# OVINE ILL-THRIFT IN NOVA SCOTIA. 13. ANOREXIA AND DIGESTIBILITY DECLINE IN FEMALE LAMBS GIVEN 3,7,11-³H₃-3-ACETOXY-7,15-DIHYDROXY-12, 3-EPOXYTRICHOTHEC-9-EN-8-ONE*

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3,7,11-³H₃-3-Acetoxy-7,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one (I, R=H, R'=Ac) was produced by *Fusarium culmorum* (CMI 14764) grown on a defined medium supplemented with 5-³H₂-mevalonic acid. The metabolite was rigorously purified and the position of the 3-³H label unequivocally determined by tritylation of the 15-hydroxy group, hydrolysis of the ester and regiospecific oxidation of the 3-hydroxy group thus generated. The metabolite (I, R=H, R'=Ac) given as a single intraruminal dose (5 mg kg⁻¹) to female lambs resulted in a 44% decline in food intake and a 5% decrease in apparent digestibility of this feed, in the 4 days following administration of the toxin (I, R=H, R'=Ac).

## Introduction

Deoxynivalenol (= vomitoxin = Rd toxin = 3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one = I, R=R'=H, Yoshizawa and Morooka, 1973) was shown to be an emetic when present in the diet of pigs (Vesonder et al., 1973) and subsequently proved to induce anorexia in mice (Pestka et al., 1986), swine (Friend et al., 1986, Prelusky, 1994) and sheep (Harvey et al., 1986). It is produced in culture by *Fusarium culmorum* and *F. graminearum* (Greenhalgh et al., 1984, 1986b) where it is invariably accompanied by a large number of congeners and indeed may well be an artifact of the isolation procedures since the esters (I, R=H, R'=Ac; I, R=Ac, R'=H) are very readily hydrolysed in mildly alkaline solution (Grove et al., 1988) or by intracellular enzymes (Yoshizawa and Morooka, 1975, Yoshizawa and Luangpitsuka, 1985). Thus there is some agreement that the alcohol (I, R=R'=H) is not normally present in plant tissues but rather occurs as the 3- or 15-acetate. It is possible that the pharmacology and hence toxicity of these esters differ from their parent alcohol and a few reports have appeared of anorexia in mice (Pestka et al., 1986) and emesis in pigs (Pestka et al., 1987) after treatment with 15-acetyl-deoxynivalenol (I, R=Ac, R'=H).

