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NOVEL 4,15-POLYETHER ANALOGUES OF MACROCYCLIC TRICHOTHECENES

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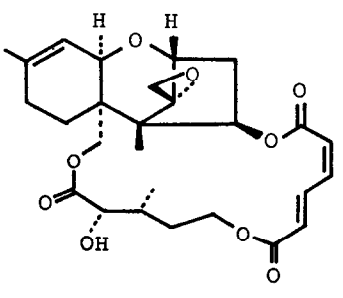
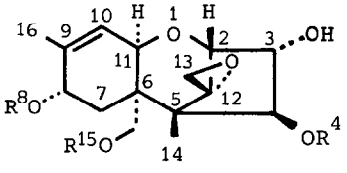
Following the development of the appropriate protecting group chemistry, the preparation of the 4,15-polyether analogues **17-20**, **23-25**, and **29-31** *of macrocyclic trichothecenes from T-2 toxin* (**2**) *and neosolaniol* (**3**) *is described.*

The trichothecenes are a family of sesquiterpenoid secondary fungal metabolites, usually characterised by the presence of a spiro-epoxy group, produced[1] by species belonging to the genera *Fusarium*, *Myrothecium*, *Stachybotrys*, *Trichoderma*, *Trichothecium*, *Cephalosporium*, and *Verticimonosporium*. They are potent inhibitors of protein synthesis in eukaryotic cells and exhibit[2] a broad spectrum of biological properties including antifungal, phytotoxic, insecticidal, and cytotoxic activities. They have been implicated[3] in various mycotoxicoses of animals and man, such as red-mould disease, alimentary toxic aleukia, and stachybotryotoxicosis. Their cytotoxic properties have also attracted[4] considerable interest as potential anti-tumour agents. The hundred or more naturally-occurring trichothecenes may be broadly classified[5] into two groups, depending on the presence of a macrocyclic ring of varying degrees of complexity linking C-4 and C-15. Many of the macrocyclic trichothecenes, such as those produced by species of *Myrothecium* and *Stachybotrys*, exhibit[6] extremely potent biological properties. Verrucarin A (**1**), for example, is toxic to mouse lymphoma cells[7] at concentrations of 1 ng ml^{-1}.

Other macrocyclic antibiotics, such as erythromycin require[8] the availability of certain inorganic cations (*e.g.* K^+ or NH_4^+) before they exhibit biological activity. Also, many naturally-occurring macrocyclic trichothecenes have polyoxygenated 18-membered rings and so bear a superficial resemblance to the well-known ionophore analogue[9], 18-crown-6 (18C6). Thus, it was of interest to synthesise some simple macrocyclic polyether derivatives[10] of the trichothecenes in order to (*i*) evaluate their abilities to complex with inorganic cations and (*ii*) assess their biological activities. Here, we report the preparation of some 4,15-polyether analogues of macrocyclic trichothecenes derived from T-2 toxin (**2**) and neosolaniol (**3**).

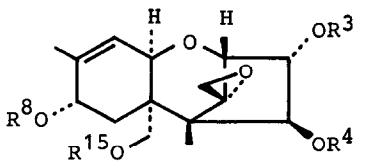


Verrucarin A (**1**)



		R^4	R^8	R^{15}
T-2 Toxin	(**2**)	Ac	Val^i	Ac
Neosolaniol	(**3**)	Ac	H	Ac
HT-2	(**4**)	H	Val^i	Ac
T-2 Triol	(**5**)	H	Val^i	H

Table 1. A list of trichothecenes derived from T-2 toxin (**2**) and neosolaniol (**3**), together with melting points, and partial ^{1}H n.m.r. spectroscopic data recorded at 250 MHz in $CDCl_3$ solution[a].



Cpd	M.p. (°C)	R^3	R^4	R^8	R^{15}	δ H-3[b]	δ H-4[c]	δ H-8[d]	δ H-15[e]
2	151-152[f]	H	Ac	Val[i]	Ac	4.17	5.30	5.29	4.07/4.28
3	171-172[g]	H	Ac	H	Ac	4.15	5.23	4.10	3.70/4.15
6	149-151	TBDMS	Ac	Val[i]	Ac	4.25	5.72	5.28	4.09/4.34
7	138-141	TBDMS	H	H	H	4.11	3.99	4.07	3.40/3.72
8	48-50	TBDMS	H	H	TBDMS	4.10	4.02	4.08	3.61/3.78
9	35-37	TBDMS	Ac	Ac	TBDMS	4.19	6.15	5.28	3.60/3.85
10	177	TBDMS	TBDMS	H	H	4.12	4.06	4.07	3.45/3.68
11	129-133	TBDMS	TBDMS	Ac	Ac	4.13	4.29	5.25	3.95/4.28
12	(Oil)	TBDMS	H	MEM	H	4.11	4.05	4.00	3.40/3.72
13	(Oil)	TBDMS	H	H	MEM	4.11	4.05	4.03	4.02/4.12
14	144-146	TBDMS	H	CH_2Ph	H	4.12	4.01	3.82	3.45/3.64
15	(Oil)	TBDMS	Ac	CH_2Ph	Ac	4.25	5.67	3.76	4.18/4.42
21	143-144	TBDMS	H	PMB	H	4.11	4.00	3.83	3.38/3.62
22	(Oil)	TBDMS	Ac	PMB	Ac	4.25	5.69	3.73	4.19/4.41
26	117-120	TBDMS	Ac	H	Ac	4.26	5.67	4.08	4.39/4.27
27	(Oil)	TBDMS	Ac	TBDMS	Ac	4.22	6.05	4.05	4.51/4.13
28	127-130	TBDMS	H	TBDMS	H	4.11	4.08	3.96	3.51/3.62

