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PLANT PATHOTOXINS FROM *ALTERNARIA CITRI*: THE MINOR ACRL TOXINS*

Y. KONO, J. M. GARDNER†‡, Y. SUZUKI and S. TAKEUCHI

The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan; ‡Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, U.S.A.

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Key Word Index—*Citrus jambhiri*; *Citrus limonia*; Rutaceae; rough lemon; Rangpur lime; ACRL-toxin; *Alternaria citri*; *Alternaria alternata* pv. *citri*; brown spot disease; α-pyrone; polyalcohol.

Abstract—Five host-specific pathotoxins, ACRL toxins II, III, III', IV and IV', were isolated from the culture broth of *Alternaria citri*, the fungus causing brown spot disease of rough lemon. These toxins are related structurally to the major ACRL toxin, toxin I, and to its derivative compound A. Chemical and spectral studies indicated that the ACRL minor toxins were a group of analogous compounds of different chain lengths all of which have a α-pyrone group, in contrast to the dihydro-α-pyrone group in toxin I. Toxin II showed a very low biological activity (ED_{50} greater than 10 μg/ml) whereas the other minor toxins had slightly higher activities ranging from 1 to 10 μg/ml. The dihydropyrone group in ACRL toxin I was correlated with high biological activity (ED_{50} = 18–30 ng/ml).

INTRODUCTION

The phytopathogenic fungus *Alternaria citri*, the causal agent causing brown spot disease of citrus, produces several host-specific toxins which specifically damage leaves of rough lemon and Rangpur lime [1, 2]. The major and most active component, ACRL toxin I, has been characterized in the previous paper [3]. In order to understand the structural parameters affecting biological activity of ACRL toxin, we have characterized five structurally analogous compounds having much less biological activity than toxin I. These minor toxins differ from each other and toxin I in chainlength and from toxin I with respect to the pyrone ring structure. In this paper, we present the complete details for the assignment of these structures and their biological activities.

RESULTS AND DISCUSSION

Methods of fungus culture and preliminary fractionation procedures were as previously described for toxin I [3]. For a typical batch representing 2–4 l. of culture filtrate, the minor ACRL toxins were isolated by in the final step by TLC ($CHCl_3$–MeOH, 85:15) in the following relative amounts: toxin II (20 mg), toxin III (12 mg), toxin III' (2 mg), toxin IV (5 mg) and toxin IV' (2 mg). UV spectra of these bands showed common absorption maxima at approximately 277 to 280 nm in methanol, which was shifted to about 285 nm after acidification. Each purified toxin was then methylated with diazomethane and each of the major methylated products were repurified by TLC. Properties and formulae of the minor toxins are listed in Table 1. Molecular formulae of the purified toxins and/or their derivatives were determined

*Part 2 in this series.

†Present address: Crop Science Laboratory, Allied Corp., P. O. Box 6, Solvay, NY 13209, U.S.A.

Table 1. Summary of formulas and properties of the major and minor toxins

Toxin	Formula (M_r)*	mp (°)	$[\alpha]_D$†	R_f‡	UV§ λ_{max} (nm)
I	$C_{19}H_{30}O_6$ (354)	192–196	—	—	245 (285)
II	$C_{17}H_{24}O_5$ (308)	67–68	+5	0.46	277 (240)
III	$C_{19}H_{28}O_6$ (352)	142–145	−17	0.34	280 242)
III'	$C_{18}H_{26}O_6$ (338)	—	+3	0.31	281 (242)
IV	$C_{21}H_{32}O_7$ (396)	102–103	−11	0.30	280 (242)
IV'	$C_{20}H_{30}O_7$ (382)	—	−20	0.17	280 (242)

*Determined by FD-MS and/or FAB-MS.

†Optical dispersion measurements at *ca* 2.5 mg/ml.

‡R_f of monomethylated ethers in $CHCl_3$–MeOH (9:1).

§UV λ_{max} of predominant tautomer (dihydro-α-pyrone for I, α-pyrone for II–IV). Approx. λ_{max} of γ-isomers in parentheses.

Table 2. Molecular ions of ACRL minor toxin derivatives

Toxin	Derivative*	Formula	Molecular ion†	
			Expected	Observed
II	IIc	$C_{22}H_{30}O_7$	406	406[1], 407 $[M+H]^{+\cdot}$[2]
II	IId	$C_{32}H_{34}O_7$	530.2285	530.2302[1]
III	IIIb	$C_{20}H_{30}O_6$	366	367 $[M+H]^{+\cdot}$[2]
III	IIIc	$C_{28}H_{35}O_7B$	494.2462	494.2474[1]
III′	IIIe	$C_{19}H_{28}O_6$	352	375 $[M+Na]^{+\cdot}$[2]
III′	IIIf	$C_{27}H_{33}O_7B$	480.2346	470.2317[1]
IV	IVb	$C_{22}H_{34}O_7$	410	433 $[M+Na]^{+\cdot}$[2]
IV	IVc	$C_{32}H_{41}O_9B$	580.2817	580.2841[1]
IV′	IVe	$C_{21}H_{32}O_7$	396	419 $[M+Na]^{+\cdot}$[2]
IV′	IVf	$C_{31}H_{39}O_9B$	566.2710	566.2686[1]

*Refer to Fig. 2 for description of derivatives.
†Method used for mass spectra: [1]EI-MS; [2]FD-MS.

by MS (HR-MS and FD-MS) (Table 2).