*Fusarium graminearum* has not been isolated from soils at Nappan, Nova Scotia, and *F. culmorum* has been found in only one root culture from many hundreds of soil samples collected from upland pastures at that Experimental Farm (Brewer et al., 1971; Brewer and Taylor 1980).
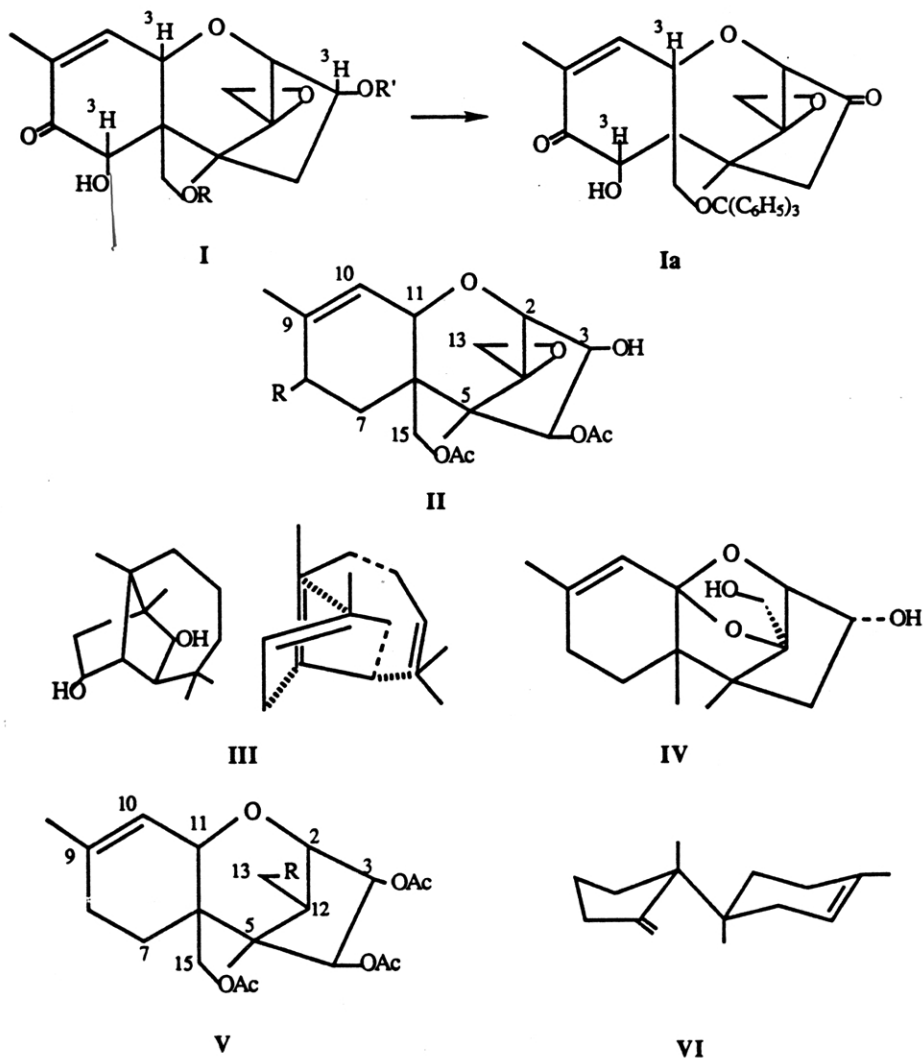
However *F. solani* is commonly found in these soil samples. For example in 1973, 71 isolates were obtained of mean frequency $2 \times 10^4$ (number of propagules $g^{-1}$ dry soil), from 197 soil samples. *F. solani* is known to produce the trichothecenes (Ishii et al., 1971), neosolaniol (**II**, R=OH), diacetoxyscirpenol (**II**, R=H) and T2 toxin (**II**, R=OCOCH$_2$CHMe$_2$) - the oral toxicity of the latter in sheep is about an order of magnitude greater than deoxynivalenol (Rukhlyada, 1983). These considerations suggested a careful examination of the effect of 3-acetyldeoxynivalenol, of known purity, on the dietary intake and digestion of female lambs. The results of such experiments are reported in this paper.

## Materials and Methods

*General* Full details of thin layer chromatography, solvents used, and methods of detection have been reported in a preceding publication (Grove et al., 1988). Infrared spectra were obtained on samples dispersed in potassium bromide using a Perkin-Elmer 283B spectrometer, and ultraviolet spectra on solutions in methyl alcohol using a Cary 14 instrument. Optical rotations (± = standard deviation) were measured using a Rudolf polarimeter. A Beckman 7500 scintillation counter was used to determine the radioactivity of samples; quenching factors were calculated by the channel ratio method and the algorithm used was written to accommodate the radioactive decay of tritium. All serum, urine and ultracentrifuged rumen fluid samples (1 mL; Brewer et al., 1990) were added to a solution (15 mL) of 2,5-diphenyloxazole (60 mg), 1,4-bis(5-phenyloxazol-2-yl)benzene (3.8 mg), naphthalene (0.9 g), ethylene glycol (0.3 mL), methyl alcohol (1.8 mL) and 1,4-dioxane. All such solutions were kept at room temperature for 24 h before measurement of their radioactivity. Samples of 3-acetyldeoxynivalenol (I, R=H, R'=Ac) and its derivatives were added to a solution (15 mL) of 2,5-diphenyloxazole (75 mg) in toluene for determination of their radioactivity. The radioactivity of the 5-$^3$H$_2$-mevalonic acid (New England Nuclear) was checked using the scintillation fluid used for the serum samples.

*Animal husbandry and digestibility studies* The husbandry of the experimental sheep (mean body weight 27.9±3.7 kg) was as described in an earlier paper (Brewer et al., 1990). The sheep pens were fitted with trays mounted on rails beneath their wooden slatted floors. These trays were dressed with coarse polyester filter cloth (Brewer et al., 1982) which retained faecal pellets and allowed urine to pass into bottles of capacity 2L. Each morning at 0600 h the trays were removed, the faeces collected and weighed and the volume of urine was measured. The filter cloth was washed, the trays reassembled and replaced under the pens. Weighed samples of urine and faeces were lyophilized to determine their dry weights. At the same time all residual feed was collected, weighed and replaced with a weighed quantity of fresh feed. Details of these dry weights are given in Tables I* and II* in the version of this paper on magnetic disc. The *in vivo* digestibility was then calculated in the usual way ((Feed consumed (g)-(faeces (g)+urine (g) excreted))x100)/Feed consumed (g). Random samples of the feed were dried in a vacuum oven at 60°; its *in vitro* digestibility was measured as described by Brewer et al., (1986). The method of collecting rumen samples has been reported (Brewer et al., 1990) and blood samples were collected by jugular venepuncture into heparinized evacuated tubes.

