The Complexation Properties of Some Unnatural and Natural Macrocyclic Trichothecenes

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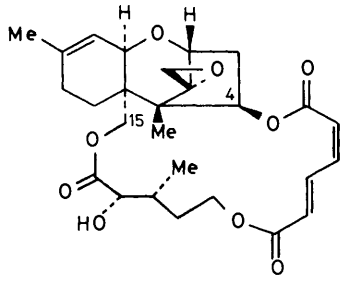
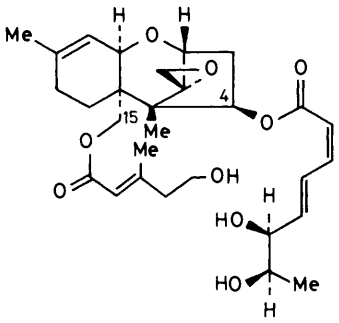
As a result of investigating the complexing properties of a series of novel polyether analogues (**3**)—(**9**) of macrocyclic trichothecenes towards alkali metal cations and RNH_3^+ ions, it has been proposed, with supporting evidence from *X*-ray crystallography on verrucarin A (**1**) and fast atom bombardment mass spectrometry of complexes involving (**1**) and the triacetate (**10**) of baccharinol B4 (**11**), that the enhanced biological activities of macrocyclic trichothecenes, such as (**1**) and (**11**), compared with non-macrocyclic trichoverroids such as (**2**), may be a consequence of their abilities to bind to NH_3^+ ionic sites on proteins, particularly those associated with the 60S ribosomes.

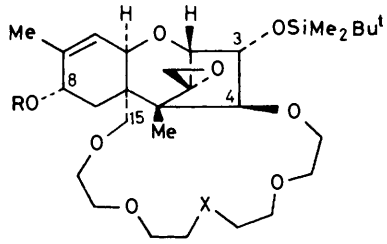
The trichothecene family of sesquiterpenoids are secondary metabolites[1—4] which exhibit potent biological activities. Recently, the macrocyclic trichothecenes, such as verrucarin A (**1**), have attracted our attention because of their high cytotoxicity and cytostaticity. Although the rôle played by the macrolide ring in determining the bioactivity of these compounds is not clear, the closely related, but non-macrocyclic trichoverroids, such as (**2**), are about two orders of magnitude less toxic than the macrocyclic trichothecenes,[5] suggesting that the macrolide ring plays an important part in optimising the activities of these compounds. This 'macrocycle effect,' in relation to biological potency, is reminiscent of the dramatic increase in the strength of the complexation of cations observed[6] on going from acyclic polyethers, such as the dimethyl ether of pentaethyleneglycol to its macrocyclic counterpart, 18-crown-6 (18C6). Other macrocyclic antibiotics such as valinomycin, nonactin, and erythromycin are known[7] to form strong complexes with M^+ and/or NH_4^+ ions: in some cases, it is thought[7] that this cationic complexation causes a conformational change in the macrocycle and so elicits the observed biological response. In an attempt to obtain information which could help elucidate the biological function, if any, of the macrocyclic ring in trichothecenes such as (**1**), we prepared a series of simple macrocyclic polyether analogues, *eg.* (**3**)—(**9**), the syntheses of which have been described elsewhere.[8] Here, we report on the complexation



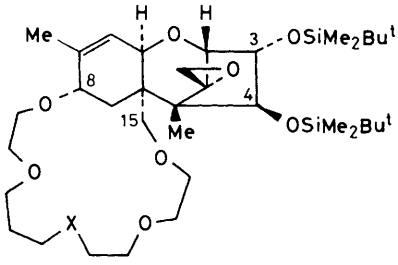
(1) Verrucarin A



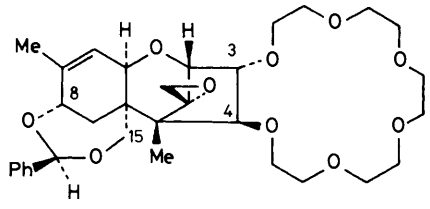
(2)



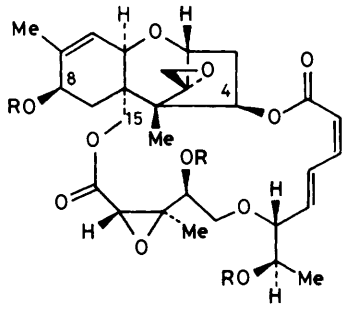
(3) R = p-$MeOC_6H_4CH_2$, X = O
(4) R = Bu^tMe_2Si, X = O
(5) R = p-$MeOC_6H_4CH_2$, X = OCH_2CH_2O
(6) R = Bu^tMe_2Si, X = O O



