

Total oocyst and ova ( $\bar{x} \pm \text{SE}$ ) produced during single and concurrent infections with *Eimeria separata* and *Nippostrongylus brasiliensis*

Group	Infection schedule (N = 5)	<i>E. separata</i> Oocysts $\times 10^4$	<i>N. brasiliensis</i> Ova $\times 10^4$
1	Single infection	—	29 $\pm$ 10
2	Single infection	280 $\pm$ 150	—
3	N.b. and E.s. simultaneous	140 $\pm$ 35	38 $\pm$ 8
4	E.s. on day 4 PI N.b.	230 $\pm$ 120	91 $\pm$ 25*
5	E.s. on day 6 PI N.b.	120 $\pm$ 60	56 $\pm$ 16*
6	E.s. on day 9 PI N.b.	115 $\pm$ 45	39 $\pm$ 8
7	E.s. on day 11 PI N.b.	121 $\pm$ 61	82 $\pm$ 21*
8	N.b. on day 2 PI E.s.	100 $\pm$ 50	55 $\pm$ 11

\*Differs significantly ( $p \leq 0.05$ ) from group 1.

fections and the following data on worm burdens have been routinely obtained on day 10 PI that had been administered  $1 \times 10^3$  larvae: total worm burden =  $271 \pm 35$ , female worms =  $154 \pm 31$  and male worms =  $117 \pm 16$ . These numbers are lower than those reported by Keymer et al.<sup>7</sup>, however, their counts were made at 7 days PI while ours were calculated at 10 days PI at which time self-cure had begun. A sex ratio of females to males  $> 1$  is consistent with that obtained previously<sup>7</sup>.

Data were determined to be parametric by the  $F_{\max}$  distribution test and the one-way analysis of variance was then used to determine statistical significance ( $p \leq 0.05$ ).

**Results and discussion.** Ova production by *N. brasiliensis* was elevated significantly during some but not all of the concurrent infections while *E. separata* fecundity was unaltered. Oocyst production was less than that of controls in all concurrently infected groups, however, statistical significance could not be demonstrated due to the large variance for the singly-infected group in this rat strain. Although each group was composed of a small number (N = 5) of rats, results of the present study are in agreement with those obtained during *E. nieschulzi*-*N. brasiliensis* concurrent infections<sup>6</sup>. Since rats were sacrificed 2 days after ova production had ceased in the present study, it is not known whether alterations in ova production resulted from *E. separata* affecting *N. brasiliensis* fecundity directly or from decreased numbers of helminths establishing in the presence of *E. separata*. At this time, all *N. brasiliensis* had been eliminated from the host intestine.

The presence of *E. nieschulzi* during a *N. brasiliensis* infection has been shown to suppress rejection of the nematode<sup>6</sup>, however, when *E. separata* was superimposed on a *N. brasiliensis* infection, the patent periods of both *E. separata* ( $4 \pm 0$  days) and *N. brasiliensis* ( $8 \pm 0.3$  days) were not significantly different from those in singly-infected control rats.

The affect of *E. separata* on *N. brasiliensis* ova production, irrespective of the mechanism, suggests that a systemic effect may be responsible since these parasites do not inhabit the same region of the gastrointestinal tract. This postulation is supported by the fact that chicken coccidia, of the genus *Eimeria*, cause pathologic changes in regions distant from the site of endogenous development<sup>8,9</sup> and the fact that the small intestine weight ratios are elevated in rats singly infected with *E. separata*<sup>10</sup>. More importantly, since *E. nieschulzi* but not *E. separata* is capable of altering the patent period length of *N. brasiliensis*, it suggests that *E. nieschulzi* may be unique among rat eimerians in its ability to alter the host's immune and/or inflammatory response<sup>3,5,11</sup> and effect rejection of *N. brasiliensis*.

