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Biologically active natural products

Kenneth L. Rinehart, Ryuichi Sakai, Tom G. Holt, Nancy L. Fregeau, Thomas J. Perun, Jr., David S. Seigler, George Robert Wilson, Lois S. Shield

Departments of Chemistry and Plant Biology, University of Illinois, Urbana, Illinois 61801, U.S.A.

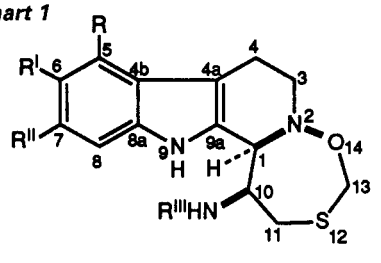
Abstract. Systematic bioassays of marine-derived extracts have led to the eudistomins (antiviral), didemnins (antiviral, antitumor, immunosuppressive), and ecteinascidins (antitumor). Extension of these bioassays to plant and insect extracts appears promising.

INTRODUCTION

For the past 15 years our research group has carried out systematic on-site bioassays of marine species in Baja California, the Caribbean, and elsewhere, searching for antimicrobial and antiviral activity and for cytotoxicity as a predictor of antitumor activity (refs. 1-3). We describe in the following some of the most promising leads developed under that program.

Eudistomins In our most extensive field testing for antiviral activity, during the 1978 Alpha Helix Caribbean Expedition (AHCE), the most active antiviral extract by far was that from the colonial tunicate *Eudistoma olivaceum* (ref. 1). In due course this extract yielded the eudistomins, which fall into three general classes--1-unsubstituted and 1-substituted β-carbolines, and oxathiazepino-tetrahydro-β-carbolines (refs. 4,5). Some antiviral and antibacterial activity is shown by all three classes, but the last compounds are clearly most active (Chart 1). While representatives of the first two classes were synthesized some time ago by our group (ref. 4), only very recently have syntheses of the more difficult fused tetracyclic system been reported (refs. 6-8). Although scarcity of the tunicate and lack of a suitable synthesis have hindered in vivo testing of the eudistomins, a preliminary mouse vaginal *Herpes* assay was promising; with syntheses in hand, testing should be resumed.

Chart 1



Eudistomin	R	R^I	R^{II}	R^{III}
C	H	OH	Br	H
E	Br	OH	H	H
K	H	H	Br	H
L	H	Br	H	H

Didemnins The most reproducible antiviral activity observed during AHCE 1978 was that of extracts of the compound colonial tunicate *Tridemnum solidum*. Since then, the antiviral/antitumor didemnins, cyclic depsipeptides isolated from *T. solidum*, have been the focus of a major research effort in our laboratory (refs. 9-17). The structures of didemnins A - E, assigned in 1981 (ref. 11), were later modified (ref. 12) in the course of synthetic studies (ref. 13); they differ from one another mainly in the substitution on the *N*-methylleucine side chain (Chart 2).

Recently, we isolated didemnins X and Y with a new *N*-terminal blocking group, a 3-hydroxydecanoyl unit (refs. 16-17). The HRFAB and tandem FAB mass spectra were instrumental in identifying the sequence shown, while hydrolysis gave Glu, plus the usual MeLeu, Leu, Thr, Pro, Me_2Tyr, and Ist. The non-polar layer yielded 3-hydroxydecanoic acid, whose stereochemistry was assigned by chiral NMR. The new didemnins have activity similar to that of didemnins D and E (Table 1).

In vivo antiviral testing of some of the early didemnins indicated protection against vaginal *Herpes* as well as Rift Valley Fever, though toxicity was a problem (refs. 14,15). We have attempted with some success to reduce toxicity by chemical modification. The relative activities of the didemnins in hand are summarized in Table 1, from which it can be seen that acyl substitution, whether the side chains be acetyl or longer (didemnins D, E), enhances cytotoxicity. On the other hand, cytotoxicity can be reduced by acylating the isostatine hydroxyl along with the free MeLeu amino group or by bridging the amino nitrogens of *N*-MeLeu and Thr with a methylene group. Unfortunately, in vivo testing has not yet been carried out on most of these promising derivatives.

The didemnins' antitumor activity is also under study. Didemnin B was determined to be the most cytotoxic vs. L1210 cells, although a number of more recently isolated didemnins have similar activity (Table 1). Didemnin B has progressed through toxicology studies and Phase I clinical trials sponsored by the U.S. National Cancer Institute (NCI) (ref. 18) and is currently undergoing Phase II trials against a variety of tumor types.

