Crystal Structures and Conformational Analysis of Ochratoxin A and B: Probing the Chemical Structure causing Toxicity

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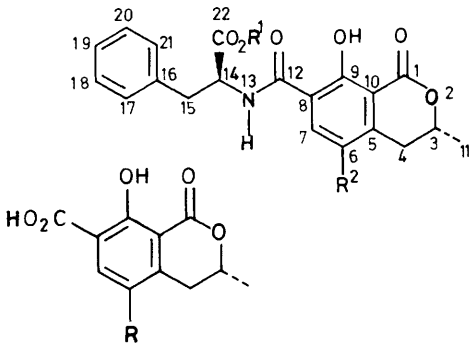
Studies performed on two metabolites from *Aspergillus ochraceus*, ochratoxin A (**1**) and B (**2**), have yielded information related to the steric, conformational, and electronic considerations that could contribute to the different toxicities of these metabolites. The relative HCl-catalysed hydrolysis rates of the toxins are reversed compared with those observed in biological systems. This reversal of relative rate is ascribed to steric hindrance due to the chloro group in ochratoxin A (**1**). Single-crystal *X*-ray structural analyses, i.r., and high field n.m.r. analysis indicate no discernible difference in conformations.

The relative difference in the toxicity of the ochratoxins, metabolites of *Aspergillus ochraceus*, is well established.[1] Ochratoxin A (**1**) is far more toxic than its dechloro analogue ochratoxin B (**2**). Similarly, ochratoxin A (**1**) displays immunosuppressive activity while ochratoxin B (**2**) under the same conditions has no influence on the natural killer cell activity of mice.[2] Various reasons have been proposed for the relative biochemical activities of these compounds and related metabolites.

Doster and co-workers suggested that the toxicity of these compounds is enhanced by increasing the molecular mass while maintaining the free phenolic hydroxy group.[1] They did, however, draw attention to the complexity of *in vitro* studies since many parameters, such as substrate metabolysis and the method of dosing, also play a role in the lifetime of the compound and hence its apparent toxicity.

The non-toxic dihydroisocoumarins ochratoxin α (**4**) and ochratoxin β (**5**) are obtained on acid hydrolysis of ochratoxins A (**1**) and B (**2**), respectively. The relative rates of hydrolysis of these compounds in biological systems have been demonstrated in a number of laboratories. Ochratoxin A (**1**) is only partially hydrolysed in the intestines of pregnant sows, whereas ochratoxin β (**5**) only was isolated in the faeces of sows fed ochratoxin B (**2**).[3] Relative to ochratoxin A (**1**), poorer absorption of ochratoxin B (**2**) from the intestines was indicated and faster enzymic hydrolysis in the liver suggested. *In vitro* kinetic studies with the enzyme bovine carboxypeptidase A showed ochratoxin B (**2**) to be hydrolysed almost one hundred times faster than ochratoxin A (**1**).[4] Tissue extracts of rat liver and intestines also hydrolysed ochratoxin B (**2**) faster than ochratoxin A (**1**). It was suggested that the lesser toxicity of the former could be due to detoxification by hydrolysis. The hydrolysis kinetics of ochratoxin A (**1**) and B (**2**) by carboxypeptidase A have been used for the determination of ochratoxin B (**2**) in the presence of ochratoxin A (**1**).[5]

Good correlation exists between the relative toxicities of ochratoxins A (**1**), B (**2**), C (**3**), α (**4**), and β (**5**) and the acidity of their phenolic protons.[6] The phenolic pK_a values of ochratoxin A (**1**) and C (**3**) are 7, that of ochratoxin B (**2**) is 8, and those of the hydrolysed compounds (**4**) and (**5**) are even higher. In biological systems a significant proportion of ochratoxins A (**1**) and C (**3**) would be dissociated to form phenoxide ions capable of interfering with biochemical processes, whereas the predominantly undissociated phenolic groups of ochratoxins B (**2**), α (**4**), and β (**5**) would be less apt to interact with molecules.



