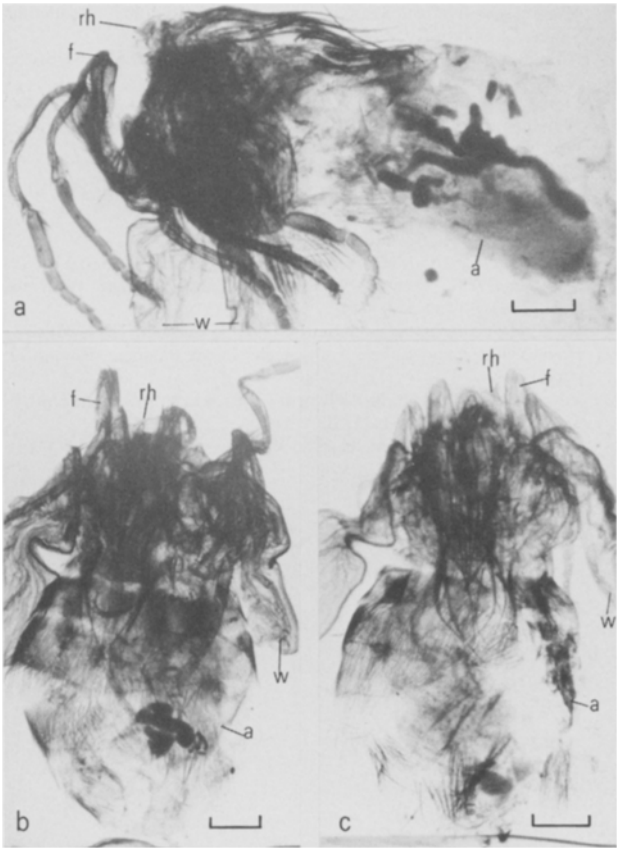

Whole mounts of headless flies from pupuria formed after treating post-feeding, final-stage larvae of *Calliphora* with 0.3 µg ivermectin in 1 µl ethanol. Scale = 1 mm. *a* Side view showing advanced thorax terminating at respiratory horns: abdomen is pupal-like. *b* Ventral view showing respiratory horn between the front femora: abdomen is more advanced and segmented. *c* Dorsal view showing complete thorax and well-developed abdomen with long setae. a, abdomen; f, front femur; rh, respiratory horn; w, wing.

This curious finding can be explained. Fraenkel[5] described vigorous peristaltic activity in newly formed pupal abdomens, activity resulting in the rapid eversion of the head from the thorax. At this early stage in metamorphosis, the peristalsis must be caused by larval muscles. Pupae dissected from the puparia of ivermectin-treated larvae do not show this muscular activity (unpublished observations). Since ivermectin has known effects upon inhibitory neuromuscular synapses[2], it is not unreasonable to attribute the lack of muscular activity to the presence of ivermectin in the treated insects. However, there are GABA binding sites in the central system of insects[6], and ivermectin might also exert its effects on muscular activity through the central nervous system.

Since headless insects develop in the wrong location in the puparium, one function of head eversion might be to align the pupa in the correct position. Even so, the insects can still progress to an advanced stage in the wrong place. Whether the 15% pharate adults that remain within the puparia is due to residual action of ivermectin on adult muscles is beyond the scope of this paper, although it is unlikely that ivermectin would prevent adult emergence without affecting head eversion at the earlier stage. In conclusion, this work demonstrates how ivermectin can prevent adult development during metamorphosis in *Calliphora*, and attempts to explain the inhibition in terms of known effects of ivermectin.

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Structure of minor host-selective toxins from *Cochliobolus victoriae*

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Summary. Structures **2**–**5** have been assigned to four minor host-selective toxins from *Cochliobolus victoriae*, namely victorin B, victorin D, victorin E, and victoricine, by using as a lead the established structure **1** of victorin C, the main component of the toxin complex.

