GRISEULIN, A NEW NITRO-CONTAINING BIOACTIVE METABOLITE PRODUCED BY *Streptomyces* spp.†

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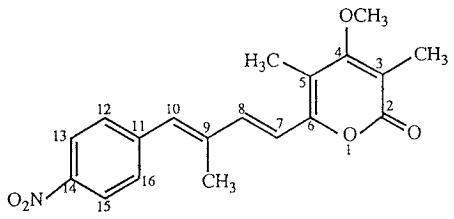
Our continued bioassay directed fractionation and purification of the mother liquor from the *Streptomyces griseus* var. autotrophicus[1] afforded an aromatic nitro compound, griseulin, **1**, with nematocidal and mosquitocidal activities.

Cultures of *S. griseus* var. autotrophicus, strain MSU32058/ATCC 53668 (12 liters), were grown in 2-liter baffle-bottomed Erlenmeyer flasks, each containing 400 ml of A-9 medium (peptone 4 g, glucose 10 g, "Brer Rabbit green label" molasses 15 g, distilled H_2O 1 liter). The inoculated flasks were placed on a rotary shaker at 150 rpm at 26°C for 7 days and centrifuged to obtain the mycelial cake. The mycelial cake was extracted with MeOH and faeriefungin crystals were removed[1]. The orange-brown powder thus obtained (16 g) was fractionated by VLC using hexane - acetone (1 : 1, v/v) on column silica gel (300 g). The six fractions, I (150 ml), II (75 ml), III (325 ml), IV (210 ml), V (125 ml) and VI (425 ml) were evaporated to dryness *in vacuo* separately. Bioassay of these fractions indicated that fractions I, V and VI were not active. The active fractions II, III and IV were combined (2.2 g) and purified further by TLC (hexane - acetone; 3 : 1 v/v) on tapered plates (2,000 μ, Analtech Uniplate, Newark, Delaware). Griseulin, **1** (250 mg, Rf = 31) was eluted by $CHCl_3$.

Griseulin, **1**. Yellow-orange solid; mp, 164~165°C; UV λ_{max}^{EtOH} nm (ε) 367 (22,417), 222 (24,256), 203 (21,971); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.95 (s, 3H, 3-CH_3), 2.11 (s, 3H, 5-CH_3), 2.14 (s, 3H, 9-CH_3), 3.93 (s, 3H, OCH_3), 6.25 (s, 1H, 8-H), 6.57 (s, 1H, 7-H), 7.1 (s, 1H, 10-H), 7.45 (d, J = 9 Hz, 2H, 12-H, 16-H), 8.19 (d, J = 9 Hz, 2H, 13-H, 15-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.40 (3a-CH_3), 14.96 (5a-CH_3), 19.82 (9a-CH_3), 56.86 (OCH_3), 94.16 (C-3), 103.67 (C-5), 124.23 (C-15), 124.25 (C-13), 127.69 (C-10), 130.40 (C-16), 130.42 (C-12), 131.36 (C-7), 136.52 (C-8), 139.08 (C-9), 144.50 (C-11), 146.88 (C-14), 160.24 (C-6), 165.35 (C-2), 166.28 (C-4); LRMS (EI, 70 eV) *m/z* (relative intensity) 341 ($C_{19}H_{19}O_5N$, M^+, 100), 326 (35), 312 (30), 298 (33), 282 (10), 266 (12), 238 (10), 205 (10), 168 (37), 152 (30), 139 (45), 128 (12), 115 (16), 83 (43).



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Griseulin was found to contain a 1,2-pyrone moiety similar to luteoreticulin[2] as indicated by the carbonyl signal at 165.35 ppm in its ^{13}C NMR. A furan ring was absent in **1** and contained 3 × CH_3 and one OCH_3 as in the case of aureothin and gave the molecular ion at *m/z* 341 with 100% intensity. This compound did not show any aliphatic methylene or methine protons in its NMR spectrum. The spectral data are in agreement with the proposed structure for **1**. *Streptomyces spectabilis* (ATCC 27465) and *Streptomyces luteoreticuli* or *Streptoverticillium mobaraense* (ATCC 25365) when fermented under the same conditions produced compound **1**. Griseulin is a novel aromatic nitro-containing compound with a 1,2-pyrone moiety.

Synthetic cholinesterase inhibiting agents such as carbamates and organophosphates are commonly used for nematocidal pest-management. Plant products with nematocidal activity have been recently reported[3~8] but these natural products did not show any potential for commercial development. Synthetic substituted phenols, phenoxyacetic acid esters and hydrazides were investigated for nematocidal activities by MALIK *et al.*[9] against seed-gall nematode (*Anguina tritica*), root-knot nematode (*Meloidogyne javanica*) and pigeon-pea cyst-nematode (*Heterodera cajani*). Phenols with electron donating substituents as well as $-NO_2$ group showed good nematocidal activity[9].

Nematocidal activity was carried out on *Panagrellus redivivus*, *Caenorhabditis elegans* and *Heterodera glycines*[1]. The nematode suspension (45 μl) containing 30~50 nematodes at various develop-

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mental stages were transferred into each well of a 96-well tissue culture plate. Test compounds in 2% DMSO (5 μl each) were added to each well and mixed gently. The inoculated plates were held in a humid chamber. Mortality was recorded after 2 and 24 hours. Compound **1** gave 100% mortality at 5 and 1 ppm, respectively, at 4 and 24 hours with all three of the nematodes assayed. Similarly, griseulin, **1**, gave 100% mortality when tested with 4th instar mosquito larvae, *A. aegypti*, reared from the mosquito eggs[1] at 24 hours.

Organophosphate nematocides are very expensive and only a limited amount can be applied annually due to environmental and toxicity concerns. There is no eradicative chemical treatment available for soil nematodes. Strategies should be developed for effective and safer compounds for the management of nematodes and natural products such as griseulin may function as a preliminary step to achieve this goal.

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