(7) X = O
(8) X = OCH_2CH_2O



(9)



(10) R = Ac
(11) R = H

properties of these novel chiral crown ethers† with M^+, NH_4^+, and RNH_3^+ ions and compare the results with those obtained for verrucarin A (**1**) and the peracetylated derivative (**10**) of another naturally-occurring compound, baccharinol B4 (**11**): the cation complexing behaviour of (**1**) is interpreted with reference to its *X*-ray crystal structure.‡

The association constants (expressed as log K_a values) determined[9] in $CDCl_3$ (by extraction of picrate salts from aqueous solution) between M^+, NH_4^+, and RNH_3^+ ions and the trichothecene macrocycles (**3**)—(**9**) are displayed graphically in Figure 1. Although the trichothecene polyether macrocycles form strong complexes with monovalent cations, they are much weaker than those formed[10] by 18C6. Assuming that the non-covalent bonds responsible for the binding of these cations are directional[11] with respect to the donor oxygen atoms, the perturbation of the polyether (*i.e.* OCH_2CH_2) repeating unit by the trichothecene nucleus in (**3**)—(**8**) impairs considerably their complexing abilities relative to those of 18C6. Whether the polyether chains bridge the 'natural' C(4) and C(15) positions or the 'unnatural' C(8) and C(15) positions, log K_a values are in the range 2.3—6.6 which

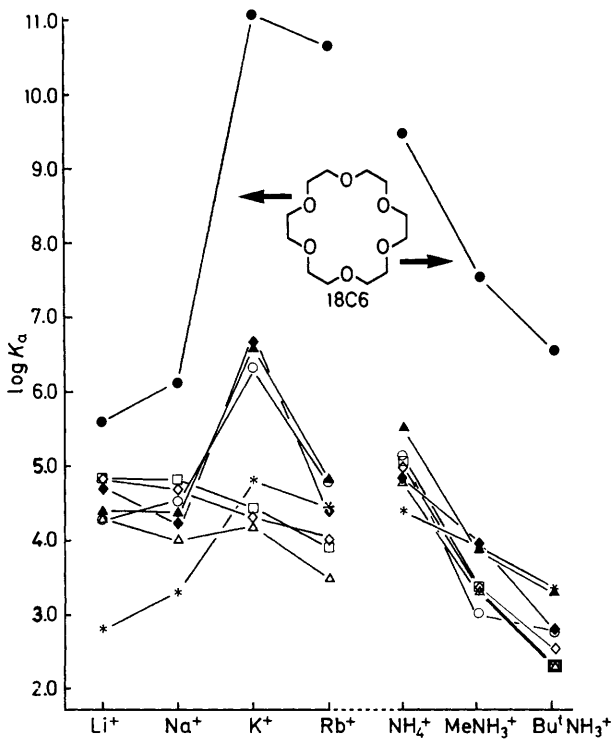


Figure 1. The association constants, expressed as log K_a values, for 1:1 complexes formed between various picrates in $CDCl_3$ and the macrocyclic trichothecenes (**3**) (◇), (**4**) (□), (**5**) (▲), (**6**) (◆), (**7**) (△), (**8**) (○), and (**9**) (*); and 18C6 (●) for comparison.

† Although numerous stereospecific syntheses of chiral crown ethers from natural products such as carbohydrates have been reported in the literature (J. F. Stoddart, *Chem. Soc. Rev.*, 1979, **8**, 85; *Top. Stereochem.*, 1987, **17**, 207) examples leading to the incorporation of terpenoids are rare; for macrocycles based on (+)- and (−)-camphane-2,3-diols, see S. Sasaki and K. Koga, *Heterocycles*, 1979, **12**, 1305.

‡ Single crystals suitable for *X*-ray crystallography were isolated from chloroform solutions of verrucarin A (**1**) on vapour diffusion with pentane.