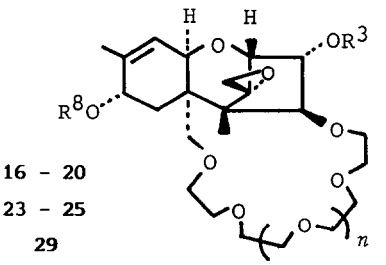
[a] Spectra recorded at ambient temperature on a Bruker AM250 spectrometer using $CDCl_3$ as lock and Me_4Si as internal standard. [b] $J_{2,3}$ 4.8-5.0 Hz. [c] $J_{3,4}$ 2.0-3.0 Hz. [d] $J_{7\beta,8}$ 4.8-6.3 Hz. [e] J_{AB} 10.0-12.8 Hz. [f] J.R Bamburg, N.V. Riggs, and F.M. Strong, *Tetrahedron*, 1968, **24**, 3329. [g] K. Ishii, K. Sakai, Y. Ueno, H. Tsunoda, and M. Enomoto, *Appl. Microbiol.*, 1971, **22**, 718.

T-2 Toxin (**2**) was isolated[11] as the major metabolite, along with neosolaniol (**3**), HT-2 (**4**), and T-2 triol (**5**) from the culture *Fusarium tricinctum* (strain NRRL 3299) grown on rice. All the new compounds[12] that have been synthesised are listed in **Tables 1** and **2** together with partial ^{1}H n.m.r. spectroscopic data and melting-points for crystalline compounds. Protection of the 3-hydroxyl group of **2** as a TBDMS-ether (TBDMS-Cl/imidazole/DMF) afforded **6** in 95% yield. De-esterification (NaOMe/MeOH/rt) of **6** to give the triol (**7**) was accomplished quantitatively. All attempts to monosilylate selectively the allylic hydroxyl group of **7** with TBDMS-Cl in the presence of a range of different bases and solvents failed. For example, with imidazole in DMF, the 3,15- and 3,4- disilyl ethers **8** and **10**, which were characterised respectively as their 4,8- and 8,15-diacetates **9** and **11** were obtained in a 4:1 ratio. Next, we explored the possibility of protecting the allylic 8-hydroxyl group as a MEM-ether, only to discover that the 4,8-diol **13** was formed as a minor product (*ca.* 8%) in the reaction (MEM-Cl/NaH/THF/0°/20 min) of **7** to give the expected 4,15-diol **12** as the major product (*ca.* 63%), which could not be separated from **13**. Finally, the desired regioselectivity was obtained on benzylation ($PhCH_2Br$/NaH/THF/reflux/1h) of **7** to give (86% yield) exclusively the 8-*O*-benzyl derivative **14**, which was characterised as its 4,15-diacetate **15**. Although treatment (NaH/THF) of **14** with triethyleneglycol bistosylate[13] did not yield[14] the 14-membered ring compound **16**, the corresponding 17-crown-5 and 20-crown-6 derivatives **17** and **18** were obtained with tetra- (TEGBT) and penta- (PEGBT) ethyleneglycol bistosylates[13] in 71 and 76% yields, respectively. Although removal ($^nBu_4NF$/THF/rt) of the silyl protecting groups at the 3-positions in **17** and **18** proceeded in excellent yields (>80%) to give the alcohols **19** and **20**, respectively, de-*O*-benzylation (*e.g.* H_2/Pd on C; Na/NH_3; AcOH/MeOH) at the 8-positions could not be achieved

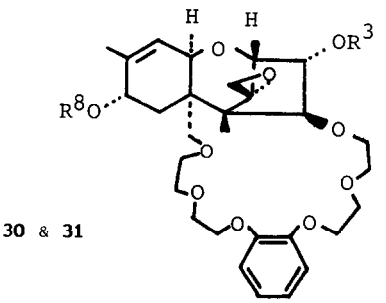
Table 2. A list of 4,15-polyether analogues of macrocyclic trichothecenes and partial ^1H n.m.r. spectroscopic data recorded at 400MHz in $CDCl_3$ solution[a].