Another notable activity of the didemnins is immunosuppression; they have been shown in some assays to be approximately 1000 times as active as cyclosporin A (ref. 19). Their mode of action, however, appears to be different from that of cyclosporin (ref. 20).

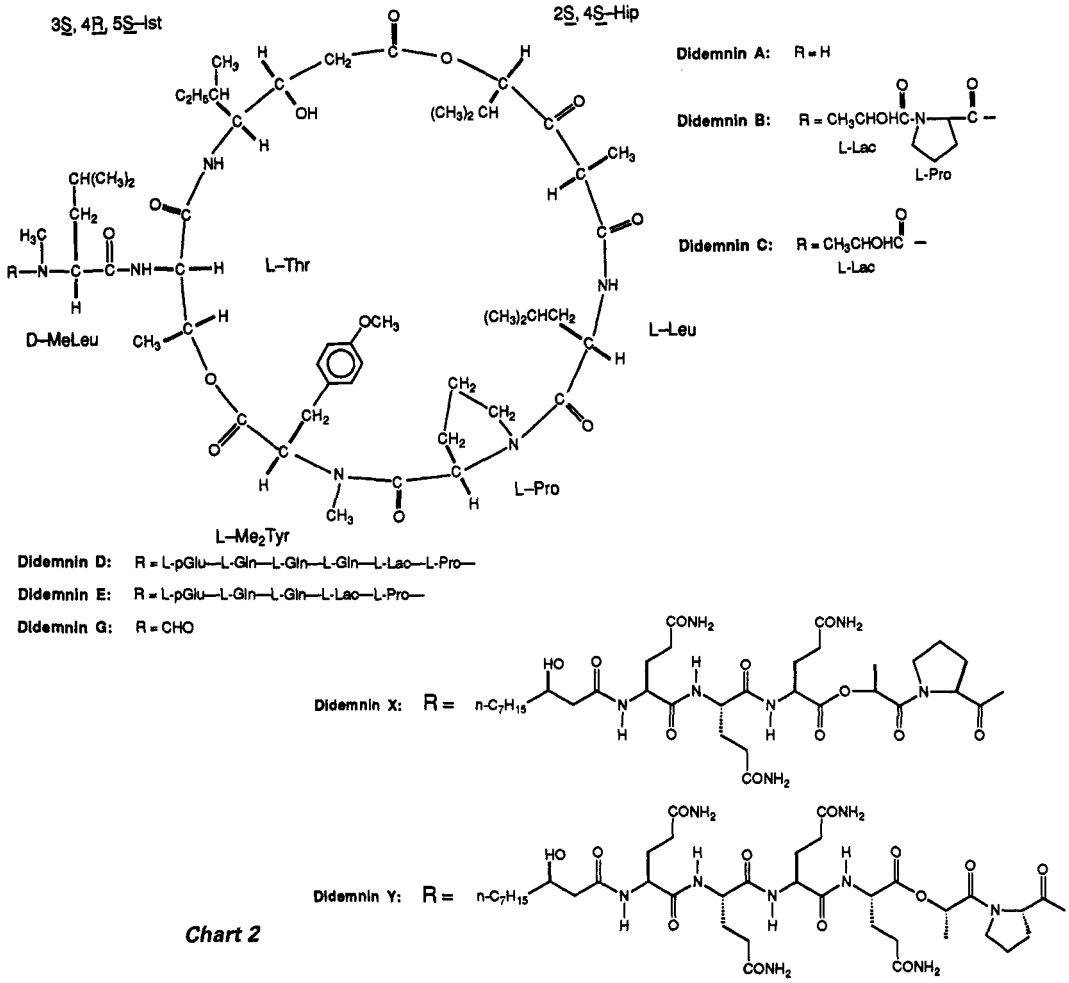


Chart 2

Table 1. *In Vitro* Bioactivities of the Didemnins and Their Derivatives

	RNA Viruses[a]				DNA Viruses[a]			L1210 Cells[b]	
Compound	PR8	COE	HA-1	E.R.	HSV-1	HSV-2	Vacc	ID_{50}	ID_{90}
Didemnin A						2/3		0.019	0.056
Didemnin B	4/0	4/0	4/0	4/0	4/0	3/3	4/0	0.0011	0.0049
Synthetic								0.0018	0.0135
Didemnin C	4/0	3/3	4/4	4/0	1/4	2/3	1/4	0.011	0.019
Didemnin D	4/0	4/0	4/0	4/4	4/0	4/0	4/0	0.0065	0.016
Didemnin E	4/0	4/0	4/0	4/4	4/0	4/0	4/0	0.0051	0.013
Didemnin G								0.006	0.038
Didemnin X								0.0048	0.017
Didemnin Y								0.0048	0.021
Methylene didemnin A	0/0	0/2	0/4	0/4	0/4	0/4	0/4	0.0065	0.023
N-Acetyldidemnin A								0.0024	0.007
Diacetyldidemnin A	0/0	0/3	0/4	0/4	1/4	1/4	1/4	0.015	0.052
Dihydrodidemnin A								0.52	>1
Nordidemnin B	4/0	4/0	4/0	4/0	4/4	4/4	3/4	0.0078	0.019
Diacetyldidemnin B	3/0	3/3	1/0	2/4	3/4	3/4	3/4	0.0016	0.0036
Prolyl-didemnin A								0.014	0.076

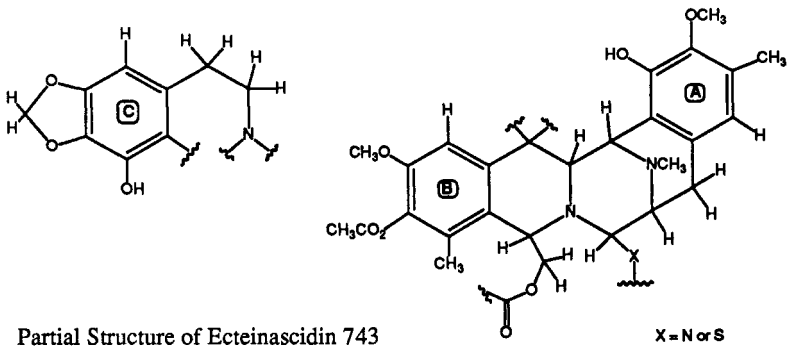
[a]Cytotoxicity/antiviral activity, 1 = 1-10, 2 = 10-20, 3 = 20-30, 4 = 30-40 mm zone of inhibition; PR8, influenza virus; COE, Coxsackie A21 virus; HA-1, parainfluenza-3 virus; E.R., equine rhinovirus; HSV-1, HSV-2, *Herpes simplex* virus, types 1 and 2; Vacc, vaccinia virus. [b]μg/mL.

