darkness. The present study further demonstrates that proline level also varies according to the maturity of the leaf.

In the present study, proline accumulation was also followed in the 3 uppermost leaves of 4 rice cultivars during their development after flowering, i.e., during monocarpic senescence. It was most interesting to note that in both Jaya and Ratna proline accumulation increased according to the chronological age of the leaf up to the 14th day from flowering, but on the 21st day the accumulation of proline was higher in the flag than in the second leaf which suggests that the accumulation pattern of proline was non-sequential (fig. 2). Unlike the above 2 cultivars, both Masuri and Kalojira showed a sequential pattern of proline accumulation, i.e., it was proportional to the chronological age of the leaf throughout development. Such an observation clearly fits the concept of the mode of senescence reported for these rice cultivars[9-11]. All these data seem thus to suggest that proline accumulation can serve as a reliable indicator of monocarpic senescence in rice.

Both cytokinin and abscisic acid were implicated in the regulation of monocarpic senescence in rice, the former retarding but the latter hastening the leaf senescence[12]. The idea that proline accumulation can be taken as a reliable indicator of senescence is further supported by the present observation that the retardation of leaf senescence with BA was also accompanied by a proportional decrease of proline accumulation and its acceleration with ABA was associated with a characteristic rise of proline accumulation compared with the untreated control (table). However, the pattern of proline accumulation in 3 leaves of the 4 cultivars (being non-sequential in Jaya and Ratna and sequential in Masuri and Kalojira) remained unaltered in BA treated plants of Jaya and Ratna suggesting that such treatment could not establish the sequential mode of proline accumulation in these cultivars where BA proportionally suppressed proline accumulation throughout plant development.

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Reciprocal biological activities of the cyclic tetrapeptides chlamydocin and HC-toxin

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Summary. Chlamydocin, a potent cytostatic agent against cultured mammalian cells, and HC-toxin, a host-specific phytotoxin, are cyclic tetrapeptides containing the same epoxide α-amino acid. We show here that these compounds have reciprocal biological activity; HC-toxin is cytostatic against cultured mastocytoma cells, and chlamydocin has host-specific toxin activity against maize. Chlamydocin and another related cyclic peptide, Cyl-2, are less host-specific than HC-toxin because maize tolerant to HC-toxin is more sensitive to chlamydocin and Cyl-2.

Key words. Chlamydocin; HC-toxin; phytotoxin; cytostatic agent.

Host-specific phytotoxins are compounds which lectively affect the same plant species, varieties, or genotypes as the pathogenic fungi that produce them[2]. *Bipolaris zeicola* (Nisak. and Miyake) Shoem. (*Cochliobolus carbonum* Nelson) race 1 produces a phytotoxin, HC-toxin, which specifically inhibits maize that is susceptible to the fungus; dicotyledons and other grasses and maize genotypes are affected only at concentrations at least 100 times higher than are needed to inhibit growth of susceptible maize[3]. Major resistance to *B. zeicola* race 1 and to HC-toxin is conditioned by a single dominant nuclear gene *Hm* which has been mapped on the first chromosome[4]. HC-toxin has recently been identified as cyclo(L-Aoe-D-Pro-L-Ala-D-Ala)[5], where Aoe stands for 2-amino-8-oxo-9,10-epoxidecanoic acid[6,7]. Its structure is similar to those of two other previously described fungal metabolites, chlamydocin and Cyl-2 (figure). All three compounds are cyclic tetrapeptides containing Aoe and either Pro or Pip. Chlamydocin, cyclo(L-Aoe-α-Aib-L-Phe-D-Pro), is produced by the cosmopolitan soil-inhabiting fungus *Diheterospora chlamydosporia* (Kamyschko) Barron and Onions[8]. Chlamydocin was originally isolated as a cytostatic agent against cultured mammalian cells[9]. It is more potent than several other common cytostatic drugs, including actinomycin D, amethopterin, colchicine, vincristine, and vinblastine, yet has a very low toxicity to rats[10]. Chlamydocin and HC-toxin have been synthesized[11,12]. Cyl-2, cyclo-(Aoe-D-O-methylTyr-L-Ile-L-Pip), produced by the phytopathogenic fungus *Cylindrocladium scoparium* Morgan, was discovered as an inhibitor of lettuce root elongation and rice seedling growth[13,14].

