Structure Elucidation of a Novel Trichothecene Glycoside using ^1H and ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The structure elucidation of a novel trichothecene glycoside, 15-acetoxy-3α-hydroxy-4β-(α-D-glucopyranosyloxy)-12,13-epoxytrichothec-9-ene, is based on a detailed study of its one- and two-dimensional ^1H and ^{13}C n.m.r. spectra, and chemical reactions. The metabolite displays a reduced toxicity when compared with other trichothecene metabolites in several biological systems.

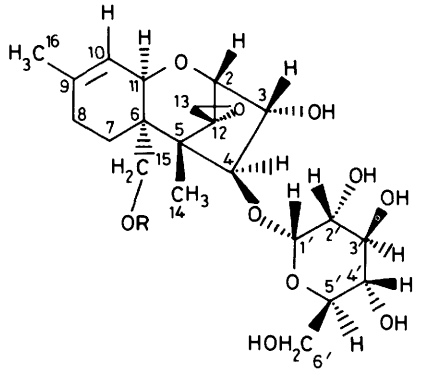
The trichothecenes form one of the most diverse and important families of mycotoxins and are produced by numerous species of *Cephalosporium*, *Fusarium*, *Myrothecium*, *Stachybotrys*, and *Trichoderma*.[1] As a consequence of the wide geographical distribution of these fungi, the mycotoxins have been implicated in a variety of human and animal diseases,[2] and as such have been the subject of numerous toxicological reports.[3] More than 60 trichothecenes are known at present. In this paper we describe the isolation and structure elucidation of the first naturally occurring trichothecene glycoside, an α-glucopyranoside derivative of monoacetoxyscirpenol, produced by a strain of *Fusarium sulphureum*, isolate MRC 514.

The structure of the metabolite was elucidated as (**1**) by ^1H and ^{13}C high-field n.m.r. spectroscopy, chemical reactions, and a knowledge of the related trichothecene metabolites produced by this strain of *F. sulphureum*.[4] Fast-atom bombardment (FAB) mass spectrometry indicated a molecular mass of 486, in accord with the molecular formula $C_{23}H_{34}O_{11}$. Absorption in the i.r. region at 1 720 cm^{-1} and 3 340 cm^{-1} was attributed to the presence of carbonyl and hydroxy groupings in compound (**1**).

Addition of D_2O to the sample caused the resonances at δ_H 5.197, 4.875, 4.770, 4.764, and 4.501 in the ^1H n.m.r. spectrum to disappear, thus identifying five hydroxy groups. The presence of a 12,13-epoxytrichothecene moiety in the molecule was suggested by the characteristic doublets at δ 2.904 and 2.688 (*J* 4.2 Hz) assigned to the C-13 geminal protons. Two-dimensional (^1H,^1H) chemical shift correlation spectroscopy using the COSY-45 pulse sequence[5] established the (^1H,^1H) connectivity pattern, and this, coupled with chemical shift criteria and coupling constant values, permitted the assignment of the complete ^1H n.m.r. spectrum of compound (**1**) (Table). Thus the anomeric proton (δ 4.717) was used as the starting point in the assignment of the protons of the glucosyl ring; the olefinic proton, 10-H (δ 5.325), in the assignment of 11-H and 16-H; and the hydroxy proton, (3-OH) (δ 5.197), in the assignment of 3-H, 2-H, and 4-H. The identification of the remaining protons, 7-H, 8-H, 14-H, and 15-H, follows from their chemical shift values.

The ^{13}C n.m.r. data for compound (**1**), collated in the Table, were obtained from proton-decoupled and single-frequency nuclear Overhauser-enhanced (n.O.e.) ^{13}C n.m.r. spectra, and revealed that the 23 carbon resonances observed in the n.m.r. spectrum of (**1**) are due to 3 methyl, 5 methylene, 10 methine, and 5 quaternary carbon atoms.

Chemical shift criteria dictate that the resonances at δ_C 170.20, 138.83, and 119.04 p.p.m. must be attributed to the acetate carbonyl carbon atom and the olefinic carbon atoms C-9 and C-10, respectively, whereas the resonance at δ_C 99.71 p.p.m. is typical of the anomeric carbon atom in α-glycosides[6]



(**1**) R = $COCH_3$
(**2**) R = H

and must thus be attributed to C-1′. The remaining eight methine carbon atoms directly bonded to one oxygen atom resonate between δ_C 86.96—67.07 p.p.m. (Table) and were individually assigned by two-dimensional (^1H,^{13}C) chemical shift correlation spectroscopy.[5a,7] Collectively they represent C-2, C-3, C-4, C-11, C-2′, C-3′, C-4′, and C-5′.