Table 1. Abundances of [Macrocycle + Metal]$^+$ or [Macrocycle + RNH_3]$^+$ ions[a] relative to the [Macrocycle + H]$^+$ ion[b] expressed as percentages.[c]

Macrocyclic trichothecene	Li^+	Na^+	K^+	Rb^+	Cs^+	NH_4^+	$MeNH_3^+$	$Bu^tNH_3^+$
Verrucarin A (**1**)	200(509)	232(525)	187(541)	—	285(635)	—	238(534)	—
Baccharinol B4 triacetate (**10**)	89(695)	181(711)	30(727)	45(773)	—	14(706)	303(720)	1430(762)

[a] Abundances are shown followed by *m/z* values for [Macrocycle + Metal]$^+$ or [Macrocycle + RNH_3]$^+$ in parentheses. [b] The *m/z* values for [(**1**) + H]$^+$ and [(**10**) + H]$^+$ are 503 and 689, respectively. [c] F.a.b. experiments were carried out using a Kratos MS80RF mass spectrometer (accelerating voltage 4 kV; resolution 1500) coupled to a DS55 data system. The atom gun was an adapted saddle-field source (Ion Tech limited) operated at *ca.* 8 keV and a tube current of *ca.* 2 mA. Xenon was used to provide the primary beam of atoms and samples were dissolved in a small amount of 3-nitrobenzyl alcohol which had been coated on to a stainless steel probe. All spectra were recorded in the positive ion mode and at a scan speed of 30 s per decade.

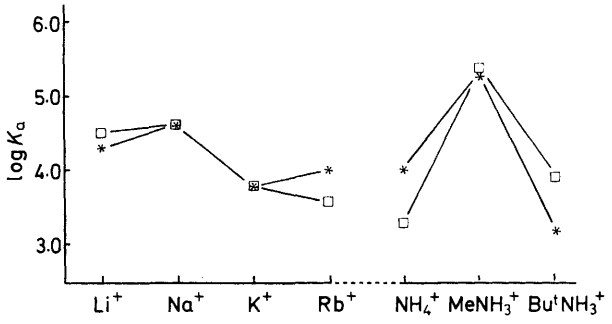


Figure 2. The association constants, expressed as log K_a values, for 1:1 complexes formed between various picrates in $CDCl_3$ and verrucarin A (**1**) (*) and baccharinol B4 triacetate (**10**) (□).

is 3—6 log K_a units lower than those observed[10] for 18C6. Although the 17-crown-5 derivatives (**3**), (**4**), and (**7**) are relatively unselective in their complexation of Li^+, Na^+, K^+, and Rb^+ ions, the 20-crown-6 derivatives (**5**), (**6**), and (**8**) exhibit modest selectivities towards K^+ ions. In both sets of derivatives, the binding to RNH_3^+ ions decreases across the series, H > Me > Bu^t for R. Despite the fact that the macrocyclic ring in (**9**) possesses an uninterrupted crown ether constitution, the *trans*-fusion to the trichothecene nucleus at C(3) and C(4) confers§ relatively low binding properties upon this receptor with its superficial resemblance to 18C6.

To our knowledge, there are no reports in the literature of the naturally-occurring macrocyclic trichothecenes functioning as molecular receptors towards cationic substrates. Since fast atom bombardment mass spectrometry (f.a.b.m.s.) has proved[12] to be such a sensitive technique for the detection of complexes between crown ethers and metal, inorganic, and organic cations, we decided to examine verrucarin A (**1**) and baccharinol B4 triacetate (**10**) in the presence of a range of picrate salts. The results (Table 1) were so encouraging that no time was lost in determining log K_a values in $CDCl_3$ for complexation with the same picrate salts as had been employed with the macrocyclic polyether derivatives (**3**)—(**9**).

The graphic display in Figure 2 shows interestingly, that, for (**1**) and (**10**), whilst their interaction with M^+ ions is relatively unselective, they both show some selectivity for $MeNH_3^+$ ions compared to NH_4^+ and $Bu^tNH_3^+$ ions.

In order to investigate whether the conformation of the macrocyclic ring in verrucarin A (**1**) is capable of presenting a convergent set of oxygen atom donor sites to a RNH_3^+ ion, we have determined the *X*-ray crystal structure¶ of (**1**). Figure 3 shows space filling representations of (a) the α-face and (b) the β-face of the macrocyclic ring in (**1**). On the β-face, two of the three inward pointing macrocyclic oxygen atoms are obscured by the quaternary methyl group on the trichothecene nucleus; the remaining hydroxyl and carbonyl oxygen atoms are directed away from the centre of the macrocycle. On the α-face, two of the carbonyl oxygen atoms‖ are oriented in the direction of that face and while the ring oxygen atom belonging to the third lactone function is directed inwards, it is shielded partly by the tertiary methyl group. Clearly, a small amount of conformational change is required to produce an optimum binding site for a RNH_3^+ ion.‖ Comparison (Figure 4) of the conformation of (**1**) with that of its *p*-iodobenzenesulphonate shows that, whereas the conjugated diene-containing segments of the macrocycles are essentially invariant, there is a significant difference in the relative orientations of the substituents in the remainder of the macrocyclic ring.