Key words. Blight of oats; host-specific phytotoxins; *Cochliobolus victoriae;* victorins, victoricine.

In a previous communication on the host-selective toxins produced by *Cochliobolus victoriae*, the causal agent of victoria blight of oats, we described the isolation of five fractions displaying host-selective toxic activity which were named victorin A, B, C, D and E, respectively, according to their order of elution from a reversed phase HPLC column[2]. Preliminary chemical examination of the most abundant compound, victorin C, which is probably identical with the toxic preparation reported by two other groups[3,4], revealed the presence of six subunits, namely 5,5-dichloroleucine (Cl_2leu)[2,5], erythro-β-hydroxyleucine (OHleu)[2,5], threo-β-hydroxylysine (OHlys)[2,5], α-amino-β-chloroacrylic acid (aClaa)[2], victalanine (victala)[2], and glyoxylic acid[2]. Further work on victorin C and its degradation products eventually led to the establishment of the unusual structure **1** for the toxin[6]. We now report on the structure of the minor toxic companions of victorin C.

The fractions from the RP-HPLC column previously designated as victorins A, B, and E were found homogeneous when analyzed by TLC and NMR techniques; in contrast, NMR analysis of fraction D revealed it to be a mixture of two components which could be resolved into the homogeneous compounds by TLC (table 1). To avoid upsetting the original alphabetical nomenclature we have retained the designation victorin D for the compound with R_f 0.59 and have named the other compound with R_f 0.83 victoricine.

The main toxin, victorin C, was obtained routinely in yields of

Table 1. Characterization of toxins from *C. victoriae*

	Victorin B	Victorin C	Victorin D	Victorin E	Victoricine
Ret. time[a]	3.75	6.5	8.0	11.6	8.0
R_f[b]	0.45	0.50	0.59	0.54	0.83
$[\alpha]_D$ (H_2O)	− 62°	− 76°	− 72°	–	− 30.5°
UV (H_2O)	256/18300	256/20300	247/21600	254/13300	290/ 2040
	sh 227/12000	sh 225/14000	sh 223/14400	sh 228/10000	224/14600
CD (H_2O)[c]	299/ − 3.6	298/ − 4.7	280/ − 11.6	–	294/ + 1.0
	255/ − 10.3	253/ − 10.8	251/ + 0.4		255/0
	214/ + 1.9	213/ + 0.9	235/ − 1.3		228/ − 14.5
			218/ + 1.3		
IR (KBr)	1675, 1610, 1385	1680, 1609, 1385	1674, 1612, 1385	–	1680, (1615), 1209
Mol. wt[d]	780	814	798	848	742
	($C_{31}H_{46}O_{13}N_6Cl_2$)	($C_{31}H_{45}O_{13}N_6Cl_3$)	($C_{31}H_{45}O_{12}N_6Cl_3$)	($C_{31}H_{44}O_{13}N_6Cl_4$)	($C_{32}H_{47}O_{12}N_6Cl$)

[a] C_{18} reversed phase HPLC column (Waters resolve) with 25% CH_3CN/0.1% TFA at 1 ml/min. [b] TLC on Merck ‹soft plus› plates with 2-propanol/CH_3CN/H_2O = 3:2:2. [c]Measured on a JOBIN'Yvon Dichrograph Mark III; amplitudes expressed as Δε in l/Mol·cm. [d] From multiple FAB-MS in NOBA (pos. mode) and gly (pos. and neg. mode). Interpretation problems caused by easy loss of water from the hydrated aldehydo forms have been discussed in extenso elsewhere[6].

