STEREOCHEMICAL STUDIES ON THE FUMONISINS,

METABOLITES OF *FUSARIUM MONILIFORME*

by

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OPSOMMING

Die hoë voorkoms van slukdermkanker onder inwoners van die Transkei, asook harsingsverweking onder perde, word toegeskryf aan *Fusarium moniliforme*, 'n algemene swam wat op mielies voorkom.

Daar is reeds vasgestel dat die fumonisiene, 'n familie van struktuurverwante mikotoksiene wat vanuit *F. moniliforme* kulture geïsoleer is, die diesters is van propaan-1,2,3-trikarboksielsuur en 2-asetielamino- of 2-amino-12,16-dimetiel-3,5,10,14,15-pentahidroksiikosaan sowel as in elke geval die C-10 deoksi analoog. Die C-14 en C-15 hidroksigroepe is in elke geval betrokke in estervorming.

In die huidige studie is die koppeling van die propaan-1,2,3-trikarboksielsuureenhede met die C-14 en C-15 hidroksigroepe bestudeer deur selektiewe reduksie van die ester funksionaliteite met natriumboorhidried, en die daaropvolgende karakterisering van die laktoon. Sodoende is daar vasgestel dat 'n terminale karboksigroep van propaan-1,2,3-trikarboksielsuur in beide gevalle betrokke is in estervorming.

Die relatiewe konfigurasie van die verskillende chirale sentra teenwoordig in die fumonisiene, is afgelei van proton-proton koppelingskonstantes en proton-proton k.O.e. studies op 2,2-dimetiel-1,3-dioksolaan-, 1,3-dioksaan-, 2,2-dimetiel-1,3-dioksaan- en 2-oksasolidinoon-derivate. Die absolute konfigurasie van die C-10 en C-5 chirale sentra is bepaal deur van Horeau se metode gebruik te maak. Die C-16 sentrum se absolute konfigurasie is vasgestel deur oksidatiewe splyting van die C-14 — C-15 diol en die daaropvolgende vergelyking van die α-metiel-*p*-nitrobensielamied derivaat van die gevormde 2-metielheksanoësuur met 'n standaard.

In hierdie stereochemiese studie is die absolute konfigurasie van fumonisien B_1 bewys as (2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R*)-1,1'-[14,15-(2-amino-3,5,10-trihidroksi-12,16-dimetielikosaandiiel)] di-(2,3—diwaterstof propaan-1,2,3-trikarboksilaat).

SUMMARY

Fusarium moniliforme, a common fungal contaminant of maize, has been implicated as the causative agent in human oesophageal cancer in the Transkei as well as equine leukoencephalomalacia in horses worldwide.

Previous studies established that the fumonisins, a family of structurally related mycotoxins isolated from cultures of *F. moniliforme*, are the diesters of propane-1,2,3-tricarboxylic acid and either 2-acetylamino- or 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyicosane as well as in each case the C-10 deoxy analogue. In all cases both the C-14 and C-15 hydroxy groups are involved in ester formation.

In the present study the mode of linkage of the propane-1,2,3-tricarboxylic acid groups to the C-14 and C-15 hydroxy groups in the fumonisin molecule was studied by selective reduction of the ester functionalities with sodium borohydride and subsequent characterization of the lactone. In this way it was established that in each case a terminal carboxy group of propane-1,2,3-tricarboxylic acid is involved in the ester linkage.

The relative configuration of the different chiral centres present in the fumonisins was deduced from proton-proton coupling constants and proton-proton n.O.e. studies on 2,2-dimethyl-1,3-dioxolane, 1,3-dioxane, 2,2-dimethyl-1,3-dioxane and 2-oxazolidinone derivatives. The absolute configuration of the C-10 and C-5 chiral centres was determined by the method of Horeau. Oxidative cleavage of the C-14—C-15 diol moiety and comparison of the *α*-methyl-*p*-nitrobenzylamide derivative of the 2-methylhexanoic acid formed with a standard, established the absolute configuration at C-16.

The stereochemical studies established the absolute configuration of fumonisin B_1 as (2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R*)-1,1'-[14,15-(2-amino-3,5,10-trihydroxy-12,16-dimethylicosandiyl)] di-(2,3—dihydrogen propane-1,2,3-tricarboxylate).

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CHAPTER 1

INTRODUCTION

Mycotoxins, toxic secondary metabolites produced by fungi, are the causative agents of various disorders in man and his domestic animals and have been part of mankind's environment throughout the ages. The diseases caused by the ingestion of foods or animal feeds contaminated by these toxic fungal metabolites are commonly called mycotoxicoses and are characterized by their sporadic regional and seasonal occurrence.

The earliest described mycotoxicosis,[1] ergotism, was one of the most prominent persistent disasters of European history for 2000 years. Grain contaminated by the parasitic fungus, *Claviceps purpurea*, is the causative agent of this disease. Toxin-producing strains of *Stachybotrys atra* are responsible for the fatal disease, stachybotryotoxicosis, which reached epidemic proportions affecting horses in the Soviet Union during the 1930's.[2] Alimentary toxic aleukia, caused by overwintered grain infected with *Fusarium sporotrichioides*, was responsible for the deaths of hundreds of thousands of people in Russia during the last years of World War II. *Fusarium sporotrichioides* strains produce trichothecene mycotoxins *e.g.* nivalenol, neosolanol, and T-2 toxin.[3] Trichothecenes are also produced by *Fusarium graminearium*, which can affect more than 30% of the national production of wheat, rye and rice in Japan. Infections are frequently associated with outbreaks of human mycotoxicoses.[3]

The current international awareness of mycotoxins is the result of the outbreak of a mycotoxicosis that caused the deaths of 100 000 turkeys and chickens in 1960 in England. The origin of this disease was traced to Brazilian peanut meal[2] contaminated by the aflatoxins, highly carcinogenic secondary metabolites of the ubiquitous fungi, *Aspergillus flavus* and *Aspergillus parasiticus*.

Since the dramatic discovery of the aflatoxins, mycotoxins and fungal related problems have been extensively investigated by *inter alia* organic chemists, mycologists, plant pathologists, toxicologists and epidemiologists. *Fusarium* species and their toxic metabolites have been the subject of several investigations and are

currently receiving considerable attention.

Fusarium moniliforme Sheldon, a common fungal contaminant of maize throughout the world, has been implicated as the possible cause of pellagra in Italy in 1881 and of equine leukoencephalomalacia in the United States in 1900 and 1978.[3] Since then it has been suspected of being involved in various human and animal diseases resulting after the ingestion of *Fusarium* contaminated foods and feeds. The causative role of *F. moniliforme* has been established beyond doubt for the mycotoxic disease, equine leukoencephalomalacia (LEM).[4] Field outbreaks of LEM occur sporadically in many countries including the United States of America, South Africa, Argentina and China.[4] This neurotoxic disease of horses and other Equidae is characterized by liquefactive necrosis of the white matter of the cerebral hemispheres.

Mycotoxins produced by *F. moniliforme* are the main suspects as causative agents of human oesophageal cancer. The highest oesophageal cancer rate in Africa occurs in the southwestern districts of the Transkei, while the rate in the northeastern region is relatively low. In a comparative study of the mycoflora of home-grown maize produced in the two areas, the most prominent difference was the significant higher incidence of *F. moniliforme* in maize produced in the high cancer rate area.[3]
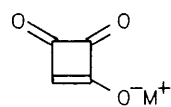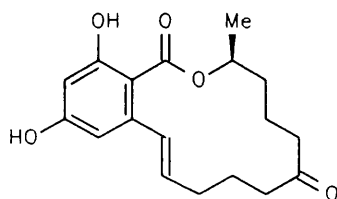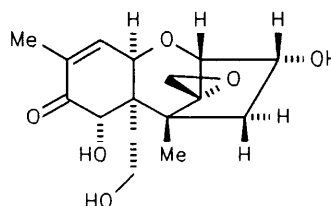
M = Na or K

(1)
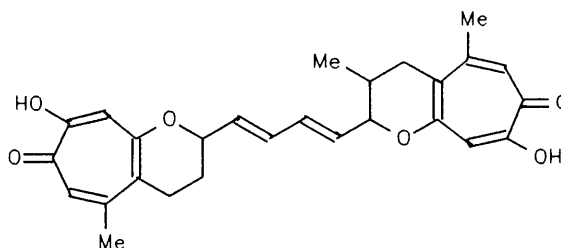
(3)

(2)

(4)

Strains of *Fusarium moniliforme* have been reported to produce a number of mycotoxins *e.g.* moniliformin (1), zearalenone (2), fusariocin A and C (3) and the trichothecenes deoxynivalenol (4), diacetoxyscirpenol, fusarin C and T-2 toxin.[3,5] Investigations proved it highly unlikely that moniliformin is responsible for leucoencephalomalacia, since the *Fusarium* strain responsible for this disease does not produce detectable levels of this mycotoxin.[3]

The chemical nature of the *F. moniliforme* mycotoxins implicated in the above mentioned diseases, remained unknown until 1988, when a family of structurally related mycotoxins, the fumonisins, was isolated from cultures of *F. moniliforme* (strain MRC 826) using a short-term cancer initiation-promotion bioassay.[6] The structures of fumonisin B_1 (5), B_2 (6), A_1 (7) and A_2 (8) were elucidated by means of high resolution nuclear magnetic resonance spectroscopy and mass spectrometry.[7] The structure of fumonisin B_1 (5) was established as 1,1'-[14,15-(2-amino-3,5,10-trihydroxy-12,16-dimethylicosandiyl)] di-(2,3-dihydrogen propane-1,2,3-tricarboxylate). The structures of fumonisin B_1 (5) and B_2 (6) differ in that the C-10 hydroxy group of fumonisin B_1 (5) is replaced by a hydrogen atom in fumonisin B_2 (6). Fumonisin A_1 (7) and A_2 (8) are the *N*-acetyl derivatives of fumonisin B_1 and B_2, respectively.
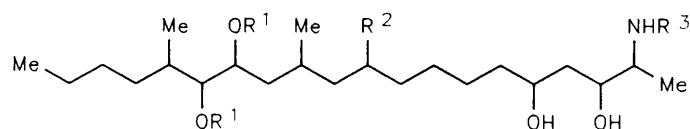
(5) R^1 = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H, R^2 = OH, R^3 = H

(6) R^1 = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H, R^2 = R^3 = H

(7) R^1 = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H, R^2 = OH, R^3 = Ac

(8) R^1 = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H, R^2 = H, R^3 = Ac

It is of interest to note that two structurally related mono-esters of propane-1,2,3-tricarboxylic acid and 1-amino-2,4,5,13,14-pentahydroxy-11,15-dimethylheptadecane (9) and (10) are host-specific phytotoxins produced by *Alternaria alternata*, the causal agent of stem canker disease of tomato.[7-9]

Since the reported isolation and structure elucidation of the fumonisins a number of biological studies have been performed to determine the natural occurrence and toxicity of fumonisin B_1 (5) and B_2 (6). The first conclusive report of the natural
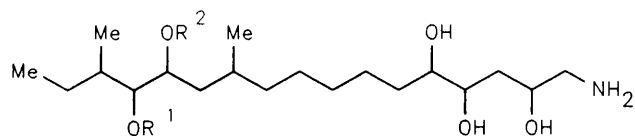
(9) R^1 = H, R^2 = CO-CH_2-CH(CO_2H)-CH_2-CO_2H

(10) R^1 = CO-CH_2-CH(CO_2H)-CH_2-CO_2H, R^2 = H

occurrence of fumonisin B_1 (5) in home-grown maize obtained from an area in the Transkei, was published by Sydenham *et. al.*[10] Recently, fumonisin B_1 (5) and B_2 (6) were also found to be causative factors in the development of leukoencephalomalacia in horses[4] and to be hepatotoxic to rats.[11]

In the present study, the mode of linkage of the propane-1,2,3-tricarboxylic acid moieties to the C-14 and C-15 hydroxy groups of the fumonisin C_{20}-chain was studied. In addition, the relative and absolute stereochemistry of the fumonisins were determined by spectroscopic and chemical methods.

CHAPTER 2

GENERAL BACKGROUND

2.1 DETERMINATION OF RELATIVE CONFIGURATION

N.m.r. spectroscopy is one of the most powerful tools for structural analysis available to the organic chemist. The detailed analyses of both the ^{1}H and ^{13}C n.m.r. spectra of fumonisin derivatives were facilitated by the results obtained from a number of n.m.r. techniques.

Assignments based on first-order analysis of the spin systems in the ^{1}H n.m.r. spectra were confirmed by homonuclear ^{1}H{^{1}H} decoupling, two-dimensional (2D) ^{1}H{^{1}H} homonuclear shift correlation (COSY), and proton-proton nuclear Overhauser effect (n.O.e.) experiments. The ^{13}C n.m.r. data, *viz.* chemical shifts and multiplicities were obtained from proton-decoupled, and proton-coupled and DEPT (distortionless enhancement by polarization transfer) spectra, respectively. The signals of the proton-bearing carbon atoms were correlated with specific proton resonances in a two-dimensional ^{13}C{^{1}H} heteronuclear shift correlation experiment utilizing the one-bond (^{13}C,^{1}H) spin-spin couplings.

2.1.1 Polarization transfer and the distortionless enhancement by polarization transfer experiment (DEPT)

The DEPT experiment was used in the present study to establish the multiplicities of the various ^{13}C resonances in the n.m.r. spectra of the fumonisins and the various derivatives.

The fundamental principle of the technique is based on polarization transfer and is demonstrated for a simple two-spin AX system of spin-½ nuclei, consisting of a ^{1}H (A part) and a ^{13}C (X part) nucleus. **Figure 1** shows the AX energy level diagram with the relative populations at thermal equilibrium and the allowed single quantum transitions, as well as the corresponding spectrum of the X part. The population difference between two energy levels, and thus the intensity of a transition, is

proportional to the gyromagnetic ratio, γ, of the nucleus that changes its spin state during the transition. The relative populations and transition intensities of an AX spin system before and after inversion of the A1 transition are collated in **Table 1**.[12]

a   b
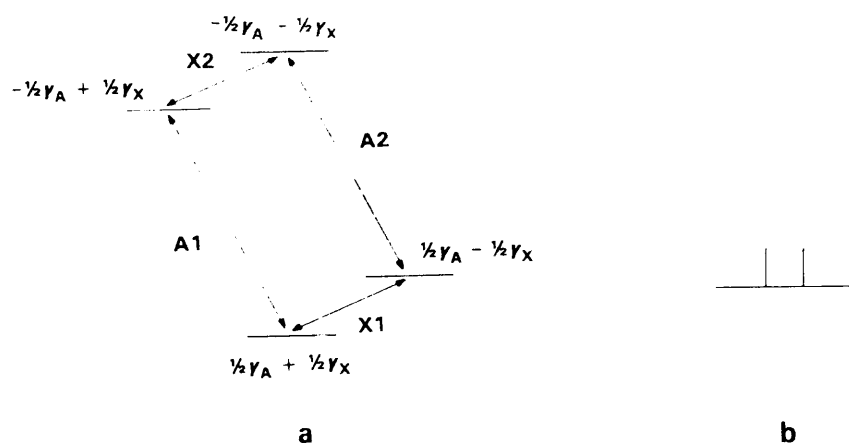
Figure 1   (a) Energy level diagram for an AX spin system at thermal equilibrium and (b) corresponding spectrum of the X part (^{13}C spectrum).

Table 1

	Populations		Transition	Intensities	
Energy level	At equilibrium	After inversion of A1 transition		At equilibrium	After inversion of A1 transition
1	$\tfrac{1}{2}\gamma_A + \tfrac{1}{2}\gamma_X$	$-\tfrac{1}{2}\gamma_A + \tfrac{1}{2}\gamma_X$	A1(1→3)	γ_A	$-\gamma_A$
2	$\tfrac{1}{2}\gamma_A - \tfrac{1}{2}\gamma_X$	$\tfrac{1}{2}\gamma_A - \tfrac{1}{2}\gamma_X$	A2(2→4)	γ_A	γ_A
3	$-\tfrac{1}{2}\gamma_A + \tfrac{1}{2}\gamma_X$	$\tfrac{1}{2}\gamma_A + \tfrac{1}{2}\gamma_X$	X1(1→2)	γ_X	$-\gamma_A - \gamma_X$
4	$-\tfrac{1}{2}\gamma_A - \tfrac{1}{2}\gamma_X$	$-\tfrac{1}{2}\gamma_A - \tfrac{1}{2}\gamma_X$	X2(3→4)	γ_X	$\gamma_A + \gamma_X$

The energy level diagram and the corresponding spectra shown in **Figure 2** result when the respective spin populations are interchanged through selective population inversion involving the A1 transition.

The result of this inversion is that the populations of the two energy levels of the A1 transition have been interchanged. For the two A transitions the population differences are the same, γ_A, except that one is now negative. For the X transitions which previously had a population difference of γ_X, we now find $-\gamma_A + \gamma_X$ for the X1 transition and $\gamma_A + \gamma_X$ for the X2 transition. This result implies that the population differences for the A transition have been transferred to the X transitions and added to the existing differences. This phenomenon is known as polarization transfer.
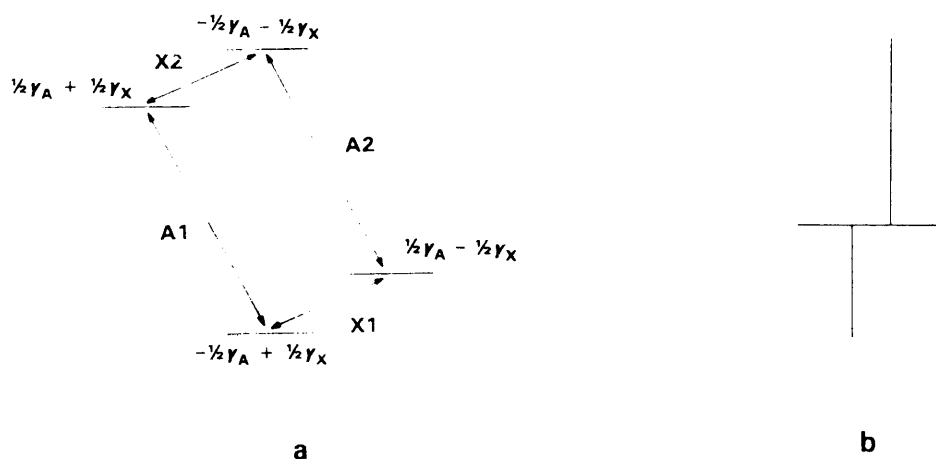
Figure 2   (a) Energy level diagram for an AX spin system after inversion of the A_1 transition and

(b) corresponding spectrum of the X-part.[12]

The DEPT experiment brings about polarization of all transitions, regardless of frequency. Signal intensities for CH, CH_2, and CH_3 are influenced by the precession of the components I, as a function of a defined angle, Θ, the polarization transfer pulse.

CH : $I \propto \sin\Theta$

CH_2: $I \propto \sin2\Theta$

CH_3: $I \propto 3/4\ (\sin\Theta + \sin3\Theta)$

Variation of the polarization transfer pulse, Θ leads to different intensities for the various multiplicities. Three DEPT spectra are recorded with a variable pulse Θ equal to $\pi/4$, $\pi/2$, and $3\pi/4$.[12,13] The $\theta = \pi/4$ spectrum contains all resonances, whereas the $\theta = \pi/2$ spectrum gives the methine resonances only. The $\theta = 3\pi/4$ spectrum once again exhibits all resonances, but with the methylene resonances inverted. Appropriate combinations of these spectra allows the generation of the CH, CH_2 and CH_3 subspectra.