Each of the minor toxins were assayed by inhibition of proline incorporation into leaf discs. Biological activities of all five toxins were approximately two orders of magnitude less active than Band I toxin [3] with ED_{50} (50% inhibition) values in the range of 1 μg/ml to greater than 10 μg/ml. None of the toxins affected nonhost (*C. reticulata* Blanco) tissues at 100 μg/ml. Results with toxin III, which was determined below to be identical in structure with toxin I except for unsaturation at the 5,6-position in the pyrone ring, indicated that biological activity was dependent on the reduced form of the pyrone ring. Although chainlength may have had some influence on biological activity since the 17-carbon toxin II had very low activity, there seemed to be no correlation between activity and chainlength from 17 to 21 carbons within the minor toxin group.

The methyl ethers of each of the toxins were produced as described for toxin I [3] and were analysed by ^{1}H NMR and ^{13}C NMR spectroscopy. Each of the methylated toxins showed many signals in common with ACRL toxin I and compound A. These common proton signals (Table 3) were found to be approximately identical to those assigned to the first 7-carbon chain from the left side (Fig. 1A); the respective ^{13}C NMR signals (Table 4) supported this observation. The first 7-carbon chain was designated partial structure A for simplicity (Fig. 1).

Another set of signals were assigned to a pyrone ring structure which was similar but not identical to that previously documented for toxin I [3]. Signals of two olefinic proton nuclei with meta coupling were observed for each toxin between δ5.42 (H-3″) and 5.96 (H-5″). Also, ^{13}C NMR spectra showed two corresponding olefinic methine carbons at approximately δ87.9 (C-3″) and 101.8 (C-5″) and three signals corresponding to quaternary carbon nuclei were observed between δ162 and 173. This indicated the presence of a methoxy-α-pyrone group for each of the methylated toxins, designated as partial structure B in Fig. 1, by comparison with NMR signals for authentic 4-methoxy-6-methyl-α-pyrone [4].

The remaining proton and carbon signals were assigned to the variable portions of the minor toxins as designated by partial structures C through E (Fig. 1). Based on proton decoupling experiments, these structures were located between partial structures A and B and were unique for each of the five minor toxins. For ACRL toxin II (IIb), methine signals at δ4.33 (1H, *dd*, J = 8 Hz, H-8) and 2.64 (1H, *m*, J = 8, 7 Hz, H-9) and a methyl signal at 1.18 (3H, *d*, J = 7 Hz, H-9) indicated the partial structure C (Fig. 1). Likewise, ACRL toxin III (IIIb) had methine signals at δ4.10 (1H, *dd*, H-8), 1.71 (1H, *m*, H-9) and 4.07 (1H, *m*, H-10), methylene signals at 2.48 (1H, ABX, H-11) and 2.80 (1H, ABX, H-11), and a methyl signal at 0.85 (3H, *d*, H-9) indicating partial structure D. For ACRL toxin III′ (IIIe), partial structure E was derived from methine signals at δ4.41 (1H, *m*, H-8), and 4.32 (1H, *m*, H-10), and methylene signals at 1.55–1.77 (2H, H-9), 2.58 (1H, ABX, H-11) and 2.61 (1H, ABX, H-11). The partial structures F and G for toxins IV and IV′, respectively, were likewise obtained by ^{1}H NMR and ^{1}H decoupling data (see Experimental). These partial structures were each supported by ^{13}C NMR spectra (Table 4).

Derivatives of each of the toxins were made by methylation, acetylation and phenylboration, or a combination of each. Assignments of derivatives were made by ^{1}H NMR as shown in Fig. 2 and molecular ions determined by MS (Table 2; Experimental). These data confirmed the proposed structures.

Tautomerization of the pyrone ring was apparent during isolation and characterization of the toxins, therefore necessitating methylation with diazomethane. For example, a minor product of toxin II (IIb′) was isolated from the reaction mixture by TLC (silica gel, $CHCl_3$–MeOH, 95:5, R_f = 0.20) and characterized. Its UV absorption maximum was at 240 nm (ε = 5800) in contrast to the UV maximum of the major isomer (280 nm). ^{1}H NMR spectra (400 MHz, $CDCl_3$) of IIb′ showed essentially the same signals (24 protons) as those of the major isomer, and decoupling experiments were also identical for IIb and IIb′. The results indicated that IIb′ was indeed a tautomer of IIb resulting from a keto-enol shift. The UV maxima and IR absorptions were compared to those for authentic pyrones and the results indicated that IIb was a 4-methoxy-α-pyrone and IIb′ a 2-methoxy-γ-pyrone [5]. Tautomers of nonmethylated toxins could be separated by HPLC (CN normal phase column) as indicated by UV maxima of individual peaks; however, instability was a problem in further analysis of these compounds. Methylation of the four other minor toxins produced minor products having 2-methoxy-γ-

