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Structure of victorin C, the major host-selective toxin from *Cochliobolus victoriae*

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Summary. The predominant host-selective toxin from *Cochliobolus victoriae*, victorin C, is a peptide with an apparent mol. wt of 796, representing a cyclic array of the subunits **1**–**6**. The structure of the toxin has now been established as in **16** through analysis of the degradation products generated by enzymic and non-enzymic partial hydrolysis. The presence of a hydrated aldehydo group requires for victorin C the composition $C_{31}H_{45}O_{13}N_6Cl_3$ with an amended mol. wt of 814, for which independent experimental support has been secured.
Key words. Blight of oats; *Helminthosporium victoriae;* phytotoxins; NMR-spectra; structural elucidation; unusual oligopeptides.

The causal agent of victoria blight of oats, *Cochliobolus victoriae* Nelson produces a host-specific toxin called victorin[2]. Victoria blight of oats became a major disease about 40 years ago as a consequence of the introduction of commercial varieties of oats carrying the Pc gene for resistance to crown rust, *Puccinia coronata*[3,4]. It was later discovered that rust resistance and susceptibility to *C. victoriae* and its toxin were either closely linked or controlled by the same genetic locus. Subsequent studies on the inheritance of susceptibility to *C. victoriae* have shown that sensitivity to the fungus and its toxin, victorin, is controlled by a single dominant gene with the homozygous dominant genotype conferring sensitivity to the toxin and the homozygous recessive genotype conditioning insensitivity[5]. Thus, victorin appears to be a gene-specific toxin affecting only those genotypes of oats that carry the dominant allele for susceptibility to *C. victoriae*. In a previous communication[6] we reported on the isolation of several homogeneous host-selective toxins from the culture filtrate of *Cochliobolus victoriae* Nelson (*Helminthosporium victoriae* Meehan and Murphy)[7] and, in addition, have shown that the most abundant compound, victorin C, (apparent mol. wt 796, corresponding to $C_{31}H_{43}O_{12}N_6Cl_3$) must represent a cyclic combination of the components glyoxylic acid, **1**, 5,5-dichloroleucine (Cl_2leu), **2**, *erythro*-β-hydroxyleucine (OHleu), **3**, victalanine, **4**, *threo*-β-hydroxylysine (OHlys), **5**, and α-amino-β-chloroacrylic acid (aClaa), **6**. We now outline new evidence which has led to the derivation of a complete structure for victorin C, thus establishing its correct composition as $C_{31}H_{45}O_{13}N_6Cl_3$ (mol. wt 814).

Materials and methods

Acid hydrolyses. a) 40 mg of victorin C were incubated in 10 ml of 6N HCl at r.t. for 21.5 h. The sample was dried in vacuo and redissolved in water, a small portion was injected onto an analytical reverse phase C_{18} column (Wa-

ters Resolve) which was eluted isocratically with 21% acetonitrile containing 0.1% TFA at 1 ml/min while monitoring at 254 nm. This analysis revealed the presence of six major UV absorbing components. In the order of their elution from the HPLC column the following compounds were obtained: **10**, **9**, an unidentified compound followed by **8**, starting material, and **7**. The remaining sample was then loaded onto a preparative reverse phase HPLC column (Waters Z module equipped with a Nova Pak C_{18} cartridge) and eluted with a gradient of water to 15% acetonitrile over 2 h at a flow rate of 3 ml/min. Individual UV absorbing fractions were analyzed for homogeneity by injection onto the analytical C_{18} column and fractions were combined based on the analysis. Fractions containing a mixture of compounds were pooled accordingly and rechromatographed until homogeneous preparations were obtained.

b) 2.5 mg of compound **9** were incubated in 0.6 ml of 6 N HCl for 15.5 h at 60 °C. The sample was dried in vacuo, redissolved in 0.15 ml H_2O, applied to the prep. HPLC C_{18} column and eluted with 2% acetonitrile containing 0.1% TFA at 2.5 ml/min. Fractions were collected and six major compounds including **4**, **10**, **12**, **13**, and **15** were separated as detected by their absorbance at 203 nm. The appropriate fractions were pooled, dried in vacuo and redissolved in 30 µl D_2O for micro NMR analysis.

