Structures of the Austalides A—E, Five Novel Toxic Metabolites from *Aspergillus ustus*

By R. MARTHINUS HORAK, PIETER S. STEYN, PETRUS H. VAN ROOYEN, and ROBERT VLEGGAAR*
(*National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box* 395, *Pretoria* 0001, *Republic of South Africa*)

and CHRISTIAAN J. RABIE
(*National Research Institute for Nutritional Diseases, Medical Research Council, P.O. Box* 70, *Tygerberg* 7505, *Republic of South Africa*)

Summary The structures of austalides A–E, five new mycotoxins isolated from cultures of *Aspergillus ustus*, are based on the *X*-ray crystallographic study of austalide A, chemical derivatization, and a correlation of their ^{13}C and ^{1}H n.m.r. spectral data.

PREVIOUS investigations of toxigenic cultures of *Aspergillus ustus* resulted in the isolation of a number of mycotoxins *viz.* the austamides,[1] austdiol,[2] the austocystins,[3] and austin.[4] We now wish to report the structure elucidation of five new toxic metabolites, austalides A–E, isolated from whole maize cultures of a toxigenic strain of *Aspergillus ustus* (Bain) Thom. and Church, isolate MRC 1163. This strain of *A. ustus*, which also produces austocystin A and D,[3] was isolated from dried fish destined for human consumption in the Bandar-Shah region of Iran.

Austalide A (**1**) was crystallized from chloroform–methanol, m.p. 212—214 °C and analysed as $C_{28}H_{36}O_9 \cdot CHCl_3$; it had $[\alpha]_D^{24}$ −84·4 ° (*c*, 1·00, $CHCl_3$); λ_{max}(MeOH) 222 and 267 nm (ϵ 35 400 and 17 140, respectively), and ν_{max} 1740 cm^{-1}. Alkaline hydrolysis of austalide A gave the alcohol (**2**), identical with austalide B, m.p. 243—245 °C. All of the compounds had spectral characteristics in accordance with the proposed structures; the ^{1}H and ^{13}C n.m.r. resonances of austalide A have been completely assigned and are collated in Table 1. These assignments are based on single frequency n.O.e. (nuclear Overhauser effect), proton-noise-decoupled (p.n.d.), single frequency off-resonance proton-decoupled spectra, selective population inversion experiments, knowledge of chemical shift data, the correlation of ^{1}H and ^{13}C n.m.r. resonances, and information derived from derivatives of (**1**). The ^{1}H and ^{13}C n.m.r. data essential for the structural elucidation of the remaining compounds are given in Table 2.

TABLE 1. ^{13}C[a] and ^{1}H[b] n.m.r. assignments of austalide A.

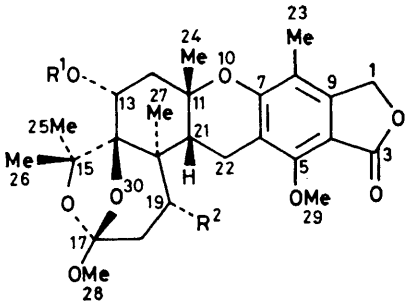
	δ_C[f]	*J*/Hz	δ_H	*J*/Hz
C(1)	67·9Tm	151·3	5·12s	
C(3)	168·9Sm			
C(4)	107·1S			
C(5)	155·1Sm			
C(6)	115·9Sm			
C(7)	157·6Sq	3·0		
C(8)	113·7Sm			
C(9)	145·3Sq	4·0		
C(11)	75·3Sm			
C(12)	37·9Tm	131·0	2·14dd	16·0, 4·3
			2·54dd	16·0, 2·1
C(13)	70·6Dd	151·0, 5·0	5·08dd	4·3, 2·1
C(14)	85·2Sm			
C(15)	84·2Sm			
C(17)	119·1Sm			
C(18)[e]	30·4Tm	127·8	1·6—2·0m	
C(19)[e]	30·1Tm	127·0		
C(20)	40·2Sm			
C(21)	35·7Dm	125·2	2·40d	8·0
C(22)	17·8Tm	129·9	2·83dd	18·8, 8·0
			2·94d	18·8
C(23)	10·2Q	127·7	2·00s	
C(24)	28·3Q	124·1	1·33s	
C(25)[d]	27·2Q	126·6	1·23s	
C(26)[d]	25·6Qd	122·7, 3·1	1·58s	
C(27)	17·8Qd	123·5, 6·0	1·00s	
C(28)	48·5Q	143·9	3·42s	
C(29)	61·6Q	145·3	4·13s	
C(31)[e]	169·1Sm			
C(32)[e]	20·8Q	129·7	2·06s	

