the conditions of the Fischer assay (500°C), the mineral matter serves as a source of NH_3. Under the higher temperatures and different oxygen tensions typical of other processes, the position of the equilibrium between NH_3 and the minerals would shift.

Stevenson pointed out that the Kjeldahl method, which traditionally has been used to determine N in both raw and spent oil shale, measures inorganic N along with organic N; thus the results of any soil or rock analysis by the Kjeldahl method should be reported not as organic N but as total N (*14*). Stevenson and Cheng measured total N and inorganic N (the sum of fixed NH_4^+-N and exchangeable NH_4^+-N) and calculated organic N by the difference (*15*). We have adopted their analytical procedures except that we determined NH_3 in the final step of each procedure by distilling it into a boric acid–indicator solution and titrating with 0.005*N* H_2SO_4 (*16*). In this study, exchangeable NH_4^+-N was insignificant for all samples and therefore is not reported.

The key to understanding the distribution of the types of N is proper determination of the fixed NH_4^+-N—that is, NH_4^+ that cannot be extracted with 1*M* KCl and that is thought to occur within silicate mineral lattices as a proxy for K^+ (*6*). The most likely source of error would seem to be reporting organic N as fixed NH_4^+-N; thus, we have incorporated several steps into the procedure to minimize this risk. (i) A pretreatment with KOBr is used to oxidize the N contained in organic matter and exchangeable NH_4^+ to elemental N (*17*). The treatment with 5*N* HF and 0.1*N* HCl used to break down the silicate lattice does not release NH_3 from organic matter, even from compounds as labile as amino acids or amino sugars (*18*). In fact, Stevenson found that organic matter not removed by oxidation tends to interfere with the fixed NH_4^+-N determination by protecting the minerals from HF-HCl, thereby preventing release of NH_4^+ rather than contributing NH_3 (*6*). (ii) The NH_3 is steam-distilled from a solution buffered at *p*H 8.8 with borate in order to avoid base-induced release of NH_3 from organic matter (*18*). Thus, we believe that the N reported here as fixed NH_4^+-N occurs as a proxy for K^+ within the crystal lattice of silicate minerals.

We determined the fixed-layer K^+ by removing exchangeable K^+ by extraction with NH_4Cl solution, breaking down the silicate lattice with HF, and analyzing the solution with an atomic absorption spectrophotometer (Perkin-Elmer model 403).

We propose that the N occurring as fixed NH_4^+-N is derived from the organic matter during early diagenesis. Oil shale of the Green River Formation is thought by some to have formed in a stratified lake with highly anoxic bottom waters (*19*). Because this is precisely the type of environment in which significant quantities of NH_3 would be generated (*20*), NH_4^+ should have been available for incorporation at the time authigenic minerals were forming. This model is consistent with the suggestion of Gulbrandsen that in the Phosphoria Formation NH_4^+ derived from the decomposition of organic matter was incorporated in feldspar as it formed from volcanic glass or intermediate alteration products of glass such as smectite or zeolites (*7*).

JAMES E. COOPER
W. SCOT EVANS*
Department of Geology, University of Texas, Arlington 76019

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* Present address: Exxon Company U.S.A., Los Angeles, Calif. 90067.

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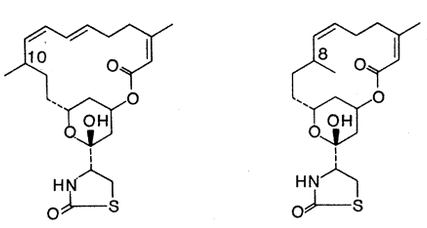
Latrunculins: Novel Marine Toxins That Disrupt Microfilament Organization in Cultured Cells

Abstract. *Two toxins, latrunculins A and B, which contain a new class of 16- and 14-membered marine macrolides attached to the rare 2-thiazolidinone moiety, were purified recently from the Red Sea sponge* Latrunculia magnifica. *The effects of these toxins on cultured mouse neuroblastoma and fibroblast cells have been evaluated. In both types of cells, submicromolar toxin concentrations rapidly induce striking changes in cell morphology that are reversible upon removal of the toxin. Immunofluorescence studies with antibodies specific for cytoskeletal proteins reveal that the toxins cause major alterations in the organization of microfilaments without obvious effects on the organization of the microtubular system.*

Among the most prominent sponges in the Gulf of Eilat (the Red Sea) are colonies of the branching red-colored *Latrunculia magnifica* (Keller), which are found at depths of 6 to 30 m and are clearly visible from long distances. Unlike most Gulf of Eilat sponges, colonies of this sponge grow exposed and appear never to be damaged or eaten by fishes. Furthermore, when squeezed manually, the sponge exudes a reddish fluid that causes fish to retreat from its vicinity. Squeezing *Latrunculia magnifica* into an aquarium is lethal, causing agitation of the fish in seconds, followed by hemorrhage, loss of balance, and, after 4 to 6 minutes, death (*1*).