- 1 This study was supported by NIH MBRS Grant RRO8012-8.
- 2 Present address: University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.
- 3 Duszynski, D.W., Russell, D., Roy, S.A., and Castro, G.A., J. Parasit. 64 (1978) 83.
- 4 Stewart, G.L., Reddington, J.J., and Hamilton, A.M., Exp. Parasit. 50 (1980) 115.
- 5 Castro, G.A., and Duszynski, D.W., J. Protozool. 31 (1984) 283.
- 6 Bristol, J.R., Piñon, A.J., and Mayberry, L.F., J. Parasit. 69 (1983) 372.
- 7 Keymer, A., Martin, J., and Wainwright, S.M., J. Helminth. 57 (1983) 225.
- 8 Bertke, E.M., and Herrick, C.A., J. Parasit., 40 suppl. (1954) 30.
- 9 Levine, L., and Herrick, C.A., J. Parasit. 40 (1954) 525.
- 10 Duszynski, D.W., J. Protozool. 19 (1972) 82.
- 11 Duszynski, D.W., Roy, S.A., and Castro, G.A., J. Protozool. 25 (1978) 226.

0014-4754/85/050689-02\$1.50 + 0.20/0

© Birkhäuser Verlag Basel, 1985

## A non-peroxide norsesterterpene from a marine sponge *Hyrtios erecta*

P. Crews, P. Bescansa and G.J. Bakus

Thimann Laboratories and Center for Marine Studies, University of California, Santa Cruz (California 95064, USA) and Allan Hancock Foundation, University of Southern California, Los Angeles (California 90007, USA), 15 March 1984

**Summary.** A new norsesterterpene, hyrtial 4, and known sesterterpenes, 1–3, have been isolated from an anti-inflammatory active crude extract of the sponge *Hyrtios erecta*.

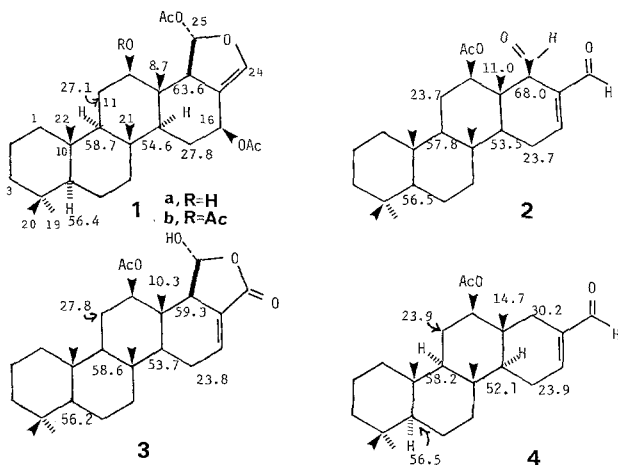
**Key words.** Sponge, marine; *Hyrtios erecta*; norsesterterpene; sesterterpenes; hyrtial.

Demosponges which are soft to the touch yet seem to repel normal sponge predators such as rasping fish or nudibranchs are of interest to us<sup>1</sup>. Several years ago we encountered an abundant black Tongan sponge, *Hyrtios erecta*, whose crude extract was ichthyotoxic and possessed anti-inflammatory activity<sup>2</sup>.

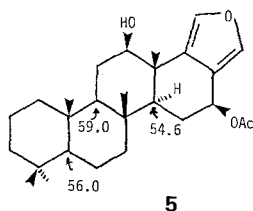
Semipure heteronemin 1a<sup>3</sup> crystallized from the crude dichloromethane extract. Interestingly, 1a was first isolated in 1976<sup>3a</sup> whereas its correct stereostructure was not described until 1981<sup>3d</sup>. Flash chromatography followed by HPLC yielded, in order of increasing polarity, hyrtial 4<sup>4</sup>, 12-epi-scalaradial 2<sup>5</sup>, and 12-epi-scalarin 3<sup>6</sup>.

