# Graminin A, a New Toxic Metabolite from *Cephalosporium gramineum* Nisikado & Ikata

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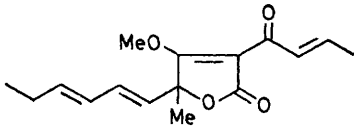
*Summary* From the culture filtrate of *Cephalosporium gramineum* a new phytotoxic compound, graminin A, has been isolated and its structure determined by spectroscopic analyses.

WE have recently reported the isolation,[1] phytotoxicity, and antibiotic activity[2] of five new tetronic acid derivatives (gregatins A, B, C, D, and E) from *Cephalosporium gregatum*. We now report on the isolation and structure characterisation of a new phytotoxic compound, graminin A, the main component from the culture filtrate of *Cephalosporium gramineum*.

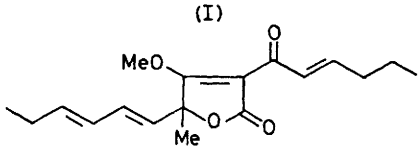
*Cephalosporium gramineum* which causes stripe disease of wheat (*Triticum aestivum*) was grown in a modified Richard medium for 4 weeks at 25 °C with occasional shaking. Extraction of the culture filtrate with ethyl acetate and chromatography of the extract over silica gel with chloroform as eluent afforded a crude gum. Further preparative t.l.c. on silica gel with chloroform–methanol (98 : 2, v/v) gave graminin A as a colourless viscous oil.

Graminin A, $C_{18}H_{24}O_4$ ($M^+$ 304); $[\alpha]_D^{20}$ −145° (*c* 0·98, $CHCl_3$), gave a characteristic lilac colour with diazotized *o*-dianisidine. Its i.r. [$\nu_{max}$ (film) 1740sh, 1705, and 1640 $cm^{-1}$] and u.v. [$\lambda_{max}$ (EtOH) 225 ($\epsilon$ 34,000), 240 sh (20,000), and 300 (15,000) nm] spectra were very similar to those of gregatin A (I). The n.m.r. spectrum of graminin A ($CCl_4$; 90 MHz) showed signals at $\tau$ 2·4—3·1 (2H, m, =CH), 3·6—4·7 (4H, m, =CH), 6·24 (3H, s, OMe), 7·61 (2H, q, *J* 7 Hz, =CH–C$H_2$–$CH_2$–), 7·86 (2H, quintet, *J* 7 Hz, =CH–C$H_2$Me), 8·38 (2H, m, –$CH_2$–C$H_2$Me), 8·52 (3H, s, tert. Me), and 9·0 (6H, *J* 7 Hz, Me). These data indicate that graminin A is closely related in structure to gregatin A.

In the n.m.r. spectrum of graminin A, compared with that of gregatin A, the two $CH_2$ signals at $\tau$ 7·61 and 8·38 and the methyl triplet at $\tau$ 9·0 are new whereas the =CMe doublet at $\tau$ 7·92 is absent. This indicates that the crotonyl side chain attached to C-3 in gregatin A should be replaced by the COCH=CH–$CH_2$–$CH_2$Me group. This was further confirmed by spin decoupling experiments. Irradiation at $\tau$ 7·61 caused the olefinic multiplet at $\tau$ 2·4—3·1 to collapse to an AB quartet (*J* 15 Hz). This coupling constant indicated that the double bond in the C-3 side chain in graminin A is *trans*. Irradiation at $\tau$ 2·93 caused the quartet at $\tau$ 7·61 to collapse to a triplet (*J* 7 Hz), indicating the presence of the CO–CH=CH–$CH_2$–$CH_2$– group. The mass spectral fragments at *m/e* 207 ($M^+$ −$C_6H_9O$) and 97




(I)




(II)

were also in good agreement with the presence of this group. Furthermore, irradiation at $\tau$ 9·0 caused the quintet at $\tau$ 7·86 to collapse to a doublet (*J* 6 Hz), indicating the presence of the =CH–$CH_2$Me group. The *trans* nature of both double bonds in the hexadiene system was also confirmed by comparison of the n.m.r. data with those for gregatin A. On the basis of these results graminin A has structure (II).

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[1] K. Kobayashi and T. Ui, *Tetrahedron Letters*, 1975, 4119.
[2] K. Kobayashi and T. Ui, *Physiological Plant Pathology*, 1977, **11**, 55,.