(**1**) Ochratoxin A, R^1 = H; R^2 = Cl
(**2**) Ochratoxin B, R^1 = R^2 = H
(**3**) Ochratoxin C, R^1 = Et; R^2 = Cl
(**4**) Ochratoxin α, R = Cl
(**5**) Ochratoxin β, R = H

Biochemical studies thus indicate[1-6] that the relative efficiencies of interaction of the respective ochratoxins with a variety of substrates in several organs may account for the differences in toxicity of the ochratoxins. The interactions are the conversion of the ochratoxins to metabolites which often are less toxic, and the interference of the ochratoxins with the normal functioning of vital processes. This physico-chemical study was conducted to probe the relationship between the structure and toxicity of ochratoxins A (**1**) and B (**2**).

As insufficient ochratoxin B (**2**) was available, ochratoxin A (**1**) was dechlorinated by treatment with 10% Pd/C and ammonium formate at room temperature for 30 min yielding ochratoxin B (**2**) quantitatively.[7]

The kinetics of the HCl-catalysed hydrolysis of (**1**) and (**2**) were investigated. The role of the aromatic chloro group in the *in vivo* hydrolysis by enzymes such as carboxypeptidase could thus be better understood. In separate reactions, 15 mg of each of ochratoxin A (**1**) and B (**2**), together with alanine as an internal standard, were suspended in DMSO (2 cm^3). The suspensions dissolved on addition of constant-boiling HCl (10 cm^3). The solutions were maintained at 80 °C and aliquots were

Table 1. Kinetics of hydrochloric acid hydrolysis of ochratoxin A (**1**) and B (**2**).

Time/ min	Concentrations[a]/ μmol dm^{-3}	k_{obs}[b]/ 10^{-6} s^{-1}	Extent of hydrolysis (%)
(*a*) Ochratoxin A			
160	39.2	2.13	2.0
240	72.0	2.64	3.7
320	99.6	2.75	5.2
400	128.8	2.87	6.7
1 360	902.2	7.70	46.7
(*b*) Ochratoxin B			
80	17.1	1.54	0.7
160	30.3	1.37	1.3
240	44.0	1.33	1.9
320	55.5	1.26	2.4
400	71.0	1.29	3.1
1 405	302.1	1.65	13.0

[a] Concentration of phenylalanine. [b] First-order rate coefficient calculated from zero to entry time (*n*).

Table 2. Side chain dihedral angles/° for ochratoxins A (**1**) and B (**2**).

Angle	(**1**)	(**2**)
C(7)–C(8)–C(12)–N(13)	−167.4(9)	−174.3(7)
C(8)–C(12)–N(13)–C(14)	−176.3(9)	−172.0(6)
C(12)–N(13)–C(14)–C(15)	178.6(9)	178.4(7)
N(13)–C(14)–C(15)–C(16)	−70.4(10)	−63.4(9)
C(14)–C(15)–C(16)–C(17)	108.0(10)	108.2(10)

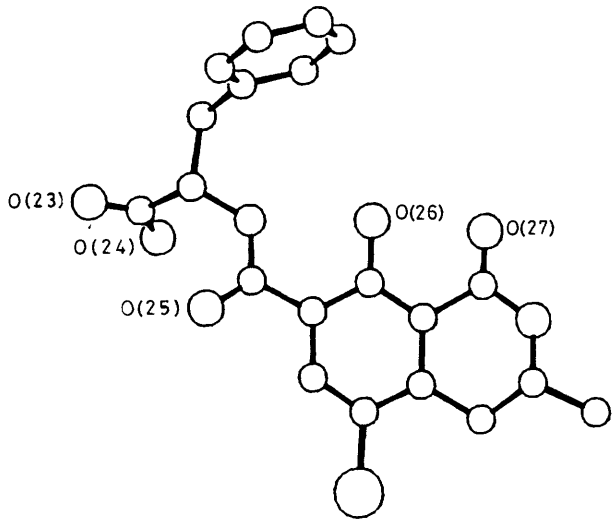
periodically taken to analyse for phenylalanine and alanine.[8] The results are presented in Table 1. The hydrolysis of both compounds was linear in the first 400 min with the rate for ochratoxin A (**1**) being twice that for ochratoxin B (**2**). Contrary to first-order kinetics [equation (1)], the hydrolysis accelerated between 400 min and 1 400 min of the reaction time.

$$\text{ochratoxin B} \longrightarrow \text{ochratoxin } \beta + \text{phenylalanine}$$