The molecular formula of 4 ( $\text{C}_{26}\text{H}_{40}\text{O}_3$ ) was deduced by considering both its mass spectrum, highest  $m/z = 340$  ( $\text{M}^+ - \text{HOAc}$ ) and  $^{13}\text{C}$ -NMR attached proton test (APT) results<sup>7</sup>. Diagnostic NMR signals revealed key structural elements such as: an equatorial  $\text{C}_{12}\text{-OAc}$  [ $^{13}\text{C}$   $\delta$  170.2 (C=O), 21.6 (Me),  $^1\text{H}$   $\delta$  4.70 (dd,  $J = 11.4$  Hz,  $\text{H}_{12}$ , 1.70 (s, Me)];  $\text{C}_{17}\text{-CHO}$  [ $^{13}\text{C}$   $\delta$  192.8 (C=O),  $^1\text{H}$   $\delta$  9.34 ( $\text{H}_{24}$ )]; a trisubstituted  $\text{C}=\text{C}$  [ $^{13}\text{C}$   $\delta$  148.6 ( $\text{C}_{16}$ ), 139.6 ( $\text{C}_{17}$ ),  $^1\text{H}$   $\delta$  6.06 (b.s.,  $\text{H}_{16}$ )]; axial methyls<sup>8</sup> at  $\text{C}_{4,8,10,13}$  [ $^{13}\text{C}$   $\delta$  20.1, 16.9, 16.7, 14.7]; and axial protons at  $\text{C}_{5,9,14}$   $J_{\text{aa+ae}} > 15$  Hz for each methine H. The appearance of four methine  $^{13}\text{C}$  signals near [ $\delta$  60, 59, 56, 54] are helpful in pinpointing a normal scalarane frame (see structures). How-

ever for **4** only three such peaks [ $\delta$  58.2, 56.5, 52.1] were observed. The  $^{13}\text{C}$ -NMR signal at  $\delta$  30.2 (t) along with a  $^1\text{H}$ -NMR clean AB doublet at  $\delta$  2.68 and 1.90 ( $J = 17.4$  Hz) was especially important and justified placing an isolated  $\text{CH}_2$  at C-18. Finally, remaining  $^{13}\text{C}$ -NMR peaks in **4**, excepting  $\text{Me}_{23}$ , were close in chemical shift to those in **2**.



At 50  $\mu\text{g/mL}$ , **4** shows comparable anti-inflammatory activity vs. a standard, indomethacin<sup>9</sup>; whereas no appreciable activity was exhibited by **1a**, **2**, synthetic scalarafuran **5**<sup>3c</sup>, or synthetic heteronemin acetate **1b**.



Sponge sesterterpenes such as **1–3** are commonly observed from the subclass Dictyoceratida of which *Hyrtios* is a member. By contrast, sponge norsesterterpenes are extremely rare and to the best of our knowledge, **4** is the first such compound to be reported from the subclass Dictyoceratida. Four other norsesterterpene peroxides which vary from monocarbocyclic to bicarbocyclic have been reported from sponges of the subclass Ceractinomorpha<sup>1b</sup> and Tetractinomorpha<sup>10</sup>.

**Acknowledgments.** Partial research support to PC was from NOAA, National Sea Grant College Program, Department of Commerce, University of California project number R/M-P-33. The US Government is authorized to produce and distribute reprints for governmental purposes. A grant to PC from the University Research Expeditions Program supported our field work in Tonga. We thank Mr Jim Loo for assistance with certain NMR experiments.