Latrunculin A (LAT-A) Latrunculin B (LAT-B)
Fig. 1. Structures of latrunculins.

Two toxins, latrunculin A (LAT-A) and latrunculin B (LAT-B) were recently isolated and purified from two *Latrunculia magnifica* species (or specimens), and their structures were determined by

spectroscopic methods and x-ray diffraction analysis (*2*). The latrunculins are the first marine macrolides known to contain 16- and 14-membered rings, and they are unusual in that they contain the rare 2-thiazolidinone moiety (Fig. 1).

In order to investigate the cellular processes affected by latrunculins and their site of action, we used cultured mouse neuroblastoma and fibroblast cells. We now report that submicromolar concentrations of latrunculins rapidly cause morphological changes in both types of cells. Immunofluorescence studies with antibodies specific for actin and tubulin reveal that the latrunculins induce profound changes in the organization of microfilaments while leaving the organization of microtubules unaltered. When the toxin is removed, both types of cells rapidly recover their normal morphology and microfilament organization.

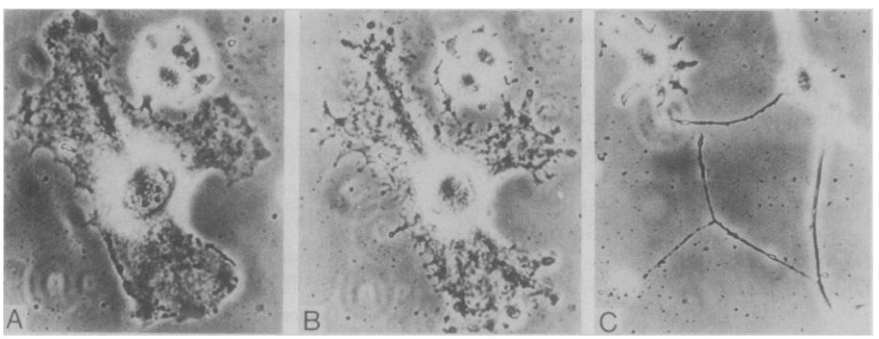


Fig. 2. Phase-contrast micrographs showing the effects of LAT-B on the morphology of living mouse neuroblastoma N1E-115 cells (A) before addition of LAT-B and (B) and (C) 60 minutes after addition of LAT-B (150 ng/ml). Note the retraction of the lamellar expansions, the formation of notches, and the rounding up of the nuclear region in (B) and the lack of effect on the long processes in (C). Cells were grown in 2 percent DMSO for 7 days. Magnification ×160.