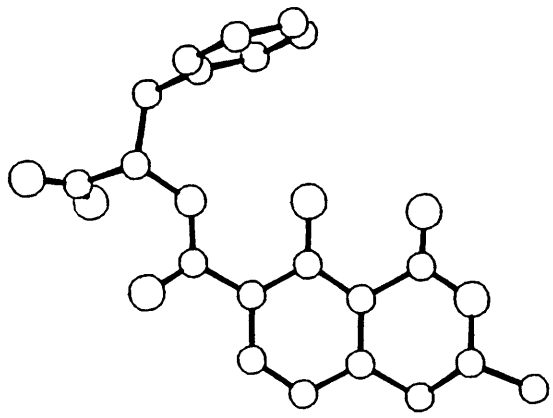
$$k_{obs}t_n = -\ln\left(\frac{[\text{ochratoxin B}]_0 - [\text{phenylalanine}]_n}{[\text{ochratoxin B}]_0}\right) \quad (1)$$

Ochratoxin A (**1**) has a 168% increase in rate, calculated for the full reaction time in both cases. The rate constant for the hydrolysis of ochratoxin A (**1**) calculated for the interval from 400 to 1 360 min is therefore even greater *i.e.* 9.72×10^{-6} s^{-1}. The deviation from first-order kinetics may be ascribed to catalysis by one of the products. What is of interest, however, is that ochratoxin A (**1**) is hydrolysed faster with HCl than ochratoxin B (**2**), contrary to the relative kinetics of hydrolysis *in vivo* and *in vitro* with enzymes.[3–5] This reversal of rates could be due to the bulky chloro group making ochratoxin A (**1**) less accessible to the active site of carboxypeptidase A, relative to ochratoxin B (**2**).

Differences in the conformation of the toxins may play a role in their relative rates of hydrolysis as well as their relative toxicities. If the amide carbonyl and the phenol groups are *syn* with respect to each other, the amide carbonyl will compete with the lactone carbonyl to hydrogen-bond the phenolic proton. Additional H-bonding by the amide *per se* would have a significant influence on the pK_a of the phenol group and thus its ability to interact with vital substrates. Single-crystal *X*-ray crystallography was employed to determine the solid-state conformations of (**1**) and (**2**).



Ochratoxin A (**1**)



Ochratoxin B (**2**)

Figure 1. Perspective drawings (ORTEP) of Ochratoxin A (**1**) and B (**2**), without hydrogen atoms.

There is little difference between the conformations of the two toxins in the solid state (Figure 1). The side chain dihedral angles are listed in Table 2, and are indicative of the similarity between the conformations. There are no significant differences in bond lengths or angles. Figure 2 shows a view of the two molecular conformations with the aromatic carbon atoms of the dihydroisocoumarins superimposed.

The amide carbonyl and phenol hydroxy groups are *anti* with respect to each other, and the phenol group is only involved in hydrogen bonding with the lactone carbonyl—the interoxygen distances are 2.542 Å for ochratoxin A (**1**) and 2.562 Å for ochratoxin B (**2**) ('β-form', see Figure 3). The amide carbonyls are, for both structures, mainly involved in intermolecular hydrogen bonding with the hydrogen atom of the acid group, with interoxygen distances of 2.702 Å for (**1**) and 2.643 Å for (**2**). The intramolecular interoxygen distances for O(24)–O(25) are 3.153 Å for (**1**) and 3.090 Å for (**2**).

Since the biological activity of the ochratoxins relates to their conformations in solution, the next step was to compare their solid-state conformations with those in solution. Hydrogen bonding of the amide and phenol protons and the orientation of the amide carbonyl in solution were of paramount interest.

Table 3. Relevant i.r. bands[a]/cm^{-1} of ochratoxin A (**1**) and B (**2**) in KBr and $CHCl_3$.

		NH stretch	Carbonyl stretch			Aromatic breathing	Amide II
			COOH	Lactone	Amide		
A (**1**)	KBr pellet	3 398	1 737		1 669[b]	1 630	1 536
B (**2**)	KBr pellet	3 372	1 728		1 664[b]	1 623	1 534
A (**1**)	$CHCl_3$ solution	3 391	1 721	1 675	1 657[c]	1 613	1 529
B (**2**)	$CHCl_3$ solution	3 384	1 718	1 670	1 655[c]	1 617	1 528

[a] L. J. Bellamy, 'The Infra-red Spectra of Complex Molecules,' Chapman and Hall, London, 1975, 3rd edn., pp. 74, 79, 204, 233, 240, 266, and 274. [b] Lactone and amide bands overlap. [c] Shoulder on lactone band.