Table 2. NMR data of the modified terminal leucine residue in victorins B(**2**), D(**3**), and E(**4**)

	^1H[a]			^{13}C[b]	
Carbon No.	**2**	**3**	**4**	**2**	**3**
1	–	–	–	174.9 s[c]	175.0 s[c]
2	4.54 (dd; 10,4)	4.62 (dd; 11, 4)	d	54.2 d	53.8 d
3	1.85 (m)	2.20 (ddd; 17, 11, 4)	2.20 (m)	36.9 t	35.9 t
	1.75 (m)	1.95 (m)	1.95 (m)		
4	1.95 (m)	2.35 (m)	2.50 (m)	34.4 d	42.6 d
5	3.60 (d; 4)	6.14 (d; 3)	–	53.7 t	81.3 d
6	1.04 (3H, d; 7)	1.20 (3H, d;7)	1.35 (3H, d; 6)	18.9 q	16.7 q

[a] D_2O/0.2% deut. TFA solutions. δ values relative to external DSS = 0 ppm; in parenthesis: number of hydrogens (if more than one), multiplicities (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplett, br. = broad signal); J values in Hz, rounded to next full number. Connectivities between H-carrying atoms were verified by COSY-2D measurements. [b] Solutions in 150 µl D_2O/0.2% deut. TFA. δ values relative to external TSP = 0 ppm; multiplicities as observed in the DEPT spectrum. [c] Tentative assignment. d, Signal hidden under waterpeak at 4.71 ppm.

Table 4. Selected NMR data[a] of victoricine (**5**)[b]

Subunit	Carbon No.	^1H		^{13}C	
		major	minor	major	minor
Leu	1			174.8[c]	
	2	4.47 (dd; 11, 3)		54.9	
	3	1.62 (2H, m)		42.2	
	4	1.68 (m)		26.8	
	5	0.91 (d; 6)		24.5	
	6	0.89 (d; 6)		23.6	23.4
2,5-DOPA	1			176.9[cc]	177.3[c]
	2	4.85 (m)	4.49	57.4	52.0
	3	3.30 (m)	3.48 (dd; 13, 6)	34.6	34.1
		3.05 (m)	2.88 (dd; 13, 3)		
	4			132.3	
	5			152.4	151.6
	6	7.12 (d; 9)	6.98	127.5	127.2
	7	6.82 (dd; 9, 3)	6.71	122.0	
	8			150.9	
	9	6.69 (d; 3)		118.0	

[a] For experimental procedures and symbols see table 2, footnotes a) and b). [b] Displaying dichotomous signals see table 3, footnote b). Data not included in the table match closely the ones described previously for victorin C^2. [c] Tentative assignment.

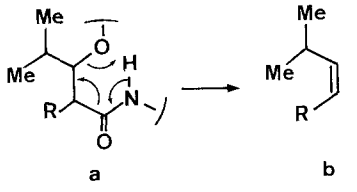
1–2 mg/l of culture fluid and accounted for 85–90% of the toxic activity; a crude estimate indicated that the concentration of the minor toxins was decreasing in the order victorin B (40–80 µg/l) > victoricine (30–50) > victorin D (20–40) > victorin E (5–10). All the minor toxins induced half-maximal inhibition of susceptible root growth in the same concentration range as victorin C^2; however, using dark CO_2 fixation as a more quantitative assay, significant differences among the activities of the toxins could be observed[8].

With the exception of victorin A, which was not available in sufficient amounts, the new toxins were characterized by the data presented in table 1, as well as by FAB-MS, ^1H-NMR, and, whenever possible, by ^{13}C-NMR and total hydrolysis. Comparison with the spectral data of victorin C, **1**, facilitated the identification of single subunits in the new toxins. For example it follows from the data in table 1 that victorins B, D, and E, but not victoricine, share with victorin C a UV spectrum which is characteristic for the presence of the victala and aClaa chromophores[2]. In addition, the NMR data provide evidence that the hydrated glyoxylic acid moiety as well as the OHlys, OHleu, and aClaa residues are common features of these three new toxins. A further analogy with victorin C, **1**, is expressed in their NMR spectra by the appearances of several redundant signals due to the dichotomous behavior of single nuclei, a feature which in the case of **1** has been interpreted as a consequence of the conformational properties of the 12-membered ring[6]. An important diagnostic clue for the derivation of specific structures for the new compounds was provided by the observation that all the victo-