Ecteinascidins Among the antitumor marine extracts studied from 1972-1980 under the NCI Natural Product Acquisition Program (ref. 21), the most promising were from the colonial tunicate *Ecteinascidia turbinata*, whose colonies look like bunches of pink- or orange-tipped white grapes. The original extracts gave T/C values as high as 270 against P388 murine leukemia and also showed extremely interesting immunological properties (refs. 22,23), but the compounds responsible for this activity were not isolated. Our studies of *E. turbinata* commenced *ca.* 1981 (refs. 24,25), and we, too, found the bioactive materials to be remarkably susceptible to decomposition and difficult to isolate. Ultimately, a system involving countercurrrent chromatography (CCC) was employed, with characterization by liquid chromatography (LC)/FABMS and bioautography (TLC/cytotoxicity), to give six closely related ecteinascidins that differ considerably in their cytotoxicity and T/C values (Table 2).

Table 2. Ecteinascidin Yields and Activities

Ecteinascidin	Yield, %	CV-1, mm zone at 1.6 μg/6.35-mm disk	P388, T/C (at μg/kg)	B16, T/C (at μg/kg)
729	1×10^{-5}	18	214 (3.8)	246 (10)
743	10×10^{-5}	28	167 (15)	
745	2×10^{-5}	14	111 (250)	
759A	1×10^{-5}	16		
759B	1×10^{-5}	22		
770	1×10^{-5}	25		

The compound we have studied most extensively is the most abundant, ecteinascidin 743. Its tandem FAB mass spectrum indicated three similar structural units, each containing one nitrogen and an aromatic ring, identified in oxygenated tetrahydroisoquinoline units. A combination of ^{1}H and ^{13}C NMR spectra, homonuclear and heteronuclear COSY, both short- and long-range, and NOE, argued for the structural units shown in Scheme 1. Location of the methylene, methine, and sulfide groups would complete the structure; thus far, its determination by X-ray methods has not been possible.