[a] Taken in $CDCl_3$ on a Varian XL-100 spectrometer at 25·1 MHz. [b] Taken in $CDCl_3$ on a Bruker WM-300 spectrometer at 300 MHz. [c] and [d] may be interchanged. [e] C(31) and C(32) are the C(:O) and methyl carbon atoms of R^1. [f] Relative to Me_4Si. Capital letters refer to the patterns resulting from directly bonded protons and lower-case letters to (^{13}C, H) couplings over more than one bond.

TABLE 2. Relevant n.m.r. data for the austalides; chemical shifts are in p.p.m. relative to Me_4Si.

	^{13}C N.m.r.[a]					^{1}H N.m.r.[b]	
	(2)	(3)	(4)	(5)	(6)	(3)	J/Hz
C(11)	78·0S	77·7S	75·5S	78·4S	75·3S		
C(12)	41·2T	40·7T	37·6T	40·8T	37·6T	2·34dd	15·8, 4·2
						2·36dd	15·8, 2·2
C(13)	69·7D	69·5D	70·7D	69·8D	70·6D	4·18m	4·2, 2·2, 7·5[c]
C(14)	86·2S	85·4S	85·7S	87·0S	85·1S		
C(15)	84·7S	85·9S	85·9S	86·2S	85·1S		
C(17)	118·8S	117·2S	118·6S	118·5S	117·7S		
C(18)	30·4T[d]	37·1T	39·0T	38·2T	36·9T	2·28dd	15·3, 6·2
						1·94d	15·3
C(19)	30·3T[d]	70·8D	70·7D	71·2D	70·8D	5·43d	6·2
C(20)	39·6S	44·8S	46·3S	45·7S	45·4S		
C(21)	36·5D	38·2D	37·6D	39·2D	37·5D	2·18d	8·4
C(22)	18·3T	19·4T	19·1T	19·6T	19·2T	2·97dd	18·9, 8·4
						3·23d	18·9
C(27)	19·0Q	13·7Q	13·4Q	14·4Q	12·7Q		

[a] Taken in $CDCl_3$ on a Varian XL-100 spectrometer at 25·1 MHz. [b] Taken in $CDCl_3$ on a Bruker WM-300 spectrometer at 300 MHz. [c] Due to spin–spin coupling with C(13)–OH at δ_H 2·62 (d, J 7·5 Hz). [d] May be interchanged.



(**1**) Austalide A: R^1 = –COMe, R^2 = H
(**2**) Austalide B: R^1 = R^2 = H
(**3**) Austalide D: R^1 = H, R^2 = –OCOMe
(**4**) Austalide E: R^1 = –COMe, R^2 = OH
(**5**) R^1 = H, R^2 = OH
(**6**) Austalide C: R^1 = –COMe, R^2 = –OCOMe