§ The relatively weak binding of M^+ and RNH_3^+ by (**9**) may be compared with the K_a value of <50 M^{-1} in $CDCl_3$ for methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-altropyranosido[2,3-*b*]-[1,4,7,10,13,16]hexaoxacyclo-octadecane (R. B. Pettman and J. F. Stoddart, *Tetrahedron Lett.*, 1979, 457), where an *anti* conformation is enforced upon the relative orientation about the C(2)–C(3) bond of the two oxygen atoms in the 18C6 unit attached to the pyranosidic rings and obliged by virtue of the *altro* configuration of the 4,6-fused pyranosidic ring to be *trans*-diaxial.

¶ *Crystal data* for verrucarin A (**1**): $C_{27}H_{34}O_9$, M = 502.6, monoclinic, space group $P2_1$, a = 10.604(6), b = 8.823(3), c = 14.309(7) Å, β = 97.90(4)°, U = 1326 Å^3, Z = 2, D_c = 1.26 g cm^{-3}, μ(Cu-K_α) = 7 cm^{-1}. 1839 Independent observed reflections [$|F_o| > 3\sigma(|F_o|)$, θ ≤ 58°] were measured on a Nicolet R3m diffractometer with graphite monochromated Cu-K_α radiation using the ω-scan measuring routine. The structure was solved by direct methods and the non-hydrogen atoms were refined anisotropically to give R = 0.067, R_w = 0.073, [$w^{-1} = \sigma^2(F) + 0.001F^2$]. The hydroxy and epoxy hydrogen atoms were located from a ΔF map and refined isotropically. The positions of the remaining H atoms were calculated (C–H, 0.96 Å), assigned isotropic thermal parameters, $U(H) = 1.2U_{eq}(C)$, and allowed to ride on parent C atoms. The methyl groups were refined as rigid bodies. 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.

‖ Amide carbonyl groups can act as hydrogen bond acceptors towards NH_3^+ centres: this is demonstrated by the formation (B. Bartman, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, 1977, **99**, 1028) of a stable complex between *cyclo*-(L-Pro-Gly)$_3$ and (*S*)-valine methyl ester hydrochloride. Also of relevance to the present investigation is the fact that the acyclic polyether antibiotic lasalocid forms (J. W. Westley, R. H. Evans, Jr., and J. F. Blount, *J. Am. Chem. Soc.*, 1977, **99**, 6057) stable and highly crystalline complexes with catecholamines.

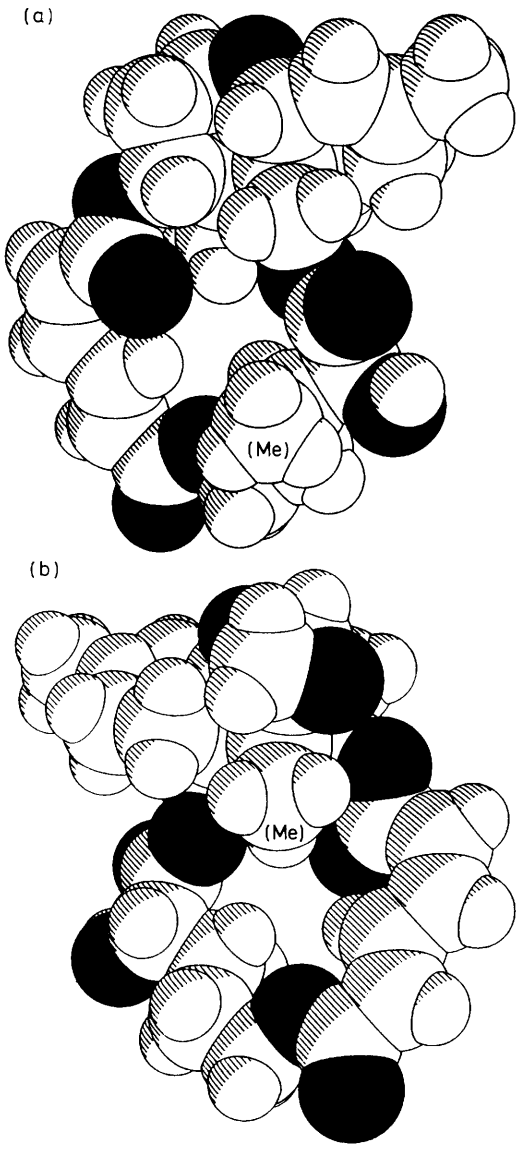


Figure 3. Space-filling representations of the *X*-ray crystal structure of verrucarin A (**1**) viewed (a) from the α-face and (b) from the β-face of the macrocycle. The oxygen atoms are shaded in black.