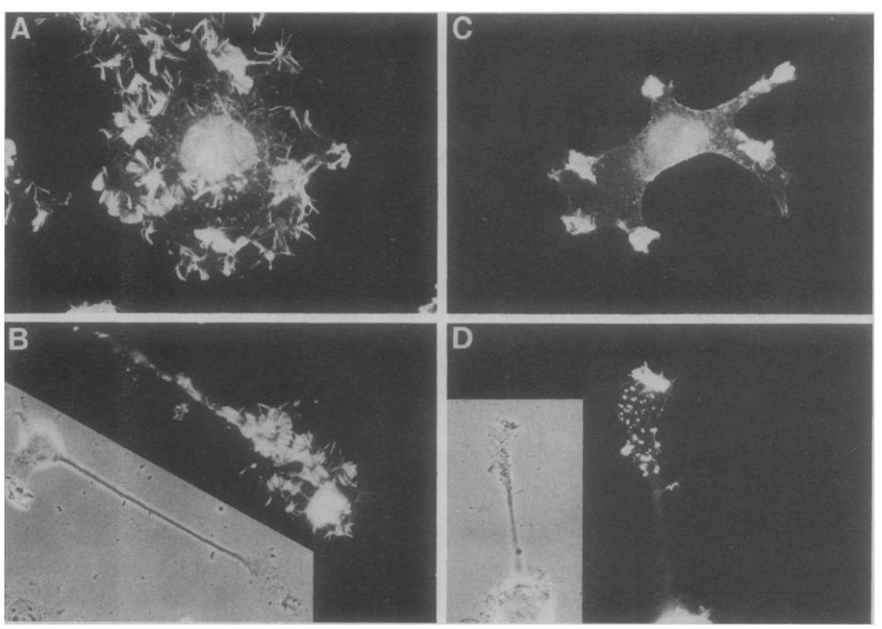


Fig. 3. Fluorescence micrographs showing the effects of LAT-A on actin organization in mouse neuroblastoma N1E-115 cells stained with purified antibody to actin. (A and B) Before and (C and D) after addition of LAT-A (35 ng/ml; 1 hour of incubation). Note the disappearance of actin filaments and bundles (C) in the cell body and (D) in the growth cone after treatment with LAT-A. Magnification ×400 in (A) and (C) and ×640 in (B) and (D). Insets in (B) and (D) show the corresponding phase micrographs at a ×400 magnification. Cells grown on glass cover slips were fixed with 3.7 percent formaldehyde in phosphate-buffered saline (PBS) for 10 minutes, washed with PBS, and permeabilized with 0.2 percent Triton X-100 in PBS for 2 minutes at room temperature. The cover slips were then placed in moist chambers and incubated with primary antibody (affinity-purified antibody to skeletal muscle actin, 20 to 50 μg/ml) for 30 minutes at 37°C. After the cells were again washed with PBS, the procedures were repeated with the secondary antibody (affinity purified rhodamine-conjugated goat antibody to rabbit immunoglobulin G, 100 μg/ml). After a final series of washings in PBS, the cover slips were mounted in Gelvatol. The labeled preparations were viewed and photographed with a Zeiss Universal microscope equipped with phase-contrast and epifluorescence illumination; oil immersion objectives (25× and 40×) were used.

Cells of mouse neuroblastoma clone N1E-115 were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 percent fetal calf serum at 37°C in an atmosphere of 10 percent CO_2 in air. Mouse fibroblasts (Swiss/3T3) were grown in DMEM supplemented with 10 percent fetal calf serum. Cells were replated into 35-mm plastic tissue culture dishes containing glass cover slips (18 mm in diameter). Differentiated neuroblastoma cells were obtained by adding 2 percent dimethyl sulfoxide (DMSO) to the growth medium (*3*).

Electrophysiological experiments did not reveal any obvious effects of LAT-A or LAT-B on electrical membrane properties (*4*). During the course of these experiments, we noticed that toxin concentrations as low as 35 ng/ml could rapidly elicit profound morphological changes in N1E-115 cells. With LAT-B at 150 ng/ml, the changes are initiated shortly after toxin application, and in less than 1 hour, well spread cells (*5*) lose their smooth contour, assume a jagged shape, and round up in the nuclear region (Fig. 2, A and B). In morphologically differentiated cells, the neuronal soma rounds up, the cells lose their numerous microspikes, and the shape of the growth cones is markedly altered. However, the long and thick processes characteristic of such cells are hardly affected, even after several hours in the presence of toxin at concentrations as high as 350 ng/ml (Fig. 2C). Alterations in cellular morphology are not restricted to neuroblastoma cells. Exposure of 3T3 fibroblasts to LAT-A at 350 ng/ml results in rounding up and arborization in less than 1 hour, whereas at concentrations of 50 to 150 ng/ml, many cells retain their normal shape.

The proposed role of cytoskeletal elements in cell motility and shape determination (*6*) led us to examine the effects of latrunculins on microfilaments and microtubules by indirect immunofluorescence microscopy with purified antibodies specific for actin and tubulin. The organization of actin in N1E-115 cells before and after exposure to LAT-A is shown in Fig. 3. The immunofluorescence labeling for actin in N1E-115 cells reveals rich and varied actin patterns similar to those described in other neuroblastoma clones (*7*). Cells at all stages of growth and differentiation are characterized by short and thin actin filaments that can form a fine network of meshes and be assembled into bundles that form a multiplicity of patterns (Fig. 3A). In well spread cells these patterns are abun-

dant at, or close to, the cell periphery. In morphologically differentiated cells, specific actin structures are easily recognized, mainly in the growth cones (Fig. 3B). After 1 hour of incubation with LAT-A or LAT-B at 35 ng/ml, most of the actin filaments and bundles disappear, and diffuse staining, dots, and patches become visible with pronounced actin accumulation in the edges of the processes (Fig. 3, C and D).