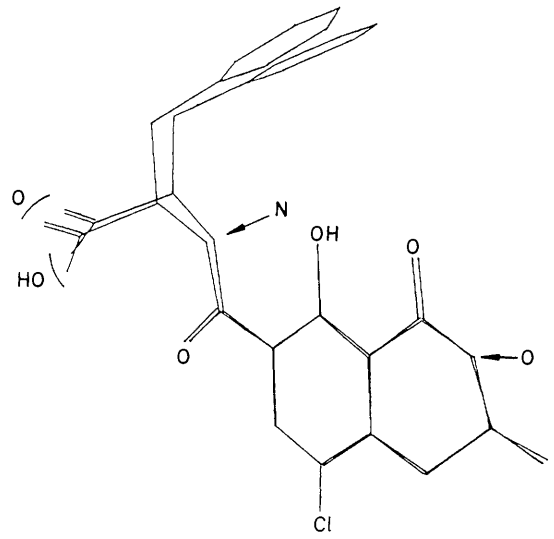


Figure 2. Fit (ALCHEMY, from TRIPOS) of ochratoxin A (**1**) and B (**2**), based on superimposed phenolic carbon atoms.

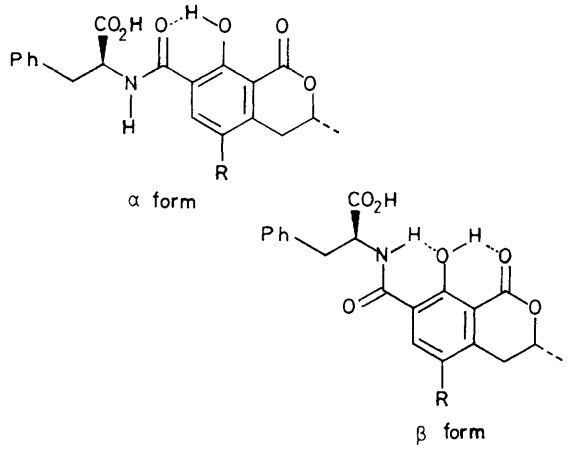
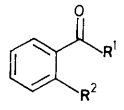


Figure 3. Two possible conformations for the ochratoxins in which the phenolic proton forms a hydrogen bond with either the amide carbonyl or the lactone carbonyl. The 'β-form' allows for further hydrogen bonding by the amide proton.

Infrared spectroscopy proved useful for the conformational study. The frequencies of the carbonyl and amide bands of ochratoxin A (**1**) and B (**2**), recorded as KBr pellets and dissolved in chloroform, are displayed in Table 3. A comparison showed a significant similarity. The frequencies of all four of the NH-stretch bands are within the range of the 'β form' of a series of halogenated salicylamides (3 352–3 408 cm^{-1}) and outside the range of the 'α-form' (3 414–3 456 cm^{-1}) (Figure 3).[9] The

Table 4. ^{13}C N.m.r. chemical shifts [δ(ppm)] of the amide and ester carbonyls of ochratoxins and model compounds (**6**).



Model compounds (**6**)

R^1	R^2 = −H[solvent]	R^2 = −OH[solvent]
−OMe	167.09 [$CDCl_3$][b]	170.1 [$(CD_3)_2SO$ or $CDCl_3$][c]
−NHMe	166.81 [$(CD_3)_2SO$][d]	169.8 [$(CD_3)_2SO$][e]

	Ochratoxins	
Carbonyl	A (**1**)[a] [$CDCl_3$]	B (**2**) [$(CD_3)_2SO$]
Lactone	169.6	169.1
Amide	163.0	163.6

[a] A. E. de Jesus, P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Perkin Trans. 1*, 1980, 52. [b] I. I. Schuster, *J. Org. Chem.*, 1985, **50**, 1656. [c] M. M. A. Hassan and M. U. Zubai, *Spectrosc. Lett.*, 1982, **15**, 533. [d] V. Mizrahi, K. R. Koch, and T. A. Modro, *S. Afr. J. Chem.*, 1983, **36**, 111. [e] δ 171.1 in $CDCl_3$, M. Kondo, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 521.

conformation and H-bonding of the 'β form' is analogous to that of the *X*-ray structure of the ochratoxins.