This report compares the effects of these cyclic peptides of diverse origins in two test systems: maize seedlings tolerant of or sensitive to HC-toxin, and mammalian cell cultures.

Materials and methods. HC-toxin, chlamydocin, and Cyl-2 were purified from culture filtrates of the respective fungi[6,9,13]. The compounds were tested in a root growth bioassay against two maize (*Zea mays* L.) hybrids that differ at only the nuclear *Hm* locus: Pr × K61 (genotype *hmhm*; susceptible to *B. zeicola* race 1 and sensitive to HC-toxin) and Pr1 × K61 (genotype

Hmhm; resistant to *B. zeicola* race 1 and tolerant to HC-toxin). Maize caryopses were germinated for 36 h in damp paper towels, and seedlings with primary roots between 15 and 40 mm in length were measured and placed on pieces of Whatman No. 1 filter paper in 100 mm glass petri plates containing the test compounds dissolved in 5 ml distilled water. Seedlings were incubated 48 h in darkness and then measured to the nearest mm[6]. Each compound was tested at six concentrations with two plates, each with four seedlings, per treatment. The data are the average of four experiments, ± one SEM.

D. chlamydosporia was obtained from Dr G. Barron, University of Guelph, Guelph, Canada, and maintained on potato dextrose agar. To test it for pathogenicity on maize, 1 ml aliquots of spore suspension (3×10^6 chlamydospores and numerous phialospores per ml) in 0.01% Tween 20 were pipetted into whorls of 25-day-old plants in the greenhouse. The plants were subsequently incubated under continuous light and 100% humidity and observed after 14 days. Under these conditions *hmhm* maize inoculated with *B. zeicola* race 1 showed substantial infection.

Mammalian cells were cultured in multititer plates (Costar) in medium RPMI 1640 in air with 5% CO_2 at 37.5 °C. Doubling time is about 14 h in these cultures. Incorporation of labeled thymidine, uridine, and leucine was determined with a 2 h pulse after 9 h preincubation in the test solution[15].

Results and discussion. The HC-toxin analogs chlamydocin and Cyl-2 differentially inhibited root growth of two maize hybrids that differ genetically only at the *Hm* locus, but chlamydocin and Cyl-2 were more active than HC-toxin against *Hmhm* maize (table 1). In some but not all experiments HC-toxin was slightly more inhibitory to *hmhm* maize than were chlamydocn or Cyl-2 (table 1). There were no consistent differences between chlamydocin and Cyl-2. Maize with genotype *HmHm* (inbred Pr1) had the same response to chlamydocin as did *Hmhm* maize (half-maximal inhibition 12 μM), indicating that insensitivity to chlamydocin, like HC-toxin, is genetically dominant. HC-toxin at sub-inhibitory concentrations could not protect *Hmhm* maize against chlamydocin (table 2).

D. chlamydosporia was unable to infect either *hmhm* or *Hmhm* maize under conditions which allowed substantial infection of *hmhm* maize by *B. zeicola* race 1. *C. scoparium* has never been reported as a pathogen of maize[16], although it is a common pathogen of many other plant species[17]. Chlamydocin and Cyl-2 are thus the first examples of compounds with 'host-specific' activity from organisms not pathogenic on the host.