2.1.2 Two-dimensional n.m.r. spectroscopy

In the present work two-dimensional (2-D) $^1H\{^1H\}$ homonuclear chemical shift correlation (COSY) and $^{13}C\{^1H\}$ heteronuclear chemical shift correlation experiments were used to correlate the chemical shift of ^1H-^1H and ^{13}C-^1H nuclei, respectively.

The concept of two-dimensional n.m.r. spectroscopy[12] is best explained by considering a sample which gives rise to only one signal with chemical shift value, v : for instance a solution of chloroform in a deuteriated solvent with proton observation.

At the start of the experiment the magnetization vector M is aligned along the z axis as indicated in **Figure 3**. Application of a $(\pi/2)_x$ pulse generates transverse magnetization (coherence) in the xy plane where it begins to precess with frequency v for a time t_1. At the end of the interval t_1, a second $(\pi/2)_x$ pulse is applied and the magnetization is measured as a normal free induction decay (FID).

$\left(\frac{\pi}{2}\right)_x$ —————————————— t_1 —————————————— $\left(\frac{\pi}{2}\right)_x$ ——————— Acquisiti·
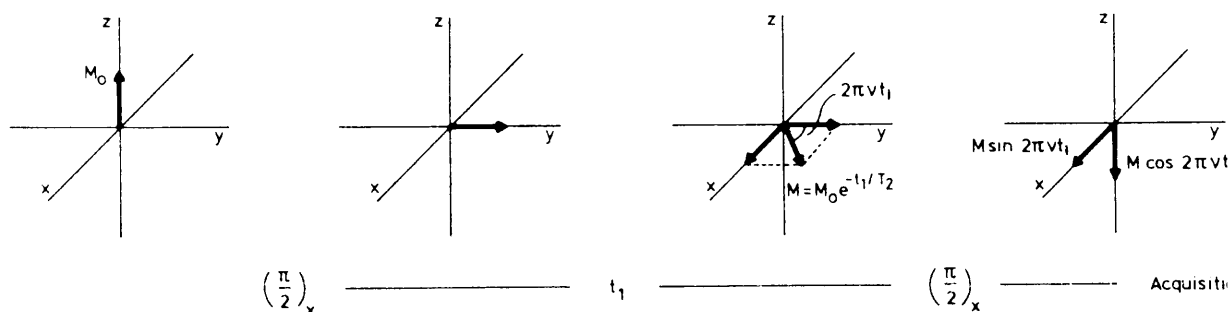
Figure 3   Influence of $(\pi/2)_x$ pulses on the magnetization vector, M_0. Amplitude modulation of an n.m.r. signal can be effected by varying the interval t_1 between two pulses.[12]

When a series of experiments with different values of t_1 is performed, a separate FID is detected in t_2 for each t_1 value.[14] Fourier transformation of each FID with respect to t_2 generates a set of spectra in which the amplitude of the signal oscillates (with frequency v) as a function of t_1 viz. M sin $2\pi v t_1$. A second Fourier transformation over t_1 of this sine function generates a signal that is centred at v_1 Hz. The two-dimensional Fourier transform converts the original dataset into a two-dimensional frequency spectrum $f(v_1,v_2)$ with v_1 and v_2 representing the chemical shift, v of the signal in the two dimensions.

In general, experiments are arranged such that the magnetization which evolves with some frequency during t_1, evolves with a different frequency during t_2; the latter frequency will invariably be the normal chemical shift value and could include spin-spin couplings.

In a coupled system of two nuclei e.g. ^{1}H and ^{13}C the first ($\pi/2$) pulse creates ^{1}H magnetization (coherence) which is transferred to the ^{13}C nucleus through (^{13}C,^{1}H) coupling by the simultaneous application of a ($\pi/2$) pulse to both the ^{1}H and ^{13}C nuclei. The two-dimensional spectrum which is obtained after Fourier transformation contains a signal at the coordinates (v_1,v_2) where v_1 represents the ^{1}H chemical shift and v_2 the ^{13}C chemical shift. This is the fundamental scheme for heteronuclear chemical shift correlation. The underlying phenomenon of the technique is the transfer of coherence amongst coupled spins.

The technique can be utilized to correlate ^{1}H and ^{13}C chemical shifts through either the one-bond or the two- and three-bond (^{13}C,^{1}H) coupling constants by changing the delay times, which are a function of $J(^{13}$C,^{1}H), in the pulse sequence.[12] Excitation of proton transitions is nonselective *i.e.* all resonances of the ^{1}H n.m.r. spectrum are excited at the same time and polarization transfer is t_1-dependent. The elegance of this type of two-dimensional correlation experiment must be seen in the fact that, using a single experiment, connections between two types of nuclei can be established. The correlation character of the method allows an assignment made for one type of nucleus to be transferred immediately to another type.

2.1.3 The Nuclear Overhauser Effect

The relative configuration of the C-2, C-3, C-5, C-14, and C-16 chiral centres in the fumonisins were determined by homonuclear ^{1}H{^{1}H} n.O.e. difference spectroscopy. The nuclear Overhauser effect (n.O.e.) is a very useful n.m.r. parameter for chemical structure elucidation and conformational analysis, as it is the only technique that does not depend on the presence of scalar coupling for its operation.[12]

The basic principle of the n.O.e. effect can be explained by considering an AX system with two spin-½ nuclei I and S in the same molecule, but with different chemical shifts and no J-coupling.[12] This system has four energy levels corresponding to the

nuclei in different spin states as illustrated in **Figure 4**.
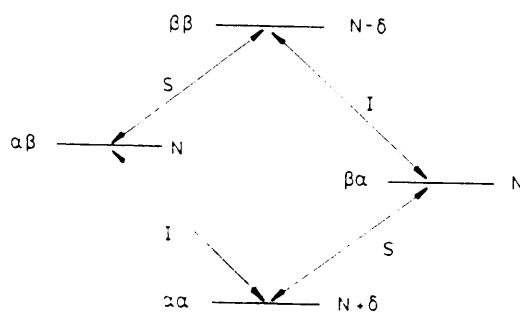
Figure 4   Energy levels and populations of a homonuclear AX system[12]

Irradiation at the resonance frequency of nucleus S produces a perturbation in the observed signal of nucleus I, because the decoupled nucleus contributes to the dipolar relaxation of the observed nucleus. Six different pathways between the four energy levels may be involved in relaxation **(Figure 5)**, but only two pathways W_2 and W_0 **(Figure 5)**, are contributing to the observed n.O.e. effect.
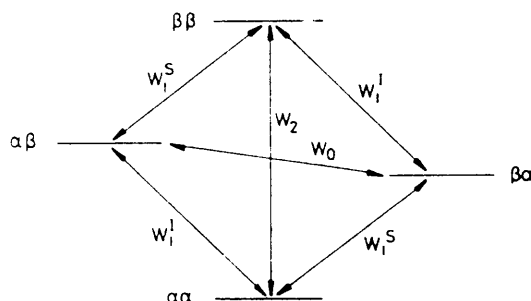
Figure 5   (a) Connections between the energy levels of an AX system, which may be involved in relaxation.

(b) Cross-relaxation after saturation of the S transition.[12]

The sign and magnitude of the n.O.e. are dependent on the equilibrium between these two cross-relaxation pathways. If W_2 is the dominant relaxation pathway, the result is a positive n.O.e. at I due to S. Dominance of the W_0 pathway results in a negative n.O.e.. The maximum positive homonuclear n.O.e. that can be obtained, is 50%.[12] When a methyl group and a methine proton are dipolar coupled, it is advantageous to irradiate the resonances of the methyl group, because fewer relaxation pathways will lead to a more significant n.O.e. effect.

Generally, n.O.e.'s are positive for small molecules in non-viscous solution, but negative for macromolecules *e.g.* proteins in viscous solution. In the event of balanced W_2 and W_0, the n.O.e. effect will be negligible. However, as the relaxation pathways are influenced by molecular motion in solution, a change in solvent or temperature may solve the problem.

Therefore, unlike chemical shifts and coupling constants that depend in part on through-bond effects, n.O.e.s are through-space effects. In an environment providing multiple dipole-dipole relaxation pathways, the n.O.e. between two protons is essentially inversely proportional to the sixth power of the distance between them. However, the ability to observe small n.O.e.s using difference techniques allows one to determine long-range through-space connections between protons and was applied with great success in the stereochemical studies on the fumonisins.

2.2   DETERMINATION OF ABSOLUTE CONFIGURATION

The 'partial kinetic resolution' method of Horeau was utilized in studies on the absolute configuration of the C-5 and C-10 centres of the fumonisins.

Stereoselective reactions can be used to correlate the configuration of chiral compounds if the course can be predicted on the basis of empirical rules or theoretical considerations. If the absolute configuration of one reactant is known, the chirality of a second can then be derived.[15] Horeau's method for the 'partial kinetic resolution' of secondary alcohols is based on this principle.[16] Although not completely unambiguous,[17] Horeau's method has been the primary chemical approach to the chirality assignment of secondary hydroxy groups for nearly three decades.[18]

The method involves esterification of a chiral alcohol with the general formula, L-CHOH-S, where L is larger than S, with an excess of racemic α-phenylbutyric anhydride. The principle of the method is based on the fact that the reaction occurs through diastereomerically related transition states which must be of different energy and the diastereomeric products will therefore be produced at different rates. As a result preferential reaction of the hydroxy group with one of the enantiomers of α-phenylbutyric acid occurs. When the excess of the anhydride is hydrolysed, the recovered α-phenylbutyric acid shows optical activity. Horeau has empirically

correlated the sign of the specific rotation of the recovered acid with the absolute configuration of the starting alcohol. In the event that the α-phenylbutyric acid has a negative optical rotation, and thus the R configuration, the starting alcohol is assigned the S configuration and *vice versa*.

The esterification reaction between the secondary alcohol and α-phenylbutyric acid can be highly stereoselective. As a result the optical yield has to be taken in consideration. The optical yield is the ratio between the optical purity of the α-phenylbutyric acid recovered from the reaction, which is measured experimentally and the optical purity which would have been realized had the reaction proceeded with complete stereoselectivity. The optical yield is given by:

$$\text{Optical yield} = [\alpha]_{D\ exp} / [\alpha]_{D\ theoretical} \times 100$$

$$\text{where} \quad [\alpha]_{D\ theoretical} = \pm 92° / 2a\text{-}1 \quad \text{and} \quad a = [\text{anhydride}] / [\text{alcohol}]$$

The value of $\pm 92°$ correspons to the specific rotation of optically pure α-phenylbutyric acid.

Values of optical yields quoted in the literature should be considered as accurate only to within about 20% due to racemization of the α-phenylbutyric acid in the presence of the anhydride. The establishment of the sign of rotation of the α-phenylbutyric acid is the more important process for the determination of absolute configuration.[19]

Since the development of the classical Horeau method, a number of modifications have been developed to extend the applicability of the method using micromolar quantities[20] and to the chirality determination of amines and carboxylic acids.[15]

Horeau's method has been applied successfully to the chirality determination of secondary hydroxy groups in a number of diverse natural products.[21-24] It should be noted that the method is not completely unambiguous as examples are known where unsatisfactory results were obtained. In studies on sterically hindered secondary alcohols,[18,25] as for instance in yohimbine, (-)-menthol (11) and the nakafuran sesquiterpenes, better results were achieved using racemic 2-phenylbutyryl chloride instead of 2-phenylbutyric anhydride. As the steric hindrance of the secondary alcohol group increases, the acid chloride gave superior yields or proved to be the only way

to effect esterification.[18] Thus the absolute configuration of the secondary alcohol in the compound, neomeranol (12), could be determined only by using 2-phenylbutyryl chloride.[18]
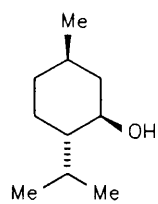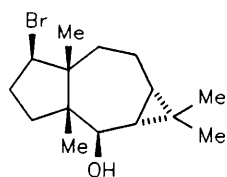

(11)   (12)

CHAPTER 3

DETERMINATION OF THE MODE OF LINKAGE OF THE PROPANE-1,2,3-TRI-CARBOXYLIC ACID MOIETIES TO THE C-14 AND C-15 HYDROXY GROUPS

The structure of fumonisin B_1 (5) has been established as 1,1'-[14,15-(2-amino-3,5,10-trihydroxy-12,16-dimethylicosandiyl)] di-(2,3-dihydrogen propane-1,2,3-tri-carboxylate).[6] The n.m.r. data for fumonisin B_1 (5) are collated in **Table 2**. The involvement of the terminal carboxy groups of the propane-1,2,3-tricarboxylic acid moieties in the ester linkage with the C-14 and C-15 hydroxy groups of the fumonisin backbone as reported in the literature[6] is based on a comparison of the n.m.r. data with that of the related phytotoxins (9) and (10) obtained from *A. alternata*. The C-14 and C-15 hydroxy groups of the fumonisins could be involved in ester formation with either the terminal carboxy group as in (13) or the central carboxy group of the propane-1,2,3-tricarboxylic acid moiety as in (14). The use of a terminal carboxy group in ester formation would generate two additional chiral centres in the fumonisin B_1 structure whereas a new prochiral centre is formed when the central carboxy group is used.

(13) R = CO–CH$_2$–CH(CO$_2$H)–CH$_2$–CO$_2$H
(14) R = CO–CH(CH$_2$–CO$_2$H)$_2$
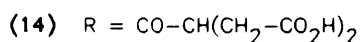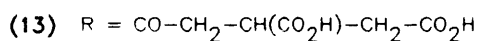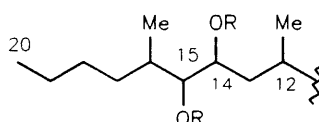
Two strategies were followed to determine the mode of linkage of the propane-1,2,3-tricarboxylic groups with the fumonisin C_{20}-chain. The first strategy involves the selective reaction of the carboxylic acid groups, followed by ester hydrolysis of the modified 1,2,3-tricarboxylic moieties. The second strategy requires the selective reduction of the ester functions in the presence of the carboxylic acid groups. The results of both strategies are the same: an ester linkage involving a terminal carboxy group would lead to the formation of a chiral C_6 compound whereas an ester linkage involving the central carboxy group would produce an achiral C_6 compound.
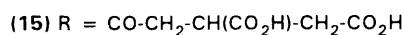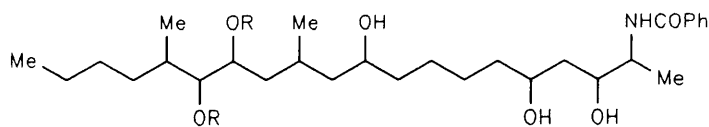
Selective reduction of the carboxylic acid groups of fumonisin B_1 with diborane in

Table 2   Relevant ^{13}C (125,76 MHz) and ^{1}H (500,13MHz) N.m.r. data for fumonisin B_1 (5)

Carbon	δ_C/p.p.m.	δ_H/p.p.m.	J(HH)/Hz
1	15,54Q	1,114d	6,6
2	51,74D	2,978qd	6,4; 6,6
3	68,44D	3,614ddd	6,2; 6,2; 6,4
4	40,75T	1,36m	
5	66,06D	3,63m	
10	67,21D	3,41m	
11	43,35T		
12	25,52T		
13	35,52T		
14	70,73D	4,978ddd	3,2; 3,7; 10,0
15	76,58D	4,795dd	3,7; 7,9
16	32,97D		
17	31,09T		
18	28,03T		
19	22,24T		
20	13,87Q	0,828t	7,1
21	15,47Q	0,844d*	6,8
22	20,16Q	0,850d*	6,5

* May be interchanged.

tetrahydrofuran (THF), following the first strategy, proved unsuccessful because of solubility problems of fumonisin B_1 (5). In order to improve the solubility of fumonisin B_1 (5), the polar amino group was selectively derivatized with benzoyl chloride. The reaction of aliphatic alcohols with benzoyl chloride in the presence of sodium hydrogen carbonate is much slower than that of amines[26] and therefore only N-benzoylfumonisin B_1 (15) [(M+H)$^{+\bullet}$ 862] was obtained from the reaction. The resonance at δ_C 167,68S in the ^{13}C n.m.r. spectrum is indicative of the carbonyl carbon atom of the newly-formed amide bond. Treatment of N-benzoylfumonisin B_1 (15) with diborane in THF gave mainly starting material and a small amount of a complex mixture. This route was not pursued any further.

Me   OR   Me   OH   NHCOPh

Me

OR   OH   OH   Me

(15) R = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H

The next approach involved the protection of the four carboxylic acid groups of fumonisin B$_1$ (5) as the methylthiomethyl ester derivatives. The methylthiomethyl ester function is stable in aqueous solutions at pH 0-15 for at least one hour at room temperature[27] and should allow selective hydrolysis of the propane-1,2,3-tricarboxylic ester groups involving the C-14 and C-15 hydroxy groups of fumonisin B$_1$ (5). The potassium salt of fumonisin B$_1$, obtained by careful treatment of fumonisin B$_1$ (5) with aqueous potassium hydroxide at pH 7,5, was reacted with chloromethyl methyl sulfide to afford fumonisin B$_1$ tetra(methylthiomethyl) ester (16) [$(M+H)^{+\bullet}$ 962].

Me   OR   Me   OH   NH$_2$

Me

OR   OH   OH   Me

(16) R = CO-CH$_2$-CH(CO$_2$-CH$_2$-S-CH$_3$)-CH$_2$-CO$_2$-CH$_2$-S-CH$_3$
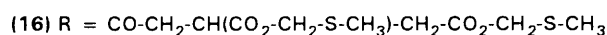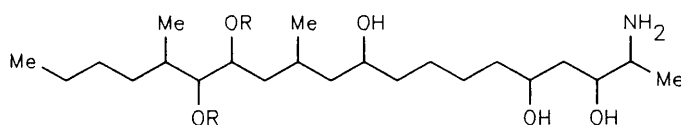
The characteristic ^{1}H resonances of the thiomethyl groups[27] appear as singlets at δ_H 2,228 (3H) and 2,235 (9H) in the ^{1}H n.m.r. spectrum. Treatment of fumonisin B$_1$ tetra(methylthiomethyl) ester (16) with sodium hydroxide (pH 10) afforded a small amount of di(methylthiomethyl) ester (17) or (18) admixed with intractable impurities which prevented analysis by ^{1}H n.m.r. spectroscopy.

CO$_2$H

CH$_3$-S-CH$_2$-CO$_2$ CO$_2$-CH$_2$-S-CH$_3$

(17)

CO$_2$-CH$_2$-S-CH$_3$

HO$_2$C   CO$_2$-CH$_2$-S-CH$_3$
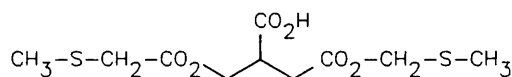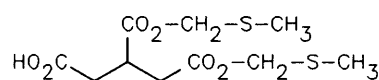
(18)

This lack of success prompted the implementation of the second strategy: selective reduction of the ester function present in fumonisin B_1 in the presence of the carboxy groups. According to the findings of Soai et al.[28] reduction of an ester group with sodium borohydride in t-butanol–methanol does occur and yields the primary alcohol. Carboxylic acids are not reduced under these conditions.
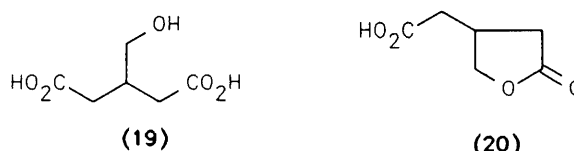
Reduction of the ester group in fumonisin B_1 involving the central carboxy group of the propane-1,2,3-tricarboxylic acid moiety will lead to the formation of an achiral compound, 3-(hydroxymethyl)glutaric acid (19) or the cyclised product 2-oxo-tetrahydrofuran-4-acetic acid (20).