Table 3. ^{1}H NMR spectral data of minor ACRL-toxins (methylated Toxins II, III, III', IV, IV') [400 MHz, $CDCl_3$, δ (ppm)]

Compounds		IIb	IIIb	IIIe	IVb	IVe
1	CH_3	1.62 *d*	1.63 *d*	1.63 *d*	1.63 *d*	1.62 *d*
2	CH	5.47 *q*	5.47 *q*	5.47 *q*	5.48 *q*	5.48 *q*
3	C					
3'	CH_3	1.60 *s*	1.60 *s*	1.60 *s*	1.60 *s*	1.61 *s*
4	CH–OH	3.67 *d*	3.66 *d*	3.66 *d*	3.67 *d*	3.67 *d*
5	CH	2.35 *m*	2.33 *m*	2.31 *m*	2.34 *m*	2.32 *m*
5'	CH_3	0.86 *d*	0.86 *d*	0.85 *d*	0.86 *d*	0.84 *d*
6	CH	5.67 *dd*	5.62 *dd*	5.64 *dd*	5.62 *dd*	5.64 *dd*
7	CH	5.55 *dd*	5.57 *dd*	5.57 *dd*	5.58 *dd*	5.61 *dd*
8	CH–OH	4.33 *dd*	4.10 *dd*	4.41 *dd*	4.03 *dd*	4.42 *dd*
9	CH	2.64 *m*	1.71 *m*	1.55–1.71 *m** (2H)	1.65 *m*	1.55–1.70 *m* (2H)*
9'	CH_3	1.18	0.85 *d*		0.78 *d*	—
10	CH–OH	—	4.07 *m*	4.32 *m*	3.94 *m*	4.19 *m*
11	CH_2	—	2.48 ABX	2.58 ABX	1.53 ABX	1.55 ABX
			2.80 ABX	2.61 ABX	1.75 ABX	1.70 ABX
12	CH–OH	—	—	—	4.29 *m*	4.31 *m*
13	CH_2	—	—	—	2.59 *d* (2H)	2.56 *d* (1H)
6"†	C					
5"	CH	5.88 *d*	5.96 *d*	5.91 *d*	5.95 *d*	5.92 *d*
4"	O C–OCH_3	3.79 *s*	3.79 *s*	3.80 *s*	3.79 *s*	3.79 *s*
3"	CH	5.42 *d*	5.42 *d*	5.43 *d*	5.43 *d*	5.42 *d*
2"	C=O					

*Tentative assignment.

†Ring carbons numbered separately for convenience.

pyrone structures as evidenced by UV spectra (λ_{max} = 241–242 nm). The effect of tautomerization on biological activity was not possible to assess due to stability problems and because the stabilized methyl ethers were inactive. Aside from the tautomerization, the methylated toxins were reasonably stable structurally and biologically as observed for toxin I [3].

Yet to be answered are the questions about the biosynthetic origins of each of the toxins and the possibility that conversions among the toxins can occur under certain conditions. Other minor toxins may well be present and their relative amounts may be affected by choice of culture media and other conditions.

EXPERIMENTAL

Analytical methods and general toxin isolation procedures were performed as previously described [3]. The pH 3.5 EtOAc extract after XAD-7 desorption contained most of the minor toxins. Batches of concd toxin extracts (800 mg from 2 to 4 l. of culture filtrate) were purified by chromatography on small columns of silica gel and fractions were separated by TLC (Merck silica gel 254; $CHCl_3$–MeOH, 85:15). From these preparations, five minor ACRL toxins were purified as follows: toxin II (18–25 mg, R_f 0.30), toxin III (10–12 mg, R_f 0.22), toxin III" (1–3 mg, R_f 0.24), toxin IV (3–6 mg, R_f 0.17) and toxin IV' (1–3 mg, R_f 0.13). The ^{14}C-proline incorporation assay [3] was used to determine their biological activities.

Methyl ester and acetate derivatives were prepared as described [3]. Phenylboronic acid (3–5 mg) was added to methylated toxins in C_5H_5N at 0°. Solns were stirred at 4° for 5 hr, evaporated *in vacuo* and purified by TLC (C_6H_6–EtOAc, 8:2). These compounds were acetylated and purified by TLC (C_6H_6–EtOAc, 9:1).

Properties of the minor toxins and their derivarives, are listed below. (Refer to Fig. 2 for designations of derivatives, i.e. IIa, IIb, etc.)

