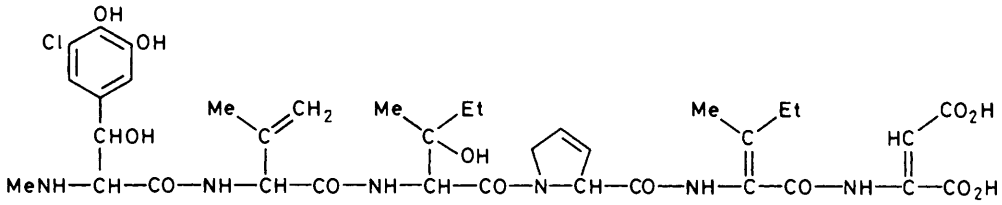
The *X*-Ray Structure of Phomopsin A, a Hexapeptide Mycotoxin

Maureen F. Mackay,*[a] Albert Van Donkelaar,[b] and Claude C. J. Culvenor[c]

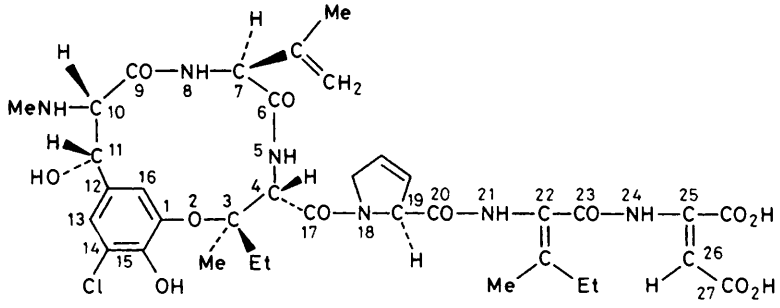
[a] *Department of Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia*
[b] *Division of Protein Chemistry, C.S.I.R.O., 343 Royal Parade, Parkville, Victoria 3052, Australia*
[c] *Division of Animal Health, C.S.I.R.O., Private Bag No. 1, Parkville, Victoria 3052, Australia*

A crystallographic study of phomopsin A, the hexapeptide mycotoxin of *Phomopsis leptostromiformis* responsible for lupinosis disease in animals, has shown that it is a linear peptide, modified by an ether bridge in place of the hydroxy groups of the *N*-methyl-3-(3-chloro-4,5-dihydroxyphenyl)-3-hydroxyalanine and 3-hydroxyisoleucine units, and thus containing a 13-membered ring.

The mycotoxin, phomopsin A, is a metabolite of *Phomopsis leptostromiformis* and the cause of lupinosis, a liver disease of sheep and cattle in Australia and other countries. Phomopsin A is a hexapeptide made up of 2,3- and 3,4-didehydro and 3-hydroxy amino acids.[1] It was initially reported to be a cyclic peptide[1] but this was changed recently to a linear structure (**1**) because fast atom bombardment (f.a.b.) mass spectral and n.m.r. evidence showed that there was no link between the substituted phenylalanine and 2,3-didehydroaspartic acid units.[2] The mass spectral data also confirmed the amino acid



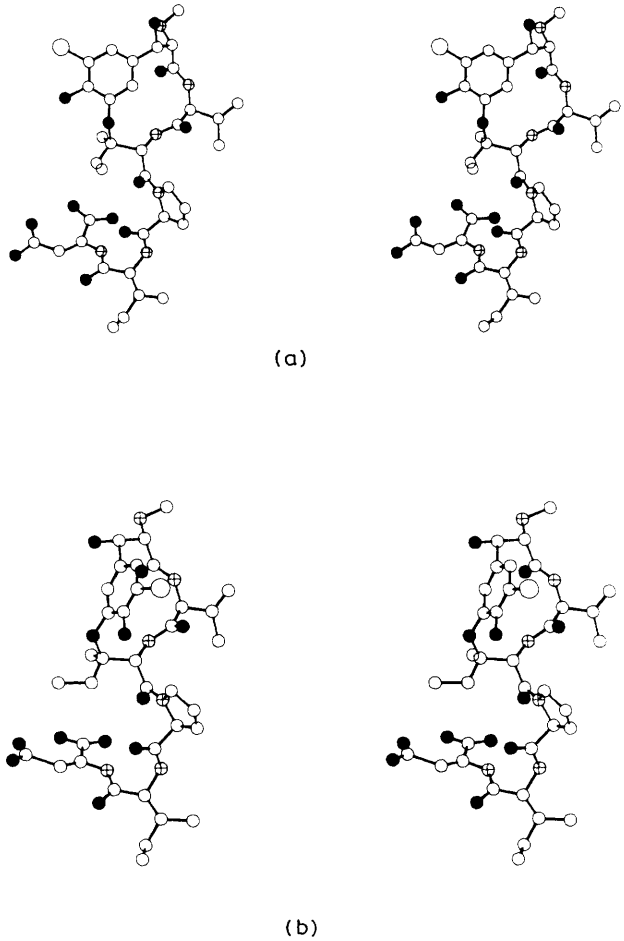
(1)



(2)

sequence. In a crystallographic study† we have now shown that the linear structure is further modified by an ether bridge replacing hydroxy groups of the substituted phenylalanine and 3-hydroxyisoleucine units. Thus phomopsin A has the structure (**2**), with a 13-membered macrocyclic ring incorporating a *meta*-fused benzene ring.