(19)   (20)

The situation is more complex when a terminal carboxy group of propane-1,2,3-tricarboxylic acid is involved in ester formation in fumonisin B_1. Reduction of the ester moiety will now lead to the formation of a chiral compound, 2-(2-hydroxyethyl)-succinic acid (21). Cyclisation of (21) could lead to the formation of either a five-membered lactone, 2-oxo-tetrahydrofuran-3-acetic acid (22) or a six-membered lactone, 2-oxo-tetrahydropyran-4-carboxylic acid (23).

(21)   (22)   (23)
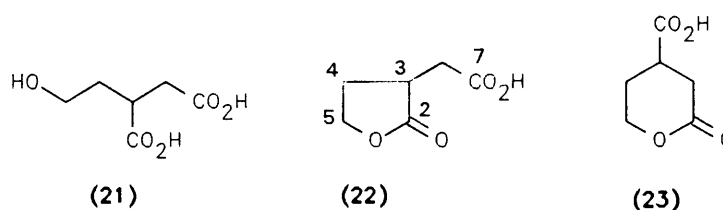
The formation of a five-membered lactone ring is thermodynamically favoured over a six-membered ring because of the greater strain energy associated with the six-membered lactone.[29] For example 2,3-dideoxyaldonolactone (24) is readily formed but the six-membered isomer has not been detected.[29] On the basis of this precedent it would appear that the formation of the five-membered lactone (22) would be favoured.

Treatment of fumonisin B_1 with sodium borohydride in t-butanol–methanol using the procedure as described by Soai[28] gave the reduction product as a colourless oil. The absorption band at 1765 cm^{-1} in the i.r. spectrum and the molecular ion at m/z 144
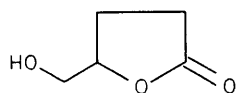
(24)

in the mass spectrum indicated that cyclisation occurs during work-up to afford a lactone.

The ^{1}H n.m.r. spectrum of the lactone recorded in deuteriochloroform ($CDCl_3$) could not be completely analysed as a number of resonances overlapped. In contrast the ^{1}H resonances in the spectrum recorded in C_6D_6 were well resolved due to diamagnetic shielding by the benzene nucleus. The values of the proton chemical shifts and proton-proton coupling constants were obtained by first-order analysis of these resonances and are collated in **Table 3**. The various proton resonances were correlated with specific proton-bearing carbon resonances in a two-dimensional (2D) $^{13}C\{^1H\}$ chemical shift correlation experiment and allowed the assignment of the proton-bearing carbon resonances as shown in **Table 3**. The proton-proton connectivity pattern for the lactone was deduced from these values and a number of homonuclear $^1H\{^1H\}$ experiments. The resonances at δ_H 3,227 and 3,484, assigned to the methylene proton situated on an oxygen-bearing carbon atom on the basis of their chemical shift values, served as starting point in determining the connectivity pattern as shown in **Figure 6**.
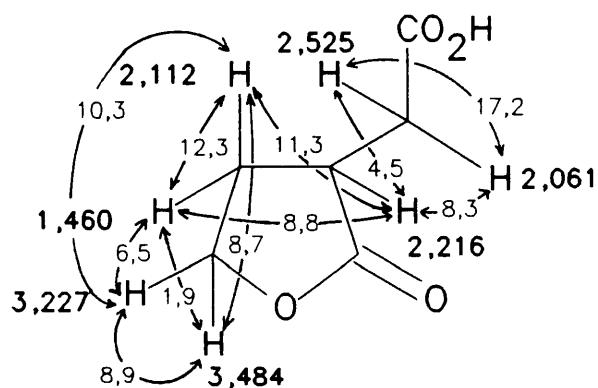
Figure 6 Connectivity pattern for **(22)**
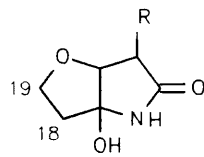
Table 3 ^{13}C (75,47 MHz) and ^{1}H (500,13 MHz) N.m.r. data for (22)

Carbon	δ_C/p.p.m.[a]	δ_H/p.p.m.[b]	J(HH)/Hz
2	178,05S		
3	35,84D	2,216m	4,5; 8,3; 8,8; 11,3
4	28,49T	1,112m	8,7; 10,3; 11,4; 12,4
		1,460m	1,9; 6,5; 8,8; 12,3
5	66,63T	3,227ddd	6,5; 8,9; 10,3
		3,484ddd	1,9; 8,7; 8,9
6	34,34T	2,061dd	8,3; 17,2
		2,525dd	4,5; 17,3
7	176,08S		

[a] Solvent: CDCl$_3$

[b] Solvent: C$_6$D$_6$.

It is immediately evident that the observed proton-proton connectivity pattern excludes the structure (20) for the lactone and is compatible only with the structures (22) and (23). A comparison of the magnitude of the geminal and vicinal coupling constants observed for the C-5 and C-4 methylene protons of the lactone with coupling constants reported for fusarin A (25)[30] (Figure 7), and (S)-4,5-dihydro-4-methyl-2(3H)-furanone[52] indicates that the lactone exists as the five-membered lactone (22).

	δ_C	δ_H	J(HH)/Hz
19	68,85	4,084	3,9; 8,6; 8,9
		3,992	6,5; 8,9; 8,9
18	37,89	2,365	8,6; 8,9; 12,8
		2,260	3,9; 6,5; 12,8

Figure 7   Coupling constants reported for the geminal and vicinal C-5 and C-4 methylene protons of fusarin A (25)

The above results establish unambiguously that reduction of fumonisin B$_1$ (5) using sodium borohydride in t-butanol-methanol leads to the formation of 2-oxo-

tetrahydrofuran-3-acetic acid (22), a compound, which, as indicated earlier, should be chiral. This is indeed the case as (22) exhibits optical activity and had a specific rotation of -1,3°. These findings furthermore prove that one of the prochiral enantiotopic carboxy groups (*i.e.* one of the terminal carboxy groups) of propane-1,2,3-tricarboxylic acid is involved in the ester linkage with the C-14 and C-15 hydroxy groups of the C_{20}-chain. In the enzyme-mediated formation of the ester linkage a chiral centre is created in each of the propane-1,2,3-tricarboxylic acid moieties of fumonisin B_1. No information on the absolute configuration of these centres is available as no physical data has been reported in the literature for either of the enantiomers of (22).

CHAPTER 4

DETERMINATION OF THE RELATIVE AND ABSOLUTE CONFIGURATION OF THE FUMONISINS

The strategy for the determination of the relative configuration of a number of chiral centres in the fumonisin molecule is based on the formation of conformationally-rigid 1,3-oxazolidinone, 1,3-dioxane, and 1,3-dioxolane derivatives. N.m.r. spectroscopic analyses of these derivatives may establish the relative configuration of the different chiral centres on the basis of proton-proton coupling constants, proton-proton n.O.e. studies and ^{13}C chemical shift values. In the present study on the fumonisins, this methodology led to the assignment of the relative configuration of six chiral centres.

4.1 DETERMINATION OF THE RELATIVE CONFIGURATION OF THE C-2 – C-3 CHIRAL CENTRES.

The relative configuration of the C-2 and C-3 chiral centres was assigned on the basis of proton-proton nuclear Overhauser effect (n.O.e.) experiments. The oxazolidinone derivative of fumonisin B$_2$ was prepared in order to lock the amino and hydroxy groups in a conformationally-rigid ring that could be readily analysed. The procedure is outlined in **Scheme 1**.

Fumonisin B$_2$ **(6)** , was hydrolysed with an aqueous methanolic solution (4:1 v/v) of potassium hydroxide (1M) to yield the aminotetrol **(26)**, which was reacted with an excess of di-*t*-butyl dicarbonate. After separation and purification by column chromatography, *N*-*t*-Boc aminotetrol **(27)** was obtained.

N-*t*-Boc-2-Amino-12,16-dimethyl-3,5,14,15-tetrahydroxyicosane **(27)** exhibits a characteristic carbonyl absorption band at 1685 cm^{-1} in the i.r. spectrum. The protons of the newly introduced *t*-butyl group appear as a nine-proton singlet at δ_H 1,414 whereas the singlet resonance at δ_C 156,45 in the ^{13}C n.m.r. spectrum was assigned to the carbonyl carbon atom of the *N*-*t*-Boc group.

1674082
1665432

(6)   R   = CO-CH$_2$-CH(CO$_2$H)-CH$_2$-CO$_2$H

(a)

(26)

(b)

(27)

(c)

(28)
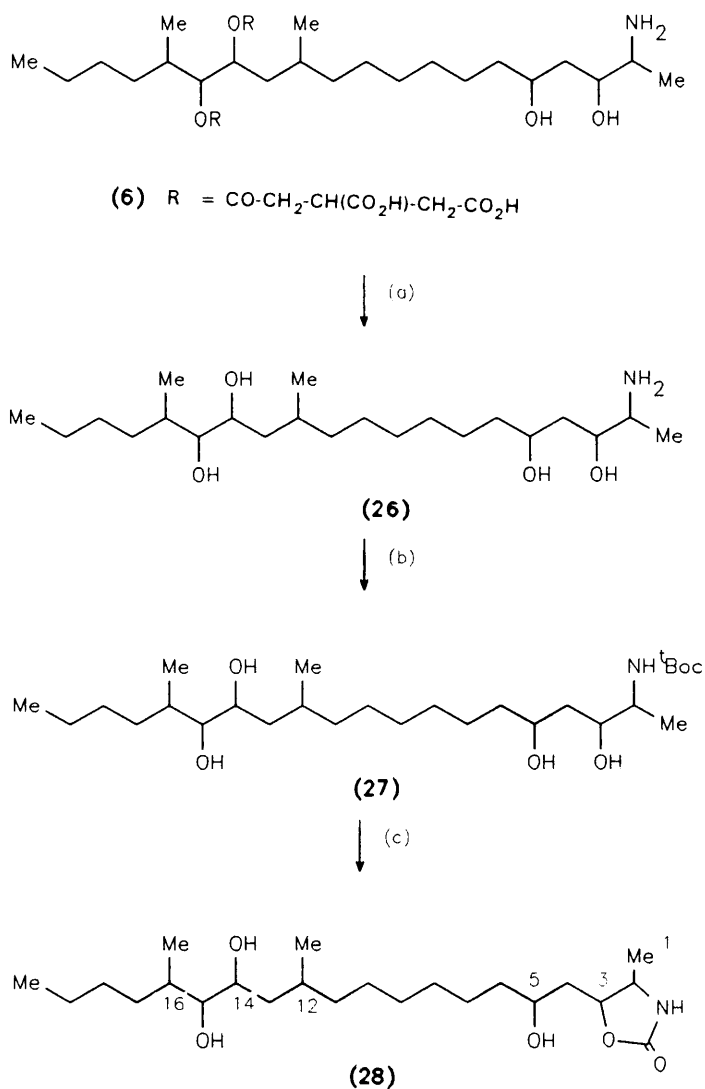
Scheme 1   (a) 1M KOH in MeOH:H$_2$O, (4:1) (b) Di-t-butyldicarbonate, KOH
(c) NaOEt

The formation of the oxazolidinone ring was effected by treatment of the N-t-Boc aminotetrol **(27)** with freshly prepared sodium ethoxide to give a moderate yield (45%) of the oxazolidinone **(28)**. Attempts to improve the yield of the reaction by changing the conditions were unsuccessful : thus the use of 1M potassium hydroxide in methanol[31] resulted in a 31% yield of the oxazolidinone **(28)**.

Fast atom bombardment (f.a.b.) mass spectrometry of the oxazolidinone **(28)** showed the protonated molecular ion at m/z 416 in agreement with the molecular formula of $C_{23}H_{45}NO_5$. The band at 1745 cm^{-1} in the i.r. spectrum is assigned to the carbonyl group of the oxazolidinone moiety. The ^{1}H and ^{13}C n.m.r. data relevant to the present discussion are collated in **Table 4**.

A number of the signals in the ^{1}H n.m.r. spectrum of the oxazolidinone exhibited fine structure. First order analysis of these multiplets in conjunction with ^{1}H{^{1}H} decoupling experiments yielded the values of the proton chemical shifts and proton-proton coupling constants. The proton-proton connectivity pattern of the oxazolidinone was established in a 2-D COSY-45 experiment and is shown in **Figure 8.**
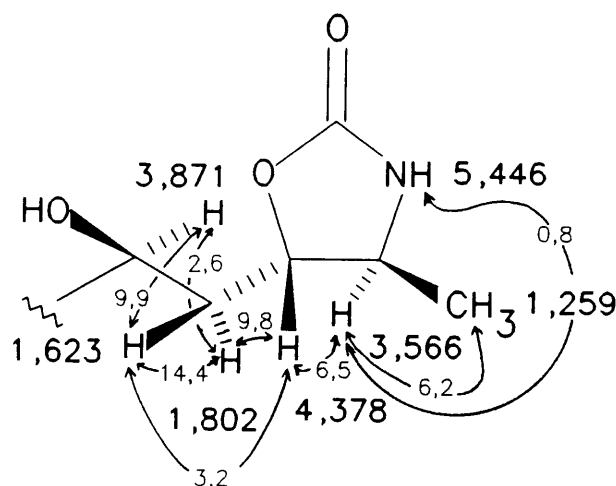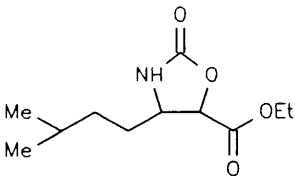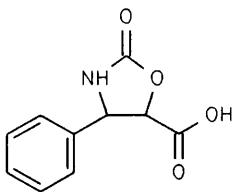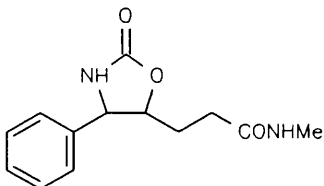
Figure 8   The proton-proton connectivity pattern of **(28)**.

Table 4   Relevant ^{13}C (125,76 MHz) and ^{1}H (500,13 MHz) n.m.r. data of **(28)**.

Carbon	δ_C/p.p.m.	δ_H/p.p.m.	J (HH)/Hz
1	20,15Q	1,259d	6,2
2	53,98D	3,566dq	0,8; 6,2; 6,5
3	81,37D	4,378m	3,2; 6,5; 9,7
4	41,59T	1,623ddd	3,2; 9,9; 14,4
		1,802ddd	2,6; 9,8; 14,4
5	67,80D	3,871m	
12	28,90D		
14	70,37D	3,770ddd	3,2; 3,7; 9,6
15	79,38D	3,340dd	3,7; 8,3
16	32,31D		
20	14,12Q	0,877t	7,0
21	15,39Q	0,821d	6,8
22	20,91Q	0,920d	6,7
NH		5,446d	0,8

The magnitude of the coupling constants (9,7 and 3,2 Hz) between the C-4 and C-3 protons is indicative of a preferred conformation for this part of the molecule and suggests dihedral angles of about 170° and 70°, respectively. A small coupling of 0,8 Hz observed between the C-2 and N-H protons is ascribed to a dihedral angle of about 90° between these protons and is in agreement with values reported in the literature.[32,33] The nitrogen atom in the oxazolidinone ring causes restriction of pseudorotation as a result of the non-ethane-like torsion barriers.[34] The bond angle is thus prevented from deviating significantly from 90° and consequently, very little interaction occurs between the two protons.

The ring-proton coupling constants of oxazolidinone rings have been utilised for the determination of the relative configuration of the two chiral centres in these compounds. A comparison of the coupling constant value of 6,5 Hz between C-2 and C-3 of (28) with literature values[32,35,36] (see **Figure 9**), does not allow an unambiguous assignment of the relative configuration at these two centres.

	J_{anti}	J_{syn}
	3,1 Hz	7,9 Hz
	5,1 Hz	8,8 Hz
	5,0 Hz	7,5 Hz

Figure 9 Coupling constants between the C-2 and C-3 protons in oxazolidinone rings.

The interpretation of J in five-membered rings is difficult, since ring substitution and pseudorotation alters the form of the Karplus equation.[37] In addition the presence of electron-withdrawing or donating groups as well as angle distortion also have an effect.[38] The oxazolidinone ring can be compared to the five-membered carbonate ring studied by Anet[38] who concluded that the *cis* isomer of a carbonate ring exists essentially in one conformation, whereas the *trans* isomer can exist in two

Protons irradiated

1-H : δ_H 1,259

2-H : δ_H 3,566

3-H : δ_H 4,378

NH : δ_H 5,579
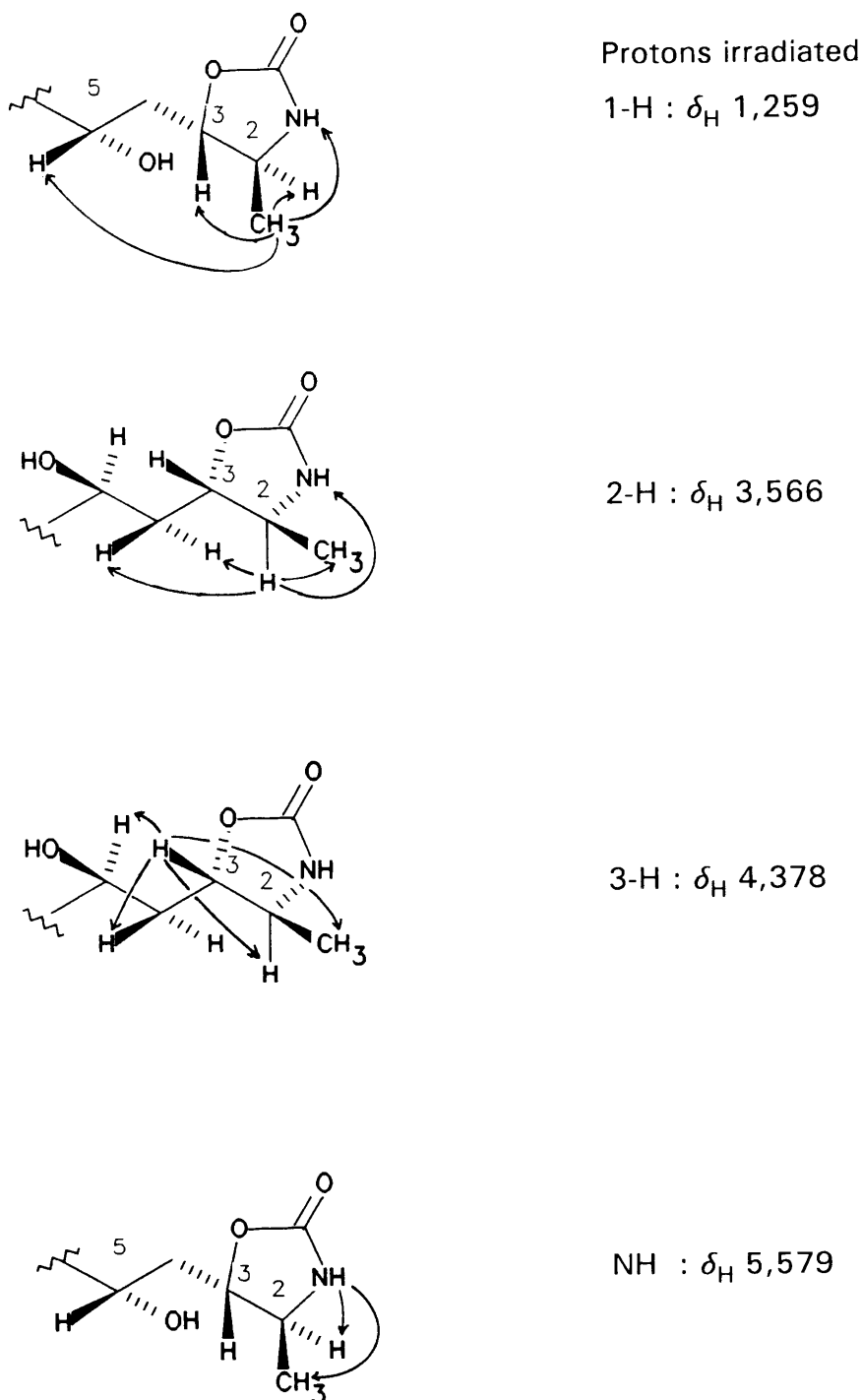
Figure 10   N.O.e. connectivity pattern for oxazolidinone **(28)**

possible conformations of about equal energy in which the methine protons can be either in the quasi-equatorial or quasi-axial positions. A coupling constant of 6,5-7,5 Hz is expected for the protons in the quasi-axial position whereas for the quasi-equatorial protons the coupling constant is about 0 Hz. The observed J-values therefore depend on the relative populations of the two conformations. All these effects have a bearing on the value of J and explain the wide range of coupling constants reported for the ring protons of oxazolidinones.

