we are still working on various structural and synthetic aspects of the dolastatins, it seems appropriate to limit further review here to only two more of the most promising, namely dolastatins 11 (**39**)[100,101] and 15 (**40**).[102] Dolastatin 11 (**39**) represents a new type of depsipeptide with good cell-growth inhibitory activity and unusual activity toward actin (a protein series that cause cells to creep). As a result, the NCI placed dolastatin 11 into preclinical development in March 1995. Dolastatin 15 (**40**)[102,103] is another very active substance with a different antineoplastic profile than that shown by dolastatins 10 and 11. In fact, the only unit common to dolastatin 10 and dolastatin 15 is the dolavaline group. Dolastatin 15 is now in clinical development, and the structural modification LU103793 (NSC D-669356, **41**)[104] has been undergoing phase I human cancer clinical trials in Europe and the U.S. phase II clinical trials of LU103793 (**41**) are nearing initiation. Present evidence suggests that the dolastatins represent an extraordinary resource for further discovery and development of potentially useful anticancer drugs.[96]

In 1968, we were collecting specimens in the upper Gulf of Mexico. During that period, a Bryozoa specimen that subsequently was found to be *Bugula neritina* was collected and gave extracts that again more than doubled the lifespan of animals with the P388 lymphocytic leukemia. We pursued that lead for 4 years, and in 1972, much to our distress, lost the activity. Meanwhile, we were conducting expeditions in the Gulf of California and collected another bryozoan, again powerfully active, that turned out to be *B. neritina*. Research was continued with that specimen, and later we collected it again off the coast of California. Extensive bioassay (P388)-directed separation of the California *B. neritina* extracts led to isolation of the first milligram of bryostatin 1 (**42**) in 1981.[105] We were able to crystallize this substance and complete the X-ray crystallographic structural determination. Bryostatin 1 was found to be a remarkable macrocyclic lactone.[106−125]

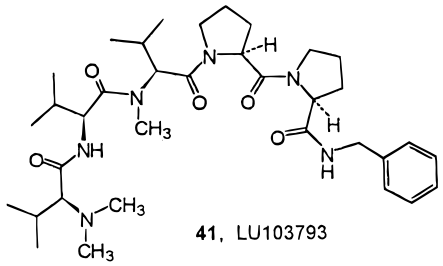
Meanwhile, we've been discovering[106] new (**43**−**45**) bryostatins and now have 20 of this series in hand from *B. neritina* collections that range from the Gulf of



