

effective residence time probably exceeds the 7-year interchange time<sup>7,8</sup> possibly being as high as 20 years. Thus the relative greenhouse effects are  $G_{\text{gas}}:G_{\text{coal}} = 0.95$  to 4.1. This calculation neglects the contributions of ethane, some 20% of North Sea gas, that need specific study. Also, from deep-mined coal in the UK, roughly  $20 \text{ m}^3 \text{ t}^{-1}$  of methane is released, discounting that drained off and burned. Including this average figure, the greenhouse factor from coal is increased to  $G_{\text{coal}} = 0.75 + 0.21\eta$

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and the relative effect to  $G_{\text{gas}}:G_{\text{coal}} = 0.8$  to 3.0.

Although the uncertainty is large, the substitution of gas-fired stations for coal is likely to worsen the greenhouse effect. If its case to the European Commission is to hold up, the Government needs to look much more carefully at the gas leakage problems.

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## Protease or protease inhibitor?

SIR—Richardson *et al.* have proposed<sup>1</sup> that the intensely sweet protein thaumatin may act as a protease inhibitor on the basis of its homology with the maize bifunctional inhibitor. We wish to point out that thaumatin also has a significant homology with a group of recently identified picor-

TRYPSIN	L	E	G	G	K	D	S	C	Q	G	D	S	G	G	P	V	C	N	G	Q	L	Q	G	I	V	S
HRV2 2A	L	I	G	E	P	C	E	P	G	D	C	G	G	K	L	L	C	K	H	G	V	I	G	I	V	T
THAUMATIN I	D	S	G	S	G	I	C	K	T	G	D	C	G	L	L	R	C	K	R	F	R	G	P	P	T	T
MAIZE 22K INHIB	A	S	G	R	G	S	C	R	T	G	D	C	G	G	V	V	Q	T	G	Y	G	R	A	P	N	T
TPR	G	S	G	R	G	N	C	E	T	G	D	C	N	G	M	L	E	C	Q	G	Y	G	K	P	N	T

Alignments of the sequences around the active site nucleophile of trypsin and the 2A protease of HRV2 with sequences from thaumatin I, the maize 22K bifunctional inhibitor and the tobacco PR protein. Residue numbers: porcine trypsin, 174-200; 2A protease, HRV2, 95-121; *thaumatococcus daniellii* Benth, 52-78; maize 22 K bifunctional inhibitor, 55-81; pathogenesis-related protein induced in tobacco by infection with TMV (TPR), 54-80. Similar residues are boxed.

naviral proteases<sup>2</sup> and that this may explain the known protease activity of thaumatin<sup>3</sup>.

The sequence surrounding the active site of the 2A protease of human rhinovirus 2 (HRV2) (see figure) was used to search the PIR and Swissprot protein data banks using the FASTP program<sup>4</sup>. Similarity was found with trypsin and other serine proteases<sup>2</sup>, but also, more surprisingly, with thaumatin I (Z values; thaumatin I, 5.8; trypsin, 5.6). In particular, the sequence around the nucleophilic active site, -GDCGG- is present in thaumatin I; all other boxed residues, with the exception of the second glycine, are typically found near the active sites of serine proteases.

We propose that the cysteine residue in the -GDCGG- sequence may be the active site of the thaumatin protease even though the crystal structure of the molecule shows that all 16 cysteines are occupied in disulphide bridges<sup>5</sup>. The protease activity of thaumatin is observed only upon addition of dithiothreitol<sup>3</sup>. As the -GDCGG- sequence is exposed on the surface of the thaumatin molecule, it is conceivable that

the cysteine residue is freed from its disulphide bridge by dithiothreitol and made available as a nucleophile in proteolytic cleavage.

Also shown in the figure are the corresponding regions of the maize bifunctional inhibitor and a tobacco protein induced on

TMV infection (TPR). The similarity of the two proteins to thaumatin has prompted the suggestion that thaumatin may function as a protease inhibitor<sup>1,6</sup>, but both proteins also have a significant similarity with the active site of HRV2 protease 2A. As the TPR protein has not yet been shown to act as a protease inhibitor (it does not, for example, inhibit trypsin), it may be of interest to examine

whether it possesses a protease activity instead.

The finding of significant similarity between thaumatin and two classes of proteins, one of proteases and one of protease inhibitors, emphasizes the care with which the results of computer searches must be treated.

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## The aroma of rice . . . and tiger

SIR—About 8 per cent of the active component of the aroma of fragrant varieties of Indian-Basmati rice has been identified as 2-acetyl-1-pyrroline<sup>1</sup>. But it may be of interest that other components of rice aroma are likely to include a constituent of the volatile fraction of the 'marking fluid' of the tiger, believed to be a source of pheromone in this animal<sup>2</sup>. We have observed the distribution pattern of 9,662 markings of 10 tigers in the open-air zoo of Nandan Kanan; the results suggest that the fluid encodes territorial and sexual connotations.

Of the volatile molecules of marking fluid we have recently studied the most elusive is one with a pleasant aroma. In its hydrochloride form, this aroma is not perceptible but the molecule can be separated from other amines such as putrescine, cadaverine and phenylethylamine by paper chromatography (solvent, *n*-butanol:acetic acid: water-4:1:1), and compared with the active component in the aroma of rice (cooked or uncooked) also in its hydrochloride form. In co-chromatograms, rice aroma and tiger aroma have the same  $R_F$ , established by cutting out the pieces of the chromatogram in an ordered sequence and moistening them with alkali. This similarity was confirmed independently. In the same chromatogram, synthetic 2-acetyl-1-pyrroline (see ref. 3) had a far higher  $R_F$  value. As habitual rice-eaters, we can detect a difference between these two aroma.

Volatiles of tiger and rice aroma also share at least one peak, as is evident from gas-liquid chromatography (with a squalane and Carbowax 20M packed column). In a tiger cub we reared, the tiger aroma was absent at an early age, but appeared later (after the aroma had become perceptible to us). Attempts to further characterize the volatile fraction (using gas chromatography and mass spectrometry) failed, possibly because by the time samples reached the analysis centres at Bangalore (India) and Swansea (UK), the minute quantities of this rather unstable substance had vanished.

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