Toxin II (IIa underivatized). $C_{17}H_{24}O_5$, M_r 308, mp 67–68°. FD-MS and FAB-MS $[M+Na]^+$, *m/z* 331. UV λ_{max}^{MeOH} 277 nm (ε = 3100); (0.1 N HCl–MeOH, 285 nm). $[\alpha]_D$ +5 (*c* 0.25; MeOH). ^{1}H NMR [400 MHz, Me_2CO-d_6, *J* (Hz)]: δ 1.58 (3H, *d*, 7 Hz), 5.40 (1H, *q*, 7), 1.57 (3H, *s*), 3.65 (1H, *d*, 9), 2.30 (1H, *m*, 9, 8, 7), 0.85 (3H, *d*, 7), 5.72 (1H, *dd*, 15, 8), 5.51 (1H, *dd*, 15, 8), 4.22 (1H, *dd*, 8, 8), 2.63 (1H, *m*, 8, 7), 1.14 (3H, *d*, 7), 5.92 (1H, *d*, 2; *meta* coupling) and 5.27 (1H, *d*, 2; *meta* coupling). ^{13}C NMR (22.5 MHz, Me_2CO-d_6): δ 13.0 (*q*), 121.5 (*d*), 138.0 (*s*), 11.6 (*q*), 82.1 (*d*), 41.2 (*d*), 17.6 (*q*), 137.0 (*d*), 132.0 (*d*), 75.1 (*d*), 45.9 (*d*), 14.7 (*q*), 169.5 (*s*), 100.7 (*d*), 170.9 (*s*), 89.9 (*d*) and 165.0 (*s*).

Toxin II (IIb; 4-methoxy-α-pyrone group). UV λ_{max}^{MeOH} 282 nm (ε = 5700). ^{1}H NMR [400 MHz, $CDCl_3$, *J* (Hz)]: δ 1.62 (3H, *d*, 7 Hz), 5.47 (1H, *q*, 7), 1.60 (3H, *s*), 3.67 (1H, *d*, 8), 2.35 (1H, *m*, 8, 7, 7), 0.86 (3H, *d*, 7), 5.67 (1H, *dd*, 15, 7), 5.55 (1H, *dd*, 15, 8), 4.33 (1H, *dd*, 8, 8), 2.64 (1H, *m*, 8, 7), 1.18 (3H, *d*, 7), 5.88 (1H, *d*, 2; *meta* coupling), 3.79 (3H, *s*) and 5.42 (1H, *d*, 2; *meta* coupling).

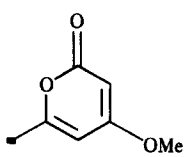
Toxin II (IIc). $C_{22}H_{30}O_7$, M_r 406. EI-MS $[M]^+$, *m/z* 406; FD-MS $[M+H]^+$, *m/z* 407. UV λ_{max}^{MeOH} 279 nm (ε = 5300).

Table 4. ^{13}C NMR of minor ACRL-toxins (methylated toxins II, III, III', IV, IV' (22.5 MHz, $CDCl_3$, ppm)

Compounds		IIb	IIb	IIIe	IVb
1	CH_3	13.0*	13.2*	12.9*	12.9*
2	CH	123.1	123.1	123.2	123.2
3	C	136.0	135.8	135.7	135.8
3'	CH_3	10.7*	10.4*	10.4*	10.4*
4	CH–OH	81.9	82.1	82.0	82.1
5	CH	40.5	40.3	40.1	39.7
5'	CH_3	17.2	17.1	16.9	17.2
6	CH	137.2	136.4	134.3	136.3
7	CH	131.4	132.7	133.5	133.1
8	CH–OH	74.4	77.7	72.6	78.3
9	CH	44.3	43.3	42.7* (CH_2)	43.8
9'	CH_3	14.6	12.9	—	12.9
10	CH–OH	—	72.6	68.4	75.2
11	CH_2	—	39.3	41.8*	38.4*
12	CH–OH	—	—	—	67.5
13	CH_2	—	—	—	40.4*
6''	C	166.6*	163.1*	162.2*	162.5*
5''	CH	100.7	101.8	101.8	101.7
4	C	171.4*	171.3*	172.5*	171.0*
O	OCH_3	55.7	55.7	55.7	55.7
3''	CH	87.9	87.7	87.8	87.7
2''	C=O	162.6*	165.1*	165.1*	165.1*

*Tentative assignment.
Ring carbons numbered separately for convenience.

A $Me^{1}CH^{2}{=}C^{3}(Me)CH^{4}(OH)CH^{5}(Me)CH^{6}{=}CH^{7}$—■

B 

C ■—$CH^{8}(OH)CH^{9}(Me)$—■

D ■—$CH^{8}(OH)CH^{9}(Me)CH^{10}(OH)CH^{11}_2$—■

E ■—$CH^{8}(OH)CH^{9}_2CH^{10}(OH)CH^{11}_2$—■

F ■—$CH^{8}(OH)CH^{9}(Me)CH^{10}(OH)CH^{11}_2CH^{12}(OH)CH^{13}_2$—■

G ■—$CH^{8}(OH)CH^{9}_2CH^{10}(OH)CH^{11}_2CH^{12}(OH)CH^{13}_2$—■

Fig. 1. Partial structures for Toxins II (A, B and C), III (A, B and D), III' (A, B and E), IV (A, B and F) and IV' (A, B and G).

