great importance to have ready in hand some simple approximate analytical solutions of LEE. In this note we present such F- and M-solutions of LEE for index 5.

$$\frac{dz}{dt} = \pm \left(2D + \frac{z^2}{4} - \frac{z^6}{12}\right)^{1/2}. \quad (1)$$

We start with the series solution[4,5]:

$$z_s(t) = \sum_{n=0}^{\infty} a_n t^n, \quad (2)$$

and present the function z (t) as Padé (2,2) approximant:

$$z_{22}(t) = \frac{A + Bt + Ct^2}{A + Et + Ft^2}. \quad (3)$$

Table 1

−t	z_{num}	z_{22}	z_{ser}
0.00	1.0000	1.00000	1.0000
	1.0000	1.00000	1.0000
0.25	0.8920	0.89297	0.8931
	0.9042	0.90514	0.9052
0.50	0.7892	0.79083	0.7941
	0.8133	0.81474	0.8174
0.75	0.6938	0.69623	0.7155
	0.7293	0.73130	0.7469
1.00	0.607	0.61014	0.679
	0.653	0.65582	0.711
1.25	0.528	0.53260	0.719
	0.586	0.58835	0.739
1.50	0.457	0.46314	0.890
	0.527	0.52846	0.873
1.75	0.393	0.40107	1.268
	0.475	0.47548	1.173
2.00	0.335	0.34562	1.956
	0.431	0.42865	1.722

Table 2

D	−t	D	−t	D	−t
0.01	4.7681	0.04	3.1343	0.07	2.4914
0.02	3.8605	0.05	2.8672	0.08	2.3512
0.03	3.4718	0.06	2.6598	0.09	2.2320
				1.00	2.1292

By usual procedure[6] we find from (3) and (2), the following expressions for the coefficients in (3):

$$\left.\begin{aligned} &A = 12,\ B = 3\psi,\ C = 2 - 15\psi^2 \\ &E = 15\psi,\ F = 2; \\ &\psi = \left(2D + \frac{1}{6}\right)^{1/2}. \end{aligned}\right\} \quad (4)$$

We have calculated the function z(t) by the methods of our approximate analytical (3, 4), numerical, and series (2) solutions. Some results for 2 values of D, $+0.01$ (F-solution, upper entries in z's) and -0.01 (M-solution, lower entries in z's) are presented in table 1.

It is evident that our approximation is far more exact than series solution and is quite close to numerical solution, and this is true for any value of D.

To show further the usefulness of present approach we have calculated, from quadratic equation $A + Bt + Ct^2 = 0$, the boundary values of t, for which $z = 0$ (table 2).

It is, of course, much more difficult to obtain these boundary values by numerical calculations from original differential equation (1)!

The more detailed results for other values of D and the next Padé approximations will be presented elsewhere.

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A crystalline, toxic, peptide metabolite of *Trichoderma* spp. isolated from soil

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Summary. Isolates of *Trichoderma* spp. from pasture soils of Nova Scotia produce at least 7 toxic peptides, probably related to alamethicin, some of which inhibit the growth of cellulase producing rumen bacteria. One of the peptides has been obtained in crystalline form and crystal data on this material is reported.

Isolates of the genus *Trichoderma* producing toxic peptides account for about 2% of the fungal propagules grown in the laboratory from soil samples taken from permanent pastures at Nappan, Nova Scotia[1]. The crude peptide metabolites affected rumen fermentation, and in particular, cellulose digestion. These metabolites are therefore implicated in the ill-thrift of pasture fed ruminants in Nova Scotia[2]. The production of toxic peptides by *Trichoderma* spp. [3-5] has been reported and these peptides receive some attention because they affect the physiological function of membranes[6,7]. At least 3 structures for these peptides have been proposed[8-10] and all have been claimed to be synthesized[11,12,18]. Unfortunately, the physical, chemical and biological properties of the synthetic materials differed from the natural products.