Enzymic hydrolyses. 10 mg of compound **8** were treated with 100 units of leucine aminopeptidase (Sigma type VI) in 2 ml 25 mM potassium phosphate buffer pH 7.1 for 1 h at r.t. The mixture was then concentrated in vacuo and separated by prep. HPLC as described above under a) while monitoring at 203 nm. This resolved, next to free OHlys, **5**, and Cl_2leu, **2**, two major new compounds designated **10** and **11**. Similar treatment of **7** led to the isolation of **9**.

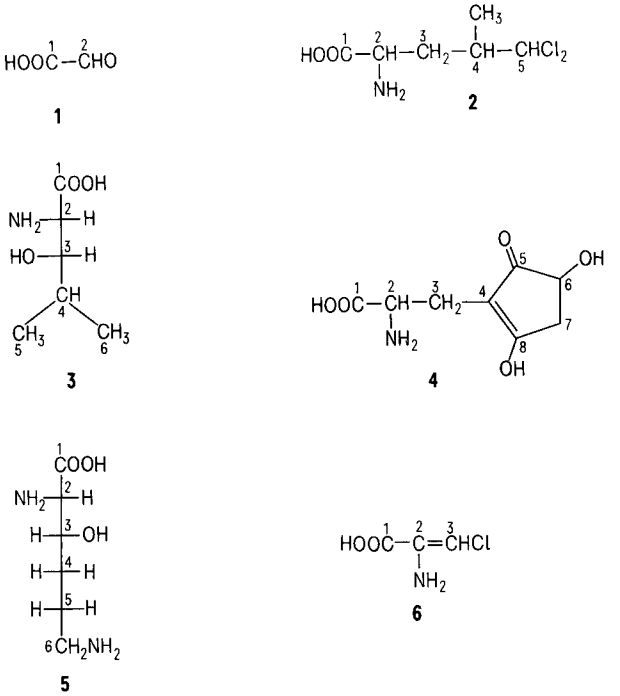


Figure 1. Carbon numbers as indicated are used for identification of NMR signals in the tables.

*Resynthesis of victorin C, **16**, from desglyovictorin C, **7**.* 140 mg of N-hydroxy-succinimide and then 400 mg of dicyclohexylcarbodiimide were added to a solution of 88 mg of glyoxylic acid in 4 ml of dioxane, the solution left at r.t. for 1 h and filtered. 0.1 ml of the filtrate was added to a solution of 300 µg of desglyovictorin C in 1 ml of potassium phosphate buffer pH 6 and left at r.t. for 1 h. The reaction mixture was then centrifuged and the supernatant chromatographed on the prep. HPLC C_{18} column equilibrated with 10 mM KH_2PO_4 and eluted with a 2-h linear gradient of 0–20% acetonitrile in 10 mM KH_2PO_4 at a flow rate of 3 ml/min. The purified victorin C was desalted with a C_{18} sep-pak (Waters), concentrated to dryness and resolubilized in 30 µl of D_2O for micro NMR analysis.

Methyl ester of victorin C. A solution of 4 mg of victorin C in 0.1 ml of DMSO was added to 1.8 ml of 14% BF_3 in methanol and incubated at r.t. for 5.5 h. The methanol was removed in vacuo and the material purified as described above for the preparation of the semisynthetic victorin C.