The ^{13}C n.m.r. chemical shifts of carbonyl carbon nuclei shift downfield when the carbonyl oxygen atom becomes H-bonded.[10] Table 4 contains the chemical shifts of model amides and esters to illustrate the effects of intramolecular *ortho*-hydroxy H-bonding. The carbonyl carbon nuclei of both the ester and the amide are deshielded by *ca.* 3 ppm when the *ortho*-hydroxy group is introduced. The chemical shifts of the ochratoxins suggest that the phenol proton H-bonds to the lactone carbonyl in both cases, and is in agreement with the *X*-ray structures and i.r. findings. This is confirmed by the $^3J_{HC}$ coupling constant of the phenol proton with C-8 and C-10 in (**1**). The respective coupling values are 5.5 and 4.6 Hz, indicating that the phenol proton is *trans* with respect to C-8 and is situated so as to H-bond the lactone carbonyl.[11]

Conclusions.—The reversal of the relative magnitudes of hydrolysis rates between ochratoxin A (**1**) and B (**2**) when catalysed by HCl compared with hydrolysis rates *in vivo*[3] and *in vitro* catalysed by carboxypeptidase A,[4,5] suggest that the bulkiness of the chloro group of ochratoxin A (**1**) may hinder the approach of the toxin to the active site of carboxypeptidase A. The difference in hydrolysis rates cannot be ascribed to conformation since both the toxins have the same conformation at

Table 5. Crystal data for compounds (**1**) and (**2**).

	Ochratoxin A (**1**)	Ochratoxin B (**2**)
Formula	$C_{20}H_{18}ClNO_6$	$C_{20}H_{19}NO_6$
Crystal size/mm	0.22 × 0.27 × 0.26	0.25 × 0.27 × 0.30
a/Å	10.690(1)	11.714(3)
b/Å	8.454(5)	8.490(1)
c/Å	11.358(5)	10.119(2)
β/°	107.069(2)	115.02(2)
V/Å^3	981.3	911.9
Space group	$P2_1$	$P2_1$
Z	2	2
Scan range	$3 \leqslant \theta \leqslant 25$	$3 \leqslant \theta \leqslant 30$
μ(Mo-K_α)/cm^{-1}	1.86	0.9
D_{calc}/g cm^{-1}	1.366	1.345
F(000)	420	376
Refl. measured	1 848	2 824
Refl. used	1 645 ($F > 0$)	1 870 ($F > 2\sigma F_o$)
Parameters refined	274	253
Residual e$^-$ density/Å^{-3}	0.36	0.35
Decay (%)	0.8	4.3
U_{iso}(H)/Å	0.116(8)	0.114(9)
$R = \Sigma\|F_o - F_c\|/\Sigma\|F_o\|$	0.083	0.087

Table 6. Fractional co-ordinates ($\times 10^4$) for ochratoxin A.

Atom	x	y	z
C(1)	4 743(8)	2 132	6 388(8)
O(2)	5 867(6)	2 886(16)	6 480(5)
C(3)	6 259(8)	4 260(18)	7 309(8)
C(4)	6 193(6)	3 811(18)	8 565(6)
C(5)	4 863(7)	3 180(18)	8 513(7)
C(6)	4 291(7)	3 265(17)	9 442(6)
C(7)	3 049(7)	2 789(17)	9 285(6)
C(8)	2 300(7)	1 993(15)	8 224(6)
C(9)	2 908(7)	1 780(17)	7 287(6)
C(10)	4 133(7)	2 389(17)	7 408(6)
C(11)	7 674(7)	4 623(18)	7 316(7)
C(12)	956(7)	1 512(16)	8 195(6)
N(13)	352(6)	511(15)	7 315(5)
C(14)	−1 004(8)	90(18)	7 211(7)
C(15)	−1 513(8)	−1 095(17)	6 074(8)
C(16)	−1 674(7)	−146(16)	4 894(6)
C(17)	−772(7)	−397(18)	4 210(7)
C(18)	−975(9)	512(18)	3 118(8)
C(19)	−1 979(10)	1 542(21)	2 724(8)
C(20)	−2 821(10)	1 800(19)	3 400(8)
C(21)	−2 654(7)	905(17)	4 484(6)
C(22)	−1 136(9)	−885(22)	8 352(7)
O(23)	−2 160(6)	−814(21)	8 537(6)
O(24)	−127(7)	−1 649(16)	9 000(5)
O(25)	418(5)	2 011(15)	8 932(4)
O(26)	2 267(6)	976(16)	6 267(5)
O(27)	4 268(6)	1 277(16)	5 542(5)
Cl(28)	5 148(2)	4 170(14)	10 817(2)