The methylene carbon atoms, C-7, C-8, C-13, C-15, and C-6′, may be assigned as follows. The carbocyclic carbon atoms, C-7 and C-8, resonate at δ_C 20.67 and 27.54 p.p.m., respectively: the high-field signal is assigned to C-7 by analogy with the ^{13}C n.m.r. spectrum of diacetoxyscirpenol,[8] in which the corresponding resonances are at δ_C 21.1 and 27.9 p.p.m., respectively. The methylene carbon atom of the oxirane ring, C-13, resonates at δ_C 46.12 p.p.m., which value is characteristic of the chemical shift found for this carbon atom in other trichothecenes.[8] The remaining methylene carbon signals, C-15 and C-6′, appear at δ_C 63.26 and 60.91 p.p.m., respectively, and were differentiated by correlation of the carbon resonances with the unambiguously assigned proton signals.

The three methyl carbon signals appear at δ_C 6.86, 20.82, and 22.85 p.p.m. The highest field signal is assigned to C-14 by comparison with the corresponding signal in other trichothecenes.[8] In the coupled ^{13}C n.m.r. spectrum of (**1**) the quartet centred at δ_C 22.85 p.p.m. shows additional fine structure in that each leg of the quartet is split into a doublet (*J* 5.8 Hz) arising from allylic coupling with 10-H. Thus this signal corresponds to C-16. The signal at δ_C 20.82 p.p.m. is therefore assigned to the acetate methyl carbon atom.

Table. ^1H and ^{13}C N.m.r. data for compounds (1) and (2)[a]

Atom	δ_H[b] (1)	δ_H[b] (2)	δ_C[c,d] (1)
2	3.302	3.248	78.81 D
3	4.071	4.000	77.42 D
3-OH	5.197	5.070	—
4	4.035	4.278	86.96 D
5	—	—	49.09 S
6	—	—	43.29 S
7(a)		1.805	20.67 T
(b)	1.925—	1.721	
8(a)	1.625	1.973	27.54 T
(b)		1.539	
9	—	—	138.83 S
10	5.325	5.285	119.04 D
11	3.836	3.783	67.07 D
12	—	—	64.54 S
13(a)	2.904	2.867	46.12 T
(b)	2.688	2.659	
14	0.719	0.763	6.86 Q
15(a)	4.026	3.465	63.26 T
(b)	3.789	3.242	
15-OH	—	4.463	
16	1.625	1.607	22.85 Q
1′	4.717	4.776	99.71 D
2′	3.215	3.213	72.12 D
2′-OH	4.501	4.373	
3′	3.399	3.407	73.07 D
3′-OH	4.764	4.766	
4′	3.070	3.055	70.32 D
4′-OH	4.875	4.874	
5′	3.572	3.562	73.07 D
6′(a)	3.440	3.421	60.91 T
(b)	3.665	3.693	
6′-OH	4.770	4.820	
C=O	—	—	170.20 S
CH_3	1.985	—	20.82 Q

[a] Recorded on a Bruker WM-500 spectrometer. [b] Relative to $[^2H_6]Me_2SO$ at δ_H 2.490. [c] Relative to $[^2H_6]Me_2SO$ at δ_C 39.50 p.p.m. [d] Capital letters refer to multiplicities arising from coupling with directly bonded protons. S = singlet, D = doublet, T = triplet, Q = quartet.

The remaining signals in the spectrum appear at δ_C 64.54, 49.09, and 43.29 p.p.m. and correspond to C-12, C-5, and C-6, respectively. Chemical shift criteria dictate that the low-field signal be assigned to the carbon atom directly bonded to oxygen, C-12, while C-5 and C-6 are assigned by comparison with the values obtained for the corresponding carbon atoms in diacetoxyscirpenol[8] (δ_C 49.1 and 44.2 p.p.m., respectively).

The ambiguities remaining in the structure related to the nature and position of the sugar moiety, and the position of the hydroxy and acetoxy functions on the trichothecene nucleus. Additions of D_2O to a sample of compound (1) in $[^2H_6]$dimethyl sulphoxide removed a 4.5 Hz coupling from the 3-H signal in the ^1H n.m.r. spectrum, thus locating the hydroxy function at C-3 of the trichothecene nucleus. Treatment of compound (1) with 2M-HCl at 100 °C for 1 h with subsequent t.l.c. against various standards (glucose, galactose, mannose, xylose, rhamnose, and fructose) in four different developers established that the sugar was glucose.[9] This was confirmed by the vicinal coupling constants in the ^1H n.m.r. spectrum of compound (1) (Figure), where the large values encountered are indicative of a diaxial antiperiplanar arrangement for 2′-H, 3′-H, 4′-H, and 5′-H. The α-glycoside linkage was inferred from the vicinal coupling constant observed for 1′-H and 2′-H (3.8 Hz),