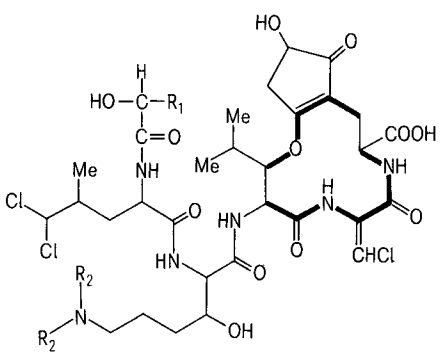
Reduction of victorin C with $NaBH_4$. a) To an ice cold solution of 5.3 mg of victorin C in 1.5 ml of 0.2 M sodium borate pH 9 were added 270 µl of a 5 mg/ml stock solution of sodium borohydride. After 2 min the mixture was neutralized by the addition of 150 µl of 1.2 N HCl and the product, **17**, purified as described for the preparation of the semisynthetic victorin C.

b) To a chilled solution of 10 mg of victorin C in 2.4 ml of 0.2 M borate buffer, pH 9.0, were added 0.3 ml of 0.4 M

7 $NH_2-Cl_2leu-OHlys-(OHleu-aClaa-victala)_{cyclo}$
8 $NH_2-Cl_2leu-OHlys-OHleu-aClaa-victala$
9 $OHlys-(OHleu-aClaa-victala)_{cyclo}$
10 $OHlys-OHleu-aClaa-victala$
11 $OHleu-aClaa-victala$
12 $OHlys-OHleu-aClaa$
13 $OHlys-OHleu$
14 $(aClaa-victala)$

15

Figure 2.



$R_1 = OH, R_2 = H$: **16**
$R_1 = R_2 = H$: **17**
$R_1 = H, R_2 = Me$: **18**
$R_1 = OMe, R_2 = H$: **19**

Figure 3.

formaldehyde. After 30 s a $NaBH_4$ solution (5 mg/ml H_2O) was added as follows: four 60 µl portions at intervals of 15 s and then finally 300 µl. After an additional min the reaction was terminated by the addition of 300 µl of 1.2 N HCl. The product, **18**, was isolated from the reaction mixture by gradient HPLC on the prep. column as described above under a), concentrating the appropriate fractions and checking them on the analytical column prior to pooling.

Total hydrolysis of 18. The sample (ca 5 mg) was dissolved in 1 ml of 6 N HCl and kept at 105 °C in a sealed tube for 15 h. After removal of the solvent in vacuo the residue was taken up in 0.4 ml of H_2O and streaked onto three prep. TLC plates (Merck 'soft plus'), eluted with isopropanol: 58% NH_4OH 7:3 and the resolved four main bands scraped and eluted with three times 20 ml of the eluent. According to their R_f values the four fractions represented, in order of increasing polarity, the known amino acids **2** and **3**, a new compound, and **4**. The two latter were rechromatographed separately as above, while for **2** and **3** the upper phase of butanol:acetic acid:H_2O = 50:10:40 was used. Bands were again scraped, eluted as above and the recovered samples purified by passing them in H_2O through a column of Biogel P-2 (Biorad) before submitting them to micro NMR analysis and FAB-MS.

Micro NMR analysis. ^{1}H-NMR spectra of limited quantities of material, 200–300 µg/30 µl, were recorded in capillary tubes (1.6–1.8 × 100 mm) placed inside a conventional 5 mm × 178 mm NMR tube.

FAB mass spectra as well as 1- and 2-dimensional NMR spectra were recorded as reported earlier[6]. Nuclear Overhauser difference spectra were obtained by standard techniques[8] using non-saturating power levels for all pre-irradiations. Selective population transfer effects were eliminated by the method of Neuhaus[9].

