arise as a consequence of callus and suspension culture[11]. The occurrence of all sorts of chromosomal variations, including numerical, ploidy, structural and also polytenic ones has been elegantly demonstrated in suspension cultures of wheat[5]. All such changes have been widely documented in the literature to account for the chromosomal basis of speciation. It was not possible to precisely study structural changes in the chromosomes in the present study on *Hyoscyamus* callus, owing to the very small size of its somatic chromosomes[12] (fig. 2). The present information is relevant for genome evolution via polyploidization – one of the major factors considered to be responsible for divergence and speciation in plants. The predominant occurrence of tetraploid cells in both 2× and 4× calli cultured over a period of time indicates that the 2× acquires the genomic dosage of 4× as a sequel to the stress enforced during artificial culture. Since this ampflified genome dosage appears to help to overcome the stress of culture, it seems reasonable to suppose that it is in order to overcome stress that the evolution of plants has taken place primarily in the forward direction by elevation of the ploidy level and enhancement of chromosome number. This theory is also consistent with the observation that polyploids are less vulnerable to induced mutagenic variation or evolutionary change compared to diploids. The present comparative study of 2× and 4× genotypes suggests that the 4× genotype is less constrained in artificial cultures, as genome multiplication brings about a buffering action. The artificial culture conditions could be assumed to resemble in some ways the stressful environment which enforces speciation and adaptation in nature.

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Sylvaticin: A new cytotoxic and insecticidal acetogenin from *Rollinia sylvatica* (Annonaceae)

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Summary. Sylvaticin (**I**), a new tetrahydroxy annonaceous acetogenin with nonadjacent tetrahydrofuran rings, has been isolated from the dried fruits of *Rollinia sylvatica* St. Hil. (Annonaceae). This compound is extremely cytotoxic to human tumor cells and shows promising insect control properties.
Key words. Sylvaticin; acetogenin; *Rollinia sylvatica*; Annonaceae; brine shrimp; cytotoxicity; insecticidal; striped cucumber beetle; European corn borer.

The tetrahydrofuran acetogenins represent a new group of diversely bioactive (antitumor, cytotoxic, antimicrobial, and antimitotic) natural compounds[1–18]. Our report of the pesticidal activity of the bistetrahydrofuran acetogenin, asimicin, from *Asimina triloba* Dunal. (Annonaceae)[7], further expanded the spectrum of biological activities of this new class of natural compounds. More recently we have reported promising pesticidal activity for the bistetrahydrofuran acetogenin, bullatacin, from *Annona bullata* Rich. (Annonaceae)[15], and lesser pesticidal activity for the monotetrahydrofuran acetogenins, goniothalamicin and annonacin, from *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae)[10]. Also, we have recently patented the pesticidal uses of the acetogenins[7].

The crude hexane extract of *Rollinia sylvatica* St. Hil. (Annonaceae) fruit produced high mortality when fed to European corn borer larvae [*Ostrinia nubilalis* (Hübner)] in an artificial diet and was very toxic to brine shrimp larvae [*Artemia salina* (Leach)]. Fractionation of the extract was guided by the brine shrimp lethality bioassay[19] and by thin-layer chromatographic monitoring. After solvent partitioning and repeated column and thin-layer chromatographic separations, sylvaticin (**I**), one of the

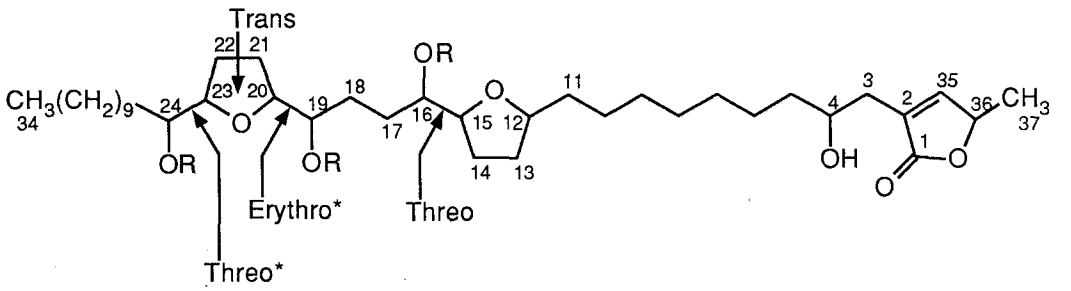


Figure 1. Sylvaticin (**I**) and its derivatives. *Indicates that, with present evidence, these two relative stereochemical assignments may be interchangeable.