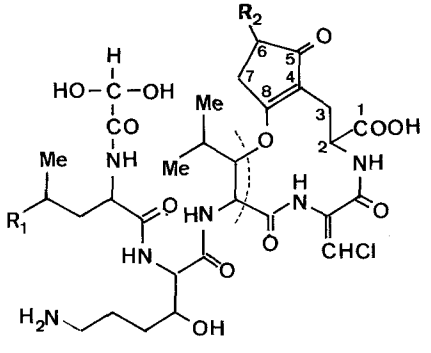
Table 3. NMR data[a] of the desoxyvictala residue in victorin D (**3**)[b]

Carbon No.	^{1}H		^{13}C	
	major	minor	major	minor
1	–	–	176.2 s[c]	–
2	4.33 (br)	4.77 (m)	55.4 d	51.6
3	2.8 (2H, m)	2.95 (br)	26.0 t	32.7
4	–	–	118.0 s	117.6
5	–	–	211.7 s	–
6[d]	2.59 (2H, s)	–	35.9 t	–
7[d]	2.95 (2H, br)	2.85 (m)	28.3 t	24.7
8	–	–	191.0 s	–

[a] For experimental procedures and symbols, see table 2, footnotes a) and b). [b] As discussed in the text some of the nuclei display dichotomous signals with different intensities; dichotomous signals for the major component are listed together with the non dichotomous ones under 'major'; additional signals due to the minor component are listed under 'minor'. [c] Tentative assignment. [d] May be interchanged.

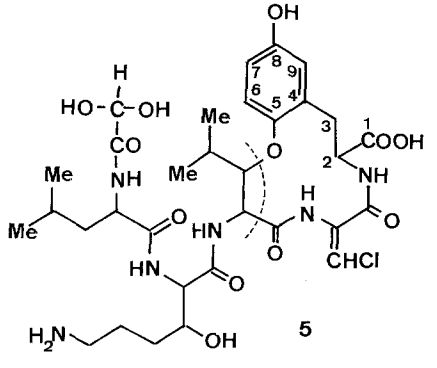


a b



1: R_1 = $CHCl_2$, R_2 = OH **2**: R_1 = CH_2Cl, R_2 = OH

3: R_1 = $CHCl_2$, R_2 = H **4**: R_1 = CCl_3, R_2 = OH



5

rins display in their positive FAB-MS spectra characteristic peaks corresponding to a fragment, and to matrix adducts thereof, generated from the protonated aldehyde form of the parent compound through the cleavage indicated by dotted lines in formulae **1–4**; a plausible mechanistic interpretation for the extrusion of this fragment is outlined in the scheme **a→b**. In the case of victorin C in a NOBA matrix the critical peaks occur at m/z 467 (weak, $C_{19}H_{33}O_5N_4Cl_2^+$) and at 520 (prominent, NOBA adduct); with a glycerol matrix the peak at m/z 467 is hardly detectable, whereas the peak of the glycerol adduct at m/z 559 is of prominent intensity.

On this basis, the FAB-MS data of victorin B (mol.wt 780, critical fragment at m/z 525, 1 Cl, corresponding to gly+$C_{19}H_{34}O_5N_4Cl^+$) can now be interpreted in terms of structure **2**. Supporting evidence for this structure is provided by the ^{1}H- and ^{13}C-NMR data of the compound, which differ from those of victorin C only in the set of signals corresponding to the new amino acid (table 2).

Similarly, structure **4** can be ascribed to victorin E by joint evaluation of the FAB-MS data (mol.wt 848, critical fragment at m/z 501, 3 Cl, corresponding to $C_{19}H_{32}O_5N_4Cl_3^+$) and the ^{1}H-NMR spectrum, which differs from the one of victorin C only in the signals belonging to the 5, 5, 5-trichloroleucine subunit (table 2).