39, Dolastatin 11

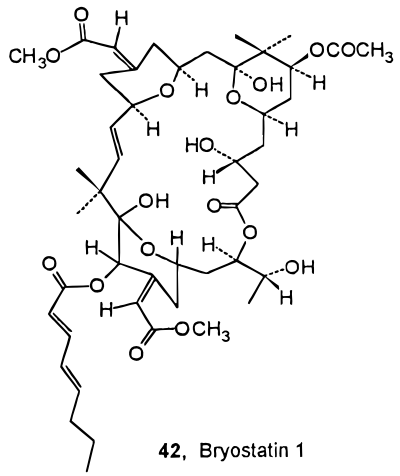


40, Dolastatin 15

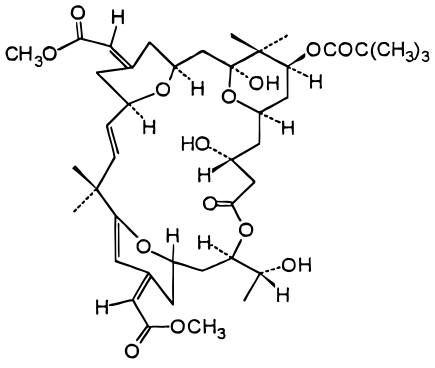


41, LU103793

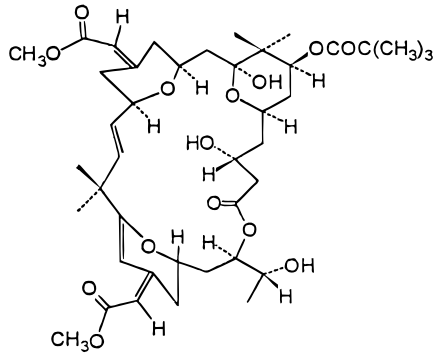
Mexico, Gulf of California, and coast of California to Japan (Gulf of Sagami).[126] More recently, we have explored *B. neritina* from two more remote areas in the Gulf of Japan.[127] *B. neritina* from one of these areas produces bryostatin 10 (**46**) in fairly abundant amounts (about 10^{-3}%). In contrast, the yields of the spongistatins range from 10^{-6} to 10^{-8}%. Dolastatin 10 was found in some 10^{-6}% yield. Some of the other dolastatins were isolated in 10^{-7}% yields. Bryostatin 1 was found in 10^{-6}% yields and some of the rarer bryostatins in yields of 10^{-8}%. Hence, bryostatin 10 being produced in 10^{-3}% yields is very interesting and potentially important for the future.



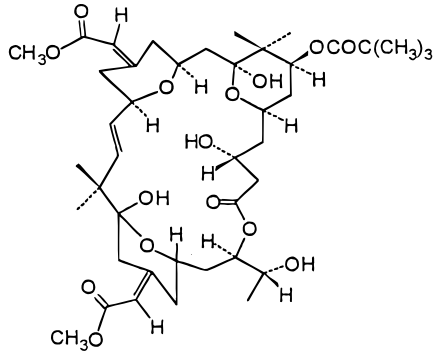
42, Bryostatin 1



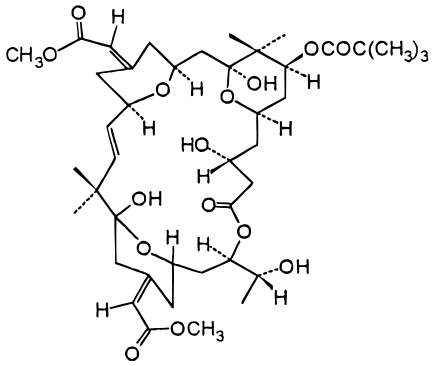
43, Bryostatin 16



44, Bryostatin 17



45, Bryostatin 18



46, Bryostatin 10

Among some of the very interesting early aspects of bryostatin 1 (**42**) was the observation that microgram doses (usually around 50 μg/kg) would double survival times against the P388 lymphocytic leukemia and later provide cures against the B16 melanoma in the mouse (particularly the variation that metastasizes very quickly to the lungs).[106] The same type of activity was found against the M5 ovary system.[106] Subsequently, we were greatly stimulated by the experiments of Dr. P. Blumberg[110,111] in the NCI, where bryostatin 1 was shown to be a powerful antitumor promoter and either an inhibi-

tor or stimulator of protein kinase C. In fact, it has a tremendous effect on the signal transduction pathway. Later, bryostatin 1 (**42**) was found to promote the normal growth of bone marrow progenitor cells.[114,120] One consequence of the hematopoietic progenitor effects was found when mice, given 1 μg of bryostatin 1, followed by a lethal dose of radiation, were found to survive.[119,121] Furthermore, bryostatin 1 was found to be an immune stimulant,[108,125] and it also stimulates the normal production of interleukin 2 and interferon.

Bryostatin 1 (**42**) was placed in clinical development in 1988 and 2 years later went into phase I human cancer trials. After nearly 5 years of clinical progress with bryostatin 1, we're beginning to see results from these first phase I trials. The first melanoma patient who was treated about 5 years ago is still alive.[123] Three years ago, the first ovarian cancer patients were entered in the phase I trials, and two who received the proper therapeutic dose are still alive.[124] The phase II clinical trials of bryostatin 1 recently started in the U.S. For example, the NCI has started six phase II human trials against non-Hodgkins lymphoma,[113] melanoma, and renal cancer. Combination anticancer drug trials with bryostatin 1 are also underway.

The firm foundation for discovery of potentially useful and hopefully curative anticancer drugs based on animal, plant, and microorganism sources is now in place. Given the necessary resources and diligence directed at discovery and development of new anticancer drugs of biosynthetic origin, the cancer problem will be controlled and the current human and economic disasters will eventually recede.

Acknowledgment. Here I am very pleased to express my grateful appreciation to the very capable members of my research group and other colleagues over the past 39 years who contributed to advancing this anticancer drug discovery research. In this regard special thanks are extended to Professor Cherry L. Herald. With pleasure I also wish to acknowledge the expert contributions of Dr. Fiona Hogan and Ms. Theresa Thornburgh to the final stages of manuscript preparation and the financial support provided by Outstanding Investigator Grant OIG CA44344-01A1-08 awarded by the U.S. National Cancer Institute, DHHS, as well as the Robert B. Dalton Endowment Fund.