*Formulation of 3-acetyldeoxynivalenol for administration to sheep* 3-Acetyldeoxynivalenol (=A, 278.5 mg; defined below) was dissolved in potable (Nova Scotia Liquor Commission) ethyl alcohol-water (19:1, 25 mL) and the solution diluted with water (20 mL). This solution (22 mL, = 5 mg kg$^{-1}$) was administered intraruminally to one animal and 23 mL to the other. The duodenal tube used for the second animal was washed with ethyl alcohol (5 mL), the solution evaporated and the radioactivity of the residue (0.91 mg; = 5 mg kg$^{-1}$ net dose) determined. The control animals were dosed similarly with ethyl alcohol-water (3:4, 25 mL).

*Preparation of 3,7,11-$^3$H$_3$-3-acetoxy-7,15-dihydroxy-12,13-epoxythricothec-9-en-8-one* An inoculum (1 L) of *Fusarium culmorum* (CMI 14764) was prepared as described (Greenhalgh et al., 1984) and added to sterile production medium (10 L) consisting of sucrose (400 g), glycerol (100 g), sodium chloride (50 g), potassium dihydrogen phosphate (35 g), diammonium hydrogen phosphate (10 g), magnesium sulfate heptahydrate (2 g) and water (Brewer et al., 1982). The culture was treated with

sterile rape-seed oil, was stirred at 300 r min$^{-1}$ at 28° and aerated (6 L min$^{-1}$). After 4 days growth, 5-$^3$H$_2$-mevalonic acid (5 µCi, 2 mg) in water (5 mL) was added and the culture was harvested and extracted 4 days later (Greenhalgh et al., 1984). The crude methyl alcohol solution obtained by this method, was evaporated, the residue dissolved in toluene (50 mL) and the solution evaporated. The resulting gum (4.49 g) was dissolved in chloroform (10 mL) and applied to a silica gel column similar to that described (Greenhalgh et al., 1984) except that the chromatogram was developed at 25° and the column was eluted as follows: chloroform-ethyl alcohol (199:1, 750 mL); chloroform-methyl alcohol (79:1, 600 mL): chloroform-methyl alcohol (39:1, 3.8 L); chloroform-methyl alcohol (4:1, 1 L) and methyl alcohol (1 L). After recycling the first 400 mL of eluate the following fractions were collected (volume of each in parenthesis): 1-3 (500 mL); 4 (250 mL); 5-14 (50 mL); 15-34 (25 mL); 35-44 (50 mL); 45-52 (100 mL); 53 (200 mL); and 54-58 (500 mL). The residues from fractions 41-46 inclusive (1.4 g) were recrystallized from diethyl ether-petroleum ether (5:1) to give colorless needles, 0.5436 g, m. p. 180-185°, 4.76 x 10$^5$ d min$^{-1}$ mmol$^{-1}$. This material (150 mg) recrystallized from diethyl ether gave needles m. p. 184-185°, 4.97 x 10$^5$ d min$^{-1}$ mmol$^{-1}$, and one further recrystallization from diethyl ether provided flat colorless blades, m. p. 185°, 4.98 x 10$^5$ d min$^{-1}$ mmol$^{-1}$, $\lambda_{max}$. 220 nm (ε 6000), [α]$^{24}_D$ +34.5±0.2°. This material (113 mg) was taken up in ethyl alcohol (2 mL) and hexane (1 mL) added. The solution was concentrated to 2 mL, hexane (1 mL) added, the solution allowed to evaporate to about 1.5 mL and then kept at -15° overnight. The supernatant mother liquors were decanted at -15° and the crystals (68 mg, m. p. 185°, 4.99 x 10$^5$ d min$^{-1}$ mmol$^{-1}$, = A) washed with ethyl alcohol-hexane (1:1, 3 x 1 mL) at this temperature. The combined washings and mother liquors were evaporated giving a colorless solid (45 mg, m. p. 185°, 4.98 x 10$^5$ d min$^{-1}$ mmol$^{-1}$). The 15-1'-naphthylurethane (m. p. 80-82°; [α]$^{24}_D$ +52.5° (c, 1.13 CHCl$_3$); Found: C, 66.4; H, 6.07; N, 2.85. C$_{28}$H$_{29}$NO$_8$ requires C, 66.3: H, 5.76; N, 2.8%; 4.95 x 10$^5$ d min$^{-1}$ mmol$^{-1}$; δ$_H$ 1.15 (3H$_{14}$); 1.84 (3H$_{16}$); 2.12 (3H); 2.02-2.38 (2H$_4$, m); 3.11 (H$_{13α}$); 3.17 (H$_{13β}$, J3 Hz); 3.85 (H, e); 3.90 (H$_2$, J3 Hz); 4.38 (H$_{15α}$, J12 Hz), 4.55 (H$_{15β}$); 4.81 (H$_{11}$); 4.85 (H$_7$); 5.22 (H$_3$), 6.59 (H$_{10}$); 6.75 (H$_{NH}$, e); 7.43-7.88 (7H, aromatic)) was prepared as described by Grove et al., (1988) for the phenylurethane derivative.