The relative configuration of the C-2 and C-3 chiral centres was deduced from the proton-proton n.O.e. connectivity pattern established in a number of homonuclear ^{1}H{^{1}H} n.O.e. difference experiments (see **Figure 10**).

Selective irradiation at the resonance frequency of the C-1 protons (δ_H 1,259) affected the resonances at δ_H 5,446 (NH), δ_H 3,566 (2-H), δ_H 4,378 (3-H) and δ_H 3,871 (5-H). The fact that an n.O.e. is observed between the C-1 protons and 3-H, but not for 4-H indicates a *trans* relationship between C-1 and C-4. This *trans* relationship was corroborated by the strong n.O.e. observed for the C-1 protons upon irradiation of the 3-H protons (see **Figure 11**) as well as the n.O.e. observed between 2-H and both of the C-4 protons. It is of interest to note the n.O.e. observed between the C-2 and C-3 protons although they are *trans* orientated.

The n.O.e. results prove that the 2-amino and 3-hydroxy groups in the fumonisins have the *syn* relative configuration.

4.2 DETERMINATION OF THE RELATIVE CONFIGURATION OF THE C-3 AND C-5 CHIRAL CENTRES.

In order to determine the relative configuration of the C-3 and C-5 chiral centres present in fumonisin B$_1$ by proton-proton n.O.e. studies it is necessary to prepare a derivative in which the 1,3-diol group forms part of a substituted 1,3-dioxane ring.

A formylal derivative of fumonisin B$_1$ was prepared as it has the advantage over the acetonide (*i.e.* 2,2-dimethyl-1,3-dioxane) derivative in that the smaller steric requirements of the formylal group allow the ring to exist in the more readily analysed chair conformation even in the case of a dioxane ring with an axial substituent (*anti* diol derivative).[39]
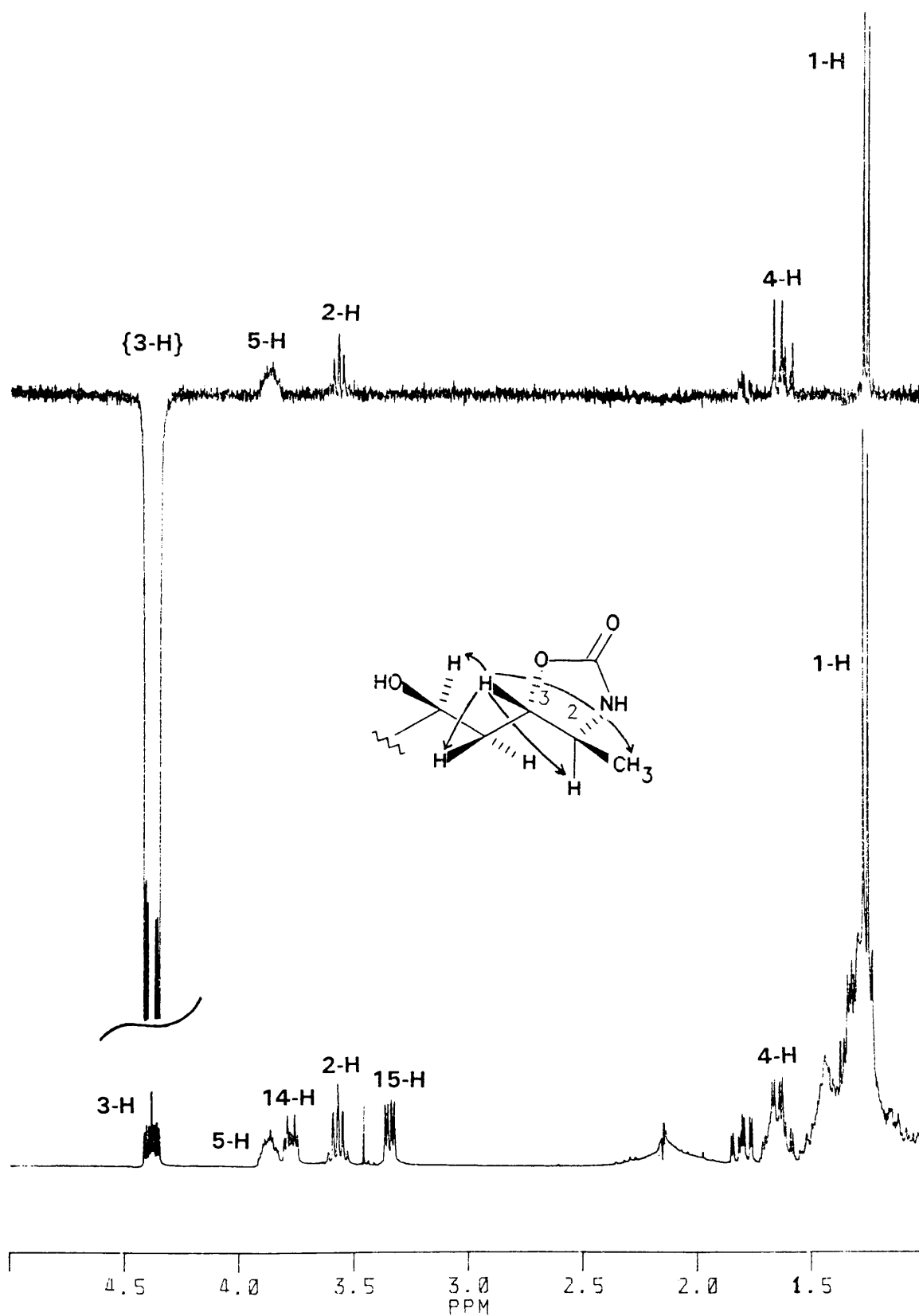
Figure 11   N.O.e.- and reference spectra of **(28)**

Irradiation of 3-H affected the resonances of 1-H, 2-H, 4-H and 5-H.

Esterification of fumonisin A_1 (7) was effected by treatment with ethereal diazomethane. The reaction of fumonisin A_1 tetramethyl ester[7] (29) with paraformaldehyde yielded the formylal derivative (30) of fumonisin A_1 tetramethyl ester $[(M+H)^{+\bullet}$ 832) (Scheme 2). Absorption bands at 3600 and 1720 cm^{-1} in the i.r. spectrum are ascribed to the hydroxy and carbonyl groups, respectively. The ^1H n.m.r. data of (30) are collated in Table 5. The signal at δ_H 5,686 in the ^1H n.m.r. spectrum, which disappeared on addition of deuterium oxide to the sample, was assigned to the amide proton. The coupling constant of 6,4 Hz for the methylene protons at δ_H 4,812 and δ_H 4,882 is characteristic of the C-2 protons of a 1,3-dioxane ring.[39]

(7)   R = $CO-CH_2-CH(CO_2H)-CH_2-CO_2H$

(a)

(29)

(b)

(30)   R = $CO-CH_2-CH(CO_2Me)-CH_2-CO_2Me$
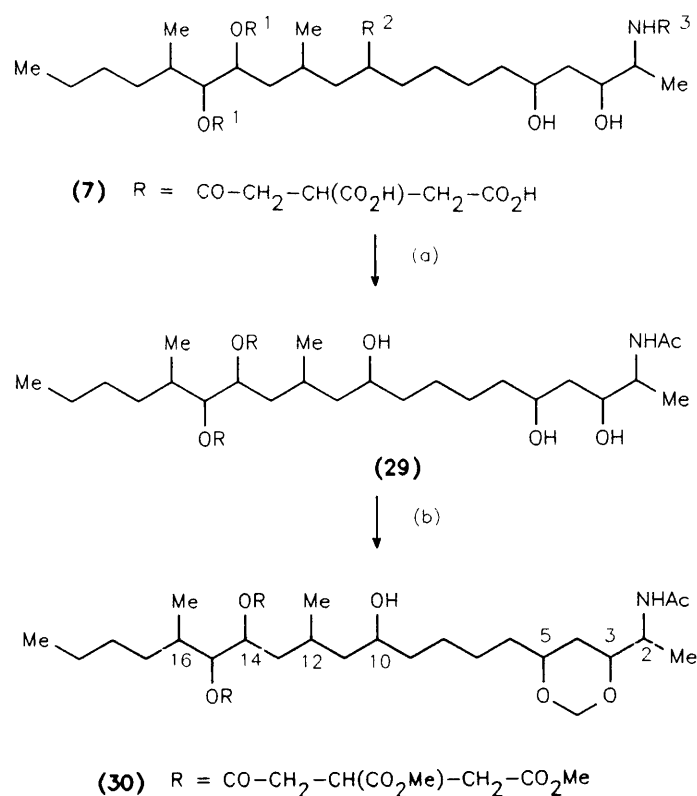
Scheme 2   (a) CH_2N_2 (b) $(CH_2O)_n$, p-toluenesulphonic acid

Inspection of the data obtained in a 2D COSY-45 experiment established the proton-proton connectivity pattern and allowed the assignment of a number of resonances in the derivative (30). Using the chemical shift of the amide proton as starting point the C-2 (δ_H 4,063), C-3 (δ_H 3,716), C-5 (δ_H 3,947), C-14 (δ_H 5,174) and C-15 (δ_H 4,900) protons could be assigned.

Table 5   Relevant ^{1}H (500,14 MHz) n.m.r. data of the formylal derivative of fumonisin A$_1$ tetramethyl ester (30).

| Carbon	δ_H/p.p.m.	J (HH)/Hz
1	1,171d	6,8
2	4,063m	
3	3,716m	
5	3,947m	
10	3,572m	
14	5,174m	
15	4,900dd	3,4; 8,6
16	1,604m	
20	0,856t	7,1
21	0,897d	6,8
22	0,941d	6,6
NH	5,686d	8,7

Assessment of the observed enhancements in a number of difference n.O.e. experiments indicated the n.O.e. connectivity pattern and configurational assignments as depicted in **Figure 12**. Irradiation at the resonance position of the axial formylal proton (δ_H 4,812) resulted in an n.O.e. for the signals corresponding to the C-2 and C-5 protons. In contrast no n.O.e. was observed when the equatorial formylal proton (δ_H 4,882) was irradiated. The implication of the above result is that the C-5 proton and carbon chain emanating from C-3 are axially orientated on the same face of the dioxane ring. Consequently an *anti* relationship is assigned to the C-3 and C-5 oxygen atoms of the fumonisins.

Rychnovsky[40] has shown that the acetonides of *syn* and *anti* 1,3-diols (4,6-dialkyl-2,2-dimethyl-1,3-dioxanes) can be easily and unambiguously distinguished by ^{13}C n.m.r. spectroscopy. Acetonides derived from *syn* 1,3-diols exist in a well-defined chair conformation with the two alkyl substituents in equatorial positions. In contrast acetonides derived from *anti* 1,3-diols exist in a twist conformation in order to avoid 1,3-diaxial interaction which would be present in a chair conformation. These two conformations and thus the relative stereochemistry of the diols can be distinguished by the ^{13}C chemical shifts of the acetonide methyl groups. The ^{13}C n.m.r. spectra of *syn* 1,3-diol acetonides show an axial methyl group carbon atom at δ_C 19,6 and the
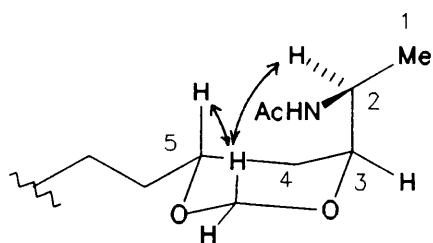
Protons irradiated

Axial formylal proton

δ_H 4,812
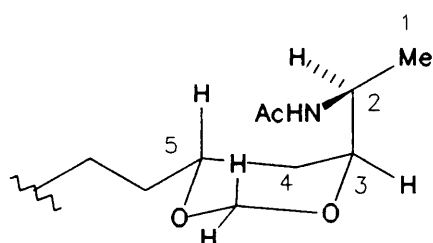
Equatorial formylal proton

δ_H 4,882

Figure 12   N.O.e. connectivity pattern observed for the dioxane ring in (30).

corresponding equatorial one at δ_C 30,0. This is in contrast to the ^{13}C n.m.r. spectrum of the *anti* 1,3-diol acetonide which shows two methyl resonances at δ_C 24,7. The acetal carbon chemical shifts are also indicative of the stereochemistry : δ_C 98,5 for the *syn* 1,3-diol acetonides and δ_C 100,4 for the *anti* stereoisomer.

The above findings have been extended by Evans[41] to more highly substituted dioxanes bearing substituents in the 5-position.

The acetonide derivative of fumonisin B_1 was prepared as outlined in **Scheme 3**. Fumonisin B_1 (5) was hydrolysed with 80% methanolic potassium hydroxide to yield the aminopentol (31).

The protonated molecular ion of the product appears at *m/z* 406 in the f.a.b. mass spectrum which is in agreement with the formation of 2-amino-3,5,10,14,15-penta-hydroxy-12,16-dimethylicosane (31). Absorption maxima at 3600 and 3400 cm^{-1} in the i.r. spectrum are consonant with the presence of hydroxy groups. The amino-pentol (31) was reacted with an excess di-*t*-butyl dicarbonate to yield, after

(5) R = CO–CH$_2$–CH(CO$_2$H)–CH$_2$–CO$_2$H

(a)

(31)

(b)

(32)

(c)

(33)

(34)
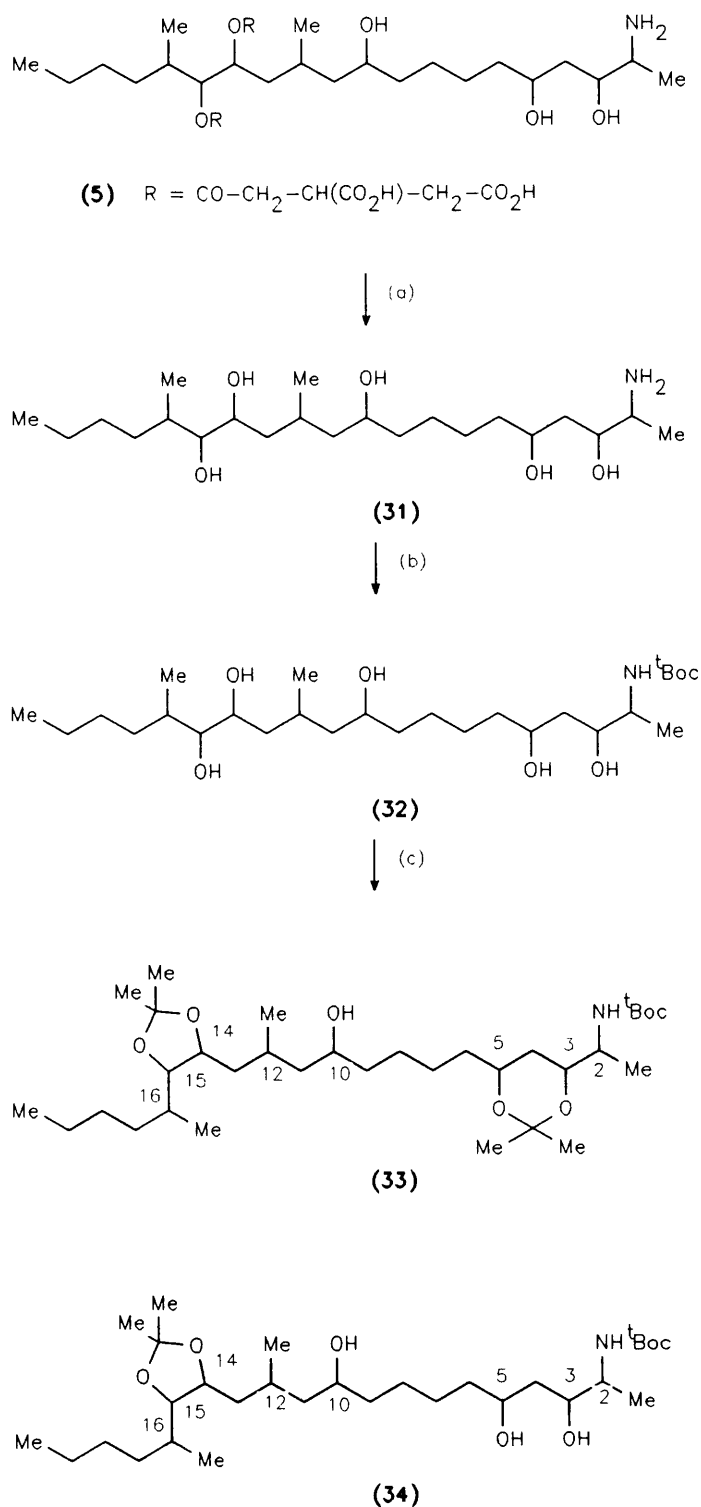
Scheme 3   (a) 1M KOH in MeOH:H$_2$O, (4:1) (b) Di-*t*-butyldicarbonate,KOH

(c) 2,2-Dimethoxypropane, *p*-toluenesulphonic acid (cat)

separation and purification by column chromatography, the *t*-Boc-derivative **(32)**. The *t*-Boc-derivative **(32)** [(*M*+H)$^{+\bullet}$ 506)] was treated with 2,2-dimethoxypropane to yield the 3,5:14,15-diacetonide **(33)** (63%) and the 14,15-acetonide **(34)** (22%).

The ^{1}H and ^{13}C data of the 3,5:14,15-diacetonide (33) are collated in **Table 6** (p 40). The ^{13}C chemical shifts observed for the methyl groups (δ_C 24,57 and 26,12) and the acetal carbon atom (δ_C 100,37) confirm the proposed *anti* relationship for the C-3 and C-5 oxygen atoms in (33) and thus the corresponding hydroxy groups in the fumonisins.