HC-toxin was cytostatic against cultured mammalian cancer cells, but was about one-eigth as active as chlamydocin when the cells were counted 9 h after drug addition. With prolonged incubation this difference in activity decreased. Both compounds inhibited thymidine, uridine, and leucine incorporation by mammalian cells (table 3). The concentrations of toxins required for half-maximal inhibition of precursor incorporation after 6 h were much higher than after 9 h (data not shown), suggesting that DNA, RNA, and protein synthesis are not primary points of attack. Cytofluorometric studies indicated that

Table 1. Comparisons of the effects of HC-toxin, chlamydocin, and Cyl-2 on primary root growth of maize susceptible (genotype *hmhm*) or resistant (genotype *Hmhm*) to *Bipolaris zeicola* race 1

Compound	Concentration causing half-maximal inhibition (μM ± SE) Maize hybrid *hmhm*	*Hmhm*	Ratio, *Hmhm*/*hmhm*
HC-toxin	1.7 ± 0.5	> 21*	> 12**
Chlamydocin	3.1 ± 0.5	10.4 ± 2.0	3.4 ± 0.3
Cyl-2	3.7 ± 0.7	7.7 ± 0.9	2.1 ± 0.4

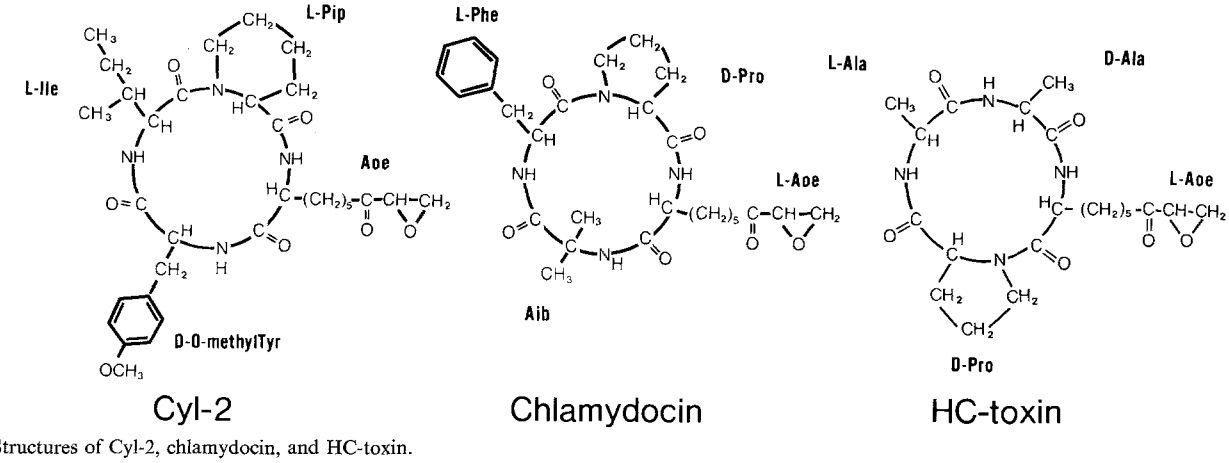
* In all four experiments, maximum inhibition of *Hmhm* maize by 21 μM HC-toxin was 15%. Published data indicate that resistant maize is half-maximally inhibited by HC-toxin at approximately 115 μM[24].
** Assuming half-maximal inhibition of *Hmhm* maize at 115 μM[24], the ratio would be 68.

Table 2. Inability of HC-toxin to protect *Hmhm* maize against chlamydocin in the root growth bioassay

Concentration of compound (μM) HC-toxin	Chlamydocin	Root growth mm ± SE (n = 4)	% of control
0	0	90.3 ± 2.9	100
0	5.1	56.5 ± 9.8	62
2.1	0	95.5 ± 7.9	106
2.1	5.1	57.3 ± 13.1	63
6.2	0	93.8 ± 1.0	104
6.2	5.1	47.0 ± 2.8	52
18.6	0	81.8 ± 2.5	91
18.6	5.1	48.6 ± 7.1	54

Table 3. Effects of HC-toxin and chlamydocin on proliferation of, and protein, RNA and DNA synthesis in, P-815 mouse mastocytoma cells grown in vitro

Effect on	Concentration causing half-maximal inhibition (μM) HC-toxin	Chlamydocin
Cell proliferation	0.010	0.0013
Thymidine incorporation	0.018	0.0023
Uridine incorporation	0.11	> 0.02
Leucine incorporation	0.12	0.019