*Isolation and radioactivity of other sesquiterpene metabolites* Fractions 49-52 from the chromatogram described in the preceding paragraph did not absorb ultraviolet light but did react with the ceric sulfate-sulfuric acid reagent (Grove et al., 1988). The fractions were combined (0.63 g) and rechromatographed on a silica gel column (20 x 7.5 cm) which was developed with chloroform-methyl alcohol (79:1, 4 L) and then with chloroform-methyl alcohol (39:1) when fractions (100 mL) were collected. Thin layer chromatography of the fractions showed that fractions 1-3 inclusive contained 3-acetyldeoxynivalenol (I, R=H, R'=Ac, 71 mg). Fractions 4-7 inclusive were combined (0.5 g), taken up in tetrahydropyran (5 mL) and hot cyclohexane (2 mL) added. The colorless solution on standing at 4° for 18 h gave culmorin (III, Ashley et al., 1937) which was recrystallized from the same solvent giving colorless plates, m. p. 183°, [α]$^{24}_D$ -15.6±0.8° (c, 0.623 MeOH), Found: C,75.4; H, 11.05; O, 12.8; m/z 238.1922. Calculated for C$_{15}$H$_{26}$O$_2$ C, 75.6; H, 11.0; O, 13.4% m/z 238.1933; 3.31 x 10$^6$ d min$^{-1}$ mmol$^{-1}$.

The residues from fractions 54 and 55 from the original chromatogram were crystalline and did not absorb in the ultraviolet, they were combined (0.2 g) and recrystallized from ethyl acetate giving needles (0.17 g) m. p. 224°, 4.12 x 10$^5$ d min$^{-1}$ mmol$^{-1}$ = sambucinol (IV, Mohr et al., 1984). The $^1$H n.m.r. and X-ray crystallography of this material have been reported (Greenhalgh et al., 1986a).