I R = H **II** R = OAc
III R = TMS **IV** R = TMS-d_9

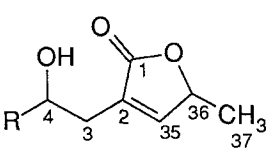
bioactive constituents of the fruit, was isolated. Sylvaticin (**I**) was toxic to brine shrimp (ED_{50} = 26 ppm) and was cytotoxic to human tumor cells: 9 KB, nasopharyngeal carcinoma, $ED_{50} < 10^{-3}$ µg/ml; A-549, lung carcinoma, $ED_{50} < 10^{-3}$ µg/ml; HT-29, colon adenocarcinoma, $ED_{50} < 10^{-3}$ µg/ml. Sylvaticin also provided protection for cantaloupe leaves from striped cucumber beetle (*Acalymma vittata* F.) nearly as effectively as that given by azadiractin, a limonoid from neem[20].

Sylvaticin (**I**) was isolated as a waxy amorphous solid, m.p. 48–50 °C, $[\alpha]_D^{23} = +5.9°$ (c = 0.524, $CHCl_3$). High resolution FAB MS analysis gave an MH^+ at m/z = 639.4836 (calc. 639.4818) corresponding to the molecular formula $C_{37}H_{66}O_8$. The IR spectrum of **I** (in dichloromethane) showed a broad hydroxyl absorption between 3300 cm^{-1} and 3700 cm^{-1}; indeed, a tetraacetate (**II**) was formed upon treatment of **I** with Ac_2O/pyridine. A single IR absorption at 1750 cm^{-1} and associated ^{1}H-NMR signals for **I** (table) at δ 7.17 (CH, C-35), 5.04 (CH, C-36), 3.73 (CH, C-4), 2.50 (CH_a, C-3), 2.38 (CH_b, C-3) and 1.41 (CH_3, C-37), specifically indicated the presence of a 4-hydroxylated α,β-unsaturated γ-lactone (subunit A)[7]. Signals at δ 174.4 (C, C-1), 151.6 (CH, C-35), 131.1 (C, C-2), 77.8 (CH, C-36), 70.0 (CH, C-4), and 19.1 (CH_3, C-37) in the ^{13}C-NMR spectrum supported these assignments. In addition, a peak at m/z 213 in the EI MS of the TMS derivative (**III**), [bis(trimethylsilyl)acetamide in pyridine] (fig. 2), which increased by 9 m.u. (mass units) in the TMS-d_9 derivative (**IV**), supported the structure of subunit A.

NMR data for sylvaticin (**I**) and its tetraacetate derivative (**II**)

	Sylvaticin (**I**) ^{1}H-NMR 300 MHz, $CDCl_3$	^{13}C-NMR 50 MHz, $CDCl_3$	Sylvaticin (**II**) tetraacetate ^{1}H-NMR 300 MHz, $CDCl_3$
1	–	174.4 (s)	–
2	–	131.3 (s)	–
3 a	2.50(dddd)	33.4 (t)	2.53 (m)
3 b	2.38(ddt)		2.53 (m)
4	3.73(m)	70.0 (d)	5.08 (m)
5	1.4–1.6	37.4 (t)[a]	1.5–2.0
6–10	1.25 (brs)	29.4–29.7	1.25 (brs)
11	1.4–1.6	31.9 (t)[b]	1.5–2.0
12	3.78–3.93	79.3 (d)	3.86 (brq)[a]
13	1.6–2.0	24.3 (t)[b]	1.5–2.0
14	1.6–2.0	26.0 (m)[b]	1.5–2.0
15	3.78–3.93	83.0 (d)[c]	3.86 (brq)[a]
16	3.48 (m)[a]	74.3 (d)[d]	4.94 (m)[b]
17	1.4–1.6	35.6 (t)[a]	1.5–2.0
18	1.4–1.6	33.2 (t)[a]	1.5–2.0
19	3.88 (m)[a]	72.5 (d)d	4.87 (m)[b]
20	3.78–3.93	81.8 (d)[c]	3.93 (m)[a]
21	1.6–2.0	25.5 (t)[b]	1.5–2.0
22	1.6–2.0	26.1 (t)[b]	1.5–2.0
23	3.78–3.93	82.5 (d)[c]	3.93 (brq)[a]
24	3.40 (m)[a]	74.1 (d)[d]	4.90 (m)[b]
25	1.4–1.6	32.4 (t)[a]	1.5–2.0
26–31	1.25 (brs)	29.4–29.7	1.25 (brs)
32	1.25 (brs)	28.5 (t)	1.25 (brs)
33	1.25 (brs)	22.6 (t)	1.25 (brs)
34	0.86 (t)	14.0 (q)	0.86 (t)
35	7.17 (d)	151.6 (d)	7.06 (d)
36	5.04 (qq)	77.8 (d)	4.99 (m)
37	1.41 (d)	19.1 (q)	1.38 (d)
4OAc	–	–	2.01 (s)
16OAc	–	–	2.04 (s)
19OAc	–	–	2.08 (s)
24OAc	–	–	2.08 (s)