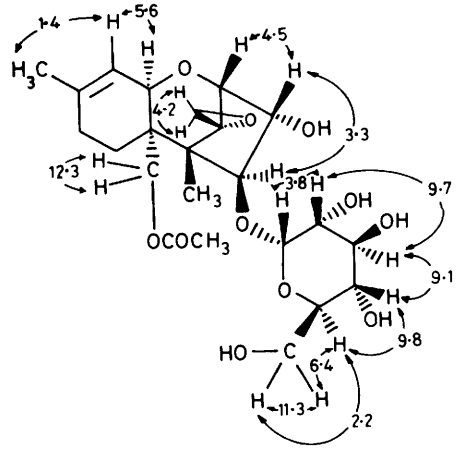


Figure. ^1H Coupling constants (Hz) for compound (1). The coupling constants to the protons of the hydroxy groups have been omitted. These are (3-H, 3-OH) 4.5 Hz; (2′-H, 2′-OH) 5.6 Hz; (3′-H, 3′-OH) 5.1 Hz; (4′-H, 4′-OH) 5.3 Hz; and (6′-H, 6′-OH) 5.2 Hz

and by the chemical shift value of C-1′ in the ^{13}C n.m.r. spectrum of glycoside (1) (δ_C 99.71 p.p.m.).[6]

Finally, base hydrolysis of compound (1) with methanolic potassium hydroxide gave the de-acetylated product (2). The upfield shift of the C-15 protons in the ^1H n.m.r. spectrum of compound (2) (Δδ 0.561 and 0.547 p.p.m.), when compared with their chemical shift values in the acetate (1), placed the acetoxy function at C-15, and thus the glucose group must be located at C-4.

Biological evaluation of compound (1) gave several interesting results. In contrast to other 12,13-epoxytrichothecenes the molecule shows no significant dermatotoxic effects, nor is it lethal to brine shrimps at levels up to 200 times greater than those found for other trichothecenes. However, *in vivo* the metabolite is toxic to rats at levels below 90 mg kg^{-1}, presumably due to hydrolysis of the glucoside in the stomach.

Experimental

I. r. spectra were measured on a Perkin-Elmer 257 spectrophotometer using KBr discs, optical rotations on a Perkin-Elmer 241 polarimeter, and u.v. absorptions on a Unicam SP8-100 spectrophotometer, both for solutions in methanol. T.l.c. was carried out on Merck precoated silica gel plates (coating thickness 0.25 mm). For column chromatography Merck silica gel, particle size 0.063—0.200 mm, and octadecyl Porasil B, prepared according to the method of Kingston and Gerhardt,[10] were used.

Isolation of Glycoside (1).—The procedures followed in the extraction and partition of maize meal (5 kg) infected with *Fusarium sulphureum* MRC 514 were identical with those described in ref. 4. The aqueous extract (300 g) was purified further by chromatography on SiO_2 (2 kg) with methanol—ethyl acetate (1:9, v/v) as eluant. After elution of the four trichothecenes characterized previously from this strain,[4] an additional fraction giving a blue colour reaction with the 4-(*p*-nitrobenzyl)pyridine spray reagent[11] was eluted. This material (450 mg) was purified on reversed-phase silica gel (100 g) with methanol–water (1:1, v/v) as eluant to yield glycoside (1) (182 mg) as a pale yellow gum, $[\alpha]_D^{20}$ +79.5° (*c* 0.585); $\lambda_{max.}$ end-absorption only; $\nu_{max.}$ 3 340, 2 890, 1 720, 1 230, and 1 015 cm^{-1}; *m/z* (FAB) 509 $[M + Na]^+$.

Acid Hydrolysis Glycoside (1).—A solution of glycoside (1) (11 mg) in water (2 ml) was treated with hydrochloric acid (2M;

2 ml) and kept at 100 °C for 1 h. The solution was cooled and extracted with ethyl acetate (2 × 5 ml). The aqueous layer was compared with standards of glucose, galactose, mannose, xylose, rhamnose, and fructose by t.l.c. in the following solvent systems: (a) n-butanol–acetic acid–water (4:1:5, v/v/v), (b) n-butanol–ethanol–water (4:1:2.2 v/v/v), (c) n-butanol–benzene–pyridine–water (5:1:3:3, v/v/v/v), and (d) phenol saturated with water. In all cases the R_F value of the unknown sugar was identical with that of glucose.

Base Hydrolysis of Glycoside (**1**).—A solution of potassium hydroxide (3 mg) in methanol (0.5 ml) was added to a stirred solution of glycoside (**1**) (20 mg) in methanol (2 ml). After 5 h at 20 °C, the solvent was removed in a stream of N_2, and the resultant gum was purified on SiO_2 (10 g) with methanol–chloroform (1:4, v/v) as eluant, to yield the hexaol (**2**) (8 mg) as a gum.

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