References and Notes

(1) Based on the Research Achievement Award address given by Professor George R. Pettit at the 36th Annual Meeting of the American Society of Pharmacognosy, University of Mississippi, Oxford, MS, July 26, 1995, and represents contribution 374 of the series "Antineoplastic Agents." Part 373: Bai, R.; Schwartz, R. E.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Cancer Res.*, manuscript in preparation.
(2) Pettit, G. R. *J. Nat. Prod.* **1995**, *58*, 359−364.
(3) Pettit, G. R. *Biosynthetic Products for Cancer Chemotherapy*; Plenum: New York, 1977; Vol. 1.
(4) Suffness, M., Ed. *Taxol: Science and Applications*; CRC Press: Boca Raton, FL, 1995.
(5) Pettit, G. R.; Klinger, H.; Jorgensen, N. O.; Occolowitz, J. *Phytochemistry* **1966**, *5*, 301−309.
(6) Pettit, G. R.; Knight, J. C. *J. Org. Chem.* **1962**, *27*, 2696−2698.
(7) Pettit, G. R.; Green, B.; Bowyer, W. J. *J. Org. Chem.* **1961**, *26*, 2879−2883.
(8) Pisha, E.; Chai, H.; Lee, I. S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Das Gupta, T. K.; Pezzuto, J. M. *Nature (Med).* **1995**, *1*, 1046−1051.
(9) Singh, G. B.; Singh, S.; Bani, S. *Drugs Future* **1994**, *19*, 450−451.
(10) Pettit, G. R.; Hartwell, J. L.; Wood, H. B. *Cancer Res.* **1968**, *28*, 2168−2169.
(11) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature* **1970**, *227*, 962−963.
(12) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482−485.
(13) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985−994.
(14) Pettit, G. R. *Pure Appl. Chem.* **1994**, *66*, 2271−2281.
(15) Pettit, G. R.; Alkalay, D. S. *J. Org. Chem.* **1960**, *25*, 1363−1365.
(16) Pettit, G. R.; Kalnins, M. V. *J. Org. Chem.* **1960**, *25*, 1365−1367.
(17) Pettit, G. R.; Freeman, S.; Simpson, M. J.; Thompson, M. A.; Boyd, M. R.; Williams, M. D.; Pettit, G. R., III.; Doubek, D. L. *Anti-Cancer Drug Design* **1995**, *10*, 243−250.
(18) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-Cancer Drug Design* **1995**, *10*, 299−309.
(19) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666−1672.
(20) Pettit, G. R.; Hofer, P. *J. Chem. Soc.* **1963**, *850*, 4439−4442.
(21) Pettit, G. R.; Hofer, P. *Experientia* **1963**, *19*, 67−69.
(22) Pettit, G. R.; Hofer, P. *Helv. Chem. Acta* **1963**, *46*, 2142−2146.
(23) Pettit, G. R.; Kamano, Y.; Inoue, M.; Komeichi, Y.; Nassimbeni, L. R.; Niven, M. L. *J. Org. Chem.* **1982**, *47*, 1503−1506.
(24) Kamano, Y.; Pettit, G. R.; Inoue, M.; Tozawa, M.; Komeichi, Y. *J. Chem. Res.* **1977**, 837−845.
(25) Kamano, Y.; Pettit, G. R.; Brown, P.; Inoue, M. *Tetrahedron* **1975**, *31*, 2359−2361.
(26) Kamano, Y.; Pettit, G. R.; Tozawa, M.; Komeichi, Y.; Inoue, M. *J. Org. Chem.* **1975**, *40*, 2136−2138.
(27) Kamano, Y.; Pettit, G. R.; Inoue, M. *J. Org. Chem.* **1974**, *39*, 3007−3010.
(28) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1974**, *39*, 3003−3006.
(29) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1974**, *39*, 2632−2634.
(30) Kamano, Y.; Pettit, G. R. *J. Org. Chem.* **1974**, *39*, 2629−2631.