^1H NMR [400 MHz, $CDCl_3$; J (Hz)]: δ1.60 (3H, *d*, 7), 5.50 (1H, *q*, 7), 1.57 (3H, *s*), 4.92 (1H, *d*, 9), 2.49 (1H, *m*, 9, 7, 7), 0.88 (3H, *d*, 7), 5.70 (1H, *dd*, 15, 7), 5.36 (1H, *dd*, 15, 9), 5.32 (1H, *dd*, 9, 8), 2.80 (1H, *m*, 8, 7), 1.17 (3H, *d*, 7), 5.81 (1H, *d*, 2; *meta* coupling), 3.80 (3H, *s*), 5.41 (1H, *d*, 2; *meta* coupling), 1.98 (3H, *s*) and 1.99 (3H, *s*).

Toxin II (*IId*). $C_{32}H_{34}O_7$, M_r 530. HR-MS, obs. 530.2285, calc. 530.2302. EI-MS, $[M]^{+\cdot}$, m/z 530. UV λ_{max}^{MeOH} 278 (ε = 4900) and 229 nm (ε = 15400). CD 280 nm ($\Delta\varepsilon$ = −4.8) and 225 nm ($\Delta\varepsilon$ = +14.5) in MeOH. ^1H NMR [400 MHz, $CDCl_3$; J (Hz)]: δ1.62 (3H, *d*, 7), 5.58 (1H, *q*, 7), 1.59 (3H, *s*), 5.18 (1H, *d*, 9), 2.67 (1H, *m*, 9, 7, 7), 0.97 (3H, *d*, 7), 5.89 (1H, *dd*, 15, 7), 5.55 (1H, *dd*, 15, 9), 5.52 (1H, *dd*, 9, 8), 2.91 (1H, *m*, 8, 7), 1.16 (3H, *d*, 7), 5.81 (1H, *d*, 2; *meta* coupling), 3.73 (3H, *s*) and 5.34 (1H, *d*, 2; *meta* coupling); 7.3–8.0 (phenyl).

Toxin II (*IIb'*) (*2-methoxy-γ-pyrone*). UV λ_{max}^{MeOH} 240 nm (ε = 5800). ^1H NMR [400 MHz, $CDCl_3$; J (Hz)]: δ1.62 (3H, *d*, 7), 5.47 (1H, *q*, 7), 1.60 (3H, *s*), 3.67 (1H, *d*, 8), 2.35 (1H, *m*, 8, 7, 7), 0.85 (3H, *d*, 7), 5.68 (1H, *dd*, 15, 7), 5.55 (1H, *dd*, 15, 8), 4.20 (1H, *dd*, 8, 8), 2.68 (1H, *m*, 8, 7), 1.18 (3H, *d*, 7), 6.07 (1H, *d*, 2; *meta* coupling), 5.47 (1H, *d*, 2; *meta* coupling) and 3.87 (3H, *s*).

Toxin III (*IIIa, underivatized*). $C_{19}H_{28}O_6$, M_r 352. Mp 142–145°. $[\alpha]_D$ −17 (*c* 0.60; MeOH). ^1H NMR [400 MHz, Me_2CO-d_6; J (Hz)]: δ1.59 (3H, *d*, 7), 5.40 (1H, *q*, 7), 1.58 (3H, *s*), 3.64 (1H, *d*, 8), 2.31 (1H, *m*, 9, 8, 7), 0.85 (3H, *d*, 7), 5.69 (1H, *dd*, 16, 9), 5.55 (1H, *dd*, 16, 8), 4.19 (1H, *dd*, 8, 8), 1.72 (1H, *m*, 8, 8, 7), 0.88 (3H, *d*, 7), 4.08 (1H, *m*, 8, 8, 7), 2.46 (1H, ABX, 15, 8), 2, 73 (1H, ABX, 15, 5), 6.02 (1H, *d*, 2; *meta* coupling) and 5.28 (1H, *d*, 2; *meta* coupling).