4.3 DETERMINATION OF THE ABSOLUTE CONFIGURATION OF THE C-2 — C-5 CHIRAL CENTRES

With the knowledge of the relative stereochemistry of the C-2 — C-5 moiety to hand, attention could now be directed towards determining the absolute configuration of one of the chiral centres in this moiety. The chirality of the C-5 hydroxy group in fumonisin B$_2$, and thus the absolute configuration of the C-2, C-3 and C-5 centres was determined by Horeau's method using the aminotetrol, 2-amino-3,5,14,15-tetrahydroxy-12,16-dimethyl-icosane (26) as a representative model of these compounds.

In order to effect esterification only at C-5 in the Horeau procedure, it is necessary to protect the other hydroxy groups present in (26). Thus the aminotetrol (26) was converted into the oxazolidinone derivative (28) as outlined in **Scheme 1**. The 14,15-diol moiety was protected as the 2,2-dimethyl-1,3-dioxolane derivative by treatment of the 2-oxazolidinone with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulphonic acid (**Scheme 4**). The positive ion f.a.b. mass spectrum of the protected 2-oxazolidinone (35) indicated a molecular mass of 456 $(M+H)^{+\bullet}$. The ^{1}H n.m.r. spectrum of (35) exhibited two three-proton singlets at δ_H1,289 and δ_H1,383 due to the two dioxolane methyl groups.

Esterification of the protected 2-oxazolidinone (35) with an excess of racemic *a*-phenylbutyric anhydride in the presence of 4-dimethylaminopyridine yielded the *a*-phenylbutyrate derivative (36). A peak appears at *m/z* 602 in the f.a.b. mass spectrum which is in agreement with the formation of a mono-ester derivative. The ^{1}H n.m.r. spectrum of (36) clearly shows the presence of two diastereomers in a ratio of 1:2. The protons of the major diastereomer appear at δ_H 5,548 (NH) and δ_H 3,356 (3'-H), whereas the corresponding protons of the minor diastereomer resonate at δ_H 5,674 (NH) and δ_H 3,388 (3'-H). The presence of two diastereomers is also evident

(28)

(a)

(35)

(b)

(36)   R =
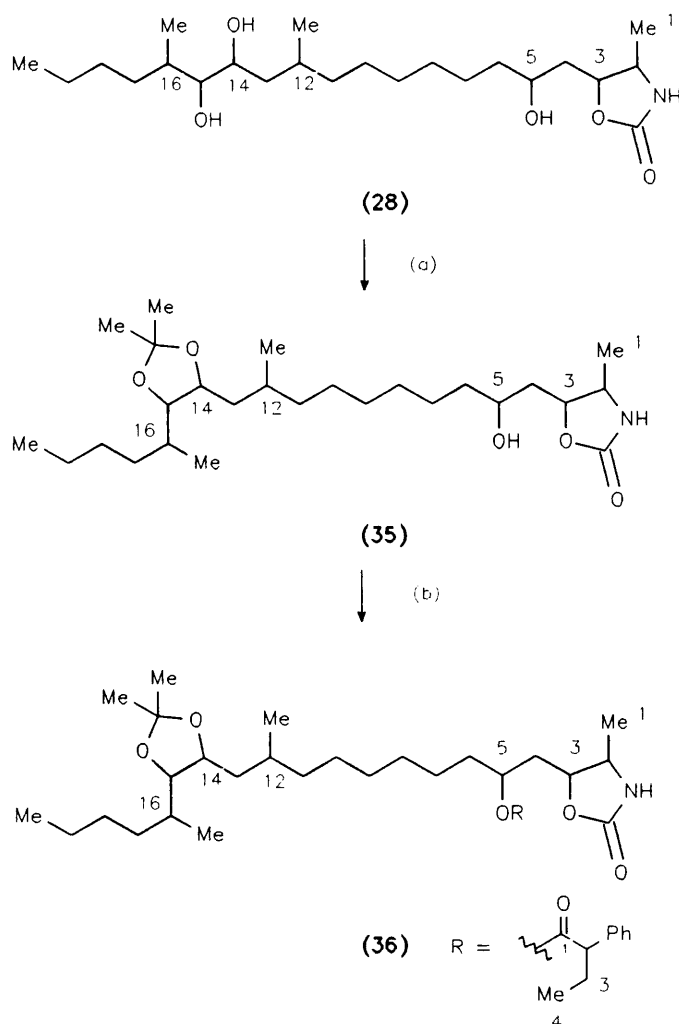
Scheme 4   (a) 2,2-Dimethoxypropane, p-toluenesulphonic acid

(b) a-Phenylbutyric anhydride, 4-dimethylaminopyridine

from the proton-decoupled ^{13}C n.m.r. spectrum which shows two signals for C-5 at δ_C 70,91 and δ_C 71,12.

The excess unreacted racemic phenylbutyric anhydride was hydrolysed and the recovered a-phenylbutyric acid had $[a]_D$ + 1,0°, which corresponds to an optical yield of 4%, and thus the S configuration. The protected 2-oxazolidinone derivative (35) and the fumonisins must therefore have the 5R configuration.

The small value of the specific rotation of the recovered a-phenylbutyric acid and the low optical yield, would indicate that there is little difference in energy between the two diastereomeric transition states as a result of the small difference in steric

hindrance between the two substituents of the chiral secondary alcohol.

In order to verify the above result it was decided to repeat the Horeau procedure using α-phenylbutyryl chloride. The ¹H n.m.r. spectrum of the resulting ester **(37)** once again indicated the presence of two diastereomers in the ratio of 2:3. The specific rotation of the recovered α-phenylbutyric acid was +4,2°, which corresponds to an optical yield of 14%.

The above results confirm the 5*R* configuration for the fumonisins and consequently on the basis of the earlier determined relative stereochemistry (Section 4.1 and 4.2), the 2*S*,3*S*,5*R* absolute configuration.

4.4 DETERMINATION OF THE C-10 ABSOLUTE CONFIGURATION

In order to determine the absolute configuration of the C-10 chiral centre use was made once again of Horeau's method but in this instance 2-acetylamino-3,5,10,14,15-pentahydroxy-12,16-dimethylicosane **(37)** served as model. As esterification in the Horeau procedure at any position other than C-10 would lead to complications, compound **(37)** was transformed by treatment with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulphonic acid into the 3,5:14,15-diacetonide derivative **(38)** (see **Scheme 5**). The positive ion f.a.b. mass spectrum of **(38)** showed a protonated molecular ion at *m/z* 528. The ¹H n.m.r. spectrum of **(38)** exhibited four three-proton singlets at δ_H 1,281, δ_H 1,294, δ_H 1,307, and δ_H 1,381 due to the four acetonide methyl groups. The ¹³C n.m.r. spectrum displayed two signals at δ_C 100,37 and 107,48 which were assigned to the two quaternary acetal carbon atoms of the 1,3-dioxane and 1,3-dioxolane moieties, respectively.

The reaction of the 3,5:14,15-diacetonide **(38)** with an excess of racemic α-phenyl-butyric anhydride proceeded smoothly to yield the 10-*O*-α-phenylbutyrate ester **(39)**, v_{max} 1710 (ester CO) and 1660 (amide CO) cm⁻¹. The f.a.b. mass spectrum of **(39)** exhibits a peak at *m/z* 634, which corresponds to the loss of a fragment of 40 mass units $(CH_2=C=CH_2)$ from the protonated molecular ion $(M+H)^{+•}$. The ¹H n.m.r. spectrum once again shows the presence of two diastereomers for the ester **(39)** with two signals for the C-2' proton at δ_H 3,413 and 3,424 in a ratio of 5:4.

(37)

(a)

(38)

(b)

(39)   R =
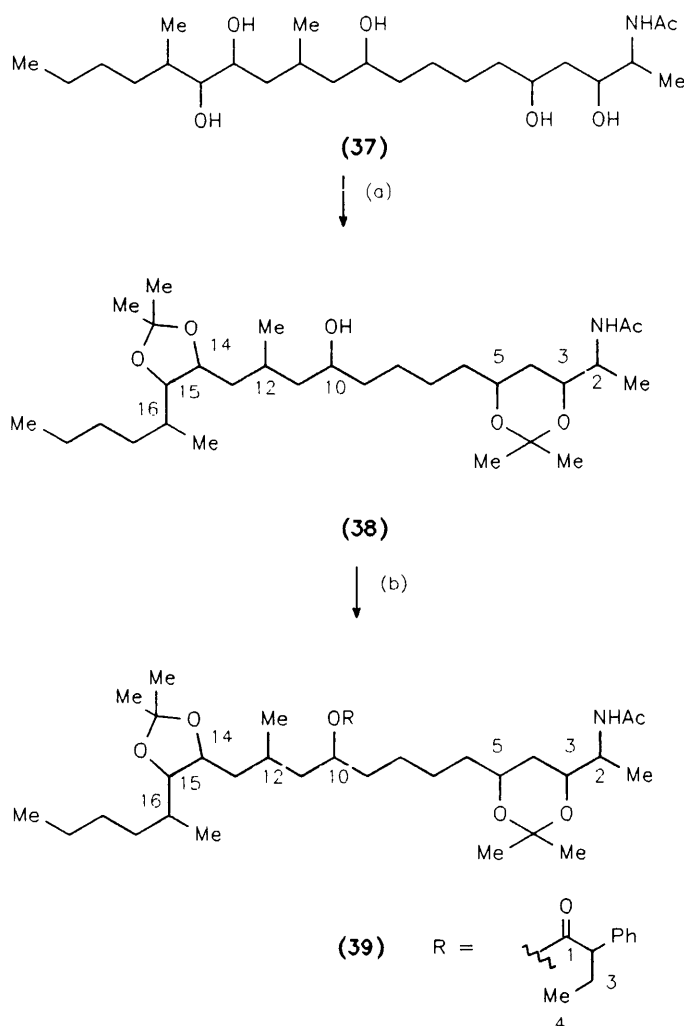
Scheme 5   (a) 2,2-Dimethoxypropane, *p*-toluenesulphonic acid

(b) *a*-Phenylbutyric anhydride, 4-dimethylaminopyridine

Hydrolysis of the excess of unreacted *a*-phenylbutyric anhydride yielded *a*-phenylbutyric acid with a specific rotation of + 2,3°, which corresponds to an optical yield of 10%, and thus the *S* configuration. The fumonisins must therefore have the 10*R* configuration.

4.5   DETERMINATION OF THE ABSOLUTE CONFIGURATION AT C-16

The strategy for the determination of the absolute configuration of the C-16 chiral centre in the fumonisins is based on the oxidative cleavage of the 1,2-diol moiety to give a single enantiomer of 2-methylhexanoic acid **(40)**. The simplest way to determine the chirality of this acid would involve the measurement of the sign and

magnitude of its specific rotation and a comparison with literature values. The limited amounts of the fumonisins available in this study precluded such an approach. Instead the 2-methylhexanoic acid was derivatized with a chiral auxiliary, (S)-α-methyl-p-nitrobenzylamine to give a single diastereomer of the (S)-α-methyl-p-nitrobenzylamide derivative **(42) (Scheme 6)**. The correlation of the HPLC retention time of this diastereomer with those of the (S)-α-methyl-p-nitrobenzylamide derivatives of each of the enantiomers of 2-methylhexanoic acid would establish the configuration of the 2-methylhexanoic acid obtained with the oxidative cleavage reaction and thus the configuration at C-16 of the fumonisins.

(37)

(40)

(41)

(42)
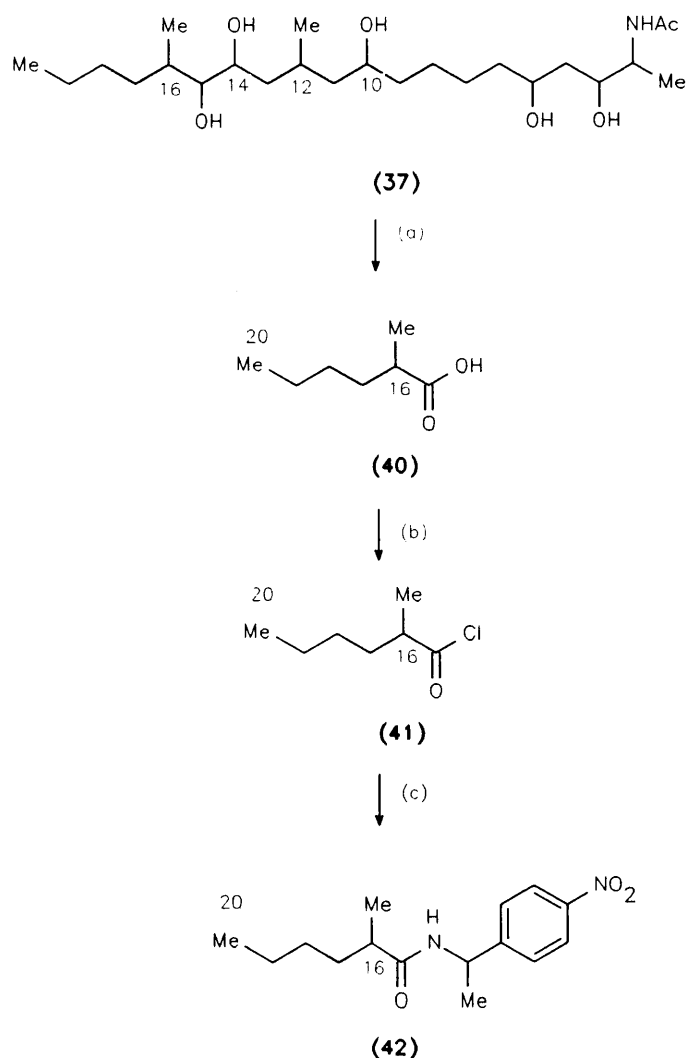
Scheme 6   (a) Kiliani reagent (CrO_3/H_2SO_4) (b) Oxalyl chloride

(c) (S)-α-methyl-p-nitrobenzylamine

Four different oxidative degradation methods to effect the cleavage of the C-14 — C-15 diol system in 2-acetylamino-3,5,10,14,15-pentahydroxy-12,16-dimethyl-icosane (37) to yield *inter alia* 2-methylhexanoic acid, were investigated *viz.* oxidation using either sodium periodate[42] or leadtetraacetate[43] followed by potassium permanganate, hydrogen peroxide,[44] and Kiliani reagent [chromium(VI)oxide in sulphuric acid].[45,46] The crude degradation products were transformed in each case to the methyl ester derivatives using diazomethane and analysed by gas chromatography for the presence of the methyl ester of 2-methylhexanoic acid using a standard of authentic methyl 2-methylhexanoate.

It was found that the Kiliani reagent was the most suitable reagent to effect the cleavage. The formation of 2-methylhexanoic acid (40) upon treatment of (37) with Kiliani reagent was confirmed by gc-ms of the reaction mixture and comparison once again with authentic 2-methylhexanoic acid. The mass spectrum of (40) showed the molecular ion at *m/z* 130 and the base peak at *m/z* 74 as a result of a McLafferty rearrangement.

The crude degradation products containing the 2-methylhexanoic acid (40) were converted to the acid chlorides (41) using oxalyl chloride and the crude acid chlorides were reacted with *S-α*-methyl-4-nitrobenzylamine (Scheme 6). A similar reaction sequence using authentic 2-methylhexanoic acid yielded a standard containing both the *RS*- and *SS*-diastereomers of the *S-α*-methyl-4-nitrobenzylamide of 2-methyl-hexanoic acid.

HPLC Analyses of the *S-α*-methyl-*p*-nitrobenzylamide derivatives were performed on a silica gel column (25x0,46 cm) using tetrahydrofuran—*n*-hexane (3:7) as mobile phase. Earlier work described in the literature established that under these conditions the *RS*-diastereomer (R_t 5,058) of the *S-α*-methyl-4-nitrobenzylamide derivative of 2-methylhexanoic acid elutes before the *SS*-diastereomer (R_t 6,849).[47]

The *S-α*-methyl-4-nitrobenzylamide (42) of 2-methylhexanoic acid obtained by oxidative degradation of the fumonisin model (37), had a retention time of 5,10 min and corresponded with the *RS*-diastereomer (Figure 13).

This result provides unambiguous proof that C-16 in the fumonisins has the *R* configuration.
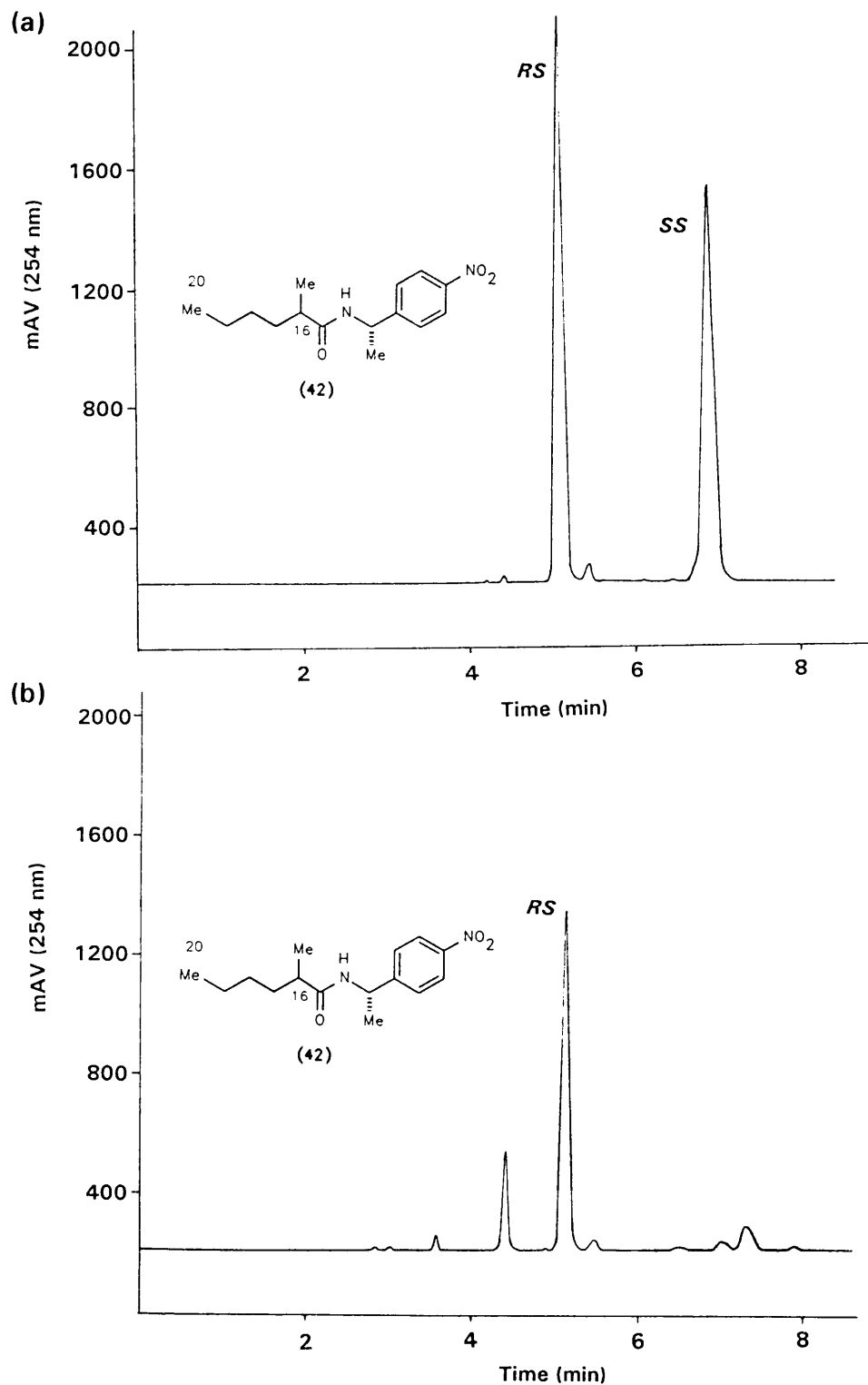
Figure 13 HPLC Chromatogram of S-α-methyl-4-nitrobenzylamide derivatives obtained from (a) racemic 2-methylhexanoic acid standard and (b) 2-methylhexanoic acid obtained by degradation from **(37)**.