Trichoderma isolates from soil samples collected from permanent pastures at Nappan, Nova Scotia were grown for 7 days at 25°C on a rotatory shaker. The cultures were

centrifuged, the supernatant liquid diluted with methanol (2 parts by volume) and chloroform (2 parts by volume), the mixture shaken, the chloroform phase collected and evaporated. The residue (150 mg) was applied to a hydrophobic hyflosupercel[13] column (20×2 cm) saturated with the lower phase of the solvents chloroform-methanol-water (2:2:1). The column was developed with the upper phase (column dead volume 63 ml; flow rate 2 ml min^{-1}; T=25±0.1°C; 1 kg cm^{-2} applied to the top of the column) and fractions (38 ml) were collected after the appearance of methyl orange in the eluate. The weight distribution obtained is shown in figure 1; 9 peaks can be discerned, those marked I, III-VI were biologically active[14]. For example, material from peak V affected the growth[15] of typical obligate anaerobic rumen bacteria as indicated in the table. The proportions of the peaks varied with respect to the producing *Trichoderma* isolate and the length of its fermentation. In some cases peaks I and III were predominent, whilst in others the composite peaks V was the most abundant. In all cases, hydrolysates of the fractions contained alanine, 2-methylalanine, glycine, glutamic acid, leucine, proline and valine. The least polar fractions contained phenylalaninol. Further chromatography of the material from peak V on a 'C_{18}Bondapack' (Waters) column (50×0.95 cm; solvent: 0.1 M ammonium acetate buffer pH 4.0-methanol 1:5; particle size < 10 µm; flow rate 3 ml min^{-1}; T=ambient) gave the weight distribution shown in figure 2. Peaks marked I-V inhibited the growth of *Sarcina lutea*[14]. Hydrolysis of the material from peak II gave: alanine (2), 2-methylalanine (8), glutamic acid (3), glycine (1), leucine (1), proline (2), phenylalaninol (1) and valine (2). The amorphous material (10 mg) was taken up in hot acetonitrile (6 ml) and the solution kept at room temperature for 6 months during which time colorless, transparent crystals grew in the solution. Some of these were tabular and very thin, growing on the walls of the vessel. Others were of a more compact habit, with a mean diameter of up to 0.5 mm. The density of the latter (measured by flotation in a solution of toluene in carbon tetrachloride) was 1.186 g cm^{-3} (±0.5%). The crystal data (from precession photographs and diffractometric measurements) were: orthorhombic, $A2_122$ (D_2^5), $a=33.77$, $b=65.31$, $c=20.56$ Å (all±0.1%). In this space group there are 8 symmetry-related positions; it can be shown that the molecular weight of the 'asymmetric unit' is 4051 (±0.8%). The crystals belonging to the tabular habit are probably structurally identical to those characterised.

The isolate of *Trichoderma* that produces alamethicin (U22324)[16] when examined as described, gave a mixture of at least 6 toxic peptides. This result may offer an explanation for the discordant observations recorded in the literature[8-10].

Organism	Minimum inhibitory concentration µg ml^{-1}
Butyrivibrio fibrisolvens	1
Megasphaera elsdenii	80
Selenomonas ruminantium	200
Succinivibrio dextrinosolvens	400

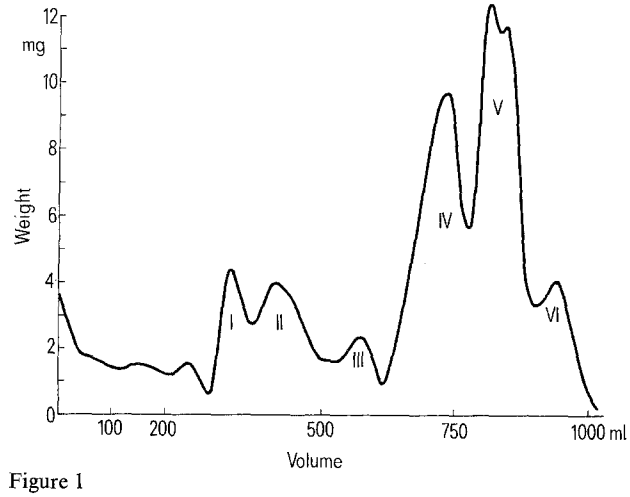

Figure 1

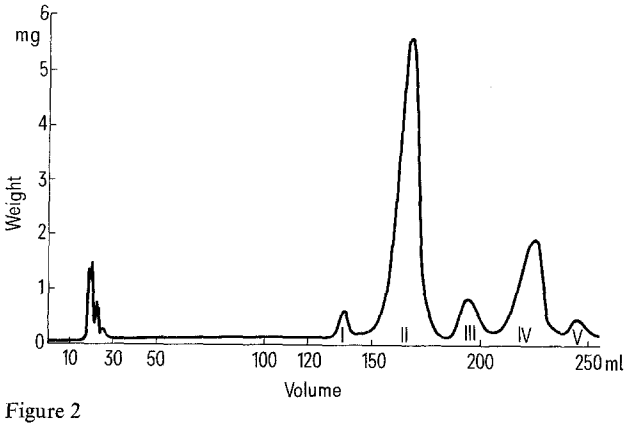

Figure 2

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