- 1 a) Myers, B.L., and Crews, P., *J. org. Chem.* **48** (1983) 3583; b) Manes, L.V., and Crews, P., *Tetrahedron Lett.* **25** (1984) 935.
- 2 This sponge, *Hyrtios (Heteronema) erecta* Keller 1889, collected in 1980/81, from Tonga was extremely abundant in the Vava'u Is. Group.
- 3 a) Kazlauskas, R., Murphy, P.T., Quinn, R.J., and Wells, R.J., *Tetrahedron Lett.* (1976) 2631; b) Kashman, Y., Rudi, A., *Tetrahedron* **33** (1977) 2997; c) Walker, R.P., Thompson, J.E., and Faulkner, D.J., *J. org. Chem.* **45** (1980) 4976; d) Yasuda, F., and Tada, H., *Experientia* **37** (1981) 110.
- 4 **4**: mass spectrum:  $m/z = 340(\text{M}^+ - \text{HOAc})$ , 325, 205, 191.  $^{13}\text{C}$ -NMR ( $\text{C}_6\text{D}_6$ ): 40.0(C-1); 18.9(C-2 or C-6); 42.5(C-3); 33.5(C-4); 56.5(C-5); 18.5(C-6 or C-2); 41.3(C-7); 37.1(C-8 or C-13); 58.2(C-9); 38.4(C-10); 23.9(C-11); 82.7(C-12); 37.7(C-13 or C-8); 52.1(C-14); 23.9(C-15); 148.6(C-16); 139.6(C-17); 30.2(C-18); 33.5(C-19); 20.1(C-20); 16.7(C-21); 16.9(C-22); 14.7(C-23); 192.8(C-4), 170.2, 21.6 (OAc). Proton NMR ( $\text{C}_6\text{D}_6$ , 360 MHz): 9.34 (s, H-24), 6.06 (m,  $w_{12} = 8$  Hz, H-16), 4.70 (dd,  $J = 11.1, 4.2$ , H-12), 2.68(d,  $J = 17.4$ , H-18a); 1.90(d,  $J = 17.4$ , H-18b, observed by difference decoupling); 1.68 (s, OAc), 0.87, 0.78, 0.76, 0.70, 0.67 (s,s,s,s,s, Me-19,20,21,22,23), 0.62 (dd,  $J = 12.7, 3.4$ , H-5), 0.56 (dd  $J = 11.9, 3.4$ , H-9), 0.53 (dd,  $J = 12.7, 4.7$ , H-14) but H-5,9,14 can be switched.
- 5 Cimino, G., De Stefano, S., and Di Luccia, A., *Experientia* **35** (1979) 1277.
- 6 Cimino, G., De Stefano, S., Minale, L., and Trivellone, E., *J. chem. Soc. Perkin I* (1977) 1587.
- 7 Patt, S.L., and Shoolery, J.N., *J. magn. Reson.* **46** (1982) 535.
- 8 Methyl stereochemistry in **1–4** was established from C-13 NMR data. Podocarpanes and kauranes, in: Wehrli, F.W., and Nishida, T., *Prog. Chem. org. nat. Prod.* **36** (1979) 2; provided base values to which were added substituent increments, in: Crews, P., and Kho-wiseman, E., *Tetrahedron Lett.* (1978) 2483. For example, in **4** an ax Me-23 is clear by comparing experimental 14.7 to calculated values: Me(ax) =  $\delta$  14, Me(q) = 20. Also, in **1a** an equatorial C-18 substituent is clear by comparing experimental Me-29 ( $\delta$  8.7) to calculated values where R-18 is ax (14), or eq (9).
- 9 We thank Prof. R. Jacobs and his research group at UCSB for this data.
- 10 Albericci, M., Braekman, J.C., Daloze, D., and Tursch, B., *Tetrahedron* **38** (1982) 1881.

0014-4754/85/050690-02\$1.50 + 0.20/0  
© Birkhäuser Verlag Basel, 1985

## Fungal competition and mycotoxin production on corn

K. Ehrlich, A. Ciegler, M. Klich and L. Lee

*Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans (Louisiana 70179, USA), 17 April 1984*

**Summary.** Aflatoxin and secalonin acid D production in corn in laboratory and field by mixed cultures of *Penicillium oxalicum* and *Aspergillus flavus* or *A. parasiticus* was lower than production by the pure cultures. However, mixed culture of these molds with *Fusarium* spp. did not affect mycotoxin production.

**Key words.** Aflatoxins; secalonin acid D; corn; fungal competition; mycotoxins; *Aspergillus flavus*, *Penicillium oxalicum*, *Fusaria*.

Fungal growth on agricultural commodities, with or without concomitant mycotoxin synthesis, usually does not occur in pure culture. Even in those cases where a single mycotoxin is present, more than one fungal species is usually isolated. The ability of different fungi to compete for a common host de-

pends on many factors, such as the sequence of infection, the optimal humidity and pH of the growth substrate, and the rate of growth after infection. The ability of a mold to elaborate secondary metabolites, mycotoxins, may give it a competitive advantage in substrate utilization<sup>1,2</sup>.