Scheme 1 Partial Structure of Ecteinascidin 743

Terrestrial plants and insects While plant antitumor activity has been studied extensively by the NCI (ref. 26) and antimicrobial activity by others (ref. 27), a systematic study of the antiviral properties of plants has apparently not been undertaken. We recently extended our systematic antiviral and cytotoxicity bioassays to 43 plant samples and detected antiviral activity in three species. Of these, the fern *Notholaena standleyi* produced the new sesterterpene notholaenic acid (Chart 3), whose pentacyclic structure, resembling that of retigeranic acid (ref. 28), was established by X-ray crystallography (ref. 29).

Finally, we are applying systematic bioassays to insects as potential sources of medicinal agents. In the first 30 species investigated, five species showed antiviral activity. The firefly *Photinus pyralis* yielded the lucibufagins (Chart 4), compounds reported earlier by Meinwald, et al. (ref. 30); they suppress *Herpes simplex* virus, type 1, plaques completely at 300 ng/mL (ref. 31) and are currently being evaluated in vivo.

Chart 3 Notholaenic acid

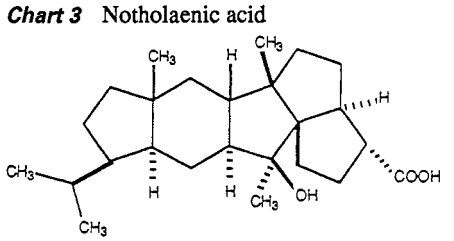
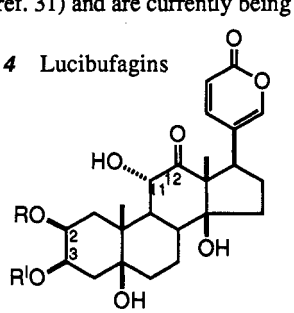


Chart 4 Lucibufagins



R = R' = Ac
R = Ac, R' = i-C_3H_7CO—
R = Ac, R' = C_2H_5CO—
R = H, R' = Ac
R = Ac, R' = H

From the above descriptions, it seems clear that systematic bioassays (especially at an early stage in the field) can lead to novel and potent pharmaceutically active compounds and, moreover, that success is limited only by one's imagination in devising new assays and exploring new classes of animals or plants.