*7,11-³H₂-12,13-epoxy-7a-hydroxy-15-trityloxytrichothec-9-en-3,8-dione* (**Ia**) - A solution (10 mL) of $3,7,11-^3H_3-3\alpha,7\alpha$-dihydroxy-12,13-epoxy-15-trityloxytrichothec-9-en-8-one (Grove et al., 1988, I, $R=(C_6H_5)_3C$, R'=H, 43.9 mg, $4.92 \times 10^5$ d min⁻¹ mmol⁻¹) in dry benzene was treated with pyridinium chlorochromate on alumina (Cheng et al., 1980, 250 mg, 0.2 mmol Cr). The mixture was stirred at room temperature for 5 h when it was filtered and the alumina washed thoroughly with diethyl ether. The filtrate and washings were evaporated and the residue (39.5 mg) applied to a silica gel plate (Merck, $60F_{254}$, 20x20x0.05 cm). The chromatogram was developed with diethyl ether-hexane (3:1) and four bands were then visible under reflected ultraviolet light. The most polar band gave the starting alcohol (2.3 mg) on elution from the silica. The main band was eluted with ethyl acetate, the eluate evaporated and the residue (37.2 mg) in diethyl ether (2 mL) on treatment with hexane gave a solid (13.5 mg, m. p. 80-85°) as a film. This solid was taken up in t-butyl alcohol (5 mL) and the solution lyophilized giving crystals of $7,11-^3H_2$-12,13-epoxy-7α-hydroxy-15-trityloxytrichothec-9-en-3,8-dione (**Ia**), m. p. 70°, $[\alpha]^{20}_D$ -49.2° (c 2.15 CHCl₃), $\nu_{max}$. 3460, 1767, 1685 cm⁻¹, $3.29 \times 10^5$ d min⁻¹ mmol⁻¹; Found: C, 74.6; H, 6.6; O, 18.3. $C_{34}H_{32}O_6 \cdot C_4H_{10}O$ requires C, 74.7; H, 6.9; O, 18.3%, $\delta_H$ (resonances of $C_4H_9OH$ omitted) 1.01 (3H₁₄), 1.80 (3H₁₆), 2.21 (H₄ᵦ, $^2J_{HH}$ 19.44 Hz), 3.13 (H₁₅α J10.7 Hz), 3.49 (H₁₅ᵦ), 3.17 (H₁₃, J 4.2 Hz), 3.27 (H₁₃), 3.30 (H₄α), 3.50 (H₂), 3.56 (H, e), 4.73 (H₁₁ J 5.96Hz), 4.86 (H₇, e), 6.47 H₁₀ $^3J_{H10H11}$ 1.5 Hz), 7.16-7.39 (15H).

## Results

$3,7,11-^3H_3$-3-Acetyldeoxynivalenol (I, R=H, R'=Ac) was purified to constant elemental analysis, optical rotation, melting point and specific molar radioactivity. It eluted from a reverse phase partition chromatography column (Synge and White, 1960) as a Gaussian distribution without detectable distortion. It was converted through the steps: I, R=H, R'=Ac → I, $R=(C_6H_5)_3C$, R'=Ac → I, $R=(C_6H_5)_3C$, R'=OH in 84% overall yield and the sequential products of these two reactions had the same specific molar radioactivity and the same level of purity as the metabolite. Regiospecific oxidation (Grove, 1994) of the alcohol (I, $R=(C_6H_5)_3C$, R'=OH) gave the ketone (**Ia**) in 87% yield (or 73% overall from 3-acetyldeoxynivalenol) whose specific molar radioactivity was, as expected (Arigoni et al., 1973, Cane and Ha, 1988), two thirds of its parent alcohol (I, $R=(C_6H_5)_3C$, R'=OH).

The $3,7,11-^3H_3$-3-acetyldeoxynivalenol purified as described in the preceding paragraphs, administered directly into the rumen of two female lambs was rapidly hydrolysed to deoxynivalenol (I, R=R'=H). Chromatography (Grove et al., 1988) of rumen fluid collected and ultracentrifuged 5 min after administration of the toxin and then kept at -78° for 6 h, indicated the presence of 3-acetyldexoynivalenol and deoxynivalenol in the ratio 1:1.

The decline in radioactivity in rumen fluid in both dosed animals was exponential and fitted the equation: $x=983.896.e^{-0.0879y}$ with an index of fit ($r^2$) of 0.918, where y=concentration (mg mL⁻¹) of 3-acetyldeoxynivalenol from the measured radioactivity and x=time in minutes. Assuming a rumen volume of 2 L, and therefore an initial concentration (~70 mg mL⁻¹) of 3-acetyldeoxynivalenol, an apparent half life of deoxynivalenol in the rumen of these sheep can be calculated from this equation as 46 min. The radioactivity of the serum of these sheep was a maximum 300-400 min after dosing and, as reported by Prelusky et al., (1985) was apparently biphasic. Twenty three percent of the radioactivity was excreted in the urine.

The mean feed (*in vitro* digestibility 58.4%) consumption of the control animals over the 12 day experimental period (26 Aug - 6 Sept) was $1626 \pm 150$ g day$^{-1}$, and the mean feed consumption of the dosed animals in the 4 days prior to dosing (26 Aug - 29 Aug) was $1678 \pm 269$ g day$^{-1}$. By contrast the mean feed consumed by the dosed animals in the 5 days after dosing was $983 \pm 124$ g day$^{-1}$ and the feed consumed by the control animals in this period was $1670 \pm 167$ g day$^{-1}$. The difference between these means (687 g) or between the statistics given above (1626g & 1678 g) is obviously real and agrees with results reported by other workers (Harvey et al., 1986). Six days after dosing the feed intake of the dosed animals returned to normal e.g. their mean daily consumption from September 4 to September 6 inclusive was 1495 g.