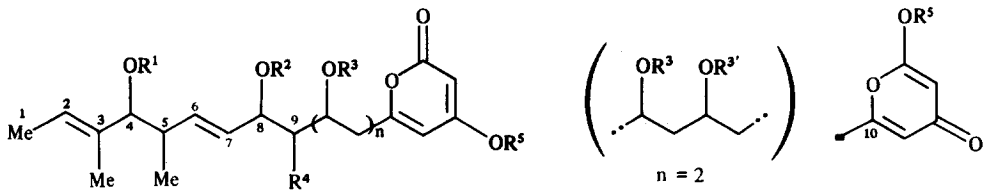
Toxin III (*IIIb; 4-methoxy-α-pyrone group*). $C_{20}H_{30}O_6$, M_r 366. FD-MS $[M+Na]^{+\cdot}$, m/z 367. UV λ_{max}^{MeOH} 280 nm (ε = 3500). ^1H NMR [400 MHz, $CDCl_3$, J (Hz)]: δ1.63 (3H, *d*, 7), 5.47 (1H, *q*, 7), 1.60 (3H, *s*), 3.66 (1H, *d*, 8), 2.33 (1H, *m*, 9, 8, 7), 0.86 (3H, *d*, 7), 5.62 (1H, *dd*, 16, 9), 5.57 (1H, *dd*, 16, 8), 4.10 (1H, *dd*, 8, 8), 1.71 (1H, *m*, 8, 8, 7), 0.85 (3H, *d*, 7), 4.07 (1H, *m*, 8, 8, 5), 2.48 (1H, ABX, 15, 8), 2.80 (1H, ABX 15, 5), 5.96 (1H, *d*, 2; *meta* coupling), 3.79 (3H, *s*), and 5.42 (1H, *d*, 2; *meta* coupling). ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 4.

Toxin III (*IIIc*). $C_{28}H_{35}O_7B$, M_r 494. HR-MS obs. 494.2462, calc. 494.2474. EI-MS $[M]^{+\cdot}$, m/z 494. ^1H NMR [400 MHz, $CDCl_3$, J (Hz)]: δ1.64 (3H, *d*, 7), 5.55 (1H, *q*, 7), 1.63 (3H, *s*), 5.01 (1H, *d*, 8), 2.55 (1H, *m*, 9, 8, 7), 0.94 (3H, *d*, 7), 5.69 (1H, *dd*, 16, 9), 5.46 (1H, *dd*, 16, 8), 4.12 (1H, *dd*, 10, 8), 1.65 (1H, *m*, 10, 10, 7), 0.98 (3H, *d*, 7), 4.16 (1H, *m*, 10, 8, 4), 2.54 (1H, ABX, 15, 8), 2.97 (1H, ABX,15, 4), 6.05 (1H, *d*, 2; *meta* coupling), 3.83 (3H, *s*), 5.47 (1H, *d*, 2; *meta* coupling) 2.07 (3H, *s*, acetyl) and 7.2–8.0 (5H, phenyl).

Toxin III' (*IIId, underivatized*). $[\alpha]_D$ +3 (*c* 0.20; MeOH); ^1H NMR [400 MHz, Me_2CO-d_6, J (Hz)]: δ1.59 (3H, *d*, 7), 5.40 (1H, *q*, 7), 1.58 (3H, *s*), 3.62 (1H, *d*, 9), 2.30 (1H, *m*), 0.82 (3H, *d*, 7), 5.73 (1H, *dd*, 15, 8), 5.57 (1H, *dd*, 15, 6), 4.43 (1H, *m*, 6, 6, 6), 1.50–1.72 (2H, *m*), 4.20 (1H, *m*), 2.60 (1H, ABX, 14, 4), 2.65 (1H, ABX, 14, 3), 6.00 (1H, *d*, 2; *meta* coupling) and 5.27 (1H, *d*, 2; *meta* coupling).

Toxin III' (*IIIe; 4-methoxy-α-pyrone*). $C_{19}H_{28}O_6$, M_r 352. FD-MS, $[M+Na]^{+\cdot}$, m/z 375. UV λ_{max}^{MeOH} 281 nm (ε = 4100). ^1H NMR [400 MHz, $CDCl_3$, J (Hz)]: δ1.62 (3H, *d*, 7), 5.47 (1H, *q*, 7), 1.60 (3H, *s*), 3.66 (1H, *d*, 8), 2.31 (1H, *m*, 9, 8, 7), 0.85 (3H, *d*, 7), 5.64 (1H, *dd*, 15, 9), 5.57 (1H, *dd*, 15, 8), 4.41 (1H, *m*, 8, 8, 5), 1.55–1.71 (2H, *m*), 4.32 (1H, *m*), 2.58 (1H, ABX), 2.61 (1H, ABX), 5.91 (1H, *d*, 2; *meta* coupling), 3.80 (3H, *s*) and 5.43 (1H, *d*, 2; *meta* coupling). ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 4.

