Table 1. The affect of iron and rock samples on the production of rhodotorulic acid and packed cell volume of cultures of *Rhodotorula pilimanae*

Substance added to culture Identity	g/l or M[a]	Rhodotorulic acid (mM)	Packed cell vol. (%)
None	NA	7.30	1.6
Fe^{+3}	10^{-8}	6.84	1.7
Fe^{+3}	10^{-7}	5.79	3.8
Fe^{+3}	10^{-6}	2.38	5.3
Fe^{+3}	10^{-5}	0.83	6.8
Fe^{+3}	10^{-4}	0.06	6.7
Fe^{+3}	10^{-3}	0.00	7.0
Mt. St. Helens' ash	40	0.17	6.6
(May 18, 1980)	5	1.62	5.5
	0.1*	4.38	3.4
Mt. St. Helens' ash	40	0.06	6.6
(June 12, 1980)	5	1.83	5.1
	0.1*	4.56	3.3
Sand	40	0.09	6.8
	5	1.82	5.5
	0.1*	2.70	4.5
Andesite	5	0.07	6.7
AGV-1	0.1*	2.02	5.4
Basalt	5	0.29	6.7
BCR-1	0.1*	1.85	5.4
Granite	5	0.92	6.5
G-2	0.1*	2.96	3.9
Grandoiorite	5	0.41	6.7
GSP-1	0.1*	2.64	4.2
Peridotite	5	0.18	6.8
PCC-1	0.1*	1.27	4.9

[a] The exponential numbers are molar while the asterisked numbers represent 300 ml cultures (see text).

purified siderophore, rhodotorulic acid, is capable of leaching iron from rock samples, but no studies have been presented on the effect of the presence of rock on siderophores production[3].

Materials and methods. The United States Geological Survey silicate rock standards[4], Mt. St. Helens' ash (May 18 and June 12, 1980, eruptions), and a local sand sample were obtained and prepared as described earlier[3]. To determine the effect of rock samples on the siderophore production by cultures of the yeast *Rhodotorula pilimanae* (ATCC 26423), weighed rock samples were autoclaved in 250 (or 2000) ml Erlenmeyer flasks, then combined with 30 (or 300) ml of sterile iron-free medium[5]. A second series of flasks contained iron added as ferric chloride. The flasks were inoculated with 2 (or 20) ml of full-grown, iron-free culture. Following incubation with shaking (200 one-inch gyrations/min) at room temperature for 14 days the packed cell volume was determined by centrifugation (15 min, 2000 × g) with graduated sedimentation tubes. The resulting supernatants were examined spectrophotometrically for the presence of rhodotorulic acid[4].

Results and discussion. The affect of iron and rock samples on the production of rhodotorulic acid and packed cell volume of cultures of *R. pilimanae* is presented in the table. Rhodotorulic acid biosynthesis is repressed in *R. pilimanae* at iron concentrations greater than micromolar levels (table and Atkin et al.[5]). Judging from the packed cell volumes, iron is the limiting nutrient in cultures containing less than micromolar concentrations of this element. An examination of the cell volume figures shows the rock samples will replace $FeCl_3$ as the limiting nutrient; in addition, increasing amounts of rock samples will repress the synthesis of rhodotorulic acid. The amounts of ash or rock required to repress the formation of rhodotorulic acid are inversely related to the amounts of iron leached by rhodotorulic acid from these rocks[3]. The lack of this latter inverse relationship with the amounts of iron leached by rhodotorulic acid-free controls[3] indicates the rhodotorulic acid is actually involved in the dissolution of the rock samples.

1 Present address: University of Texas Medical School, San Antonio, Texas 78284, USA.
2 Neilands, J.B. (ed.), Microbial iron metabolism: A comprehensive treatise. Academic Press, New York 1974.
3 Akers, H.A., and Magee, K.P., Experientia *41* (1985) 522.
4 Flanagan, F.J., Geochim. cosmochim. Acta *31* (1967) 289.
5 Atkin, C.L., Neilands, J.B., and Phaff, J.J., J. Bact. *103* (1970) 722.

0014-4754/85/101354-02$1.50 + 0.20/0

Latrunculin-A, ichthyotoxic constituent of the nudibranch *Chromodoris elisabethina*

R.K. Okuda and P.J. Scheuer

Department of Chemistry, University of Hawaii at Manoa, Honolulu (Hawaii 96822, USA), 20 December 1984

Summary. Latrunculin-A, an ichthyotoxin previously described from a Red Sea sponge, *Latrunculia magnifica*, has been isolated from a Pacific nudibranch, *Chromodoris elisabethina*, for which it serves as a defense allomone.
Key words. Nudibranch; latrunculin-A; sponge; *Chromodoris elisabethina;* defense allomone.

In 1980 Kashman et al.[1] reported the structure of latrunculin-A (**1**), isolated from a Red Sea sponge, which in its natural habitat is not predated by fishes. Subsequent biological evaluation of latrunculin-A (**1**) revealed[2] that the compound disrupts microfilament organization in cultured cells. This activity is analogous, albeit at far lower concentrations, to that of the mold derived cytochalasins.