The calculated digestibility of the feed consumed by the control animals and that consumed by the dosed animals prior to administration of the toxin, taking the mean value of the dry weight of feed consumed and the dry weight of faeces and urine excreted was about 54%. The mean digestibility calculated in the same way for the dosed animals between 30 August and September 2 was 48.7%. During the same time period a calculation of the digestibility of the same feed in the case of the control animals gave the result 52%. On a daily basis the calculated digestibilities of the feed on the day of dosing (=30 August) and for the next 3 days were 37%, 44%, 51% and 61% while the corresponding figures for the control animals were 51%, 56%, 54%, and 46%.

## Discussion

It is widely believed that the acetates of deoxynivalenol (I, R=R'=H) are the major metabolites produced by *Fusarium culmorum*, but this is not the case, as was clearly illustrated by the isolation of culmorin by Raistrick and his co-workers (Ashley et al., 1937) almost 60 years ago. In the present work culmorin, sambucinol and 3-acetyldeoxynivalenol were isolated in the ratio 3:1:7. Their separation is not simple and crystalline samples of each are often contaminated with the others. Such contamination is difficult to detect using for example, high pressure reverse phase partition chromatography because only the trichothecene absorbs ultraviolet light. These facts are toxicologically significant because the sesquiterpene 3,4,15-triacetoxy-12-hydroxytrichothec-9-ene (V, R=OH) is 200 times less toxic than 3,4,15-triacetoxy-12,13-epoxytrochothec-9-ene (V, R=O-C$_{12}$; Grove and Mortimer, 1969). It may be concluded that culmorin and/or sambucinol admixed with deoxynivalenol or its esters will appear to reduce the toxicity of the latter.

By the analytical methods at our disposal the $^3$H-3-acetyl-deoxynivalenol dosed to our experimental sheep was not contaminated by culmorin, sambucinol or any of the other 11 or 12 known metabolites of *F. culmorum* (Greenhalgh et al., 1986a; Zamir et al., 1990, 1992). The sheep after dosing differed from the control animals only in that they appeared to be somewhat lethargic, but their decreased food consumption was very obvious as was their gradual return to normality. This single dose did not affect their weight gain and the following season all four experimental animals produced healthy lambs. The anorexia observed in the dosed sheep is a well documented phenomenon for the effect of low doses of trichothecenes to mammals but so far as we are aware, a concomitant decline in digestibility of fodder by ruminants has not been reported. Although *F. culmorum* is not a common species in the fungal flora of pasture soil at Nappan (Brewer and Taylor, 1980) other *Fusarium* species are. In the seasons 1973-1975 inclusive, 272 isolates of *Fusarium* spp. were isolated from 504 soil

samples collected. A random sample of 11 of these 272 isolates revealed that 8 (73%) of them produced toxic metabolites (Brewer and Taylor, unpublished work). It suggests that these Fusaria, *a priori*, are a parameter in the ill-thrift of lambs on pasture at Nappan.

The radioactivity data given in the Experimental section, indicate that the specific molar radioactivity of sambucinol is less than that of 3-acetyldeoxynivalenol, thus confirming the suggestion of Zamir et al., (1990) and the more recent molecular biology of Hohn et al., (1995) that there are different pathways to these two metabolites from trichodiene (**VI**). However the specific molar radioactivity of culmorin was about 7 times that of 3-acetyl-deoxynivalenol and 8 times that of sambucinol. Biosynthetically this might be expected for its formation from mevalonic acid can be envisaged as shown (**III**). In this diagram the dotted bonds are those formed in the biosynthesis of farnesyl pyrophosphate and the hatched bonds are those formed by cyclization mechanisms well-charted in terpene biochemistry. The rearrangement of the allylic pyrophosphate double bond has also been reported (Cane and Ha, 1988).

From an agricultural point of view there is therefore hope that this efficient path of mevalonate metabolism can be enhanced at the expense of that of trichodiene and recent mapping of the genes involved (Hohn et al., 1995) lends substance to this expectation.

