MARINE ANIMAL BIOSYNTHETIC CONSTITUENTS FOR CANCER CHEMOTHERAPY[1]

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ABSTRACT.—A fifteen year investigation of marine animal components as sources for new and potentially useful cancer chemotherapeutic drugs has led to our discovery of a number of such valuable substances. The especially productive Indian Ocean sea hare *Dolabella auricularia* has yielded (100 kg→~1 mg each) a series of very potent cell growth inhibitory substances designated dolastatins 1-9. The first member of this new series, dolastatin 1, may represent the most potent anticancer agent so far uncovered with, e.g., a curative response (33%) using a dose of 11 μg/kg (T/C 240, to T/C 139 at 1.37 μg/kg) in the National Cancer Institute's murine B16 melanoma. Structural elucidation of the new antineoplastic agents is underway, and recent progress is illustrated with the peptide dolastatin 3 (P388 ED_{50} 2.7 x 10^{-7} μg/ml).

Fifteen years ago we began the first systematic study of marine animals and plants as a vast untapped resource for discovering new drugs necessary to improve human cancer treatment. After some four years of evaluating a large number of extracts from species representing (on a world-wide basis) various marine phyla our original expectations were amply confirmed and summarized in 1970 (2). Subsequently a number of new cytotoxic and/or antineoplastic marine animal constituents were isolated and characterized (3). For the past eleven years considerable efforts have been devoted to isolating the very active, albeit trace, constituents of certain exceptionally promising marine animal extracts. A preliminary report of success with one such problem follows.

The great Roman natural scientist Gaius Plinius Secundus (Pliny the Elder) in his comprehensive study (4) of about 60 A.D. first described a most potent Indian Ocean sea hare[2] of the genus *Dolabella*. Extracts from this animal and two related *Aplysia* species from the Mediterranean were well known for their toxic properties during the reign of Nero (4, 5). By 150 A.D. Nicander (5) recognized the possibility of using such extracts for treatment of certain diseases. However, the potential of the Indian Ocean *Dolabella* with respect to modern medical problems was not recognized until we uncovered evidence for extremely active anticancer constituents in the Indian Ocean *Dolabella auricularia*[3] (3c).

We have now completed the isolation and preliminary characterization of an exceptionally promising series of cancer chemotherapeutic agents designated dolastatins 1-9 from *D. auricularia*. The dolastatins most probably correspond to the potent *D. auricularia* constituents recognized from ancient to fairly recent (7) times. Since dolastatin 1 has been shown (by the U.S. National Cancer

[1]The present contribution is part 72 in the series Antineoplastic Agents. For part 71 refer to (1).

[2]The Romans first designated Mollusca of the family Aplysiidae in this fashion due to a similarity between the ears of a hare and the auriculate tentacles of these gastropods, consult (5).

[3]The *D. auricularia* was probably that first described by Pliny and the minor variations recorded in subsequent literature as, e.g., *D. andersoni*, *D. californica*, *D. ecaudata*, and *D. scapula* are actually one species, namely *D. auricularia*, see (6).

Institute) to cause an 88% life extension (at a dose of 11 μg/kg) with the murine P388 lymphocytic leukemia and found to afford a curative response (33%) with a dose of 11 μg/kg (with T/C 240, to T/C 139 at 1.37 μg/ml) against the murine B16 melanoma, it may represent the most active (lowest dose) presently known antineoplastic agent.

A methylene chloride solvent partition (3i,j) fraction from an ethanol extract of *D. auricularis* was very carefully separated by use of an extensive sequence of chromatographic techniques (guided by bioassay) utilizing LH-20 Sephadex and silica gel column methods. By this general approach some 100 kg of the wet sea hare afforded about 1 mg each of nine very potent cell growth inhibitory substances designated dolastatins 1–9. Each dolastatin was obtained as an amorphous colorless solid: D-1, mp 105–110°; D-2, mp 118–121°; D-4, mp 102–109°; D-5, mp 52–56°; D-6, mp 57–58°; D-7, mp 142–145°; D-8, mp 72–88°; and D-9, mp 149–152°. Structural determination of the new antineoplastic peptides is well underway and current progress with dolastatin 3 provides an illustration.

Results of field desorption mass spectrometry and elemental analyses led to tentative molecular formula $C_{29}H_{40}N_8O_6S_2$ (M^+ 660.2512) for dolastatin 3 (P388 ED_{50} 2.7 x 10^{-7} μg/ml); mp 133–137°; $[\alpha]^{26}D$ −35.5° (c, 0.09, methanol). Acid hydrolysis experiments combined with interpretation of the ^{13}C nmr, ^1H nmr (at 400 MHz), infrared and ultraviolet (fig. 1) spectral measurements revealed