(31) Kamano, Y.; Pettit, G. R. *Can. J. Chem.* **1973**, *51*, 1973−1976.
(32) Kamano, Y.; Pettit, G. R. *J. Org. Chem.* **1973**, *38*, 2202−2204.
(33) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1972**, *37*, 4040−4044.
(34) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1974**, *39*, 3003−3006.
(35) Pettit, G. R.: Houghton, L. E.; Knight, J. C.; Bruschweiler, F. *J. Org. Chem.* **1970**, *35*, 2895−2898.
(36) Akizawa, T.; Mukai, T.; Matsukawa, M.; Yoshioka, M.; Morris, J.; Butler, V. P. *Chem. Pharm. Bull.* **1994**, *42*, 754−756.
(37) Krenn, L.; Kopp, B.; Deim, A.; Robien, W.; Kubelka, W. *Planta Med.* **1993**, *60*, 63−69.
(38) Numazawa, S.; Honma, Y.; Yamamoto, T.; Yoshida, T.; Kuroiwa, Y. *Leukemia Res.* **1995**, *19*, 945−953.
(39) Pettit, G. R.; Gupta, S. K.; Whitehouse, P. A. *J. Med. Chem.* **1967**, *10*, 692−696.
(40) Pettit, G. R.; Smith, R. L. *Can. J. Chem.* **1964**, *42*, 572−578.
(41) Pettit, G. R.; Chamberland, M. R.; Green, B. *Can. J. Chem.* **1967**, *45*, 1555−1560.
(42) Pettit, G. R.; Chamberland, M. R.; Blonda, D. S.; Vickers, M. A. *Can. J. Chem.* **1964**, *42*, 1699−1706.
(43) Pettit, G. R.; Settepani, J. A. *J. Org. Chem.* **1962**, *27*, 1714−1717.
(44) Pettit, G. R.; Das Gupta, A. K. *Chem. Ind.* (*London*) **1962**, 1016.
(45) Pettit, G. R.; Settepani, J. A. *Chem. Ind.* (*London*) **1964**, 1805.
(46) Pettit, G. R.; Gieschen, D. P.; Pettit, W. E. *J. Pharm. Sci.* **1979**, *68*, 1539−1542.
(47) Pettit, G. R.; Gieschen, D. P.; Pettit, W. E.; Rawson, T. E. *Can. J. Chem.* **1981**, *59*, 217−221.
(48) Verweij, J.; Planting, A. S. Th.; de Boer, M.; van der Burg, M. E. L.; Stoter, G. *Ann. Oncol.* **1992**, *3*, 241−242.
(49) van der Burg, M. E. L.; Planting, A. S. Th.; Stoter, G.; McDaniel, C.; Vecht, C. J.; Verweij, J. *Eur. J. Cancer* **1991**, *27*, 1635−1637.
(50) Tagliabue, G.; Filippeschi, S.; Hendricks, H.; D'Incalci, M. *Ann. Oncol.* **1992**, *3*, 233−236.
(51) Pettit, G. R.; Einck, J. J.; Herald, C. L.; Ode, R. H.; Von Dreele, R. B.; Brown, P.; Brazhnikova, M. G.; Gause, G. F. *J. Am. Chem. Soc.* **1975**, *97*, 7387−7388.
(52) Pettit, G. R.; Von Dreele, R. B.; Herald, D. L.; Edgar, M. T.; Wood, H. B., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 6742−6743.
(53) Pettit, G. R.; Nelson, P. S. *J. Org. Chem.* **1986**, *51*, 1282−1286.
(54) Pettit, G. R.; Nelson, P. S. *J. Org. Chem.* **1983**, *48*, 741−744.
(55) Pettit, G. R.; Cragg, G. M.; Herald, C. L. *Biosynthetic Products for Cancer Chemotherapy*; Elsevier Scientific: Amsterdam, 1985; Vol. 5.
(56) Sakai, R.; Stroh, J. G.; Sullins, D. W.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 3734−3748.
(57) Sakai, R.; Rinehart, K. L.; Guan, Y.; Wang, A. H.-J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11456−11460.
(58) Pettit, G. R.; Cragg, G. M. *Experientia* **1973**, *29*, 781.
(59) Pettit, G. R.; Budzinski, J. C.; Cragg, G. M.; Brown, P.; Johnston, L. D. *J. Med. Chem.* **1974**, *17*, 1013−1016.
(60) Von Dreele, R. B.; Pettit, G. R.; Cragg, G. M.; Ode, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 5256−5259.