## Acknowledgements

## References

Arigoni, D., Cane, D.E., Müller, B. and Tamm, C. 1973. The mode of incorporation of farnesyl pyrophosphate into verrucarol. *Helv. Chim. Acta*, 56: 2946-2949.

Ashley, J.N., Hobbs, B.C. and Raistrick, H. 1937. The biochemistry of microörganisms. LIII. The crystalline coloring matters of Fusarium culmorum (W.G. Smith) Sacc. and related forms. *Biochem. J.* 31: 385-397.

Brewer, D., Calder, F.W., Jones, G.A., Tanguay, D. and Taylor, A. 1986. Effect of nickelous and other metal ions on the inhibition of rumen bacterial metabolism by 3-(3'isocyanocyclopent-2'-enylidene)propionic acid and related isocyanides. *Applied and Environmental Microbiology*, 51: 138-142.

Brewer, D., Calder, F.W., MacIntyre, T.M. and Taylor, A. 1971 Ovine ill-thrift in Nova Scotia. I. The possible regulation of the rumen flora in sheep by the fungal flora of permanent pasture. *J. agric. Sci. (Camb.)*, 76: 465-477.

Brewer, D., Feicht, A., Taylor, A., Keeping, J.W., Taha, A.A. and Thaller, V. 1982 Ovine ill-thrift in Nova Scotia. 9. Production of experimental quantities of isocyanide metabolites of Trichoderma hamatum. *Can. J. Microbiol.*, 28: 1252-1260.

Brewer, D., Parkinson, V.O. and Taylor, A. 1990. A note on the antibacterial properties of 3-(3'-isocyanocyclopent-2'-enylidene)propionic acid in the ovine rumen. *J. Applied Bacteriology*, 69: 701-704.

Brewer, D. and Taylor, A. 1980. Ovine ill-thrift in Nova Scotia. 6. Quantitative description of the fungal flora of soils of permanent pasture. *Proc. N.S. Inst. Sci.*, 30: 101-133.

**Cane, D.E.** and **Ha, H-J.** 1988. Trichodiene biosynthesis and the role of nerolidyl pyrophosphate in the enzymic cyclization of farnesyl pyrophosphate. *J. Am. Chem. Soc.*, 110: 6865-6870.

**Cheng, Y.**, **Liu, W-C.** and **Chen, S.** 1980. Pyridinium chlorochromate on alumina as a selective oxidant for primary and secondary alcohols. *Synthesis*, 223-224.

**Friend, D.W.**, **Trenholm, H.L.**, **Thompson, R.H.**, **Fiser, P.S.** and **Harten, K.E.** 1986. Effect of feeding diets containing deoxynivalenol (vomitoxin) contaminated wheat or corn on the feed consumption, weight gain, and sexual development of male and female pigs. *Can. J. Animal Sci.*, 66: 765-776.

**Greenhalgh, R.**, **Hanson, A.W.**, **Miller, J.D.** and **Taylor, A.** 1984. Production and X-ray crystal structure of $3\alpha$-acetoxy-$7\alpha$,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one. *J. agric. Food Chem.*, 32: 945-948.

**Greenhalgh, R.**, **Levandier, D.**, **Adams, W.**, **Miller, J.D.**, **Blackwell, B.A.**, **McAlees, A.J.** and **Taylor, A.** 1986a. Production and characterization of deoxynivalenol and other secondary metabolites of *Fusarium culmorum* (CMI 14764, HLX 1503). *J. agric. Food Chem.*, 34: 98-102.

**Greenhalgh, R.**, **Meier, R.**, **Blackwell, B.A.,**, **Miller, J.D.**, **Taylor, A.** and **ApSimon, J.W.** 1986b. Minor metabolites of *Fusarium roseum* (CMI 28114). *J. agric. Food Chem.*, 34: 115-118.

**Grove, J.F.** 1994. Regiospecific reactions with derivatives of the trichothecene mycotoxins nivalenol and vomitoxin. *J. Natural Products*, 57: 1491-1497.

**Grove, J.F.** and **Mortimer, P.H.** 1969. Cytotoxicity of some transformation products of diacetoxyscirpenol. *Biochem. Pharmacol.*, 18: 1473-1478.

**Grove, J.F.**, **McAlees, A.J.** and **Taylor, A.** 1988. Preparation of 10-g quantities of 15-O-acetyl-4-deoxynivalenol. *J. Org. Chem.*, 53: 3860-3862.