[a, b, c, d] Indicate that assignments of similar signals may be interchangeable.



A

Three additional signals in the ^{13}C-NMR spectrum of sylvaticin due to hydroxyl-bearing carbons occurred at δ 72.5, 74.1, and 74.3; the ^{13}C-NMR showed four resonances at δ 79.3, 81.8, 82.5, and 83.0 also due to oxygen-bearing carbons. These ^{13}C-NMR resonances and their corresponding ^{1}H-NMR resonances at δ 3.78–3.93 (4 H, m), 3.40 (1 H), 3.48 (1 H), and 3.88 (1 H) were analogous to similar signals in gigantecin[17]. It was apparent that sylvaticin possessed two tetrahydrofuran rings and that these rings were not adjacent since three of the tetrahydrofuran oxygen-bearing carbons were adjacent to secondary hydroxyl-bearing carbons (subunit B).

To determine the placement of subunits A and B and the lengths of the hydrocarbon chains attached to these subunits, mass spectral studies were undertaken. A fragmentation scheme which was derived from the EI MS of

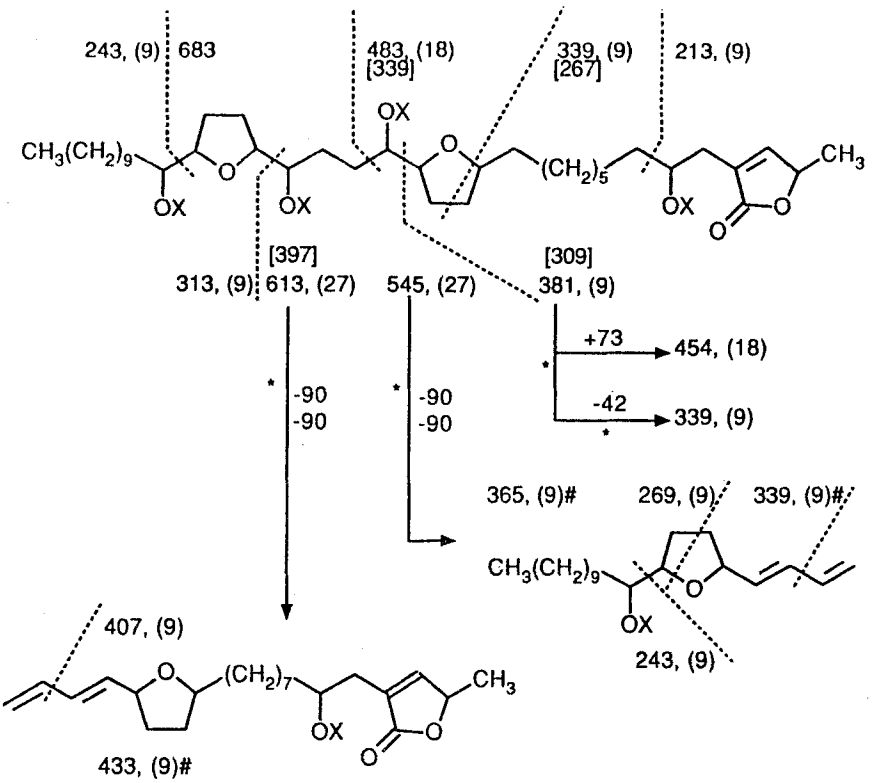
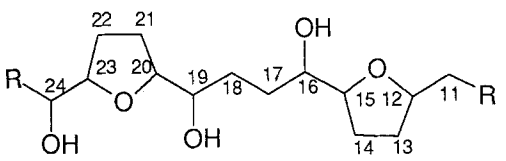


Figure 2. Diagnostic EI MS fragment ions of sylvaticin (**I**), sylvaticin-TMS derivative (**III**), and sylvaticin-TMS-d_9 derivative (**IV**). The * refers to fragments verified by MS/MS. High resolution MS (within 3 nm) confirmed each fragment composition (with the exception of the fragments marked with an #). The numbers 9, 18, or 27 in parentheses refer to mass shifts of the TMS-d_9 derivative (**IV**). The numbers in brackets refer to the mass shifts of the underivatized **I**.