Results and discussion

To obtain information on the linkage of the subunits victorin C was submitted to mild acid hydrolysis. This yielded glyoxylic acid, **1**, and a set of degradation products which, after purification by HPLC, were analyzed by FAB-MS and NMR techniques (cf. tables 1 and 3). One of the degradation compounds (mol. wt 740, 3 Cl atoms, corresponding to $C_{29}H_{43}O_{10}N_6Cl_3$) was recognized as desglyovictorin C, **7**, i.e. the product of hydrolytic removal of glyoxylic acid from the original toxin. It is noteworthy that a number of signals in the NMR spectra of **7** retain the dichotomous nature previously detected for corresponding signals of victorin C[6]. A second compound, (mol. wt 758, 3 Cl atoms, $C_{29}H_{45}O_{11}N_6Cl_3$) could be identified as a linear peptide, **8**, which still contains all the fragments **2–6** and therefore must arise from desglyovictorin C, **7**, through hydrolytic cleavage of its macrocyclic ring (cf. below). The composition of a third degradation product, **9**, was established as $C_{23}H_{34}O_9N_5Cl$ by high resolution FAB-MS (calc. for $[M+H]^+$ 560.212, found 560.218); in accordance with its NMR spectra this compound must have been generated from its immediate precursor **7** through hydrolytic removal of Cl_2leu with maintenance of the macrocyclic ring. A fourth compound was found to be a linear tetrapeptide, **10**, (mol. wt 577, $C_{23}H_{36}O_{10}N_5Cl$), related to **9** in the same way as **8** is to **7**.

Table 1. ^{1}H-NMR data* of hydrolysis compounds from victorin C

Subunit	Carbon No.***	7** Major	7** Minor	8	9** Major	9** Minor	10
Cl_2leu (**2**)	2	4.26 (dd,8,6)		4.24 (dd,8,6)			
	3	2.25 (m)		2.28 (m)			
		2.0 (m)		1.91 (m)			
	4	2.40 (m)		2.40 (m)			
	5	6.15 (d,3)		6.13 (d,3)			
	6	1.23 (3H,d,7)		1.21 (3H,d,7)			
OHlys (**5**)	2	4.54 (d,7)		4.52 (d,7)	4.00 (br)		4.0 (m)
	3	3.98 (m)		4.00 (d,t,8,4)	3.96 (br)		4.0 (m)
	4	1.60 (2H,m)		1.6 (2H,m)	1.66 (2H,m)		1.67 (2H,m)
	5	1.9 (m)		1.91 (m)	1.94 (m)		1.95 (m)
		1.8 (m)		1.86 (m)	1.77 (m)		1.75(m)
	6	3.03 (2H,m)		3.03 (2H,t,9)	3.05 (2H,m)		3.06 (2H,t,7)
OHleu (**3**)	2	5.10 (d,8)	5.04	4.67 (d,9)	5.14 (d,7)	5.08	ca 4.7
	3	4.85 (m)	4.98	3.80 (dd,9,4)	4.86 (m)	4.86	3.82 (dd,8,4)
	4	2.3 (m)	?	2.00 (m)	2.28 (m)	2.10	1.95 (m)
	5	1.12 (3H,d,7)	0.96	1.04 (3H,d,7)	1.14 (3H,d,7)	0.97	1.07 (3H,d,7)
	6	1.09 (3H,d,7)	0.92	0.93 (3H,d,7)	1.12 (3H,d,7)	0.95	0.98 (3H,d,7)
aClaa (**6**)	3	7.60 (s)	7.73	7.15 (s)	7.61 (s)	7.74	7.14 (s)
Victala (**4**)	2	4.38 (m)	?	4.66 (dd,6,6)	4.38 (br)	?	ca 4.7
	3	2.85 (2H,m)	2.95	2.74 (2H,m)	2.87 (2H,m)	?	2.77 (dd,15,6)
							2.70 (dd,15,7)
	6	4.45 (dd,7,2)	?	4.65 (dd,7,2)	4.45 (dd,7,2)	?	ca 4.6
	7	3.43 (dd,18,7)	3.33	2.9 (dd,18,7)	3.44 (dd,18,6)	3.35	2.98 (dd,18,6)
		2.72 (dd,18,2)	2.58	2.44 (dd,18,2)	2.70 (d,18)	2.55	2.43 (dd,18,2)

*D_2O/0.2% TFA solutions. δ values relative to external 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) = 0 ppm; in parentheses: number of hydrogens (if more than one), multiplicities (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad signal). J values in Hz, rounded to next full number. ** As discussed in the text some of the atoms 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'. *** Connectivities as obtained from COSY 2D spectra of **7** and **8** are indicated by lines linking the corresponding carbon Nos.