4.6 DETERMINATION OF THE RELATIVE AND ABSOLUTE CONFIGURATION OF THE C-14 — C-16 CHIRAL CENTRES.

The relative configuration of the C-14 — C-15 chiral centres of fumonisin B_1 was deduced from the magnitude of the proton-proton coupling constants observed for the 14,15-acetonide derivatives (33) and (34) of the N-t-Boc-aminopentol (32). The characteristic J values for the C-4 and C-5 protons in a 1,3-dioxolane of 8,35-8,45 Hz for trans and 4,72-5,85 Hz for cis substitution have been employed to establish the stereochemistry of highly substituted dioxolanes.[48,49]

The N-t-Boc derivative (32) [$(M+H)^{+\bullet}$ 506] of the aminopentol (31) was treated with 2,2-dimethoxypropane to yield the 3,5:14,15-diacetonide (33) (63%) and the 14,15-acetonide (34) (22%). A number of signals in the ^1H n.m.r. spectrum of (33) exhibited fine structure and first-order analysis of these multiplets yielded the values of the proton-proton chemical shifts and proton-proton coupling constants (Table 6). The coupling constant of 5,3 Hz for the C-14 and C-15 protons of (33) is in good agreement with literature values[48,49] for the cis relationship for these protons. As a consequence the C-14 and C-15 hydroxy groups involved in ester formation in the fumonisins have the $14S^*,15R^*$ configuration.

The absolute configuration of the C-14 and C-15 chiral centres would follow once the stereochemical relationship between 16-H and 15-H is established.

The value of 9,7 Hz for the coupling constant between the C-15 (δ_H 3,691) and C-16 (δ_H 1,542) protons in (33) points to a preferred conformation in which these two protons have a nearly anti- or synperiplanar arrangement i.e. dihedral angles of 160-180° or 0-20°, respectively, according to the Karplus equation.[50] This requirement in conjunction with the known 16R configuration allows for two diastereomers: a diastereomer with the 14S,15R,16R configuration and an antiperiplanar conformation or the 14R,15S,16R diastereomer with a synperiplanar conformation for the C-15 and C-16.

In order to distinguish between the two possible diastereomers n.O.e. studies were performed on (34) as the resonances of the protons of the dioxolane methyl groups are well separated (δ_H 1,282 and 1,378, respectively) and can be selectively irradiated. The n.O.e. connectivity pattern determined for (34) is collated in Figure 14.

Table 6 ^{13}C (125,76 MHz) and ^{1}H (500,14 MHz) n.m.r. data of the 3,5:14,15-diacetonide (33)

Carbon	δ_C/p.p.m.	δ_H/p.p.m.	J (HH)/Hz
1	18,35Q	1,122d	6,7
2	49,11D	3,651m	
3	68,99D	3,651m	
4	39,40T	1,753m	9,6
5	67,00D	3,651m	
10	69,89D	3,651m	
14	75,32D	4,093m	2,6; 5,2; 11,4
15	82,71D	3,691dd	5,3; 9,7
16	32,10D	1,542m	
20	14,15Q	0,870t	6,9
21	15,85Q	0,790d	6,5
22	21,37Q	0,957d	6,7
Dioxane Me:	24,56Q	1,282s	
	26,12Q	1,423s	
Dioxolane Me:	28,43Q	1,282s	
	28,64Q	1,378s	
NH		4,708d	

Irradiation of the 14-H resonance resulted in enhancements of the resonances assigned to 15-H (δ_H 3,691), 13-H$_a$ (δ_H 1,114), 13H$_b$ (δ_H 1,503), 21-H (δ_H 0,790) and most importantly the dioxolane methyl group which resonates at δ_H 1,282. The n.O.e.s observed for 14-H, 21-H and once again the δ_H 1,286 dioxolane methyl protons upon irradiation of 15-H, indicate that this methyl group is *cis* orientated with respect to both the C-14 and C-15 protons. The chemical shift of 16-H (δ_H 1,542), obtained from a COSY-45 experiment was confirmed by the n.O.e. observed for this resonance upon irradiation of the C-21 protons (δ_H 0,790).

The fact that n.O.e.s are observed for both the C-14 ad C-15 protons with the C-21 protons as well as for the C-14 proton and the protons of the C-13 methylene group suggests a preferred conformation for the pendant alkyl chains of the dioxolane moiety. The above results in conjunction with the absence of an n.O.e. for the C-16

Protons irradiated

14-H : δ_H 4,096

15-H : δ_H 3,692

21-H : δ_H 0,790

Dioxolane Me : δ_H 1,282
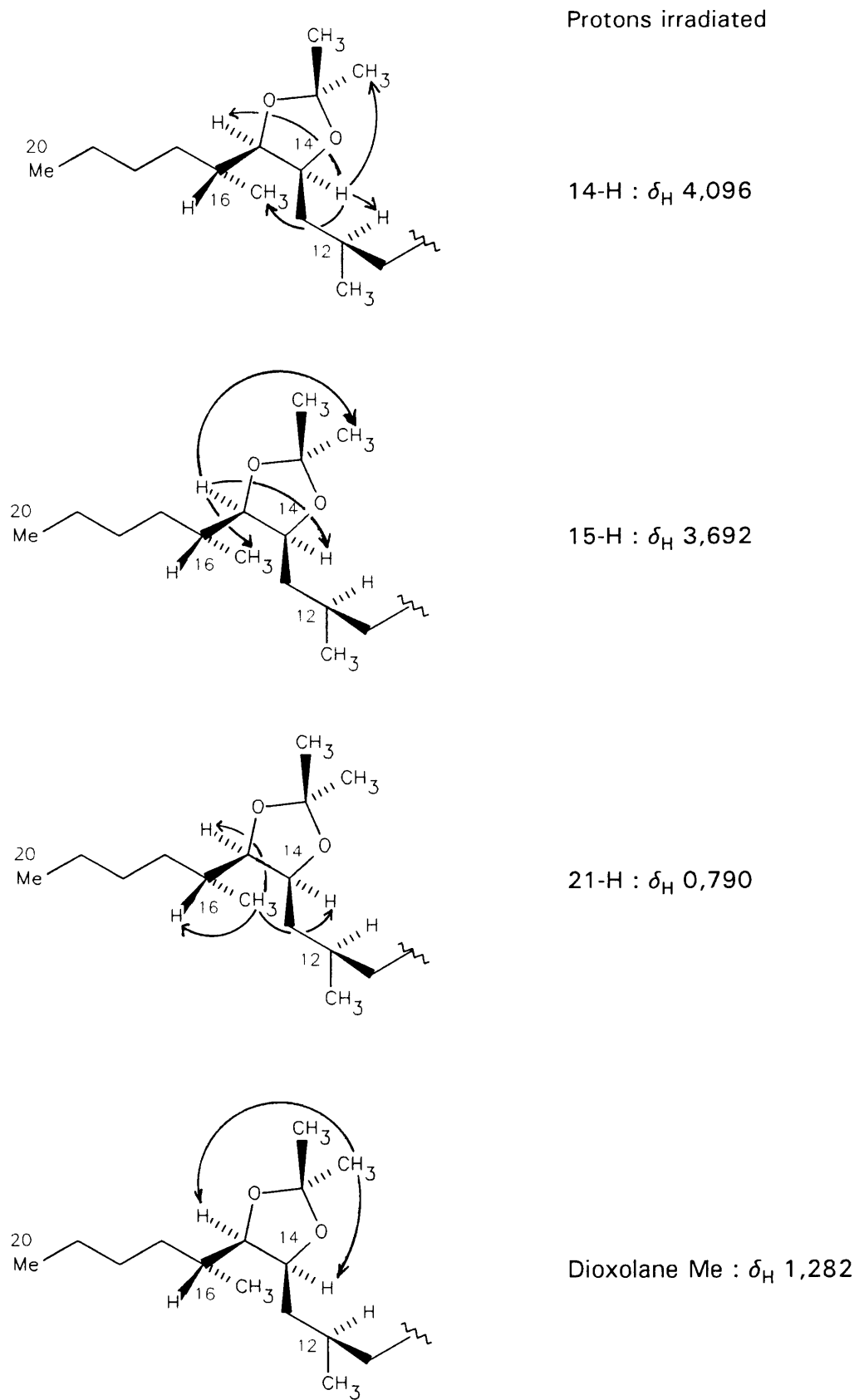
Figure 14 N.O.e. connectivity pattern of **(34)**

proton upon irradiation of either 15-H or 14-H suggests an antiperiplanar confor-
mation for 15-H and 16-H and therefore the 14*S*,15*R*,16*R* configuration.

Final confirmation of this result must await the availability of a suitable crystal of one
of the fumonisins or their derivatives for X-ray crystallography.

4.7 THE CONFIGURATION OF C-12

The relative and absolute configuration of the C-12 chiral centre were not determined
in the present study. Enzymatic methylation at the different active methylene sites
of a polyketide probably follows the same stereochemical course.[51] As a
consequence the absolute configuration of the C-12 and C-16 methyl groups should
be the same and thus C-12 must have the *S* configuration.

4.8 CONCLUSION

Based on the experimental results discussed in this chapter and keeping in mind the
assumption concerning the chirality of C-12, the proposed absolute configuration of
the fumonisins is presented in **Figure 15** as (2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R*).
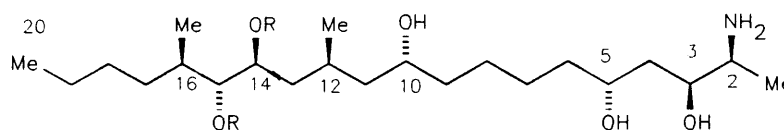
Figure 15   Proposed absolute configuration of fumonisin B_1

CHAPTER 5

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer 237 spectrometer. Fast atom bombardment (f.a.b.) mass spectra were taken on a Finnigan MAT 90 high-resolution mass spectrometer with double-focussing and reverse geometry configuration. Nuclear magnetic resonance spectra were recorded on a Bruker WM-500 spectrometer operating at 500,13 MHz for ^{1}H and 125,76 MHz for ^{13}C nuclei or a Bruker AM-300 spectrometer operating at 300,13 MHz for ^{1}H and 75,47 MHz for ^{13}C nuclei. Spectra were recorded for solutions in deuteriochloroform, unless indicated otherwise. Chemical shifts are reported in p.p.m. relative to tetramethylsilane (δ 0,000). The abbreviations s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad are used in connection with ^{1}H n.m.r. data and S = singlet, D = doublet, T = triplet and Q = quartet for ^{13}C n.m.r. data. ^{1}H-^{1}H Coupling constants J are given in Hertz (Hz). Optical rotations were measured at 25°C on a Perkin-Elmer 241 polarimeter.

Thin layer chromatography (t.l.c.) was carried out on precoated silica gel plates (thickness 0,25 mm). T.l.c. plates were sprayed after development with a solution of p-anisaldehyde (0,5 ml) in methanol (85 ml), acetic acid (10 ml) and concentrated sulphuric acid (5 ml) or ninhydrin in pyridine and heated at 120°C to make spots visible. Merck silica gel 60 (particle size 0,063-0,200 mm) was used for column chromatography.

A Varian 3400 gas chromatograph with a flame ionization detector and a fused silica capillary column [0,1 μm film thickness DB-5 bonded (internal diameter 0,32 mm, length 15 m)] were used for gas chromatographic analyses. Gas chromatography-mass spectrometry analysis was carried out on a Varian 3400 gas chromatograph, connected to a Finnigan MAT 90 high resolution mass spectrometer with a double-focussing and reverse geometry configuration.

A Hewlett-Packard 1090M HPLC with a diode array detector was used for liquid chromatographic analyses. Scanning was done from 200 to 400 nm. A Beckman Si 60 column (5 μm, 25x0,46 cm) was used at room temperature for analysis.

As it was difficult to obtain the fumonisins in pure form, a number of reactions were performed on impure fractions. Purification was subsequently performed on less polar derivatives.

5.1 DETERMINATION OF THE MODE OF LINKAGE OF THE PROPANE-1,2,3-TRICARBOXYLIC ACID SIDE CHAINS

5.1.1 *Diborane reduction of fumonisin B_1* **(5)** .—A suspension of fumonisin B_1 **(5)** (5 mg) in dry tetrahydrofuran (THF) (1 ml) at $0°C$ was treated with a solution of diborane in THF (1M, 0,3 ml). The reaction mixture was stirred at $0°C$ for 1h and then for 4h at room temperature. Water (5 ml) was cautiously added and the mixture was stirred for 1h at room temperature. The solvent was evaporated and the aqueous residue extracted with ethyl acetate. The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated to dryness. No reaction had occurred and fumonisin B_1 **(5)** was recovered unchanged.

5.1.2. N-*Benzoylfumonisin B_1* **(15)**.—Fumonisin B_1 **(5)** (225 mg) was dissolved in an aqueous sodium hydrogen carbonate solution (6M, 15 ml). Benzoyl chloride (247 mg) was added and the reaction mixture stirred for 4h at room temperature. Starting material was detected with t.l.c. and a second portion of benzoyl chloride (247 mg) was added. After 1h at room temperature the unreacted benzoyl chloride was extracted with ethyl acetate. The aqueous phase was acidified to pH 3.5 with hydrochloric acid (11,6 M) and extracted with ethyl acetate. The ethyl acetate extract was dried ($MgSO_4$), evaporated to dryness and purified by column chromatography. Elution with ethyl acetate–acetic acid (9:1) yielded N-benzoylfumonisin B_1 **(15)** as an oil (199 mg, 69%) [Found : $(M+H)^{+•}$, 826. $C_{41}H_{63}NO_{16}$ requires $M+H$, 826]; v_{max} ($CHCl_3$) 3670 (NH), 3030 (OH), 1710 (C=O), 1000 and 920 cm^{-1} (Ph).

δ_H 0,861 (3H, t, J 7,1, 20-H), 0,935 (6H, d, 21-H, 22-H),1,074 (2H, m), 1,19-1,65 (17H, m), 1,251 (3H, d, J 6,8, 1-H), 1,726 (3H, m), 1,912 (2H, m), 2,49-2,89 (8H, m, 24-H, 26-H, 30-H, 32-H), 2,722 (2H, m, 25-H, 31-H), 3,635 (2H, m, 3-H, 10-H), 3,656 (1H, m, 5-H), 3,962 (1H, m, 2-H), 4,160 (1H, m, 15-H), 4,991 (1H, m, 14-H), 5,185 (1H, br s, NH), 7,431 (2H, m, 2xPh-H), 7,497 (1H, m, Ph-H), 7,878 (2H, m, Ph-H).

δ_C 14,34Q (C-20), 15,89Q (C-21), 17,73Q (C-1), 20,63Q (C-22), 23,49T (C-19), 26,03D (C-12), 26,49T (C-8), 26,50T (C-7), 29,04T (C-18), 32,71T (C-17), 34,42D (C-16), 35,46T (C-30), 36,07T (C-26), 36,25T (C-24), 36,61T, 38,00T, 38,30T, 38,73D (C-31), 39,34D (C-25), 41,69T (C-4), 43,52T (C-11), 51,18D (C-2), 69,93D (C-3*), 69,30D (C-5*), 71,76D (C-10*), 71,95D (C-14*), 78,06D (C-15), 128,10D (C-3'), 128,10D (C-5'), 129,15D (C-6'), 129,15D (C-2'), 131,96D (C-4'), 135,99S (C-1'), 167,68S (NHCO), 171,98S 171,98S, 173,14S, 173,14S, 174,61S, 175,23S.

* May be interchanged.

5.1.3 *Diborane reduction of* N-*Benzoylfumonisin* B_1 **(15)** .— Diborane (1M, 0,24 ml) in tetrahydrofuran was added dropwise to N-benzoylfumonisin B_1 **(15)** (40 mg) in tetrahydrofuran (2 ml) at 0°. The reaction mixture was stirred at 0° for 1h and then for 4h at room temperature. Water (5 ml) was cautiously added and the mixture was stirred for 1h at room temperature. The solvent was removed under reduced pressure and the aqueous residue extracted with ethyl acetate. The combined ethyl acetate extracts were dried (MgSO$_4$) and evaporated to dryness. Thin layer chromatographic analysis of the fraction indicated the formation of a number of minor products.

5.1.4 *Preparation of fumonisin* B_1 *tetramethylthiomethyl ester* **(16)**.— Potassium hydroxide (0,1M) was added to fumonisin B_1 **(5)** (40 mg) in water (3 ml) until pH 7,4. The dry fumonisin B_1 potassium salt was obtained through freeze-drying of the fraction. Chloromethyl methyl sulfide was added to a suspension of fumonisin B_1 potassium salt, sodium iodide (8 mg) and 18-crown-6 (12 mg) in benzene (10 ml). The reaction mixture was heated under reflux for 16h after which it was cooled to room temperature and extracted with sodium hydrogencarbonate (6M) and sodium chloride (6M). The organic solution was dried (MgSO$_4$) and evaporated to dryness. The residue was purified by column chromatography with chloroform-methanol (4:1) as eluent to yield fumonisin B_1 tetramethylthiomethyl ester **(16)** (15 mg, 30%) [Found : $(M+H)^{+\bullet}$, 962. $C_{42}H_{75}NO_{15}S_4$ requires M+H 962]; ν_{max} (CHCl$_3$) 1720 (C=O) and 1155 cm^{-1} (C-O).

δ_H (CO(CD$_3$)$_2$) 0,876 (3H, t, J 6,9, 20-H), 0,949 (3H, d, J 6,7, 21-H), 0,958 (3H, d, J 6,7, 22-H), 1,136 (3H, d, 1-H), 1,10-1,60 (19-H, m), 1,970 (1H, m), 2,228 (3H, s, SCH$_3$), 2,235 (9H, s, 3xSCH$_3$), 2,5-2,85 (8H, m, 24H, 26H, 30H, 32H), 3,291 (2H, m, 25H, 31H), 3,5-3,7 (4H, m, 2-H, 3-H, 5-H,

10-H), 4,964 (1H, dd, J 3,2, 8,9, 15-H), 5,166 (1H, m, 14-H), 5,195 (4H, s, 2xCH$_2$S), 5,205 (2H, s, CH$_2$S), 5,220 (2H, s, CH$_2$S).