Nudibranchs are opisthobranch mollusks that lack a shell and hence obvious physical defenses. Numerous investigations in recent years[3] have shown that these marine invertebrates owe their survival to various chemical defensive strategies. In many instances the responsible agents originate in the nudibranchs' selective diet, predominantly sponges and cnidarians, and are sequestered and stored near the body surface.

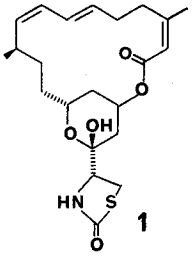
The variably colored striped *Chromodoris elisabethina* were collected on Guam and later at Enewetak in the Marshall islands, where the animals were observed while feeding on a sponge, *Heteronema* sp.[4]. The nudibranchs were either extracted with isopropyl alcohol immediately (Enewetak) or they were first frozen, then extracted (Guam). TLC of the alcoholic residue indicated a single major organic constituent. Successive solvent partition (hexanes/10% aq MeOH; CCL_4/20% aq MeOH; and $CHCl_3$/40% aq MeOH) furnished the principal metabolite in the carbon tetrachloride fraction. HPLC (Si-60, Knauer-Unimetrics, petroleum ether/EtOAc, 1:1) of this fraction led to a colorless oil of composition $C_{22}H_{31}NO_5S$ by high resolution mass spectrometry. UV (λ_{max}^{MeOH} 219 nm, ε 18,700), IR ($\nu_{max}^{CH_2Cl_2}$ 3670, 3560, 3400, 1690 br cm^{-1}) and initial ^{13}C-NMR data provided few structural clues. The richly detailed ^1H-NMR spectrum in chloroform-d and benzene-d_6, decoupling experiments of the diene portion of the molecule, and particularly a COSY plot[5] made it apparent that we were dealing with a compound that either was closely related or identical to latrunculin-A (**1**). A ^1H-NMR spectrum of **1**, kindly provided by Professor Kashman, confirmed identity.

In our antimicrobial screen latrunculin-A showed strong activity

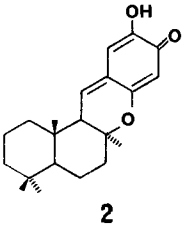
against *Candida albicans*, but no activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Eschericia coli*, or *Pseudomonas aeruginosa*. In fish toxicity studies with goldfish at a concentration of 1 mg of latrunculin-A per liter of water the goldfish exhibit increased activity within 5 min of exposure; within 15–35 min the assay animals become disorientated and progressively paralyzed; within 50 min all assay animals are dead, while all control fish behave normally.

Examination of the brown encrusting sponge, *Heteronema* sp., on which *C. elisabethina* were feeding, yielded not even traces of latrunculin-A (**1**). Instead, the hexane fraction of our routine solvent partition after Sephadex LH 20 and silica gel HPLC purification led to a yellow solid, which by spectral comparison proved to be puupehenone (**2**), which we had previously isolated from an unidentified Enewetak sponge[6].

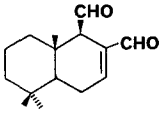
Concentration of latrunculin-A in *C. elisabethina* is high, ranging from 0.27 to 1% of wet animal. A rapid 5-min alcoholic extraction led to virtually pure latrunculin-A, which suggests that the compound is stored close to the surface of the animal. What is the biological origin of latrunculin-A? While Cimino et al.[7] have shown that the nudibranch *Dendrodoris limbata* can synthesize the well-known antifeedant polygodial (**3**) from mevalonic acid, diet-derived defensive agents seem to be employed more frequently[3]. We tend to believe – though we have no proof – that *C. elisabethina* acquires latrunculin-A (**1**) from an occasional food source, yet to be discovered.



1



2



3

Acknowledgment. We thank Mr Scott Johnson, Drs C. Ireland and G. Schulte for the collections and the National Science Foundation for financial support.

1 Kashman, Y., Groweiss, A., and Shmueli, U., Tetrahedron Lett. *21* (1980) 3629.
2 Spector, I., Shochet, N.R., Kashman, Y., and Groweiss, A., Science *219* (1983) 493.
3 Schulte, G.R., and Scheuer, P.J., Tetrahedron *38* (1982) 1857.
4 We thank Drs C. Ireland and G.R. Schulte for the June 1981 Guam collection and Mr Scott Johnson for the 1982/83 collections from Enewetak.
5 For spectral details see R.K. Okuda, Ph.D. Dissertation, University of Hawaii, 1983.
6 Ravi, B.N., Perzanowski, H.P., Ross, R.A., Erdman, T.R., and Scheuer, P.J., Pure appl. Chem. *51* (1979) 1893.
7 Cimino, G., Rosa, S. De, De Stefano, S., Sodano, G., and Villani, G., Science *219* (1983) 1237.

0014-4754/85/101355-02$1.50 + 0.20/0

Correction

G.A. Schuiling, N. Pols-Valkhof and T.R. Koiter: Clomiphene citrate can mimic the augmentative (positive) but not the depressing (negative) effect of estradiol on the LHRH-stimulated release of LH and FSH by the pituitary gland of the long-term ovariectomized rat, Experientia *41* (1985) 1060–1063. We regret that due to a production error, the placement of Fig. 1B and Fig. 2B was inadvertently reversed.