Table 2. ^{1}H-NMR data* of hydrolysis compounds from **9**

Subunit	Carbon No.	**11**	**12**	**13**	**15****
OHlys (**5**)	2		3.92 (m)	3.96 (m)	
	3		3.92 (m)	3.96 (m)	
	4		1.5–1.8 (2H,m)	1.6 (2H,m)	
	5		1.5–1.8 (2H,m)	1.75 (2H.m)	
				1.9 (2H,m)	
	6		3.02 (2H,m)	3.03 (2H,m)	
OHleu (**3**)	2	4.27 (d,6)	4.65 (d,7)	4.62 (d,6)	
	3	3.81 (dd,6,6)	3.77 (dd,9,4)	3.62 (dd,6,7)	
	4	2.10 (qqd,7,7,6)	1.9 (m)	1.9 (m)	
	5	1.16 (3H,d,7)	1.01 (3H,d,7)	0.99 (3H,d,7)	
	6	1.11 (3H,d,7)	0.91 (3H,d,7)	0.93 (3H,d,7)	
aClaa (**6**)	3	7.45 (s)	7.37 (s)		
Victala (**4**)	2	4.18 (dd,10,4)			4.60 (dd,8,5)
	3	2.65 (dd,15,4)			2,78 (dd,15,8)
		2.55 (dd,15,10)			2.72 (dd,15,5)
	6	4.45 (dd,7,2)			4.68 (dd,6,2)
	7	2.83 (dd,17,7)			2.95 (dd,18,6)
		2.29 (dd, 17,2)			2.40 (dd,18,2)

* For experimental conditions and abbreviations cf. table 1. ** The moiety generated from the aClaa unit is responsible for an AB-systems at δ 3.71 and 3.75 (J = 11.7 Hz).

These relationships were supported experimentally by submitting the individual compounds to the same hydrolytic conditions and observing the following transformations: **7**→**8**→**10** and **7**→**9**→**10**. A further important clue for the interpretation of these results was provided by the observation that the enzyme leucineaminopeptidase (Sigma, type VI) did not affect victorin C but caused a rapid transformation of **7** to **9** and of **8** to **10**. Upon prolonged contact with the enzyme **10** was converted further with release of **5** into the linear tripeptide **11** (mol. wt 433, $C_{17}H_{24}O_8N_3Cl$) containing the three fragments **3**, **4** and **6** (for ^{1}H-NMR spectrum cf. table 2). These results secure the partial structure NH_2-Cl_2leu-OHlys for desglyovictorin C, **7**; in addition, they prove that the Cl_2leu subunit is not involved in the formation of the macrocyclic ring and they point to the amino group of this subunit as the site for attachment of the glyoxylic acid moiety in the intact toxin. Further support for such