Victorin D was found to represent yet another variation in the substitution pattern of the basic framework. According to its molecular weight this toxin contains one oxygen less than victorin C and the appearance in its FAB-MS spectrum of a critical fragment at m/z 467 ($C_{19}H_{33}O_5N_4Cl_2^+$) points, by difference, to the presence of a desoxyvictala moiety as in **3.** In keeping with this structure the ^{1}H-NMR spectrum lacks one of the characteristic ABX systems of victala and is consistent with the presence of a new $-CH_2-CH_2-$ unit, which was verified by COSY-2D measurements and by the ^{13}C-NMR spectrum (table 3). Total hydrolysis of victorin D gave a mixture which, when analyzed on a Technicon TMS Automated Amino Acid Analyzer[9], was shown to contain OHleu (retention time (r.t.) 58 min), Cl_2leu (r.t. 81 min), and OHlys (r.t. 88 min), but not victala (expected r.t. 28 min); a new unidentified peak with a r.t. of 47 min is likely to correspond to 6-desoxyvictala.

The most interesting among the new toxins from *C. victoriae* is victoricine, mol.wt 742, $C_{32}H_{47}O_{12}N_6Cl$, which lacks the characteristic UV chromophore of the victala unit. The combined NMR evidence (table 4) verifies the presence in the compound of the glyoxylic acid, OHlys, OHleu, and aClaa subunits; in addition, a new set of NMR signals is easily assigned to a non modified leucine residue. Both the operation of the characteristic fragmentation scheme (FAB-MS: m/z 399, corresponding to $C_{19}H_{35}O_5N_4^+$) and the dichotomous behavior detectable in the NMR spectra for single nuclei pertaining to the OHleu and aClaa residues strongly suggest that victoricine embodies a similar 12-membered ring as the other toxins, in spite of the absence of the victala component. From a consideration of the molecular weight and from the nature of the identified fragments it can be concluded that the new amino acid substituting for victala possesses the composition $C_9H_{11}O_4N$. Residual signals in the NMR spectra of victoricine stemming mostly from dichotomous nuclei (table 4) are in good accord with the presence of a 1, 2, 4 trisubstituted benzene ring bearing two hydroxyl groups and an alanyl chain; clearly, this chromophoric group must be the one responsible for the appearance in the UV spectrum of the compound of maximum at 290 nm which is shifted to 315 nm upon addition of base. Among the remaining alternatives a choice in favor of a 2, 5-DOPA unit embedded in a 12-membered ring as shown in **5** can easily be met on the basis of nuclear Overhauser difference spectra, which disclose interactions between (C-9)-H and the (C-3)-methylene protons of the alanyl side chain as well as between (C-6)-H and the methine proton in the isopropyl group of the OHleu residue. In support of structure **5** equimolar amounts of leu and of OHlys could be detected in the total hydrolysate. The trace from the amino acid analyzer provided no evidence for the presence of OHleu; new peaks with r.t. of 26 and 82 min in this trace, while still awaiting identification, are likely to correspond to 2, 5-DOPA and to its 2-leucyl ether, from which the former might be derived in a β-elimination process leading to the destruction of the OHleu component.

The detection of a bridged 2, 5-DOPA unit in the structure of victoricine is of particular interest in connection with spec-

ulations concerning the biosynthetic origin of victala[2] and puts severe restrictions on the choice of a mechanism for the formation of the unusual 12-membered ring in this toxin and in its victorin cognates.

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Abbreviations used: FAB, fast atom bombardement; NOBA, 3-nitrobenzylalcohol; gly, glycerol; TFA, trifluoroacetic acid.

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Okinonellins A and B, two novel furanosesterterpenes, which inhibit cell division of fertilized starfish eggs, from the marine sponge *Spongionella* sp.[1]

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Summary. Two novel furanosesterterpenes, okinonellins A (**1**) and B (**2**), have been isolated from the sponge *Spongionella* sp. Both compounds inhibit cell division of starfish embryos.
Key words. Spongionella sp.; porifera; cell division inhibitor; starfish embryos, furanosesterterpene.