5.1.5 *Hydrolysis of fumonisin B$_1$ tetramethylthiomethyl ester* **(16)**.—Fumonisin B$_1$ tetramethylthiomethyl ester (13 mg) **(16)** was treated with 1M potassium hydroxide solution (3 ml) in MeOH:H$_2$O: (4:1). After 4,5h the solvent was evaporated and the aqueous residues extracted with ethyl acetate. The ethyl acetate extracts were combined, dried and evaporated to dryness to yield **(31)** (7 mg) [Found : $(M+H)^{+\bullet}$, 406. C$_{22}$H$_{47}$NO$_5$ requires $M+H$, 406]; v_{max} (CHCl3) 3420 (OH) and 1550 cm^{-1} (NH).

δ_H (CD$_3$SOCD$_3$) 0,793 (3H, d, J6,8 21-H), 0,851 (3H, t, J6,9 20-H), 0,856 (3H, d, J6,7 22-H), 0,911 (1H, m, 1-H), 1,017 (2H, m), 1,101 (3H, m), 1,120-1,1571 (22H, m), 1,576 (1H, m, 16-H), 1,862 (1H, m, 12-H), 2,914 (1H, m, 2-H), 2,995 (1H, dd, J 5,1 and 9,7, 15-H), 3,385 (1H, ddd, J 2,6, 5,2 and 11,3, 14-H), 3,452 (1H, m, 10-H), 3,593 (1H, m, 3-H), 3,624 (1H, m, 5-H).

δ_C (CD$_3$SOCD$_3$) 14,05Q (C-20), 15,87Q (C-1), 16,53Q (C-21), 21,14Q (C-22), 22,64T (C-19), 25,47D (C-12), 25,47T, 25,57T, 29,05T (C-18), 30,12T (C-17), 34,08D (C-16), 38,11T, 38,50T, 40,83T, 40,86T (C-4), 43,80T (C-11), 51,72D (C-2), 66,02D (C-5), 67,41D (C-10), 68,77D (C-3), 68,81D (C-14), 78,60D (C-15).

The aqueous residue was acidified to pH 3 and extracted with ethyl acetate. The combined ethyl acetate extracts were dried and evaporated to dryness to give an impure mono-acid di-methylthiomethyl ester compound **(17)** or **(18)** (5 mg) as product. The small amount of product, as well as the presence of impurities did not allow unambiguous assignments based on the ^{1}H n.m.r. data.

5.1.6 *Selective reduction of the ester functions in fumonisin B$_1$ with sodium borohydride* **(5)**.—Methanol (1,6 ml) was added dropwise over a period of 15 min to a mixture of fumonisin B$_1$ **(5)** (120 mg) and sodium borohydride (47 mg) in t-butanol heated under reflux. The reaction mixture was heated under reflux for 16h. Acetone was added to quench the reaction and solvents were removed under reduced pressure. The residue was dissolved in aqueous hydrochloric acid (pH 3) and extracted with ethyl acetate. Ethyl acetate extracts were dried and evaporated to dryness to yield unreacted fumonisin B$_1$ **(5)**. The aqueous layer was treated with

sodium hydroxide (1M) to a pH of 10 and extracted with ethyl acetate. Ethyl acetate extracts were dried and evaporated to dryness to afford the aminopentol (31) (21 mg). The aqueous layer was acidified with hydrochloric acid (1M) and evaporated to dryness. The residue was dissolved in methanol and filtered to remove salts. The resulting filtrate was evaporated to dryness and the residue chromatographed using chloroform-methanol (94:6) as the eluent. 2-Oxo-tetrahydrofuran-3-acetic acid (22) (7,5 mg) was obtained in 31% yield [Found: $(M+H)^{+\bullet}$, 128. $C_6H_8O_4$ requires $M+H$, 144]; v_{max} (CHCl$_3$) 1765 cm^{-1} (C=O).

δ_H (C$_6$D$_6$) 1,112 (1H, m, J 8,7, 10,3, 11,4 and 12,4, 4-H$_a$), 1,460 (1H, m, J 1,9, 6,5, 8,8, 12,3, 4-H$_b$), 2,061 (1H, dd, J 8,3 and 17,2, 6-H$_a$), 2,216 (1H, m, J 4,5, 8,3, 8,8 and 11,3, 3-H), 2,525 (1H, dd, J 4,5 and 17,3, 6-H$_b$), 3,227 (1H, ddd, J 6,5, 8,9 and 10,3, 5-H$_a$), 3,484 (1H, ddd, J 1,9, 8,7 and 8,9, 5-H$_b$).

δ_C (CDCl$_3$) 28,49T (C-4), 34,34T (C-6), 35,84D (C-3), 66,63T (C-5), 176,08S (C-7), 178,05S (C-2).

5.2 DETERMINATION OF THE RELATIVE AND ABSOLUTE CONFIGURATION THE FUMONISINS

5.2.1 *Alkaline hydrolysis of* (6).—Fumonisin B$_2$ (6) (100 mg) was hydrolysed in a 1M potassium hydroxide (solution in MeOH-H$_2$O (4:1). After 16h, the solvent was evaporated and the aqueous residue extracted with ethyl acetate. The ethyl acetate extracts were combined, dried and evaporated to dryness to yield aminotetrol (26) (50 mg) [Found : $(M+H)^{+\bullet}$, 390. $C_{22}H_{47}NO_4$ requires $M+H$, 390]; v_{max} (CHCl$_3$) 3420 (OH) and 1550 cm^{-1} (NH).

δ_H 0,798 (3H, d, J 6,8, 21-H), 0,853 (3H, d, J 6,7, 22-H), 0,853 (3H, t, J 6,9, 20-H), 0,992 (3H, d, J 6,5, 1-H), 2,733 (1H, qd, J 6,2 and 6,5, 2-H), 2,995 (1H, dd, J 5,2 and 6,0, 15-H), 3,59 (1H, m, 5-H), 3,386 (1H, ddd, J 2,1, 6,1 and 10,2, 14-H), 3,446 (1H, ddd, J 4,9, 6,2 and 7,1, 3-H).

δ_C 14,06Q (C-20), 16,52Q (C-21), 17,74Q (C-1), 21,06Q (C-22), 22,65T, 25,33T, 26,24T, 28,72D (C-12), 29,04T, 29,30T, 29,60T, 30,11T, 34,10D

(C-16), 35,31T, 38,11T, 39,95T, 41,08T, 51,42D (C-2), 66,41D (C-5), 68,88D (C-14), 70,37D (C-3), 78,64D (C-15).

5.2.2 *Protection of the aminotetrol* (26) *with the t-butyloxycarbonyl (t-Boc) group*

.—The aminotetrol (26) (100 mg) was suspended in a mixture of aqueous 0,5 M sodium hydroxide (8 ml) and *t*-butyl alcohol (2 ml). Di-*t*-butyl dicarbonate (150 mg) was added as a solid. The reaction mixture was stirred at room temperature for 16h and extracted with ethyl acetate. The extracts were combined, dried ($MgSO_4$), and evaporated to dryness. Chromatography of the residue with chloroform–methanol (94:6) as eluent afforded the *N-t*-Boc-aminotetrol derivative (27) (88 mg, 70%) [Found : $(M+H)^{+ \cdot}$, 490. $C_{27}H_{55}NO_6$ requires $M+H$, 490]; v_{max} ($CHCl_3$) 3600 and 3420 (OH) and 1685cm^{-1} (C = O).

δ_H 0,814 (3H, d, *J* 6,8, 21-H), 0,873 (3H, t, *J* 7,1, 20-H), 0,912 (3H, d, *J* 6,7, 22-H), 1,314 (3H, d, *J* 6,8, 1-H), 1,21-1,52 (19H, m), 1,414 (9H, s, C(C*H*$_3$)$_3$), 1,662 (4H,m), 3,332 (1H, dd, *J* 3,7, 8,4, 15-H), 3,611 (1H, m, 5-H*), 3,75-3,80 (2H, m, 2-H*, 3-H*), 3,871 (1H, m, 14-H*), 4,837 (1H, d, *J* 9,0, NH).

* May be interchanged.

δ_C 14,10Q (C-20), 15,37Q (C-21), 16,18Q (C-1), 20,91Q (C-22), 23,04T (C-19), 25,63T (C-8), 26,52T (C-7), 28,37Q (C-(CH$_3$)$_3$), 28,88D (C-12), 29,08T, 29,29T, 29,61T, 32,34D (C-16), 35,10T, 35,32T, 37,19T, 37,23T, 39,88T (C-4), 50,92D (C-2), 69,16D (C-5*), 70,34D (C-3*), 71,92D (C-14), 77,21S (*C*(CH$_3$)$_3$), 79,34D (C-15), 156,45S (C = O).

* May be interchanged.

5.2.3 *Preparation of the oxazolidinone derivative of the* N-t-*Boc aminotetrol*

(27).—The *N-t*-Boc aminotetrol (27) (29 mg) was heated under reflux with freshly prepared sodium ethoxide (0,2 ml, 4 eq.) in ethanol (2 ml) for 1h. Water (2 ml) was added to the reaction mixture and the ethanol was evaporated in a stream of nitrogen. The aqueous layer was acidified with hydrochloric acid to pH 4 and extracted with ethyl acetate. The extract was dried ($MgSO_4$), evaporated to dryness and the residue chromatographed with chloroform–methanol (94:6) to yield the oxazolidinone (28) (11 mg, 45%) [Found : $(M+H)^{+ \cdot}$, 416. $C_{23}H_{45}NO_5$ requires $M+H$, 416]; v_{max} (CHCl3) 3580 (OH), 3440 (NH), and 1740 cm^{-1} (C = O).

δ_H 0,821 (3H, d, J 6,8, 21-H), 0,877 (3H, t, J 7,0, 20-H), 0,920 (3H, d, J 6,7, 22-H), 1,259 (3H, d, J 6,2, 1-H), 1,23-1,51 (20H, m), 1,623 (1H, ddd, J 3,2, 9,9, 14,4, 4-H_a), 1,675 (1H, m), 1,802 (1H, ddd, J 2,6, 9,8, 14,4, 4-H_b), 3,340 (1H, dd, J 3,7, 8,3, 15-H), 3,566 (1H, dq, J 0,8, 6,2 and 6,5, 2-H), 3,770 (1H, ddd, J 3,2, 3,7 and 9,6, 14-H), 3,873 (1H, m, 5-H), 4,378 (1H, ddd, J 3,2, 6,5 and 9,7, 3-H), 5,446 (1H, br s, NH).

δ_C 14,12Q (C-20), 15,39Q (C-21), 20,15Q (C-1), 20,91Q (C-22), 23,06T (C-19), 25,32T (C-8), 26,58T (C-7), 28,90D (C-12), 29,16T, 29,27T, 29,67T, 32,31D (C-16), 35,13T, 35,40T, 37,26T, 37,96T, 41,59T (C-4), 53,98D (C-2), 67,80D (C-5), 70,37D (C-14), 79,38D (C-15), 81,37D (C-3), 158,83S (C = O).

5.2.4 *Preparation of the oxazolidinone derivative of the* N-t-*Boc aminotetrol* **(30)** *by treatment with sodium hydroxide.*—The *N-t*-Boc aminotetrol **(27)** (115 mg) was treated with 1M potassium hydroxide in MeOH:H_2O (4:1) (10 ml). The reaction was monitored with t.l.c. and diluted with H_2O after 5 days. The methanol was evaporated under reduced pressure and the aqueous residue was acidified with phosphoric acid to pH 4. The solution was extracted with diethyl ether. Ether extracts were combined, dried (MgSO$_4$) and evaporated to dryness to yield **(28)** (30 mg, 31%).

5.2.5 *Preparation of the formylal derivative of the fumonisin* A$_1$ *tetramethyl ester* **(29)**.—Paraformaldehyde (1,1 g) was heated to 120°C under vacuum. The resulting monomer was collected in a flask containing fumonisin A$_1$ tetramethyl ester **(29)** (70 mg) and a catalytic amount of p-toluenesulphonic acid in benzene at –78°C. After the complete conversion of paraformaldehyde to the monomer, the reaction mixture was left at room temperature for 1h and then heated under reflux. The residue was chromatographed on silica gel with chloroform-methanol (95:5) as eluent. The compound eluting first was the formylal derivative **(30)** (12 mg, 16%) [Found : $(M+H)^{+\bullet}$, 832. $C_{41}H_{69}NO_{16}$ requires $M+H$, 832]; ν_{max} (CHCl$_3$) 3600 (OH), 3420 (NH), 1720 (C = O), and 1150 cm^{-1} (C-O).

δ_H 0,856 (3H, t, J 7,1, 20-H), 0,897 (3H, d, J 6,8, 21-H), 0,941 (3H, d, J 6,6, 22-H), 1,104 (2H, m, 11-H), 1,12-1,15 (10H ,m), 1,171 (3H, d, J 6,8, 1-H), 1,604 (1H, m, 16-H), 1,677 (2H, m, 13-H), 1,79-2,10 (4H, m), 1,978 (3H,

s, COCH_3), 2,425 (2H, dd, J 5,7, 16,6, 24-H*), 2,711 (6H, m, 26-H*, 30-H*, 32-H*), 3,253 (2H, m, 25-H, 31-H), 3,572 (1H, m, 10-H), 3,691 (3H, s, CO$_2$CH_3), 3,664 (3H, s, CO$_2$CH_3), 3,671 (6H, s, 2xCO$_2$CH_3), 3,706 (1H, m, 3-H), 3,974 (1H, m, 5-H), 4,063 (1H, m, 2-H), 4,812 (1H, dd, J 6,4, O$_2$CH_2), 4,882 (1H, dd, J6,4 O$_2$CH_2) 4,900 (1H, dd, J 3,4, 8,6, 15-H), 5,174 (1H, m, 14-H), 5,686 (1H, d, J 8,7, NH).

* May be interchanged.

5.2.6 Alkaline hydrolysis of fumonisin B_1 (5).—Fumonisin B_1 (5) (606 mg) was hydrolysed under the same conditions as in section 5.2.1. The crude product was purified by column chromatography with ethyl acetate–acetic acid–water (7:2:1) as eluent to give the aminopentol (31) (242 mg, 70%).

5.2.7 Protection of the aminopentol (31) with the t-butyloxycarbonyl group (31).—The aminopentol (31) (200 mg) was treated with di-t-butyl dicarbonate (312 mg) as in section 5.2.2. Chromatography of the reaction mixture with chloroform-methanol (94:6) as eluent afforded N-t-Boc-aminopentol (32) (188 mg, 76%) [Found : (M+H)$^{+*}$, 506. C$_{27}$H$_{55}$NO$_7$ requires M+H, 506]; v_{max} (CHCl$_3$) 3600 and 3400 (OH) and 1658 cm^{-1} (C=O).

δ_H 0,797 (3H, d, J 6,8, 21-H), 0,869 (3H, t, J 7,2, 20-H), 0,939 (3H, d, J 6,7, 22-H), 1,135 (3H, d, J 6,7, 1-H), 1,19-1,70 (21H, m), 1,408 (9H, s, C(CH_3)$_3$), 1,891 (1H, m), 3,348 (1H, dd, J 3,7, 8,4, 15-H), 3,654 (2H, m, 5-H*, 10-H*), 3,769 (2H, m, 2-H*, 3-H*), 3,847 (1H, m, 14-H*), 4,975 (1H, d, NH).

* May be interchanged.

δ_C 14,15Q (C-20), 15,40Q (C-21), 18,27Q (C-1), 21,44Q (C-22), 23,08T (C-19), 25,28T (C-8), 25,48T (C-7), 25,57D (C-12), 28,45Q (C(CH_3)$_3$), 28,91T, 32,40D (C-16), 34,97T, 36,95T, 37,29T, 38,46T, 40,44T (C-4), 42,57T (C-11), 50,96D (C-2), 68,31D (C-5*), 68,95D (C-10*), 69,50D (C-3*), 71,38D (C-14), 79,08D (C-15), 79,15S (OC(CH$_3$)$_3$), 156,45S (C=O).

* May be interchanged.

5.2.8 Acetonide formation of N-t-Boc aminopentol (32).—The N-t-Boc aminopentol derivative (32) (190 mg) was treated with freshly distilled 2,2-dimethoxypropane (0,7

ml) and a catalytic amount of *p*-toluenesulphonic acid. The reaction mixture was stirred at room temperature for 6h, neutralized with aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The combined organic extracts were combined and evaporated to dryness. Chromatography of the resulting fraction on silica gel with *n*-hexane–ethyl acetate (75:25) as eluent yielded the 3,5:14,15-diacetonide derivative (33) (138 mg, 63%) [Found : $(M+H)^{+\bullet}$, 486, $C_{33}H_{63}NO_7$ requires $M+H$, 586]; v_{max} (CHCl$_3$) 3440 (NH), 1700 (C=O), and 1755 cm^{-1} (C-O).

δ_H 0,790(3H, d, *J* 6,5, 21-H), 0,870 (3H, t, *J* 6,9, 20-H), 0,957 (3H, d, *J* 6,7, 22-H), 1,08 (20H, m), 1,122 (3H, d, *J* 6,7, 1-H), 1,282 [6H, s, $O_2C(CH_3)_2$], 1,293 [3H, s, $O_2C(CH_3)_2$], 1,387 [3H, s, $O_2C(CH_3)_2$], 1,423 [9H, s, $C(CH_3)_3$], 1,753 (1H, m, *J* 9,6, 4-H), 1,882 (1H, m, *J* 6,7, 12-H), 3,651 (4H, m, 2-H, 3-H, 5-H, 10-H), 3,691 (1H, dd, *J* 5,3 and 9,7, 15-H), 4,093 (1H, m, *J* 2,6, 5,2, 11,4 and 14-H), 4,708 (1H, d, NH).

δ_C 14,15Q (C-20), 15,85Q (C-21), 18,35Q (C-1), 21,37Q (C-22), 23,02T (C-19), 24,56Q [C(CH$_3$)$_2$], 25,44T, 25,65T, 26,12Q [C(CH$_3$)$_2$], 28,43Q [C(CH$_3$)$_3$], 28,54T, 28,64Q [C(CH$_3$)$_2$], 32,10D (C-16), 33,50T 35,26T, 35,84T, 37,18T, 39,40T (C-4), 43,50T (C-11), 49,11D (C-2), 67,00D (C-5), 68,99D (C-3), 69,89D (C-10), 75,32D (C-14), 79,15S [OC(CH$_3$)$_3$], 82,71D (C-15), 100,33S [O$_2$C(CH$_3$)$_2$, 3,5-acetonide], 107,51S [O$_2$C(CH$_3$)$_2$, 14,15-acetonide], 155,93S (CO).

and the 14,15-acetonide (34) (46 mg, 22%) (Found : $(M+H)^{+\bullet}$, 486. $C_{30}H_{59}NO_7$ requires $M+H$, 546); v_{max} (CHCl$_3$) 3440 (NH), 1700 (C=O), and 1170 cm^{-1} (C-O).

δ_H 0,795 (3H, d, *J* 6,6, 21-H), 0,874 (3H, t, *J* 7,1, 20-H), 0,960 (3H, d, *J* 6,8, 22-H), 1,259 (3H, d, *J* 6,7, 1-H), 1,127 (2H, m, 13-H), 1,16-1,67 (19H, m), 1,283 [3H, s, C(CH$_3$)$_2$], 1,38 [3H, s, C(CH$_3$)$_2$], 1,449 [9H, s, C(CH$_3$)$_3$], 1,673 (2H, m), 1,901 (1H, m, 12-H), 3,645 (1H, m, 10-H), 3,695 (1H, dd, *J* 5,2 and 9,8, 15-H), 3,851 (1H, m, 3-H), 3,922 (1H, m, 2-H), 4,099 (1H, ddd, *J* 2,6, 5,2 and 11,4, 14-H), 6,013 (1H, d, NH).