Table 3. ^{13}C-NMR data* of selected hydrolysis compounds from victorin C

Subunit	Carbon No.	**7**** Major	Minor	**8**	**9**** Major	Minor	**11**
Cl_2leu (**2**)	1	173.0***		173.2***			
	2	54.0		54.4			
	3	36.2		36.4			
	4	42.2		42.5			
	5	80.5		80.7			
	6	17.5		17.7			
OHlys (**5**)	1	172.7***		172.6***	?		
	2	61.1		61.1	60.7		
	3	72.8		73.0	72.3		
	4	25.7		26.0	25.9		
	5	32.6		32.6	32.4		
	6	42.0		42.0	41.8		
OHleu (**3**)	1	175.1***	176.0***	176.9***	?		170.0***
	2	56.9	58.2	58.4	57.1	58.2	58.0
	3	87.8	86.3	77.7	85.6	84.0	77.6
	4	34.7	31.4	32.3	31.3	34.8	32.4
	5	19.6	21.1	21.7	21.0	19.6	21.5
	6	19.0	16.9	17.7	16.7	19.0	19.1
aClaa (**6**)	1	164.8	162.0	166.7	?		166.5
	2	136.7	132.0	133.7	?		132.9
	3	134.5		128.0	135.6		129.2
Victala (**4**)	1	172.4***		174.3***	?		176.8***
	2	56.0	51.0	54.2	51.7	53.7	55.0
	3	24.7	26.0	25.7	27.0	25.3	25.3
	4	116.0	117.6	115.2	116.4	?	115.2
	5	209.0	208.8	202.0	205 ?	?	201.7
	6	72.3	72.7	73.0	72.3	?	71.0
	7	37.2	37.0	43.3	37.0	?	43.0
	8	187.8		197.1	194 ?		198.5

* Solutions in 150 µl D_2O/0.2% deut. TFA. δ values relative to external trimethylsilylpropionate (TSP) = 0 ppm.** Displaying dichotomous signals, cf. corresponding footnote in table 1. *** Tentative assignment.

an attachment is provided by the finding that the only ^{1}H-NMR signal affected to a major extent during the transformation of victorin C to the desglyo derivative **7** is the one belonging to C-2 of the Cl_2leu moiety, which shifts its position from δ 4.57 to 4.26.

Further and conclusive insight into the concatenation of the residual units in the linear peptide form was obtained by submitting the cyclic tetrapeptide **9** to partial hydrolysis (6N HCl, 60 °C, 15.5 h). Purification of the resulting mixture by HPLC afforded small amounts of **10**, **11**, and victalanin, **4**, as well as three new compounds which were analyzed by FAB-MS and by ^{1}H-NMR (cf. table 2). The first of these was a tripeptide, **12**, (mol. wt 394) embodying the three subunits **5**, **3**, and **6**. The second compound was identified as a dipeptide, **13**, built by the union of **5** and **3**. The third compound proved of particular interest; its mol. wt of 305 and the appearance of a strong $[M + H]^+ + 2$ ion in the FAB-MS spectrum pointed to the composition $C_{11}H_{12}O_7NCl$. The presence of a victala unit is certified by the characteristic UV absorption and by the appearance in the ^{1}H-NMR spectrum of signals closely matching the spectrum of **4**. In addition, an AB system at δ 3.71/3.75 (J = 11.7 Hz) is highly suggestive of the presence of a CH_2Cl group situated like the one in the hemiacetal of chloropyruvamide[10]. These stringent requirements are uniquely met by assigning structure **15** to the compound. Clearly, the open form of this degradation product must have arisen from the hydrolysis of an intermediate labile dipeptide, **14**, and the isolation of **15** therefore provides a welcome independent support for the structure of the fragment **6** which so far had escaped chemical detection. Combination of all the results from the partial hydrolysis experiments secure for the linear pentapeptide the sequence already indicated in **8**, and the order of the subunits in the degradation compounds **9**–**14** can be formulated accordingly.