Treatment with latrunculins also disrupts the organization of actin in 3T3 cells. One hour after addition of LAT-A or LAT-B (350 ng/ml), most of the long actin bundles ("stress fibers") (*8*) are no longer visible, and intense labeling of actin is found in the cytoplasm or in the ruffling membranes. With lower concentrations (50 to 150 ng/ml), many cells display only partial disruption of their actin cables.

The effect of latrunculins on cell morphology and actin distribution is reversible, and within 1 hour after toxin removal, both N1E-115 and 3T3 cells regain their normal shape and actin structures.

Latrunculins did not have any obvious effects on the integrity of the microtubular network in either N1E-115 or 3T3 cells. In N1E-115 cells, dense aggregates of microtubule bundles appeared to run from the perinuclear region toward the edges of the cells and to extend throughout the processes, both in the absence and presence of latrunculins. Regrowth of microtubules after reversal of treatment with the depolymerizing agent colcemid (*9*) was also unaffected by the presence of latrunculins in N1E-115 and 3T3 cells, although the newly formed microtubular network conforms to the altered cell shape.

Our results show that the latrunculins represent a new class of highly potent compounds that disrupt microfilament organization in cultured cells. The mode of action of these unusual marine natural products is still unknown, but their effects resemble those of the mold metabolites, cytochalasins, the only class of drugs known to bind to actin filaments and to specifically disrupt microfilamentous structures (*10*). The latrunculins, however, exert their effects at concentrations 1/10 to 1/100 that of the cytochalasins (*11*). Furthermore, unlike the cytochalasins, the latrunculins do not alter the rate of polymerization of purified actin filaments (*12*), suggesting that the site of action of these two classes of drugs is different. Since the assembly state of actin can be modulated by various factors including actin-binding proteins (*13*), it would be important to determine whether the latrunculins act directly on actin or whether they affect this modulation process. In either case, the latrunculins should be of great value in elucidating the molecular mechanisms underlying the regulation of motile processes by the actin-microfilament systems.

ILAN SPECTOR*
NAVA R. SHOCHET†
Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205
YOEL KASHMAN
AMIRAM GROWEISS
Department of Organic Chemistry, Tel-Aviv University, Tel-Aviv, Israel

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* Present address and address for reprint requests: Department of Anatomical Sciences, State University of New York, Stony Brook 11794.
† Present address: Department of Chemistry, State University of New York, Stony Brook 11794.

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Clay Mineralogy of the Cretaceous-Tertiary Boundary Clay

Abstract. *Analyses of the clay mineralogy of samples from the Cretaceous-Tertiary boundary layer at four localities show that the boundary clay is neither mineralogically exotic nor distinct from locally derived clays above and below the boundary. The significant ejecta component in the clay that is predicted by the asteroid-impact scenario was not detected.*

Alvarez *et al.* (*1*) and other investigators (*2, 3*) have cited an anomalous iridium-rich layer at the Cretaceous-Tertiary (K/T) boundary as evidence for the collision of a large asteroid with the earth. The boundary, as determined from a change in planktonic fauna and flora, is commonly marked by a thin clay layer (1 cm to several 10's of centimeters thick) that is enriched in trace metals and 20 to 160 times above background levels in iridium. In their view, the boundary clay, or some fraction of it, was produced by the impact event and consists of a mixture of terrestrial ejecta and meteoritic material (*4*). Kyte *et al.* (*2*) have calculated that the inferred meteoritic component makes up 21 percent of the boundary clay layer in Denmark, 1.6 percent in Italy, and 9 percent in Spain (on a carbonate-free basis). Recent studies (*5*) suggest that the early high-speed ejecta from an asteroid impact could have a ratio of terrestrial to meteoritic components in this range, although earlier estimates (*1*) had suggested ratios of 100:1 or greater. The boundary clay layer should, therefore, contain a large fraction of material from the asteroid and target area.

Evidence to support the asteroid-impact hypothesis should be found in the mineralogy of the boundary layer. First, the boundary clay layer, especially the basal part of the boundary clay, should contain a mixture of meteoritic and terrestrial ejecta; since this material would be well mixed in the impact process, the boundary layer should be relatively homogeneous and mineralogically similar