A single crystal X-ray structure determination of austalide A was carried out. The crystals of austalide A are orthorhombic, space group $P2_12_12_1$ with a = 16·57(1), b = 18·54(1), c = 10·25(1) Å, Z = 4, and included chloroform of crystallization ($C_{28}H_{36}O_9$·$CHCl_3$). Intensity measurements were made with Cu-K_α radiation (λ = 1·5418 Å; graphite monochromator) on a modified Hilger and Watts four-circle diffractometer in the ω-2θ mode to θ = 55°. A total of 2290 unique reflections was measured. The structure was solved by direct methods and refined by blocked least-squares techniques using the SHELX computer program.[5] Hydrogen atoms were included at calculated positions with a common isotropic temperature factor. During refinement the hydrogen atom co-ordinates were constrained to maintain constant bond lengths and appropriate bond angles. Convergence, with anisotropic temperature factors for all non-hydrogen atoms, was reached at R = 0·150 using all data and unit weights. The high thermal vibration of most of the atoms, as indicated by their thermal parameters, results in the high value for the final R-factor. The difference electron density map based on the final atomic parameters showed no maxima greater than 0·43 e Å^{-3}. The resulting structure is illustrated in the Figure.† On the basis of the puckering parameters defined by Cremer and Pople,[6] and the exact description of ring conformations by Boeyens,[7] the seven-membered ring is a TB($\overline{15}$) conformation, while the six-membered ring formed by C(14)–C(20)–C(19)–C(18)–C(17)–O(30) is a hybrid ($^{18}C_{14}$ + ^{30}E + $^{30}H_{17}$) conformation.

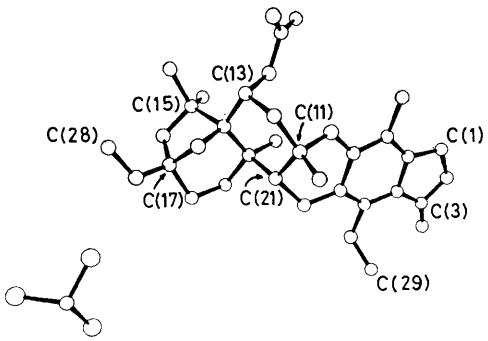


FIGURE. Perspective drawing of austalide A. Hydrogen atoms have been omitted for clarity and no absolute stereochemistry is implied.

Alkaline hydrolysis of austalide D (**3**), m.p. 259—261 °C, and austalide E (**4**), m.p. 262—264 °C (both compounds were analysed as $C_{28}H_{36}O_{10}$), gave in each case the diol (**5**). Additional chemical evidence for the structural isomerism of these two metabolites was provided by acetylation; treatment of (**3**) with acetic anhydride–perchloric acid and of (**4**) using acetic anhydride–pyridine resulted in each case in the formation of the same diacetate derivative, austalide C (**6**). With the knowledge of the structure of (**1**), the structure elucidation of (**3**) [and (**4**)] is therefore reduced to determining the location of the secondary hydroxy- and

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

acetoxy-functions in the molecule. The structures of (**3**) and (**4**) were deduced from a comparison of the relevant n.m.r. data (see Table 2 for ^{13}C data).

Although the austalides represent a new structural type among mycotoxins, biosynthetically these metabolites are related to 6-farnesyl-5,7-dihydroxy-4-methylphthalide, a known intermediate in the biosynthesis of mycophenolic acid.[8] The toxic effects of the austalides are under investigation.

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[1] P. S. Steyn, *Tetrahedron*, 1973, **29**, 107; P. S. Steyn and R. Vleggaar, *Phytochemistry*, 1976, **15**, 355.
[2] R. Vleggaar, P. S. Steyn, and D. W. Nagel, *J. Chem. Soc., Perkin Trans.* 1, 1974, 45; P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans.* 1, 1976, 204.
[3] P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans.* 1, 1974, 2250; *J. S. Afr. Chem. Inst.*, 1975, **28**, 375.
[4] K. K. Chexal, J. P. Springer, J. Clardy, R. J. Cole, J. W. Kirksey, J. W. Dorner, H. G. Cutler, and B. J. Strawter, *J. Am. Chem. Soc.*, 1976, **98**, 6748.
[5] G. M. Sheldrick, SHELX program system 1976, University of Cambridge.
[6] D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1358.
[7] J. C. A. Boeyens, *J. Cryst. Mol. Struct.*, 1978, **8**, 317; I. K. Boessenkool and J. C. A. Boeyens, *ibid.*, 1980, **10**, in the press.
[8] L. Bowen, K. H. Clifford, and G. T. Philips, *J. Chem. Soc., Chem. Commun.*, 1977, 949, 950; L. Colombo, C. Gennari, D. Potenza, C. Scolastico, and F. Aragozzini, *ibid.*, 1979, 1021.