B

compounds **I**, **III**, and **IV** is given in figure 2. The exact masses for the fragments associated with **I**, **III**, and **IV** were determined to give the elemental compositions for each illustrated fragment. The mass shift (9, 18, 27) found in the fragmentation of the TMS-d_9 derivative (**IV**) differentiates ions which bear one, two, or three hydroxyls, respectively. The fragment ions with TMS groups frequently undergo loss of TMSOH (90 m.u.), forming new ions which undergo further fragmentation. This was verified from the daughter ion spectra (E/B linked scans) of several structurally diagnostic fragments, as indicated by the * in figure 2. For example the daughter ion spectrum of the fragment at m/z 545 shows two consecutive losses of TMSOH to give the ion at m/z 365, as well as further losses to give predictable ions at m/z 339, 269, and 243.

Sylvaticin (**I**) has nine independent asymmetric centers. The problem of selecting the correct representative among the 512 possible stereoisomers is of considerable complexity and could not be solved up to now. A comparison of the NMR data of **I** with the spectra of related compounds led to the following tentative conclusions: a) the substitution pattern of the tetrahydrofuran ring containing C(20–23) is most likely trans, b) there is some evidence that the relative stereochemistry around C(15–16) is of the threo type.

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Secondary metabolites of the chemically rich ascoglossan *Cyerce nigricans*

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Summary. Two new metabolites of an apparent propionate origin have been isolated from the organic extract of the ascoglossan mollusc *Cyerce nigricans*. The proposed structures for the new natural products are based on interpretation of their physical and spectral properties. The new compounds isolated lacked the potent ichthyodeterrent properties of the whole animal extract suggesting that other molecules are involved in the defense of this shell-less mollusc.

Key words. Ascoglossa; chemical defense; *Cyerce nigricans*; polypropionate metabolites.

Shell-less gastropods such as nudibranchs, ascoglossans, and sea hares are known to be rich sources of unique secondary metabolites, most of which are sequestered from their chemically-rich prey[2]. Sequestering of these metabolites is widely viewed as a gastropod adaptation to acquire chemical defenses[2,3]; however, this has rarely been tested under ecologically relevant conditions[4]. In this paper we describe the structure of two new pyrones isolated from the Australian ascoglossan *Cyerce nigricans* and evaluate these metabolites as potential defenses against predatory reef fish.

Cyerce nigricans is a black and orange, aposematically colored ascoglossan that specializes on the chemically-rich green alga *Chlorodesmis fastigiata*. Previous ecological and preliminary chemical investigations on this species demonstrated that the live ascoglossan was repellant to coral reef fish as was its organic crude extract; however, the repellant nature of the extract was not due to the cytotoxic diterpenoid chlorodesmin (**3**), which is at least partially sequestered from its algal food[5]. We reasoned that other metabolites which were noted in the extract by TLC, but not identified, might be responsible for the deterrent nature of the *C. nigricans* extract. We therefore initiated additional chemical investigations of the chemistry of this ascoglossan.

Materials and methods

Twenty animals (420 mg, total dry mass) were collected from reefs near Lizard Island, Australia, and soaked in a mixture of MeOH/$CHCl_3$ (1/3). The extract was reduced in vacuo and the residue (120 mg) was fractionated on silica gel (flash chromatography). Proton NMR analysis of the column fractions revealed the presence of two unique metabolites (**1** and **2**), as well as minor quantities of chlorodesmin (**3**), fats, and sterols. The pyrones **1** (4 mg) and **2** (2 mg) were eluted from the column with 60% and 80% EtOAc in isooctane, respectively, and were further purified by HPLC (silica) using the same solvent mixtures.

The ability of these metabolites to deter feeding by a predatory reef fish was tested using the common Pacific wrasse *Thalassoma lunare* and methods that had proven successful in previous similar assays[5,6]. Each pure metabolite and a mixture of both **1** and **2** were dissolved in purified diethyl ether and injected into freeze-dried krill at concentrations ranging from approximately 2 to 23 times their natural yield. After allowing the solvent to evaporate, feeding on these krill was compared with feeding on krill injected with only solvent. Fish were held in individual aquaria and 1 to 3 pairs of treatment and control krill were offered to 2–3 fish at each concentra-