Toxin III' (*IIIf*). $C_{27}H_{33}O_7B$, M_r 480. HR-MS, obs. 480.2346, calc. 480.2317. ^1H NMR (400 MHz, $CDCl_3$: J (Hz)]: δ1.62 (3H, *d*, 7), 5.56 (1H, *q*, 7), 1.61 (3H, *s*), 4.98 (1H, *d*, 9), 2.50 (1H, *m*, 9, 8, 7), 0.87 (3H, *d*, 7), 5.70 (1H, *dd*, 16, 8), 5.55 (1H, *dd*, 16, 7), 4.56 (1H, *m*), 1.7–2.0 (2H), 4.55 (1H, *m*), 2.70 (1H, ABX, 15, 5), 2.77 (1H, ABX, 15, 7), 6.01 (1H, *d*, 2; *meta* coupling), 3.83 (3H, *s*) and 5.47 (1H, *d*, 2;



		R^1	R^2	R^3	$R^{3'}$	R^4	R^5 4 - keto isomer
n = 0	IIa (Toxin II)	H	H	—	—	Me	H
	IIb	H	H	—	—	Me	Me (IIb′)
	IIc	Ac	Ac	—	—	Me	Me
	IId	Bz	Bz	—	—	Me	Me
n = 1	IIIa (Toxin III)	H	H	H	—	Me	H
	IIIb	H	H	H	—	Me	Me(IIIb′)
	IIIc	Ac	B - Ph*		—	Me	H
	IIId (Toxin III′)	H	H	H	—	H	H
	IIIe	H	H	H	—	H	Me (IIe′)
	IIIf	Ac	B - Ph*		—	H	H
n = 2	IVa (Toxin IV)	H	H	H	H	Me	H
	IVb	H	H	H	H	Me	Me (IVb′)
	IVc	Ac	B - Ph*		Ac	Me	Me
	IVd (Toxin IV′)	H	H	H	H	H	H
	IVe	H	H	H	H	H	Me (IVe′)
	IVf	Ac	B - Ph*		Ac	H	Me
	IVf′	Ac	Ac	B - Ph*		H	Me

*phenylboronate

Fig. 2. Structures of Toxins II, III, III′, IV and IV′ and their derivatives.

meta coupling); 2.07 (3H, *s*, acetyl) and 7.2–8.0 (5H, phenyl).

Toxin IV (*IVa, underivatized*). $C_{21}H_{32}O_7$, M_r 396. Mp 102–105°. $[\alpha]_D - 11$ (*c* 0.30; MeOH). ^{1}H NMR [400 MHz, Me_2CO-d_6; *J* (Hz)]: δ1.59 (3H, *d*, 7), 5.40 (1H, *q*, 7), 1.58 (3H, *s*), 3.64 (1H, *d*, 8), 2.31 (1H, *m*, 9, 8, 7), 0.85 (3H, *d*, 7), 5.66 (1H, *dd*, 16, 9), 5.53 (1H, *dd*, 16, 8), 4.05 (1H, *dd*, 8, 8), 1.65 (1H, *m*, 10, 8, 7), 0.80 (3H, *d*, 7), 3.99 (1H, *m*, 10, 7, 3), 1.82 (1H, ABX_n, 15, 4, 3), 1.55 (1H, ABX_n, 15, 8, 7), 4.22 (1H, *m*, 8, 8, 4, 4), 2.53 (1H, ABX, 15, 8), 2.65 (1H, ABX, 15, 4), 6.02 (1H, *d*, 2; *meta* coupling) and 5.28 (1H, *d*, 2; *meta* coupling).

Toxin IV (*IVb; 4-methoxy-α-pyrone*). $C_{22}H_{34}O_7$, M_r 410. FD-MS $[M+Na]^+$, *m/z* 433. UV λ_{max}^{MeOH} 281 nm (ε = 5600). ^{1}H NMR [400 MHz, $CDCl_3$; *J* (Hz)]: δ1.63 (3H, *d*, 7), 5.48 (1H, *q*, 7), 1.60 (3H, *s*), 3.67 (1H, *d*, 9), 2.34 (1H, *m*, 9, 7, 7), 0.86 (3H, *d*, 7), 5.62 (1H, *dd*, 14, 7), 5.58 (1H, *dd*, 14, 8), 4.03 (1H, *dd*, 8, 8), 1.65 (1H, *m*, 8, 8, 7), 0.78 (1H, *d*, 7), 3.94 (1H, *m*, 8, 8, 3), 1.53–1.75 (2H, *m*), 4.29 (1H, *m*), 2.59 (2H, *d*, 6), 5.95 (1H, *d*, 2; *meta* coupling), 3.79 (3H, *s*) and 5.43 (1H, *d*, 2; *meta* coupling). ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 4.

Toxin IV (*IVc*). $C_{32}H_{41}O_9B$, M_r 580. HR-MS obs. 580.2817, calc. 580.2841. EI-MS $[M]^+$, *m/z* 580. ^{1}H NMR [400 MHz, $CDCl_3$; *J* (Hz)]: δ1.63 (3H, *d*, 7), 5.54 (1H, *q*, 7), 1.62 (3H, *s*), 5.01 (1H, *d*, 9), 2.55 (1H, *m*, 9, 8, 7), 0.95 (3H, *d*, 7), 5.67 (1H, *dd*, 16, 8), 5.46 (1H, *dd*, 16, 7), 4.10 (1H, *dd*, 10, 7), 1.65 (1H, *m*, 10, 10, 7), 0.91 (3H, *d*, 7), 3.97 (1H, *m*, 10, 8, 3), 1.87 (1H, ABX_n, 15, 8, 3), 2.14 (1H, ABX_n, 15, 8, 5), 5.55 (1H, *m*, 8, 7, 5, 5), 2.87 (1H, ABX, 18, 7), 2.98 (1H, ABX, 18, 5), 5.87 (1H, *d*, 2; *meta* coupling), 3.79 (3H, *s*), 5.43 (1H, *d*, 2; *meta* coupling), 2.06 (3H, *s*, acetyl), 2.07 (3H, *s*, acetyl) and 7.2–8.0 (5H, phenyl).