δ_C 14,14Q (C-20), 15,84Q (C-21), 18,28Q (C-1), 21,34Q (C-22), 23,01T (C-19), 25,63T, 25,67T, 25,99D (C-12), 26,19Q [C(CH$_3$)$_2$], 28,46Q [C(CH$_3$)$_3$], 28,52T, 28,64Q [C(CH$_3$)$_2$], 32,08D (C-16), 33,49T, 37,18T, 37,62T,

38,41T, 39,34T (C-4), 43,45T (C-11), 57,49D (C-2), 68,42D (C-5*), 69,76D (C-10*), 75,30D (C-3*), 78,74 (C-14), 79,05S [OC(CH$_3$)$_3$], 82,71D (C-15), 107,51S [O$_2$$C$(CH$_3$)$_2$], 152,11S (CO).

* May be interchanged.

5.2.9 *Acetonide formation of the oxazolidinone derivative* **(28)**. —To a solution of the oxazolidinone derivative **(28)** (50 mg) in dimethylformamide at 0°C was added freshly distilled 2,2-dimethoxypropane (0,2 ml) and a catalytic amount of *p*-toluenesulphonic acid. The reaction mixture was treated as in Section 4.2.8. Chromatography of the resulting crude product with chloroform–acetone (88:12) yielded the 14,15-acetonide 2,3-oxazolidinone derivative **(35)** (53,8 mg, 99%) [Found : (M+H)$^{+\bullet}$, 456. C$_{26}$H$_{49}$NO$_5$ requires M+H, 456]; ν_{max} (CHCl$_3$) 3600 (OH), 3440 (NH), 1755 (C=O), and 920 cm^{-1} (NH).

δ_H 0,793 (3H, d, *J* 6,6, 21-H), 0,867 (3H, t, *J* 7,1, 20-H), 0,891 (3H, d, *J* 6,7, 22-H), 0,95-1,45 (18H, m), 1,245 (3H, d *J* 6,2, 1-H), 1,289 [3H, s, O$_2$C(CH_3)$_2$], 1,383 [3H, s, O$_2$C(CH_3)$_2$], 1,626 (1H, m, *J* 3,0, 9,9, 4-H$_b$), 1,662 (4H, m), 1,787 (1H, m, *J* 2,5, 9,9, 14,5, 4-H$_a$), 2,144 (1H, m), 3,555 (1H, dq, *J* 6,3, 2-H), 3,681 (1H, dd, *J* 5,1, 9,7, 15-H), 3,841 (1H, m, 5-H), 4,055 (1H, ddd, *J* 2,6, 5,2 and 11,3, 14-H), 4,364 (1H, ddd, *J* 3,1, 6,6 and 9,7, 3-H), 5,949 (1H, br s, NH).

δ_C 14,14Q (C-20), 15,87Q (C-21), 20,09Q (C-1), 20,93Q (C-20), 23,01T (C-19), 25,40T, 26,25Q [O$_2$C(CH_3)$_2$], 26,57T, 28,54T, 28,67Q [O$_2$C(CH_3)$_2$], 29,08D (C-12), 29,49T, 29.92T, 32,14D (C-16), 33,48T, 35,41T, 36,76T, 38,05T, 41,60T (C-4), 54,02D (C-2), 67,76D (C-5), 75,85D (C-14), 81,35D (C-3), 82,73D (C-15), 107,25S [O$_2$$C$(CH$_3$)$_2$], 159,12S (CO).

5.2.10 *Preparation of the α-phenylbutyrate ester of the 14,15-acetonide 2,3-oxazolidinone derivative* **(35)** *by reaction with α-phenylbutyric anhydride.* —The 14,15-acetonide 2,3-oxazolidinone derivative **(35)** (50 mg) was dissolved in dichloromethane (5 ml). Dimethylaminopyridine (61 mg) and α-phenylbutyric anhydride (71 mg) was added and the reaction mixture was stirred at room temperature for 1,5 h. Unreacted anhydride was hydrolysed by stirring the reaction mixture with water (5 ml) for 2h. Sodium hydrogencarbonate was added and the mixture was extracted with dichloromethane. The organic layer was washed with hydrochloric acid (5x10^{-5}M),

dried and evaporated to dryness to yield the α-phenylbutyrate ester **(36)** (35 mg, 55%) [Found : $(M+H)^{+\bullet}$, 602. $C_{36}H_{59}NO_6$ requires $M+H$, 602]; v_{max} (CHCl$_3$) 3440 (NH), 1740 (C=O), and 1170 cm^{-1} (C-O).

δ_H 0,785 (3H, d, J 6,6, 21-H), 0,823-0.891 (9H, m, 22-H, 20-H, 4'-H), 1.01-1.82 (23H, m), 1,111 (3H, d, J 6,2, 1-H), 1,279 [3H, s, $O_2C(CH_3)_2$], 1,374 [3H, s, $O_2C(CH_3)_2$], 2,048 (2H, m, 3'-H), 2,072 (2H, dt, 3'-H), 3,261 (1H, m, 2-H), 3,356 (1H, t, J 7,7, 2'-H), 3,388 (1H, t, J 7,7, 2'-H), 3,490 (1H, m, 3-H), 3,667 (1H, dd, J 5,2 and 9,8, 15-H), 4,055 (1H, ddd, J 2,6, 5,2 and 11,3, 14-H), 4,957 (1H, m, 5-H), 5,548 (1H, br s, NH), 5,674 (1H, br s, NH), 7,232 (5H, m, Ph-H).

δ_C 12,10Q (C-4'), 14,14Q (C-20), 15,89Q (C-21), 19,87T , 20,16T, 20,93Q (C-1), 23,02T (C-19), 24,64T, 25,02T, 26,01T (C-3'), 26,08T (C-3'), 26,26Q [$O_2C(CH_3)_2$], 26,59T, 28,56T, 28,67Q [$O_2C(CH_3)_2$], 29,10D (C-12), 29,34T, 29.42T, 29,86T, 29,93T, 32,17D (C-16), 33,90T, 34,40T, 34,77T, 35,50T, 36,78T, 38,88T (C-4), 39,05T (C-4), 53,42 (C-2'), 53,64D (C-2), 53,84D (C-2'), 70,91D (C-5), 71,12D (C-5), 75,89D (C-14), 80,50D (C-3), 80,85D (C-3), 82,76D (C-15), 107,26S [$O_2H(CH_3)_2$], 127,21D (Ph), 127,91D (Ph), 127,91 (Ph), 128,50D (Ph), 128,58D (Ph), 139,00S, (Ph), 139,43S (Ph), 158,67S (NC=O), 173,24 (C-1'), 173,32 (C-1').

* May be interchanged.

The sodium hydrogen carbonate layer was acidified (pH 1), and extracted with dichloromethane. The dichloromethane fraction was dried and evaporated to dryness to give α-phenylbutyric acid $[a]_D$ +0,95° (c 2,1 in toluene) (optical yield 4%).

δ_H 0,839 (3H, t, J 7,4, 4-H), 1,742 (2H, m, 3-H), 2,037 (2H, m, 3-H), 3,388 (1H, t, J 7,7, 2-H), 7,187 (5H, m, Ph).

5.2.11 *Preparation of the α-phenylbutyrate ester of the 14,15-acetonide 2,3-carbamate derivative* **(35)** *by reaction with 2-phenylbutyryl chloride* **(35)**.—Thionyl chloride (2 ml) was added to a solution of racemic 2-phenylbutyric acid (52 mg) in 1 ml of dichloromethane. The mixture was heated under reflux for 3h. The solvents were evaporated under reduced pressure and the residue was dissolved in dry pyridine (2 ml). A solution of the 14,15-acetonide 2,3-carbamate derivative **(35)** (53

mg) and dimethylaminopyridine (1,8 mg) in dry benzene was added. The reaction mixture was kept at 55° for 4h, and then left at room temperature for 16h. The solvents were evaporated under reduced pressure and the residue partitioned between diethyl ether and sodium hydrogen carbonate (6M). The organic solution was dried and evaporated to dryness. The residue was purified by column chromatography with chloroform–methanol (95:5) as eluent to yield the butyrate ester derivative **(36)** (45 mg, 62%). The sodium hydrogen carbonate solution was acidified (6M HCl) and extracted with dichloromethane to give α-phenylbutyric acid (19 mg), $[\alpha]_D$ +4,2° (c 1,9 in toluene), (optical yield 14%).

5.2.12 *Alkaline hydrolysis of fumonisin A_1* **(7)**.—Fumonisin A_1 **(7)** (200 mg) was treated with potassium hydroxide solution in MeOH-H_2O as in Section 5.2.1 to yield the *N*-acetylaminopentol **(37)** (112 mg, 90%) [Found :$(M+H)^{+\bullet}$, 448. $C_{24}H_{49}NO_6$ requires $M+H$, 448]; v_{max} (CHCl$_3$) 3260 (OH), 1710 (C=O), 1365 (C-N), and 910 cm^{-1} (NH).

δ_H 0,799 (3H, d, J 6,7, 21-H), 0,873 (3H, t, J 7,1, 20-H), 0,940 (3H, d, J 6,5, 22-H), 1,01-1,07 (2H, m), 1,143 (3H, d, J 6,7, 1-H), 1,7-1,69 (20H, m), 1,961 (3H, s, COCH_3), 3,349 (1H, m, 15-H), 3,645 (1H, m, 10-H*), 3,796 (3H, m, 2-H*, 3-H*, 5-H*), 3,901 (1H, m, 14-H*), 6,393 (1H, br s, NH).

* May be interchanged.

δ_C 14,16Q (C-20), 15,42Q (C-21), 17,90Q (C-1), 21,42Q (C-22), 23,09T (C-10), 23,31Q (COCH_3), 25,29T (C-8), 25,47T (C-7), 25,56D (C-12), 28,93T (C-18), 32,43D (C-16), 34,99T, 36,92T, 37,40T, 38,48T, 40,66T (C-4), 42,55T (C-11), 49,86D (C-2), 68,15D (C-5*), 68,95D (C-10*), 69,43D (C-3*), 71,03D (C-14), 79,14D (C-15), 170,96S (COCH$_3$).

* May be interchanged.

5.2.13 *Acetonide formation of the* N-*acetylaminopentol* **(37)**.—The *N*-acetylpentol 3,5:14,15-diacetonide derivative **(38)** was prepared from the *N*-acetylaminopentol **(37)** (270 mg) and 2,2-dimethoxypropane using the procedure described earlier (Section 5.2.8). The crude product was purified by chromatography with chloroform-methanol (95:5) as eluent to give **(38)** (227 mg, 71%) [Found : $(M+H)^{+\bullet}$, 528. $C_{30}H_{57}NO_6$ requires $M+H$ 528]; v_{max} (CHCl$_3$) 3440 (NH), 1700 (C=O), 1460 (NH), 1375 (CN), and 1165 cm^{-1} (C-O).

δ_H 0,791 (3H, d, J 6,5, 21-H), 0,870 (3H, t, J 7,1, 20-H), 0,957 (3H, d, J 6,7, 22-H), 1,132 (3H, d, J 6,8, 1-H), 1,09-1,61 (23H, m), 1,281 [3H, s, $O_2C(CH_3)_2$], 1,294 [3H, s, $O_2C(CH_3)_2$], 1,307 [3H, s, $O_2C(CH_3)_2$], 1,38 [3H, s, $O_2C(CH_3)_2$], 1,677 (1H, m, 4-H), 3,687 (1H, dd, 15-H), 3,707 (1H, m, 3-H), 3,942 (1H, ddd, J 2,4, 6,7, 2-H), 4,090 (1H, ddd, J 2,4, 5,2, 11,5, 14-H), 5,668 (1H, d, J 8,8, NH).

δ_C 14,14Q (C-20), 15,84Q (C-21), 18,30Q (C-1), 21,36Q (C-22), 23,01T (C-19), 23,53Q [COCH_3], 24,57Q [$O_2C(CH_3)_2$], 25,41Q [$O_2C(CH_3)_2$], 25,61D (C-12), 26,09T, 26,19Q [$O_2C(CH_3)_2$], 28,53T (C-18), 28,64Q [$O_2C(CH_3)_2$], 28,64T, 32,08D (C-16), 33,49T, 35,38T, 35,79T, 37,17T, 38,39T (C-4), 43,49T (C-11), 47,45D (C-2), 67,01D (C-5*), 68,94D (C-3*), 69,84D (C-10*), 75,30D (C-14), 82,69D (C-15), 100,37S [$O_2C(CH_3)_2$], 107,48S [$O_2C(CH_3)_2$], 169,73S (COCH$_3$).

* May be interchanged.

5.2.14 *Preparation of the α-phenylbutyrate ester of the acetyl-3,5:14,15-diacetonide derivative* (38).—The α-phenylbutyrate ester of the 3,5:14,15-diacetonide *N*-acetylaminopentol derivative (39) was prepared from (38) (86 mg) and 2-phenylbutyric anhydride (126 mg) as described previously (*cf.* Section 5.3.11). The reaction yielded the α-phenylbutyrate ester of the 3,5:14,15-diacetonide *N*-acetylaminopentol derivative (39) (39 mg, 68%) [Found : $(M+H)^{+\bullet}$, 634. $C_{40}H_{67}NO_7$ requires $M+H$, 673]; v_{max} (CHCl$_3$) 2420 (NH), 1710 (C=O), 1660 (C=C), 1500 (NH), 1360 (C-N), and 1165 cm^{-1} (C-O).

δ_H 0,737 (3H, d, J 6,7, 21-H), 0,764 (3H, d, J 6,5, 22-H), 0,803 (3H, t, J 7,3, 20-H), 0,901 (3H, t, 4'-Me), 1,0-1,8 (23H, m), 1,169 (3H, d, J 6,9, 1-H), 1,280 [3H, s, $O_2C(CH_3)_2$], 1,305 [3H, s, $O_2C(CH_3)_2$], 1,314 [3H, s, $O_2C(CH_3)_2$], 1,378 [3H, s, $O_2C(CH_3)_2$], 1,978 [3H, s, CO_2CH_3], 2,048 (2H, m, 3'-H), 3,413 (1H, t, J 7,8, 2'-H), 3,424 (1H, t, J 7,8, 2'-H), 3,672 (1H, m, 3-H), 3,671 (1H, m, 15-H), 3,816 (1H, m, 2-H), 3,928 (1H, m, 14-H), 4,988 (1H, m, 10-H), 5,853 (1H, d, NH), 7,276 (5H, m, Ph).

δ_C 12,22Q (C-4'), 14,16Q (C-20), 15,90Q (C-21), 18,17Q (C-1), 20,39Q (C-22), 20,81Q (C-22), 23,03T (C-19), 23,44Q (COCH_3), 24,55Q [$O_2C(CH_3)_2$], 24,90T, 25,11Q [$O_2C(CH_3)_2$], 25,26T, 25,48D (C-12), 25,67Q [$O_2C(CH_3)_2$],

26,30T (C-3'), 26,39T (C-3'), 28,57T, 28,64Q [O$_2$C(CH$_3$)$_2$], 28,70T, 32,14D (C-16), 33,50T, 35,11T, 36.76T, 36,94T, 37,22T, 39,95T, 40,40T, 40,64T, 49,76D (C-2), 53,74D (C-2'), 54,11D (C-2'), 69,07D (C-5), 71,87D (C-3), 71,87D (C-10), 75,40D (C-14), 82,73D (C-15), 100,35S [O$_2$C(CH$_3$)$_2$], 107,49S [O$_2$C(CH$_3$)$_2$], 127,04D (Ph), 128,01D (Ph), 128,01D (Ph), 128,44D (Ph), 128,44D (Ph), 139,00S (Ph), 169,73S (COCH$_3$), 173,45S (C-1').

The recovered α-phenylbutyric acid (83 mg) had $[\alpha]_D$ +2,3° (c 8,3 in toluene) which corresponded to an optical yield of 10%.

5.2.15 *Oxidative cleavage of the C-14 – C-15 diol moiety of the N-acetylaminopentol* **(37)**. — Kiliani reagent (prepared from 530 mg chromiumtrioxide and 800 mg sulphuric acid in 4 ml distilled water) was added to a solution of the N-acetylaminopentol **(37)** (63 mg) in acetic acid (2 ml) at 0°C. After 1,5h at room temperature, methanol (0,5 ml) was added and the reaction mixture was left until it was dark green (0,5h). Solvents were removed under reduced pressure to yield 2-methylhexanoic acid **(40)** and a mixture of degradation products. The degradation products (10 mg) were treated with an excess of ethereal diazomethane. The resulting products were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The GC retention time of methyl 2-methylhexanoate: 10,42 min. GC-MS: m/z 101 (M$^+$, 3%), 87 (23), 74 (100), 43 (20).

5.2.16 *Methylation of authentic 2-methyl hexanoic acid for analysis.* — An authentic sample of 2-methyl hexanoic acid **(40)** (15 mg) was treated with an excess of ethereal diazomethane. The resulting methyl 2-methylhexanoate was analysed by (GC) and (GC-MS). GC-retention time : 10,42 min. GC-MS: m/z 101 (M$^+$, 4%), 87 (23), 74 (100), 43 (22).

5.2.17 *Derivatization of the crude degradation products with* (S)-α-methyl-4-nitrobenzylamine **(40)**. — Oxalyl chloride (0,1 ml) was added to a solution of the crude degradation products (50 mg), containing 2-methylhexanoic acid **(40)**, in benzene. The reaction mixture was heated under reflux for 45 min. Unreacted oxalyl chloride and benzene were removed under reduced pressure to yield a mixture, containing 2-methylhexanoylchloride **(41)**. The mixture was dissolved in diethyl ether and cooled to 0°C. (S)-α-Methyl-4-nitrobenzylamine (215 mg) in diethyl ether was added dropwise and after 1h at 0-5°C, the reaction mixture was diluted with diethyl ether.

Excess (S)-α-methyl-4-nitrobenzylamine was removed by washing the organic layer with hydrochloric acid (1M) and saturated sodium hydrogen carbonate. The HPLC analysis was performed on the crude (S)-α-methyl-4-nitrobenzylamide derivative **(42)** after drying and evaporation of diethyl ether.

5.2.18 *Derivatization of 2-methylhexanoic acid with (S)-α-methyl-4-nitrobenzylamine.*—An authentic sample of racemic 2-methylhexanoic acid (10 mg) was converted to the corresponding acid chloride and derivatized with (S)-α-methyl-4-nitrobenzylamine as described earlier (*cf.* Section 4.2.16) to yield *N*-(S)-α-methyl-4-nitrobenzyl (2*RS*)-2-methylhexanamide **(42)**.

5.2.19 *HPLC analysis of* (S)-α-*methyl-4-nitrobenzyl amide derivatives.*—Samples (20μl) of (S)-α-methyl-4-nitrobenzylamide derivatives **(42)**, dissolved in the mobile phase [hexane–tetrahydrofuran (70:30)], were injected in separate experiments. The (*SR*) and (*SS*) diastereomers of the reference material eluted at 5,06 and 6,85 min respectively. The (S)-α-methyl-4-nitrobenzyl amide derivative **(42)**, obtained from fumonisin B$_1$ **(5)**, eluted after 5,10 minutes.

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