We previously reported the isolation and structural elucidation of two bioactive furanosesterterpenes, spongionellin (**3**) and dehydrospongionellin (**4**), from a marine sponge *Spongionella* sp.[2]. We have now separated two more novel sesterterpenes, named okinonellins A and B, form another species of *Spongionella*. Both compounds inhibit the development of fertilized starfish eggs.

The sponge (800 g, wet weight) was collected by SCUBA (−20 m) at Okinoshima Island in Uwa Sea, 700 km southwest of Tokyo, and stored frozen at −20 °C until processed. The frozen sponge was extracted with ethanol, whose extract was partitioned between water and ether. The organic phase was subjected to low pressure column chromatography on silica gel with dichloromethane-ethyl acetate systems. The active materials

^{1}H and ^{13}C NMR shifts for **1** and **2**

C	**1** ^{1}H δ($CDCl_3$)	^{13}C δ($CDCl_3$)	**2** ^{1}H δ($CDCl_3$)	^{13}C δ($CDCl_3$)
1	7.25 (1H, d, 1.8 Hz)	140.7 d	7.33 (1H, brs)	142.6 d
2	6.18 (1H,d, 1.8)	110.2 d	6.25 (1H, brs)	111.1 d
3		116.8 s		124.9 s
4		154.5 s	7.19 (1H, brs)	138.8 d
5	2.42 (2H, m)	22.6 t	2.39 (2H, t, 7.5)	24.9 t
6	1.72 (2H, m)	20.1 t	1.54 (2H, m)	27.8 t
7	1.79 (1H, m)	38.5 t[a]	1.35 (2H, q, 7.5)	36.9 t[c]
	1.63 (1H, m)			
8		38.7 s	2.19 (1H, m)	36.8 d
9	1.36 (3H, s)	25.8 q	1.00 (3H, d, 6.7)	20.8 q
10	5.62 (1H, brd, 15.3)	139.0 d	5.45 (1H, dd, 15.1, 7.9)	138.5 d
11	5.97 (1H, dd, 15.3, 10.7)	124.9 d	6.19 (1H, dd, 15.1, 10.7)	124.9 d[d]
12	5.79 (1H, d, 10.7)	124.9 d	5.79 (1H, d, 10.7)	125.2 d[d]
13		137.1 s		136.0 s
14	1.65 (3H, brs)	16.6 q	1.73 (3H, brs)	16.5 q
15	2.01 (2H, t, 7.7)	39.3 t[a]	2.06 (2H, m)	39.3 t[c]
16	1.55 (2H, m)	25.8 t	1.61 (2H, m)	25.8 t
17	2.04 (2H, t, 7.7)	35.9 t[a]	2.06 (2H, m)	36.1 t[c]
18		144.4 s		144.8 s
19	4.92 (1H, brs)	113.1 t	4.92 (2H, brs)	112.3 t
	4.90 (1H, brs)			
20	2.65 (1H, dd, 15.0, 3.8)	38.4 t[a]	2.63 (1H, dd, 15.0, 7.2)	34.7 t[c]
	2.27 (1H, dd, 15.0, 8.5)		2.52 (1H, dd, 15.0, 7.2)	
21	4.79 (1H, brm)	76.8 d	4.46 (1H, dt, 3.3, 7.2)	80.8 d
22		175.8 s[b]	4.36 (1H, brdd, 3.3, 4.5)	71.7 d
23		97.4 s	2.73 (1H, dq, 4.5, 7.3)	41.9 d
24	1.70 (3H, s)	6.0 q	1.27 (3H,d, 7.3)	8.0 q
25		174.6 s[b]		177.6 s

[a–d] Assignments may be interchanged.