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REFERENCES

1. K.L. Rinehart, Jr., P.D. Shaw, L.S. Shield, J.B. Gloer, G.C. Harbour, M.E.S. Koker, D. Samain, R.E. Schwartz, A.A. Tymiak, D.L. Weller, G.T. Carter, M.H.G. Munro, R.G. Hughes, Jr., H.E. Renis, E.B. Swynenberg, D.A. Stringfellow, J.J. Vavra, J.H. Coats, G.E. Zurenko, S.L. Kuentzel, L.H. Li, G.J. Bakus, R.C. Brusca, L.L. Craft, D.N. Young and J.L. Connor, *Pure Appl. Chem. 53*, 795-817 (1981).
2. K.L. Rinehart, *Pure Appl. Chem. 61*, 525-528 (1989).
3. K.L. Rinehart, *in* Biomedical Importance of Marine Organisms, Memoirs of the California Academy of Sciences Number 13, D.G. Fautin, Ed., California Academy of Sciences, San Francisco, 1988, pp. 13-22.
4. K.L. Rinehart, Jr., J. Kobayashi, G.C. Harbour, J. Gilmore, M. Mascal, T.G. Holt, L.S. Shield and F. Lafargue, *J. Am. Chem. Soc. 109*, 3378-3387 (1987).
5. K.L. Rinehart, Jr., G.C. Harbour and J. Kobayashi, U.S. Patent No. 4,631,149, Dec. 23, 1986.
6. M. Nakagawa, J.-J. Liu and T. Hino, *J. Am. Chem. Soc. 111*, 2721-2722 (1989).
7. I.W.J. Still and J.R. Strautmanis, *Tetrahedron Lett. 30*, 1041-1044 (1989).
8. M.P. Kirkup, B.B. Shankar, S. McCombie, A.K. Ganguly and A.T. McPhail, *Tetrahedron Lett. 30*, 6809-6812 (1989).
9. K.L. Rinehart, Jr., U.S. Patent Nos. 4,493,796, Jan. 15, 1985, *Chem. Abstr. 103*, 76241v (1985); 4,548,814, Oct. 22, 1985; and 4,782,135, Nov. 1, 1988.
10. K.L. Rinehart, *in* Proceedings, 10th Am. Peptide Symposium, St. Louis, MO, May 23-28, 1987, ESCOM Science Publishers, The Netherlands, 1988, pp. 626-631.
11. K.L. Rinehart, Jr., J.B. Gloer, J.C. Cook, Jr., S.A. Mizsak and T.A. Scahill, *J. Am. Chem. Soc. 103*, 1857-1859 (1981).
12. K.L. Rinehart, V. Kishore, K.C. Bible, R. Sakai, D.W. Sullins and K.-M. Li, *J. Nat. Prod. 51*, 1-21 (1988).
13. K.L. Rinehart, Jr., V. Kishore, S. Nagarajan, R.J. Lake, J.B. Gloer, F.A. Bozich, K.-M. Li, R.E. Maleczka, Jr., W.L. Todsen, M.H.G. Munro, D.W. Sullins and R. Sakai, *J. Am. Chem. Soc. 109*, 6846-6848 (1987).
14. K.L. Rinehart, Jr., J.B. Gloer, R.G. Hughes, Jr., H.E. Renis, J.P. McGovren, E.B. Swynenberg, D.A. Stringfellow, S.L. Kuentzel and L. H. Li, *Science 212*, 933-935 (1981).
15. K.L. Rinehart, Jr., J.B. Gloer, G.R. Wilson, R.G. Hughes, Jr., L.H. Li, H.E. Renis and J.P. McGovren, *Fed. Proc. 42*, 87-90 (1983).
16. R. Sakai and K.L. Rinehart, 197th ACS National Meeting, Dallas, TX, April 9-14, 1989, Abstract ORGN 171.
17. K.L. Rinehart, R. Sakai and J.G. Stroh, U.S. Patent Appl. Ser. No. 335,903, Apr. 10, 1989.
18. H.G. Chun, B. Davies, D. Hoth, M. Suffness, J. Plowman, K. Flora, C. Grieshaber and B. Leyland-Jones, *Invest. New Drugs 4*, 279-284 (1986).
19. D.W. Montgomery, A. Celniker and C.F. Zukoski, *Transplantation 43*, 133-139 (1987).
20. D.H. Russell, A.R. Buckley, D.W. Montgomery, N.A. Larson, P.W. Gout, C.T. Beer, C.W. Putnam, C.F. Zukoski and R. Kibler, *J. Immunol. 138*, 276-284 (1987).
21. M. Suffness and J.E. Thompson, *in* ref. 3, pp. 151-157.
22. M.M. Sigel, L.L. Wellham, W. Lichter, L.E. Dudeck, J.L. Gargus and A. H. Lucas, *in* Food-Drugs from the Sea, H.W. Youngken, Jr., Ed., Marine Technology Society, Washington, D.C., 1970, pp. 281-294.
23. M.M. Sigel, L.J. McCumber, J.A. Hightower, S.S. Hayasaka, E.M. Huggins, Jr., and J.F. Davis, *Am. Zool. 23*, 221-227 (1983).
24. T.G. Holt, Ph.D. Dissertation, University of Illinois, Urbana, 1986.
25. K.L. Rinehart, Jr., and T.G. Holt, U.S. Patent Appl. Ser. No. 006,395, Jan. 23, 1987.
26. G.R. Pettit, Y. Kamano, Y. Fujii, C.L. Herald, M. Inoue, P. Brown, D. Gust, K. Kitahara, J.M. Schmidt, D.L. Doubek and C. Michel, *J. Nat. Prod. 44*, 482-485 (1981).
27. L.A. Mitscher, S. Drake, S.R. Gollapudi and S. Okwute, *J. Nat. Prod. 50*, 1025-1040 (1987).
28. M. Kaneda, R. Takahashi, Y. Iitaka and S. Shibata, *Tetrahedron Lett.* 4609-4611 (1972).
29. T.J. Perun, Jr., M.Sc. Thesis, University of Illinois, Urbana, 1990.
30. J. Meinwald, D.F. Wiemer and T. Eisner, *J. Am. Chem. Soc. 101*, 3055-3060 (1979).
31. G.R. Wilson and K.L. Rinehart, U.S. Patent No. 4,847,246, Jul. 11, 1989.