the peptide linkage in both the crystal structure and in chloroform solution.

The relative toxicities of ochratoxin A (**1**) and B (**2**) may be ascribed to the relative hydrolysis rates of the toxins[3–5] by enzymes such as carboxypeptidase A, and the pK_a of the phenol groups.[1,6] Conformational considerations apparently play no role.

Experimental

Instrumentation.—^{1}H and ^{13}C n.m.r. spectra of ochratoxin B (**2**) were run on a Bruker WM 500 spectrometer. The solvent was $(CD_3)_2SO$ which also served as the internal reference (δ_H 2.49 and δ_C 39.5). The probe temperature was 303 K. A Beckman Acculab 8 spectrophotometer was used to record all i.r. spectra. A Varian MAT 212 was used for the recording of the mass spectrum of ochratoxin B (**2**). The specific rotation of ochratoxin B (**2**) was determined on a Perkin-Elmer 241 polarimeter. The mean of ten determinations was taken.

Analysis.—Amino acid analyses were performed on a Waters amino acid analyser with a Waters amino acid column using post-column derivatisation with *o*-phthalaldehyde as the detection method.[8]

Solvents.—DMSO was distilled under vacuum and stored 4 Å molecular sieves. All other solvents were unprocessed commercial A.R. solvents.

Reagents.—Ochratoxin A (**1**) was obtained from previous extractions of *Aspergillus ochraceus* Wilh.[12] Ammonium formate and 10% Pd on carbon were commercial reagents.

Preparation of Ochratoxin B (**2**) *by Catalytic Dechlorination of Ochratoxin A* (**1**)[7].—10% Pd/C (204 mg) was suspended in a solution of ochratoxin A·C_6H_6 (**1**) (396 mg, 822 μmol) and ammonium formate (314 mg, 4.97 mmol) in methanol (10 cm^3). The reaction mixture was stirred at room temperature for 30 min, then filtered and the filtrate was evaporated under reduced pressure. The residue was suspended in chloroform (20 cm^3) and shaken with dilute HCl (pH 3, 10 cm^3). The aqueous phase was extracted twice more with chloroform (2 × 10 cm^3), and the combined chloroform extracts were evaporated and azeotroped (benzene–ethanol) under reduced pressure to yield ochratoxin B (**2**) (310 mg, quant.), m.p. 218–222 °C (from MeOH) (lit.,[12] 221 °C); $[\alpha]^{23.4}$ −50.1 ±0.6° (*c* 0.69 in MeOH) (lit.,[12] −35°; *c* 0.15) (Found: C, 64.9; H, 5.1; N, 3.9%. Calc. for $C_{20}H_{19}NO_6$: C, 65.0; H, 5.2; N, 3.8%); $\nu_{max.}$ (Table 3) [lit.,[12] (KBr disc) 1 730, 1 680, and 1 535 cm^{-1}]; δ_H(500 MHz) 1.42 (3 H, d, $J_{11,3}$ 6.3 Hz, 11-H), 2.97 (1 H, dd, $J_{4,4}$ 16.8 and $J_{4\text{-}pro\text{-}R,3}$ 11.3 Hz, 4-*pro*-*R*-H), 3.08 (1 H, dd, $J_{4,4}$ 17.2 and $J_{4\text{-}pro\text{-}S,3}$ 3.8 Hz, 4-*pro*-*S*-H), 3.08 (1 H, dd, $J_{15,15}$ 13.5 and $J_{15\text{-}pro\text{-}S,14}$ 7.5 Hz, 15-*pro*-*S*-H), 3.20 (1 H, dd, $J_{15,15}$ 13.9 and $J_{15\text{-}pro\text{-}R,14}$ 5.0 Hz, 15-*pro*-*R*-H), 4.72 (1 H, td, $J_{14,15\text{-}pro\text{-}S}$ $J_{14,13}$ 7.5, and $J_{14,15\text{-}pro\text{-}R}$ 4.9 Hz, 14-H), 4.83 (1 H, dqd, $J_{3,4\text{-}pro\text{-}R}$ 11.3, $J_{3,11}$ 6.3, and $J_{3,4\text{-}pro\text{-}S}$ 3.4 Hz, 3-H), 6.95 (1 H, d, $J_{6,7}$ 8.0 Hz, 6-H), 7.18–7.28 (5 H, m, 17–21-H), 8.07 (1 H, d, $J_{7,6}$ 7.9 Hz, 7-H), and 8.48 (1 H, d, $J_{13,14}$ 7.4 Hz, 13-H); δ_C(126 MHz) 20.1 (11-C), 33.6 (4-C), 36.7 (15-C), 53.8 (14-C), 76.1 (3-C), 109.3 (10-C), 118.3 (8-C), 118.5 (6-C), 126.6 (19-C), 128.2 (18, 20-C), 129.2 (17, 21-C), 136.9 (7-C), 137.0 (16-C), 144.7 (5-C), 159.4 (9-C), 163.6 (12-C), 169.1 (1-C), and 172.5 (22-C) (confirmed by HETCORR);[12] *m*/*z* (electron impact) 369 (M^+, 2%), 221 (57), and 205 (100).