**Harvey, R.B.**, **Kubena, L.F.**, **Corrier, D.E.,**, **Witzel, D.A.**, **Philips, T.D.** and **Heidelbaugh, N.D.** 1986. Effects of deoxynivalenol in a wheat ration fed to growing lambs. *Am. J. Vet. Res.*, 47: 1630-1632.

**Hohn, T.M.**, **Desjardins, A.E.** and **McCormick, S.P.** 1995. The TRI4 gene of *Fusarium sporotrichioides* encodes a cytochrome P450 mono-oxygenase involved in trichothecene biosynthesis. *Molecular and General Genetics*, 248: 95-102.

**Ishii, K.**, **Sakai, K.**, **Ueno, Y.**, **Tsunoda, H.** and **Enomoto, M.** 1971. Solaniol, a toxic metabolite of *Fusarium solani*. *Applied Microbiology*, 22: 718-720.

**Mohr, P.**, **Tamm, C.**, **Zürcher, W.** and **Zehnder, M.** 1984. Sambucinol and sambucoin, two new metabolites of Fusarium sambucinum possessing modified trichothecene structures. *Helv. Chim. Acta*, 67: 406-412.

**Pestka, J.J.**, **Lin, W.S.** and **Forcall, J.H.** 1986. Decreased food consumption and body weight gain in the $B_6C_3F_1$ mouse after dietary exposure to 15-acetyldeoxynivalenol. *Biol. Abs.* 83: 81362.

**Pestka, J.J.**, **Lin, W.S.** and **Miller, E.R.** 1987. Emetic activity of the trichothecene 15-acetyldeoxynivalenol in swine. *Food Chem. Toxicol.*, 25: 855-858; *Biol. Abs.*, 85: 63915.

**Prelusky, D.B.** 1994. The effect of DON (deoxynivalenol) on serotonigenic neurotransmitter levels in pig blood. *J. Environ. Sci. and Health*, B. 29: 1203-1218; *Biol. Abs.* 99: 42418.

**Prelusky, D.B.**, **Veira, D.M.** and **Trenholm, H.L.** 1985. Plasma pharmacokinetics of the mycotoxin deoxynivalenol following oral and intravenous administration to sheep. *J. Environ. Sci. Health*, B, 20: 603-624.

**Rukhlyada, V.V.** 1983. Effect of T2 toxin on sheep. *Veterinariya* (Moscow), 61-62; *Chem. Abs.*, 99: 65586.

**Synge, R.L.M.** and **White, E.P.** 1960. Photosensitivity diseases in New Zealand. XXIII. Isolation of sporidesmin, a substance causing lesions characteristic of facial eczema from *Sporidesmium bakeri* Syd. *New Zealand J. agric. Res.*, 3: 907-921.

**Vesonder, R.F.**, **Ciegler, A.** and **Jensen, A.H.** 1973. Isolation of the emetic principle from *Fusarium* infested corn. *Applied Microbiology*, 26: 1108-1110.

**Yoshizawa, T.** and **Luangpitsuka, P.** 1985. Microbial transformation of trichothecene mycotoxins by deoxynivalenol producing strains of Fusarium graminiarum. *Maikotokishin* (Tokyo), 21: 6-8; *Chem. Abs.*, 103: 210639.

**Yoshizawa, T.** and **Morooka, N.** 1973. Deoxynivalenol and its monoacetate new mycotoxins from *Fusarium roseum* and moldy barley. *Ag. Biol. Chem.*, 37: 2933-2934.

**Yoshizawa, T.** and **Morooka, N.** 1975. Biological modification of trichothecene mycotoxins: acetylation and deacetylation of deoxynivalenols by *Fusarium* species. *Applied Microbiology*, 29: 54-58.

**Zamir, L.O.**, **Devor, K.A.**, **Nikolakakis, A.** and **Sauriol, F.** 1990. Biosynthesis of Fusarium culmorum trichothecenes: the roles of isotrichodermin and 12,13-epoxytrichothec-9-ene. *J. Biol. Chem.*, 265: 6713-6725.

**Zamir, L.O.**, **Devor, K.A.**, **Nikolakakis, A.**, **Nadeau, Y.** and **Sauriol, F.** 1992. Structures of new metabolites from Fusarium species: an apotrichothecene and oxygenated trichodienes. *Tetrahedron Letters*, 5181-5184.