Next, it was necessary to locate the bond in the macrocyclic ring which is severed during the generation of the *seco*-compounds **8** and **10** by hydrolysis of **7** and **9**, respectively. In this context the following facts are relevant: 1) victorin C contains a free carboxyl group, as evidenced by the formation of a monomethyl ester (apparent mol. wt 810; IR in KBr: sh at 1730 cm^{-1}; NMR: $-OCH_3$ signal at δ 3.68) upon treatment of the toxin with BF_3 in methanol; it can be concluded from the known sequence of the linear pentapeptide **8** that this carboxyl group belongs to the victala moiety; 2) the linear peptides **8**, **10** and **11**, all of which include the victala unit, display a characteristic UV behavior similar to the one of free victalanin, **4**, ($\lambda_{max} \cong 270$ nm in 0.01 N NH_4OH, shifting to $\lambda_{max} \cong 250$ nm upon addition of TFA), whereas no such shift is observed for the UV maximum at $\lambda = 256$ nm of victorin C and of its derivatives **7** and **9** which retain the macrocyclic ring; 3) victorin C, as well as **7** and **9**, displays characteristically dichotomous NMR signals for the atoms belonging to the OHleu, aClaa and victala moieties; in contrast, this dichotomy is absent in the spectra of the linear counterparts **8** and **10**; 4) when the NMR spectra of **7** and **9** are compared with those of their *seco* counterparts **8** and **10** (cf. tables 1 and 3) it is seen that cleavage of the macrocyclic ring affects significantly the chemical shift of dichotomous protons only and, specifically, causes a major upfield shift (ca 1 ppm) for the signal of the C-3 proton in the OHleu component, together with a 10 ppm upfield shift of the corresponding ^{13}C signal. It can be concluded from the combined evidence that the missing bond must link the C-3 oxygen of the OHleu unit with the chromophoric group of the victala component as indicated in the complete structure **16** for victorin C. An alternative mode of ring closure involving the tautomeric form of the victala chromophore would require a 1,3 rather than a 1,2 relationship between the hydroxyl group and the carbonyl group in the 5-membered ring; this possibility was dismissed after evaluation of a nuclear Overhauser difference spectrum of victorin C, in which a significant positive signal was observed at δ 3.38 for one of the diastereotopic protons of the critical methylene group[6] upon specific irradiation of the methyl group at δ 1.09 which belongs to the OHleu residue. The hitherto unexplained characteristic dichotomous NMR behavior exhibited by atoms associated with the 12-membered ring can now be rationalized by inspection of models in terms of the existence of two relatively stable conformers differing in the relative orientation of the two rigid ring segments (cf. heavy lines in **16**) which stretch clockwise from C-3 of OHleu to C-3 of victala and from C-2 of victala to C-2 of OHleu, respectively.

A final point in defining the structure of the intact toxin concerns the form and the mode of attachment of the glyoxylic acid residue which, as shown above, must be linked with the amino group of the Cl_2leu subunit. The NMR data of victorin C[6] are compatible only with the presence of a masked rather than a free aldehydo group in this compound; yet, attachment of the critical residue to the rest of the molecule cannot be mediated by the potential aldehydo group, or at least not by this group alone, since victorin C readily gives a 2,4-dinitrophenylhydrazone, mol. wt 976, shown by UV difference spectroscopy to retain the original chromophore. Independent evidence confirming that attachment of the glyoxylic acid residue involves formation of an amide bond was obtained as follows. Treatment of victorin C with $NaBH_4$ in buffered solution gave a reduced compound **17** (mol. wt 798, $C_{31}H_{45}O_{12}N_6Cl_3$) displaying in its ^{1}H-NMR spectrum a new signal at δ 4.13 (s, 2 H) for a $-CH_2OH$ group but lacking the victorin C signal at δ 5.36 which is characteristic for the masked aldehydo function; in the ^{13}C-NMR spectrum this change is manifested by the appearance of a new signal, δ 63.5, at the expense of the victorin C signal at δ 87.1. When a similar reaction was carried out in the presence of an excess of formaldehyde a different reduction compound, **18** (mol. wt 826, $C_{33}H_{49}O_{12}N_6Cl_3$) was formed, which contained two additional N-methyl groups responsible for a new NMR signal at δ 2.92 (s, 6 H). The intensities of this signal and of the signal for the $-CH_2OH$ group of **18** (δ 4.19, s, 2 H) were reduced according to expectations when the reaction was carried out with $NaBD_4$. Total hydrolysis of **18** gave, next to the known degradation products **2**–**4**, a new amino acid, mol. wt 190, which could be identified as the ε-N,N-dimethyl derivative of **5**. These results are best explained by the presence in **18** of an acyl group which protects the nitrogen function of the Cl_2leu component from reductive alkylation and is easily removable by acid hydrolysis. In the event, conclusive proof for this view was obtained by showing that victorin C displaying full