Although this observation demonstrates the flexibility of the ring and hence the potential of (**1**) for providing a suitable receptor geometry for binding of an RNH_3^+ ion, to date, no crystalline complex has been obtained.

The demonstrated complexing ability of the natural macrocyclic trichothecenes for RNH_3^+ ions may be a reflection of their affinity to bind with NH_3^+ ionic sites on proteins[14] and may provide a clue to the reasons for the enhanced biological activities of these macrocyclic trichothecenes. In particular, strong binding to certain amino acid residues in proteins associated with the 60S ribosome of eucaryotic cells could orientate other functional groups on the trichothecene skeleton advantageously with respect to an appropriate receptor site and so maximise their biological activities. This hypothesis might account for the observation that the macrocyclic trichothecenes, *e.g.* (**1**) and (**11**), display higher biological activities than their non-macrocyclic analogues, *e.g.* (**2**), and even, in some instances, appear[15] to bind irreversibly to the 60S ribosome.

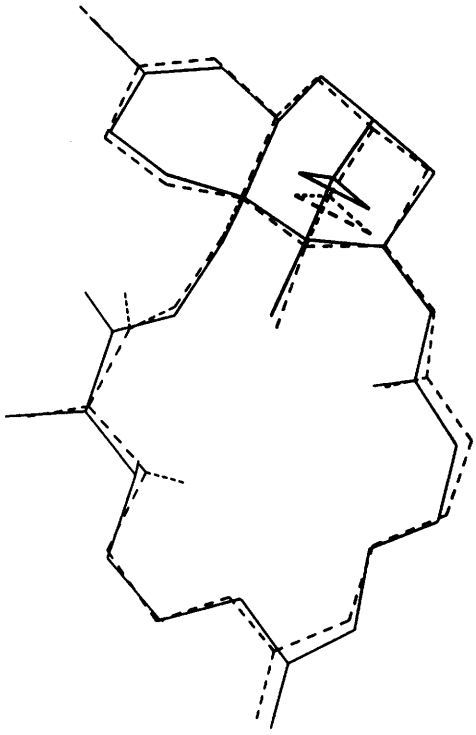


Figure 4. Comparison of the solid state structures of verrucarin A *p*-iodobenzenesulphonate (ref. 13) (solid lines with the ester group omited) and verrucarin A (**1**) (dashed lines). A similar comparison with the solid state structure of verrucarin B (W. Breitenstein, Ch. Tamm, E. V. Arnold, and J. Clardy, *Helv. Chim. Acta*, 1979, **62**, 2699), which contains an epoxide ring fused to the macrocyclic ring [*cf.* (**10**) and (**11**)], leads to a very similar result and conclusion.

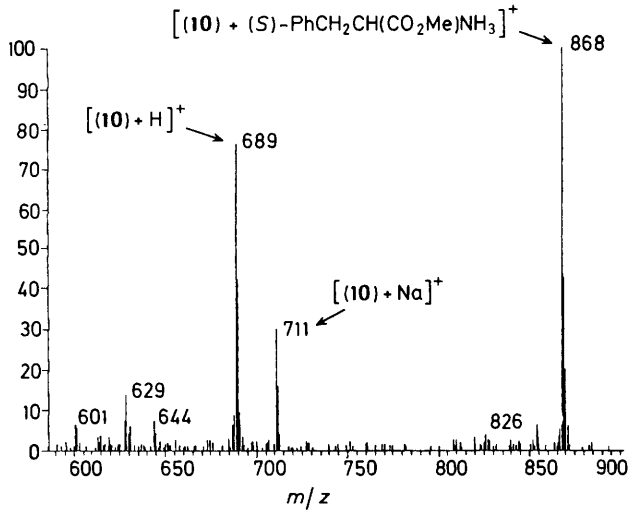


Figure 5. F.a.b. mass spectrum (positive ion detection) of baccharinol B4 triacetate (**10**) and (*S*)-phenylalanine methyl ester hydroperchlorate.

Evidence supporting the formation of a complex between an amino acid ester cation and a macrocyclic trichothecene has been obtained from the f.a.b. mass spectrum (Figure 5) performed on an equimolar solution of verrucarin A (**1**) and the methyl ester hydroperchlorate of (*S*)-phenylalanine.

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