Toxin IV′ (*IVd, underivatized*). $C_{20}H_{30}O_7$, M_r 382. $[\alpha]_D = -20$ (*c* 0.24; MeOH). ^{1}H NMR [400 MHz, Me_2CO-d_6; *J* (Hz)]: δ1.59 (3H, *d*, 7), 5.40 (1H, *q*, 7), 1.58 (3H, *s*), 3.62 (1H, *d*, 8), 2.30 (1H, *m*), 0.83 (3H, *d*, 7), 5.69 (1H, *dd*, 16, 8), 5.55 (1H, *dd*, 16, 6), 4.32 (1H, *m*), 1.45–1.75 (2H, *m*), 4.12 (1H, *m*), 1.45–1.75 (2H, *m*), 4.20 (1H, *m*), 2.53 (1H, ABX, 14, 8), 2.62 (1H, ABX, 14, 5), 6.02 (1H, *d*, 2; *meta* coupling) and 5.28 (1H, *d*, 2; *meta* coupling).

Toxin IV′ (*IVe; 4-methoxy-α-pyrone group*). $C_{21}H_{32}O_7$, M_r 396. FD-MS $[M+Na]^+$, *m/z* 419. UV λ_{max}^{MeOH} 280 nm (ε = 4400). ^{1}H NMR [400 MHz, $CDCl_3$; *J* (Hz)]: δ1.62 (3H, *d*, 7), 5.48 (1H, *q*, 7), 1.61 (3H, *s*), 3.67 (1H, *d*, 8), 2.32 (1H, *m*, 8, 7, 7), 0.84 (3H, *d*, 7), 5.64 (1H, *dd*, 16, 7), 5.61 (1H, *dd*, 16, 7), 4.42 (1H, *m*, 8, 7, 3), 1.55–1.70 (2H, *m*), 4.19 (1H, *m*, 8, 8, 3, 3), 1.55–1.70 (2H, *m*), 4.31 (1H, *m*), 2.56 (1H, ABX, 16, 5), 2.61 (1H, ABX, 16, 7), 5.92 (1H, *d*, 2; *meta* coupling), 3.79 (3H, *s*) and 5.42 (1H, *d*, 2; *meta* coupling).

Toxin IV (*IVf and IVf′*). $C_{31}H_{39}O_9B$, M_r 566. HR-MS obs. 566.2710, calc. 566.2686. EI-MS $[M]^+$, *m/z* 566. ^{1}H NMR of IVf [main isomer; 400 MHz, Me_2CO-d_6, *J* (Hz)]: δ1.63 (3H, *d*, 7), 5.55 (1H, *q*, 7), 1.62 (3H, *s*), 4.98 (1H, *d*, 9), 2.51 (1H, *m*, 9, 7, 7), 0.93 (3H, *d*, 7), 5.68 (1H, *dd*, 15, 7), 5.55 (1H, *dd*, 15, 6), 4.56 (1H, *m*, 10, 5, 5, 4), 1.67–2.07 (2H, *m*), 4.30 (1H, *m*, 10, 7, 4, 4), 1.89–2.04 (2H, *m*), 5.46 (1H, *m*), 2.89 (2H, *d*, 6), 5.86 (1H, *d*, 2; *meta* coupling), 3.82 (3H, *s*) and 5.44 (1H, *d*, 2; *meta* coupling) and 7.0–8.5 (5H, phenyl).

Toxin IV (*IVf′, minor isomer*). ^{1}H NMR δ1.63 (3H, *d*, 7), 5.55 (1H, *q*, 7), 1.62 (3H, *s*), 4.94 (1H, *d*, 9), 2.51 (1H, *m*), 0.88 (3H, *d*, 7), 5.73 (1H, *dd*, 15, 7), 5.55 (1H, *dd*, 15, 6), 5.54 (1H, *m*), 1.89–2.04 (2H, *m*), 4.30 (1H, *m*), 1.67–2.07 (2H, *m*), 4.53 (1H, *m*, 10, 7, 5, 5), 2.70 (1H, ABX, 15, 5), 2.76 (1H, ABX, 15, 7), 6.00 (1H, *d*, 2; *meta*

coupling), 3.82 (3H, *s*) and 5.46 (1H, *d*, 2; *meta* coupling); 2.0–2.2 (6H, 2 × acetyl) and 7.0–8.5 (5H, phenyl).

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