biological activity can be regenerated from the non toxic desglyo-compound **7** and glyoxylic acid using the N-hydroxysuccinimide acylation method[11]. Resynthesis of the natural toxin from **7** proves that the necessary masking of the aldehydo group is a spontaneous process. Possible candidates fitting this requirement are a hemiacetal generated by intramolecular addition of an appropriate nucleophilic center and the hydrate indicated in formula **16**[12]. Though only the former possibility can be reconciled with the putative mol. wt of 796, it was, paradoxically, in favor of the alternative hydrate form **16** that subsequent evidence could be obtained. Thus, addition of small amounts of CH_3OD to a D_2O solution of the toxin caused the progressive disappearance of the NMR signal at δ 5.37 coupled with the appearance of a new signal of complementary intensity at δ 5.06. This behavior parallels that of glyoxylic acid under the same conditions and testifies for the formation of increasing amounts of the hemiacetal **19**; moreover, it is interesting to note that in a mixed methanol-water system with both glyoxylic acid and victorin C addition of methanol is favored approximately 20-fold on a molar basis.

The structure so established for victorin C requires a mol. wt 814 which is 18 units higher than the apparent one originally assigned to the compound. Indeed, corresponding m/z peaks of weak intensity had been detected, along with many others, in the FAB spectra of the toxin when using a glycerol matrix, but were then discarded from further consideration since they could not be observed when the spectra were measured in the NOBA matrix[6]. Reinvestigation of the problem using a more refined scanning technique revealed that with both the glycerol and the NOBA matrix the appearance of the peak of the protonated hydrate form, m/z 815, is critically dependent on time and concentration of the sample in the matrix. For dilute samples in NOBA when the normal conditions of maximum ion flux are reached the intensity of this peak has already decreased to a large extent with concomitant increase of the peaks at m/z 950 (protonated adduct from aldehyde and matrix) and at m/z 797 (protonated aldehyde). Apparently, water is quickly replaced by NOBA under the experimental conditions and the aldehyde ion is easily formed from the matrix adduct by desolvation, as certified by a metastable peak at m/z 668,6 for the corresponding transition. With this additional insight corroborative clues could be obtained in favor of a mol. wt of 814 for the toxin as required by the hydrate form **16**. A concentrated solution of a thoroughly dried sample of victorin C in 99% enriched $H_2{}^{18}O$ was analyzed by multiscan FAB-MS and it was found that both in the glycerol and in the NOBA matrix the $[M + H]^+$ peaks of the aldehydo and the hydrate forms were shifted by two and by four mass units, respectively; when a 50% enriched $H_2{}^{18}O$ solution was used, the predicted oxygen distribution patterns (1:1 for the aldehydo form, 1:2:1 for the hydrate form) could be discerned in the spectrum, notwithstanding complications arising from the superimposed isotope pattern due to the presence of the 3 Cl atoms. It is worthwhile stressing that the mol. wt revision imposed by the new evidence merely reflects the addition of H_2O to a carbonyl group and, therefore, does not invalidate the arguments which were based on the apparent mol. wt for deriving the cyclic nature of the compound.

While more work is needed to settle some of the remaining stereochemical ambiguities, it is felt that the establishment of structure **16** for victorin C paves the way for the chemical study of its minor companions[6] and for a rational approach towards the understanding of its biological mode of action.

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Abbreviations used: FAB, fast atom bombardement; NOBA, 3-nitro-benzylalcohol; DMSO, dimethylsulfoxide; TFA, trifluoroacetic acid.

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