The absolute configuration as depicted in (**2**) is determined by prior evidence that the 3,4-didehydroproline and 3-hydroxyisoleucine units are of L-configuration.[3] The stereochemical specification of phomopsin A is 22*E*,25*E*,3*R*,4*S*,7*S*,10*S*,11*S*,19*S*, the configuration of the double bonds at C(22) and C(25) also having been determined previously.[1] The two molecules (A and B) in the asymmetric unit adopt different conformations, the major difference being in the relative orientation of the 3-chloro-4,5-dihydroxyphenyl moiety in the macrocyclic ring. The face of the ring which is towards the observer in molecule (A) (Figure 1a) has turned, in molecule (B) (Figure 1b), to be face-on to the C(6)–C(7) segment of the macrocyclic ring. The aromatic ring is apparently able to flip over by rotation about an axis joining



(a)

(b)

Figure 1. The molecular structures of phomopsin A; (a) molecule (A), (b) molecule (B).

† *Crystal data:* Hydrated crystals were grown from ethanol–methanol–water; $2(C_{36}H_{45}ClN_6O_{12})\cdot 5H_2O$, M = 1666.6, orthorhombic, space group $P2_12_12_1$, a = 18.756(3), b = 22.321(3), c = 23.940(6) Å, U = 10023(5) Å^3, $F(000)$ = 3528, D_c = 1.10 g cm^{-3}, Z = 4, μ(Cu-K_α) = 11.0 cm^{-1}. The structure was solved by direct methods (MULTAN) with 1.1 Å diffractometer data measured with Cu-K_α radiation ($\bar{\lambda}$ = 1.5418 Å) at 288(1) K. Structure solution, however, was not straightforward as the site of the chlorine of only one phomopsin molecule in the asymmetric unit could be readily located. The site of the chlorine of the second molecule was located only after molecular fragments corresponding to *ca.* 40% of the scattering matter were included to initiate phase refinement. Refinement with anisotropic thermal parameters for the Cl atoms and isotropic thermal parameters for C, N, and O converged at R = 0.13 for 3633 observed terms ($I > \sigma_I$). The parameters were refined in two blocks with unit weights. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1, 1986.

O(2) and C(11). Phomopsin is present in the crystal as a zwitterion, with ionization at the didehydroaspartic acid residue and the *N*-methyl nitrogen atom of the substituted phenylalanine moiety protonated.

The structure (**2**) accounts well for certain properties of phomopsin A (derivatisation and lack of borate complexing) which are consistent with one rather than two phenolic hydroxy groups.[3] It is also consistent with n.m.r. and mass spectra. The highest mass peak in the latter[2] now represents the molecular ion. The main n.m.r. evidence[1] is equally interpretable on the basis of structure (**2**), although the δ 19 peak is now known to be due to the mono-anion of the terminal didehydroaspartic acid unit,[2] and there is a need to reconsider the lack of detection of a 2-bond C,H coupling in the group *H*–N–*C*H_3 (which originally suggested that the N–CH_3 was present in an amide linkage).

Phomopsin A resembles the cyclopeptide group of alkaloids,[4] in that the latter are tetra- or penta-peptides with a modified (decarboxylated and dehydrated) 3-(*p*- or *m*-hydroxyphenyl)-3-hydroxyalanine unit linked through an ether bridge to a 3-hydroxy amino acid, forming a 13- or 14-membered ring. However, in the alkaloids, the peptide chain is connected through the amino group of the phenylalanine unit rather than through the carboxy group as in phomopsin A. The cyclopeptide alkaloids occur in the plant family Rhamnaceae and some other families, and have an ability to bind calcium, magnesium, and lithium ions which suggests a function as ionophores.[5] Phomopsin A has given preliminary evidence of a similar capability,[6] in addition to its strong tubulin-binding action.[7]

Received, 25th February 1986; Com. 254

References

1 C. C. J. Culvenor, P. A. Cockrum, J. A. Edgar, J. L. Frahn, C. P. Gorst-Allman, A. J. Jones, W. F. O. Marasas, K. E. Murray, L. W. Smith, P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Chem. Commun.*, 1983, 1259.
2 J. A. Edgar, J. L. Frahn, P. A. Cockrum, and C. C. J. Culvenor, in 'Mycotoxins and Phycotoxins,' ed. P. S. Steyn, Elsevier, Amsterdam, 1986, in the press.
3 J. A. Edgar, C. C. J. Culvenor, J. L. Frahn, A. J. Jones, C. P. Gorst-Allman, W. F. O. Marasas, P. S. Steyn, R. Vleggaar, and P. L. Wessels, in 'Trichothecenes and other Mycotoxins,' ed. J. Lacey, Wiley, Chichester, 1985, p. 317.
4 R. Tschesche and E. U. Kaussmann, in 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1975, vol. 15, p. 165.
5 J. C. Lagarias, R. A. Houghten, and H. Rapoport, *J. Am. Chem. Soc.*, 1978, **100**, 8202.
6 R. N. LePage and N. Osman, personal communication.
7 E. M. Tönsing, P. S. Steyn, M. Osborn, and K. Weber, *Eur. J. Cell Biol.*, 1984, **35**, 156.