Structures of Cyl-2, chlamydocin, and HC-toxin.

the distribution of cells among different phases of the cell cycle (as calculated from their DNA content) was not greatly affected during the first 9 h of drug exposure[18]. These cyclic peptides thus appear not to act on a process directly involved in entry into or preparation for cell division. Microscopic observation of cells treated at higher drug concentrations revealed vacuolisation, enlargement, and, starting at 9 h, a progressive destruction of the cells and a decrease in the mitotic count. Cyl-2 has not yet been tested against mammalian cells because of insufficient toxin supplies.

Chlamydocin and HC-toxin have several biological and chemical similarities, besides both being cyclic tetrapeptides and containing Aoe. Both require an intact epoxide for activity[9,19]. Both affect growth of mouse mastocytoma cells and *hmhm* maize at low concentrations. Neither compound causes rapid death of mammalian cells or nondividing leaf mesophyll protoplasts[20]. Both have the same conformation in chloroform, with four *transoid* amide bonds and a bis-γ-turn[7,21]; this may be significant because the correct conformation is required for biological activity of tentoxin, another phytotoxic cyclic tetrapeptide[22]. (However, a synthetic analog of Cyl-2, cyclo(D-O-methylTyr-L-Ile-L-Pro-L-Leu), has a trans-trans-cis-trans conformation, so Cyl-2 might differ in this respect from HC-toxin and chlamydocin[23]). Because of these similarities, HC-toxin and chlamydocin, and perhaps also Cyl-2, may have the same cellular site of action, a site which would be common to animal and plant cells.

One or both of two structural differences must be responsible for the biological differences reported here between HC-toxin and chlamydocin. Whereas chlamydocin and Cyl-2 contain the amino acid sequence Pro-Aoe or Pip-Aoe, HC-toxin has the reverse sequence Aoe-Pro (figure). Chlamydocin and Cyl-2 also contain an aromatic amino acid instead of alanine and are thus larger and more hydrophobic.

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Sodium deoxycholate promotes the absorption of heparin administered orally, probably by acting on gastrointestinal mucosa, in rats

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Summary. Sodium deoxycholate (DOC), selected as a promoter of gastrointestinal absorption of heparin, was administered orally to rats, followed, at increasing intervals, by heparin. Maximal plasma clearing activity (PC) was obtained with a 60-min interval, though PC was still elicited after 24 h, suggesting that DOC acts on the gastrointestinal mucosa. Inhibition of blood coagulation was also observed after oral heparin. The suggestion that DOC increases heparin absorption is supported by increased plasma levels of heparin. No signs of several gastrointestinal damage were seen.
Key words. Deoxycholic acid; heparin; enteral absorption.

It is widely accepted that heparin cannot be absorbed unaltered after oral administration. In fact, when introduced by this route it fails to evoke either blood plasma clearing activity (PC) or inhibition of blood coagulation, the two most typical pharmacological effects elicited by its parenteral injection, and this failure cannot be ascribed to enteral inactivation, either spontaneous[1] or enzymatic. Because of the interest of pharmaceutical oral heparin for therapeutic purposes, various procedures have been devised and tested in order to obtain PC and the anticoagulant effect after enteral application of heparin. In particular, the enteral coadministration of heparin and certain surfactants or bile acids[1-6] has been exploited.

It has previously been shown that pharmacologically active quantities of heparin are absorbed into the systemic circulation after intraesophageal administration[5], injection into the small intestine[3,7] and insertion in the colon[8], when administered together with certain bile salts. It has also been demonstrated that bile salts greatly improve enteral absorption of a number of chemically unrelated drugs[9]. However this evidence did not allow any firm inference about whether sodium deoxycholate (DOC), in particular, and surfactants in general, acted on the heparin molecule or affected the gastrointestinal mucosa, although the bulk of the evidence rather favors an influence on the enteral mucous membrane[10,11]. In order to obtain some in-