Hydrolysis Kinetics of Ochratoxin A (**1**) *and B* (**2**)*.—Ochratoxin A·C_6H_6 (**1**) (11.2 mg, 28.2 μmol) [10.3 mg, 27.9 μmol] and (±)-alanine (1.9 mg, 21 μmol) [2.2 mg, 25 μmol] were dissolved in DMSO (2 cm^3) and then diluted with constant-boiling aq. HCl (5.5 mol dm^{-3}; 10 cm^3). The mixture was then heated at 80 °C and 100 mm^3 aliquots were taken every 80 min up to 400 min and one the following day. On removal, each aliquot was evaporated to dryness on a high vacuum pump, and then subjected to amino acid analysis as described above. The results are presented in Table 1.

* Ochratoxin B (**2**) values in square brackets.

Table 7. Fractional co-ordinates (× 10^4) for ochratoxin B.

Atom	*x*	*y*	*z*
C(1)	1 660(7)	3 059	9 859(8)
O(2)	1 684(5)	2 193(13)	10 945(6)
C(3)	2 540(8)	867(16)	11 471(9)
C(4)	3 856(8)	1 393(17)	11 695(8)
C(5)	3 782(6)	1 978(15)	10 265(7)
C(6)	4 706(6)	1 746(16)	9 769(8)
C(7)	4 545(6)	2 316(15)	8 426(8)
C(8)	3 495(6)	3 166(14)	7 542(7)
C(9)	2 560(6)	3 470(14)	8 052(7)
C(10)	2 713(6)	2 849(15)	9 385(7)
C(11)	2 470(10)	304(16)	12 853(10)
C(12)	3 406(6)	3 613(15)	6 073(7)
N(13)	2 434(5)	4 569(14)	5 245(6)
C(14)	2 193(6)	4 888(16)	3 717(8)
C(15)	1 025(7)	5 956(15)	3 036(9)
C(16)	−136(6)	5 133(15)	2 993(8)
C(17)	−669(8)	5 585(18)	3 915(10)
C(18)	−1 742(10)	4 723(20)	3 837(12)
C(19)	−2 261(9)	3 543(21)	2 836(13)
C(20)	−1 739(8)	3 109(19)	1 931(13)
C(21)	−669(7)	3 911(16)	1 986(10)
C(22)	3 318(7)	5 720(17)	3 583(9)
O(23)	3 464(7)	5 497(16)	2 492(7)
O(24)	3 983(5)	6 642(13)	4 653(6)
O(25)	4 158(5)	3 164(13)	5 619(6)
O(26)	1 535(5)	4 316(13)	7 219(6)
O(27)	824(5)	3 990(14)	9 275(6)

X-*Ray Crystallography*.—Ochratoxin A (**1**) was recrystallized from xylene by dissolving a sample at 100 °C and allowing the solution to cool to 50 °C, at which temperature it was left overnight. Suitable crystals were thus obtained, without the inclusion of solvents of crystallisation, as is the case when crystallisation is from benzene.[12] Suitable single crystals of B (**2**) were obtained on recrystallisation from MeOH.