(61) Chen, C.-H.; Yang, L.-M.; Lee, T. T.-Y.; Shen, Y.-C.; Zhang, D.-C.; Pan, D.-J.; McPhail, A. T.; McPhail, D. R.; Liu, S.-Y.; Li, D.-H.; Cheng, Y.-C.; Lee, K.-H. *BioMed. Chem. Lett.* **1994**, *2*, 137−145.
(62) Pettit, G. R.; Herald, C. L.; Judd, G. F.; Bollinger, G.; Thayer, P. S. *J. Pharm. Sci.* **1975**, *64*, 2023−2024.
(63) Pettit, G. R.; Herald, C. L.; Gust, D.; Herald, D. L.; Vanell, L. D. *J. Org. Chem.* **1978**, *43*, 1092−1095.
(64) Pettit, G. R.; Houghton, L. E. *Can. J. Chem.* **1968**, *46*, 2471−2472.
(65) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kefauver, D. F.; Pettit, G. R.; Groszek, G.; Hollingshead, M.; Kirsi, J. J.; Shannon, W. M.; Schubert, E. M.; DaRe, J.; Ugarkar, B.; Ussery, M. A.; Phelan, M. J. *J. Nat. Prod.* **1992**, *55*, 1569−1581.
(66) Gabrielsen, B. J.; Monath, T. P.; Huggins, J. W.; Kirsi, J. J.; Hollingshead, M.; Shannon, W. M.; Pettit, G. R. In *Natural Products as Antiviral Agents*; Chu, C. K., Cutler, H. G., Eds.; Plenum Press: New York, 1992; pp 121−135.
(67) Pettit, G. R.; Cragg, G. M.; Suffness, M. I.; Gust, D.; Boettner, F. E.; Williams, M.; Saenz-Renauld, J. A.; Brown, P.; Schmidt, J. M.; Ellis, P. D. *J. Org. Chem.* **1984**, *49*, 4258−4266.
(68) Pettit, G. R.; Cragg, G. M.; Suffness, M. *J. Org. Chem.* **1985**, *50*, 5060−5063.
(69) Pettit, G. R.; Houghton, L. E.; Rogers, N. H.; Coomes, R. M.; Berger, D. F.; Reucroft, P. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B., Jr. *Experientia* **1972**, *28*, 382.
(70) Pettit, G. R.; Ode, R. H. *Lloydia* **1976**, *39*, 129−133.
(71) Pettit, G. R.; Hogan-Pierson, F.; Herald, C. L. *Anticancer Drugs from Animals, Plants, and Microorganisms;* John Wiley-Interscience: New York, 1994.
(72) Pettit, G. R.; Herald, C. L.; Smith, C. R. *Biosynthetic Products for Cancer Chemotherapy;* Elsevier Scientific: Amsterdam, 1989.
(73) Attaway, D. H.; Zaborsky, O. R. *Marine Biotechnology*; Plenum: New York, 1993; Vol. 1.
(74) Pettit, G. R.; Ode, R. H.; Harvey, T. B., III. *Lloydia* **1973**, *36*, 204.
(75) Pettit, G. R.; Ode, R. H. *J. Pharm. Sci.* **1977**, *66*, 757.
(76) Pettit, G. R.; Hasler, J. A.; Paull, K. D.; Herald, C. L. *J. Nat. Prod.* **1981**, *44*, 701−704.
(77) Pettit, G. R.; Herald, C. L.; Allen, M. S.; Von Dreele, R. B.; Vanell, L. D.; Kao, J. P. Y.; Blake, W. *J. Am. Chem. Soc.* **1977**, *99*, 262−263.
(78) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006−2007.
(79) Pettit, G. R.; Kamano, Y.; Inoue, M.; Dufresne, C.; Boyd, M. R.; Herald, C. L.; Schmidt, J. M.; Doubek, D. L.; Christie, N. D. *J. Org. Chem.* **1992**, *57*, 429−431.
(80) Pettit, G. R.; Xu, J.-P.; Schmidt, J. M.; Boyd, M. R. *BioMed. Chem. Lett.* **1995**, *5*, 2027−2032.
(81) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707−6716.
(82) Pettit, G. R.; Herald, C. L.; Cichacz, Z. A.; Gao, F.; Schmidt, J. M.; Boyd, M. R.; Christie, N. D.; Boettner, F. E. *J. Chem. Soc., Chem. Commun.* **1993**, 1805−1807.
(83) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem.* **1993**, *58*, 1302−1304.