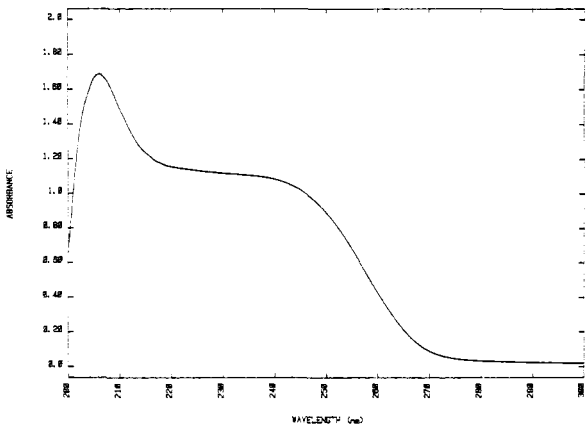
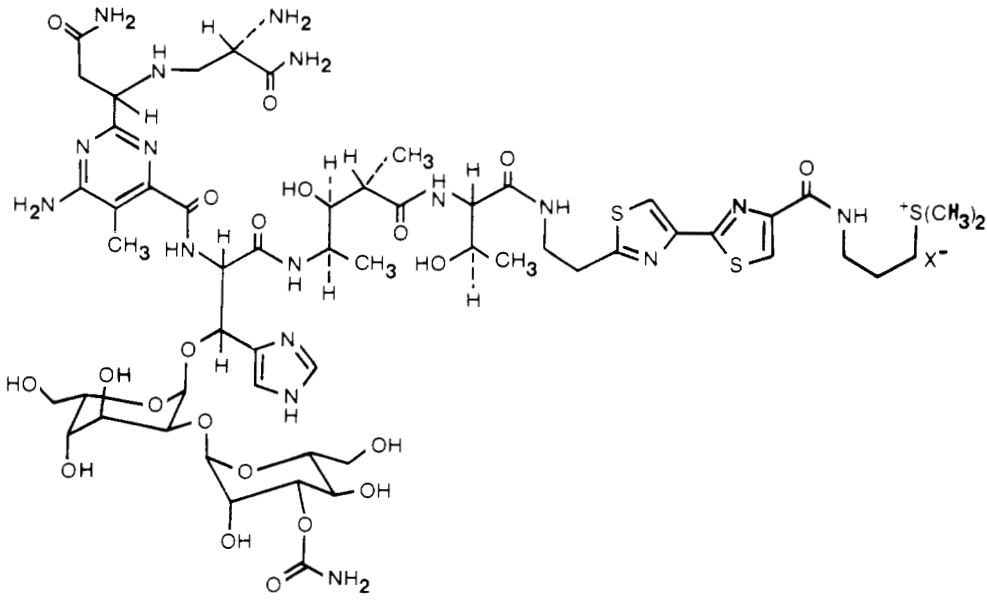


FIG. 1. Ultraviolet spectrum of dolastatin 3.

that dolastatin 3 was a peptide containing leucine, proline, valine, and two thiazole amino acids. Experiments directed at defining the amino acid sequence and remaining structural features are now in progress.

Discovery of the thiazole ring system in dolastatin 3 was especially interesting. Such biosynthetic products are relatively rare, but usually very active biologically. For example, among the lower plant components bleomycin (**1**) (8, 9, 10) in combination with vinblastine produces a significant level of complete remissions (some curvative) in patients with testicular cancer (11, 3h) and thiostrepton (**2**) (12), a



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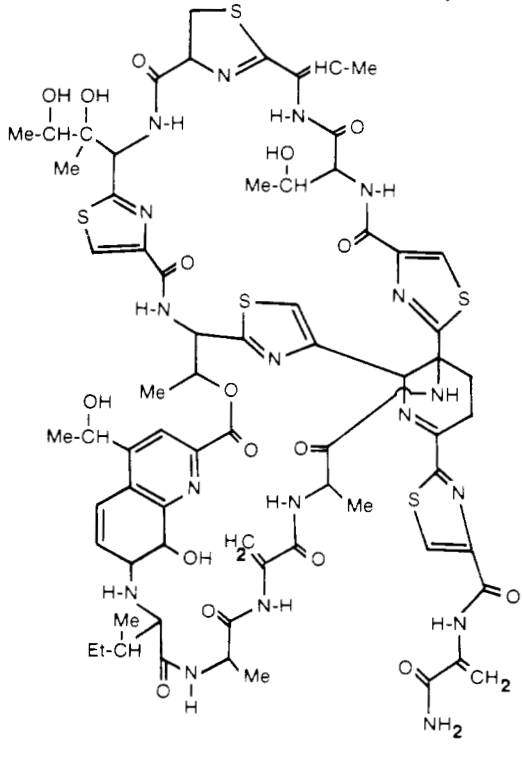

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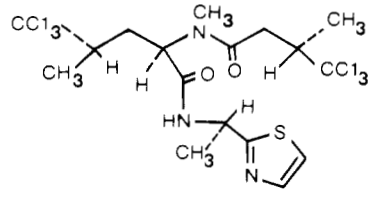

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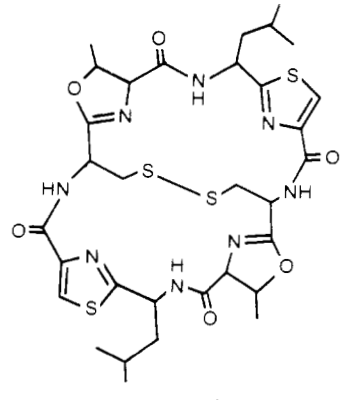

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broad spectrum antibiotic, also has antineoplastic activity. Recently the Western Pacific sponge *Dysidea herbacea* Keller (13) and ascidian *Lissoclinum patella* (14) have been found to produce, respectively, the thiazoles dysidenin (**3**) and ulithiacyclamide (**4**). The latter two marine animal thiazoles may also possess useful biological activity. Isolation of the dolastatins and completion of their structural elucidation should significantly enhance our insight into the possible role of cysteine derived thiazoles (and/or dehydro amino acids and thiazolidines) in the development of curative cancer chemotherapeutic drugs.

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