The relevant crystallographic data for ochratoxin A (**1**) and B (**2**) are given in Table 5. Diffraction intensities were measured at 20 °C with an Enraf–Nonius CAD4 diffractometer using graphite monochromated Mo–K_α radiation (λ 0.710 73 Å). The ω:2θ motor movements were fixed to a 3:2 ratio for (**1**) and 1:1 for (**2**). The ω-angle changed as 0.62 + 0.35 tan θ for (**1**) and 0.59 + 0.34 tan θ for (**2**). A variable scan speed was employed for both data collections with a maximum of 5.49° min^{-1} and a minimum corresponding to 50 s measuring time per reflection. The stability of the crystals was tested every 2 h and the data for (**2**) were corrected for decay. Both data sets were corrected for Lorentz and polarization effects. Direct methods[13] and subsequent Fourier synthesis revealed the positions of all the non-hydrogen atoms, and these were refined anisotropically until no significant shifts occurred.[14] The standard neutral-atom scattering factors supplied with SHELX76[14] were used.

The positions of some hydrogen atoms could be located from the difference maps, although the majority of the hydrogen atoms were placed in their expected positions and constrained to ride upon their associated non-hydrogen atoms. Common isotropic thermal parameters were refined for all the hydrogen atoms in each structure.

The fractional atomic co-ordinates of the non-hydrogen atoms of (**1**) and (**2**) are tabulated in Tables 6 and 7, respectively. Full lists of bond lengths, bond angles, thermal parameters and non-hydrogen atom co-ordinates have been deposited at the Cambridge Crystallographic Data Centre (CCDC).*

* For details of the CCDC deposition scheme see 'Instructions for Authors (1989),' *J. Chem. Soc., Perkin Trans. 2*, in the January issue.

References

1 R. C. Doster, R. O. Sinnhuber, and N. E. Pawlowski, *Food Cosmet. Toxicol.*, 1974, **12**, 499, and references cited.
2 M. I. Luster, D. R. Germolec, G. R. Burleson, C. W. Jameson, M. F. Ackermann, K. R. Lamm, and H. T. Hayes, *Cancer Res.*, 1987, **47**, 2259.
3 D. S. P. Patterson, B. A. Roberts, and B. J. Small, *Food Cosmet. Toxicol.*, 1976, **14**, 439.
4 R. C. Doster and R. O. Sinnhuber, *Food Cosmet. Toxicol.*, 1972, **10**, 389.
5 K. Hult, E. Hökby, and S. Gatenbeck, *Appl. Environ. Microbiol.*, 1977, **33**, 1275.
6 F. S. Chu, *CRC Crit. Rev. Toxicol.*, 1974, **2**, 499; F. S. Chu, I. Noh, and C. C. Chang, *Life Sci.*, 1972, **11**, Part I, 503.
7 M. K. Anwer and A. F. Spatola, *Tetrahedron Lett.*, 1985, **26**, 1381.
8 K. S. Lee and D. G. Drescher, *Int. J. Biochem.*, 1978, **9**, 457.
9 D. Welti, *Spectrochim. Acta*, 1966, **22**, 281.
10 E. Breitmaier and W. Voelter, 'Carbon-13 NMR Spectroscopy,' VCH Verlagsgesellschaft, New York, 1987, 3rd edn., p. 117.
11 P. L. Wessels, personal communication.
12 K. J. van der Merwe, P. S. Steyn, and L. Fourie, *J. Chem. Soc.*, 1965, 7083.
13 P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declerq, and M. M. Woolfson, 'MULTAN80, A System of Computer Programs for the Automatic Solution of Structures from *X*-Ray Diffraction Data,' Universities of York (England) and Louvain (Belgium), 1980.
14 G. M. Sheldrick, 'SHELX76, A Program for Crystal Structure Determination,' University of Cambridge, England, 1976.

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