(84) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R. *J. Chem. Soc., Chem. Commun.* **1993**, 1166−1168.
(85) Pettit, G. R.; Herald, C. L.; Cichacz, Z. A.; Gao, F.; Boyd, M. R.; Christie, N. D.; Schmidt, J. M. *Nat. Prod. Lett.* **1993**, *3*, 239−244.
(86) Pettit, G. R.; Cichacz, Z. A.; Herald, C. L.; Gao, F.; Boyd, M. R.; Schmidt, J. M.; Hamel, E.; Bai, R. *J. Chem. Soc., Chem. Commun.* **1994**, 1605−1606.
(87) Bai, R.; Taylor, G. F.; Cichacz, Z. A.; Herald, C. L.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Biochemistry* **1995**, *34*, 9714−9721.
(88) Bai, R.; Cichacz, Z. A.; Herald, C. L.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1993**, *44*, 757−766.
(89) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A.; Rützler, K. C. *J. Med. Chem.* **1991**, *34*, 3339−3340.
(90) Pettit, G. R.; Tan, R.; Gao, F.; Williams, M. D.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J. N. A.; Tackett, L. P. *J. Org. Chem.* **1993**, *58*, 2538−2543.
(91) Pettit, G. R.; Gao, F.; Doubek, D. L.; Boyd, M. R.; Hamel, E.; Bai, R.; Schmidt, J. M.; Tackett, L. P.; Rützler, K. *Gazz. Chim. Ital.* **1993**, *123*, 371−377.
(92) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883−6885.
(93) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151−9170.
(94) Pettit, G. R.; Singh, S. B.; Hogan, F.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463−5465.
(95) Pettit, G. R.; Srirangam, J. K.; Singh, S. B.; Williams, M. D.; Herald, D. L.; Barkóczy, J.; Kantoci, D.; Hogan, F. *J. Chem. Soc., Perkin Trans. 1* **1996**, *8*, 859−863.
(96) Pettit, G. R. The Dolastatins. In *Progress in the Chemistry of Organic Natural Products;* Herz, W., Kirby, G. W., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, in press.
(97) Pettit, G. R.; Srirangam, J. K.; Barkóczy, J.; Williams, M. D.; Durkin, K. P. M.; Boyd, M. R.; Bai, R.; Hamel, E.; Schmidt, J. M.; Chapuis, J.-C. *Anti-Cancer Drug Des.* **1995**, *10*, 529−544.
(98) Bai, R.; Taylor, G. F.; Schmidt, J. M.; Williams, M. D.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1995**, *47*, 965−976.
(99) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Hamel, E. *J. Org. Chem.* **1994**, *59*, 6127−6130.
(100) Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R. J.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553−557.
(101) Bates, R. B.; Bontems, R.; Brusoe, K. G.; Caldera, S.; Cui, W.; Gangwar, S.; Gramme, M. R.; Jianxing, Z.; McClure, K. J.; Pettit, G. R.; Rouen, G. P.; Schadow, H.; Stessman, C. C.; Taylor, S. R. *211th ACS National Meeting*, New Orleans, LA, 1996.
(102) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005−6006.
(103) Pettit, G. R.; Herald, D. L.; Singh, S. B.; Thornton, T. J.; Mullaney, J. T. *J. Am. Chem. Soc.* **1991**, *113*, 6692−6693.
(104) de Arruda, M.; Cocchiaro, C. A.; Nelson, C. M.; Grinnell, C. M.; Janssen, B.; Haupt, A.; Barlozzari, T. *Cancer Res.* **1995**, *55*, 3085−3092.
(105) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846−6848.
(106) Pettit, G. R. The Bryostatins. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, 1991; Vol. 57, pp 153−195.