				δ		
Cpd[b]	*n*	R^3	R^8	H-3[c]	H-4[d]	H-8[e]
16	0	TBDMS	CH_2Ph	-	-	-
17	1	TBDMS	CH_2Ph	4.16	3.42	3.76
18	2	TBDMS	CH_2Ph	4.15	3.42	3.76
19	1	H	CH_2Ph	4.26	3.55	3.77
20	2	H	CH_2Ph	4.28	3.54	3.76
23	1	TBDMS	PMB	4.15	3.42	3.75
24	1	TBDMS	H	4.25	3.43	4.05
25	1	H	H	4.27	3.55	4.04
29	1	TBDMS	TBDMS	4.17	3.42	3.99
30	-	TBDMS	TBDMS	4.15	3.43	3.99
31	-	H	H	4.23	3.55	4.03

[a] Spectra recorded at ambient temperature on a Bruker WH 400 spectrometer using $CDCl_3$ as lock and internal reference. Since the signals for H-3, H-4, and H-8 were masked by the resonances for the OCH_2 protons in the polyether chains, double resonance difference spectroscopy had to be employed to identify the signals. [b] All compounds are oils and were purified by column chromatography (SiO_2) using eluants such as $CHCl_3$, CH_2Cl_2, or Et_2O to which, in most cases, 2-10% MeOH had been added. [c] $J_{2,3}$ 4.8-5.0 Hz. [d] $J_{3,4}$ 2.0-2.3 Hz. [e] $J_{7\beta,8}$ 5.0-5.3 Hz.



16 - 20
23 - 25
29



30 & 31

because of competitive reactions at either the 9,10-double bond or the 12,13-epoxide. This problem was overcome by *para*-methoxybenzylation (*p*-$MeOC_6H_5CH_2Cl$/NaH/THF) of **7** to afford (80%) **21**, which was characterised as its 4,15-diacetate **22**. Conversion of **21** to the 17-crown-5 derivative **23** proceeded as described for the conversion of **14** to **17**. Deprotection (DDQ/H_2O/CH_2Cl_2 /rt/2h) of **23** to give **24**, followed by treatment of **24** with fluoride ion, afforded the unprotected 17-crown-5 derivative **25**. Clearly, this 3,8-diol could serve as a precursor to a range of modified (*e.g.* acylated, alkylated, oxidised, *etc.*) macrocyclic trichothecenes.

The use of the *para*-methoxylbenzyl function as a labile protecting group was exploited[15] to achieve a six-step conversion [**2** → **6** → **7** → **21** → **22** → **26** → **3**] of T-2 toxin (**2**) to neosolaniol (**3**). The availability of neosolaniol (**3**) from the original culture medium afforded us with the opportunity to make the unprotected 17-crown-5 macrocycle **25** by a much shorter alternative route [**3** → **27** → **28** → **29** → **25**] where the yields were >70% at all four steps. Reaction (NaH/THF) of the 4,15-diol **28** with 1,2-bis-(5-*p*-toluenesulphonyloxy-3-oxapentoxy)benzene[16] gave (73%) the benzo-20-crown-6 derivative **30** which was deprotected with fluoride ion to afford (85%) the 3,8-diol **31**.

The relative locations of different protecting groups at C-3, C-4, C-8, and C-15 on the trichothecene nucleus were deduced from ^1H and ^{13}C chemical shift data. For example, free primary and secondary hydroxyl functions were most easily identified (**Table 1**) by acetylating (Ac_2O/C_5H_5N) the diols (*e.g.* **10**, **14**, **21**, **28**) and noting the substantial downfield shifts ($\Delta\delta$ = 0.50 - 2.02 ppm) of the appropriate methylene and methine protons in the diacetates (*e.g.* **11**, **15**, **22**, **27**).

The complexing abilities and biological activities of the novel macrocyclic trichothecene analogues **17 - 20**, **23 - 25**, and **29 - 31** are currently under investigation.

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10. With the biological properties of the macrocyclic trichothecenes in mind, a series of lariat ethers based on the 18C6, 15C5, and 12C4 constitutions, and bearing pendant epoxide groups have recently been synthesised and evaluated (B.B. Jarvis, V.M. Vrudhula, D.M. Dishong, and G.W. Gokel, *J. Org. Chem.*, 1984, **49**, 2423).
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12. The compositions of all new compounds were confirmed by either elemental analysis or by high resolution mass spectrometry. Structural assignments were based upon results of low resolution mass spectrometry and high field ^{1}H and ^{13}C n.m.r. spectroscopy.
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14. Inspection of CPK space-filling molecular models suggests that at least a 16-membered ring is required to bridge C-4 and C-15 on the trichothecene nucleus.
15. The fact that silylation (imidazole/DMF) of **3** with TBDMS-Cl yields the 3-*O*-TBDMS ether **26** preferentially means that a three-step conversion [**3** → **26** → → **2**] of neosolaniol (**3**) to T-2 toxin (**2**) can be achieved (D.W. Anderson and R.M. Black, Unpublished results) by subsequent acetylation (i-ValCl, C_5H_5N) at the 8-position and deprotection (n-Bu_4NF/THF/rt) at the 3-position.
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