(107) Maki, A.; Diwakaran, H.; Redman, B.; Al-Asfar, S.; Pettit, G. R.; Mohammad, R. M.; Al-Katib, A. *Anti-Cancer Drugs* **1995**, *6*, 392−397.
(108) Eisemann, K.; Totola, A.; Jurcic, K.; Pettit, G. R.; Wagner, H. *Pharm. Pharmacol. Lett.* **1995**, *1*, 45−48.
(109) Patella, V.; Casolaro, V.; Ciccarelli, A.; Pettit, G. R.; Columbo, M.; Marone, G. *Blood* **1995**, *85*, 1272−1281.
(110) Kazanietz, M. G.; Lewin, L. E.; Gao, F.; Pettit, G. R.; Blumberg, P. M. *Mol. Pharmacol.* **1994**, *46*, 374−379.
(111) Szallasi, Z.; Denning, M. F.; Smith, C. B.; Dlugosz, A. A.; Yuspa, S. H.; Pettit, G. R.; Blumberg, P. M. *Mol. Pharmacol.* **1994**, *46*, 840−850.
(112) Stanwell, C.; Gescher, A.; Bradshaw, T. D.; Pettit, G. R. *Int. J. Cancer* **1994**, *56*, 585−592.
(113) Mohammad, R. M.; Diwakaran, H.; Maki, A.; Emara, M. A.; Pettit, G. R.; Redman, B.; Al-Katib, A. *Leukemia Res.* **1995**, *19*, 667−673.
(114) Berkow, R. L.; Schlabach, L.; Dodson, R.; Benjamin, W. H., Jr.; Pettit, G. R.; Rustagi, P.; Kraft, A. S. *Cancer Res.* **1993**, *53*, 2810−2815.
(115) Al-Katib, A.; Mohammad, R. M.; Khan, K.; Dan, M. E.; Pettit, G. R.; Sensenbrenner, L. L. *J. Immunother.* **1993**, *14*, 33−42.
(116) Prendiville, J.; Crowther, D.; Thatcher, N.; Woll, P. J.; Fox, B. W.; McGown, A.; Testa, N.; Stern, P.; McDermott, R.; Potter, M.; Pettit, G. R. *Br. J. Cancer* **1993**, *68*, 418−424.
(117) Szallasi, Z.; Smith, C. B.; Pettit, G. R.; Blumberg, P. M. *J. Biol. Chem.* **1994**, *269*, 2118−2124.
(118) Prendiville, J.; McGown, A. T.; Gescher, A.; Dickson, A. J.; Courage, C.; Pettit, G. R.; Crowther, D.; Fox, B. W. *Br. J. Cancer* **1994**, *70*, 573−578.
(119) Grant, S.; Traylor, R.; Pettit, G. R.; Lin, P.-S. *Blood* **1994**, *83*, 663−667.
(120) Gebbia, V.; Testa, A.; Pettit, G. R.; Sensenbrenner, L. L.; May, W. S. *Cell Pharmacol.* **1993**, *1*, 31−35.
(121) Grant, S.; Traylor, R.; Pettit, G. R.; Lin, P.-S. *Cytokine* **1993**, *5*, 490−497.
(122) Stewart, J. P.; McGown, A. T.; Prendiville, J.; Pettit, G. R.; Fox, B. W.; Arrand, J. R. *Cancer Chemother. Pharmacol.* **1993**, *33*, 89−91.
(123) Philip, P. A.; Rea, D.; Thavasu, P.; Carmichael, J.; Stuart, N. S. A.; Rockett, H.; Talbot, D. C.; Ganesan, T.; Pettit, G. R.; Balkwill, F.; Harris, A. L. *J. Natl. Cancer Inst.* **1993**, *85*, 1812−1818.
(124) Jayson, G. C.; Crowther, D.; Prendiville, J.; McGown, A. T.; Scheid, C.; Stern, P.; Young, R.; Brenchley, P.; Chang, J.; Owens, S.; Pettit, G. R. *Br. J. Cancer* **1995**, *72*, 461−468.
(125) Scheid, C.; Prendiville, J.; Jayson, G.; Crowther, D.; Fox, B.; Pettit, G. R.; Stern, P. L. *Cancer Immunol. Immunother.* **1994**, *39*, 223.
(126) Pettit, G. R.; Gao, F.; Blumberg, P. M.; Herald, C. L.; Coll, J. C.; Kamano, Y.; Lewin, N. E.; Schmidt, J. M.; Chapuis, J.-C. *J. Nat. Prod.* **1996**, *59*, 286−289.
(127) Kamano, Y.; Zhang, H.-P.; Morita, H.; Itokawa, H.; Shirota, O.; Pettit, G. R.; Herald, D. L.; Herald, C. L. *Tetrahedron* **1996**, *52*, 2369−2376.

NP9604386