# Isolation and Characterization of Phytotoxins Associated with *Streptomyces scabies*

**Russell R. King,*[a] C. Harold Lawrence,[a] Merv C. Clark,[a] and Larry A. Calhoun[b]**

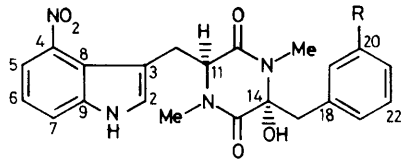
[a] *Research Station, Research Branch, Agriculture Canada, Fredericton, New Brunswick, Canada E3B 4Z7*
[b] *Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E2*

Two phytotoxins capable of inducing scab-like lesions on immature potato tubers were isolated from field grown and aseptically cultured potato tubers that had been infected by *Streptomyces scabies*; the compounds were characterized by spectral methods and partial syntheses as unique 2,5-dioxopiperazines.

Because of its detrimental effect on appearance, grade and quality, common scab of potato caused by *Streptomyces scabies* (Thaxt.) Waksman and Henrici is considered a serious disease in many potato producing areas of the world. Attempts at this institution to elucidate the host–parasite interaction have demonstrated that ng levels of toxins produced extracellularly by pathogenic strains will induce the development of scab-like lesions on immature potato tubers.[1] This communication describes procedures utilized in the isolation and characterization of the two main phytotoxins. These compounds are provisionally named thaxtomin A (**1**) and thaxtomin B (**2**) and were accumulated in μg quantities from scab lesions in both field-grown and aseptically cultured potato tubers (their presence in liquid cultures was not readily discernible[2]). Although a number of other indole containing 2,5-dioxopiperazines have been characterized,[3] we are unaware of any previous examples displaying the inclusion of nitroindol-3-yl units.

The isolation procedure involved initial acetone extraction of infected tuber tissue, and subsequent fractionation of the chloroform soluble extracts by preparative t.l.c. This procedure (0.5 mm silica gel 60 with chloroform/methanol 9 : 1) yielded two impure components that induced scab-like lesions on immature potato tubers. Further purification of the phytotoxins was achieved by successive fractionations on 0.2 mm RP-$C_{18}$ t.l.c. plates (acetone/water 60 : 40). The major product, thaxtomin A (**1**) crystallized from methanol/acetone as pale orange rosettes, m.p. 230 °C (decomp.); $M^+$ 438.1708; $\lambda_{max.}$ (EtOH) 398 (ε 4050), 343 (3220), 279 (5830), 249 (15 070), and 220 (27 700) nm; $\nu_{max}$ (Nujol) 3200—3380, 1650, 1600, and 1510 $cm^{-1}$. This compound yielded a monoacetate derivative with acetic anhydride and a diacetate with acetic anhydride–pyridine.

The molecular formula of (**1**) was deduced as $C_{22}H_{22}N_4O_6$ from high resolution fast atom bombardment (f.a.b.) mass spectra and DEPT $^{13}C$ n.m.r. experiments [6 $sp^3$ (1s, 1d, 2t,



(**1**) R = OH

(**2**) R = H

and 2q) and 16 $sp^2$ (8s and 8d) hybridized carbon signals observed]. $^1H$ N.m.r. indicated the presence of 19 unexchangeable protons, 8 of which were of an aromatic or alkenic nature.†

The presence of a nitroindol-3-yl group in (**1**) was first suggested from high resolution mass spectral analysis (electron impact, EI) of intense fragments at *m/z* 175 ($C_9H_7N_2O_2$), *m/z* 162 ($C_8H_6N_2O_2$), and *m/z* 116 ($C_8H_6N$). Regiochemical assignment of the nitro substituent to the 4-position in the indole ring was made from comparison with u.v. and $^1H$ n.m.r. literature data on nitroindoles[4,5] and $^1H$ homonuclear shift-correlation spectroscopy (COSY). Subsequent comparative n.m.r. studies with a synthetic sample of a 4-nitrotryptophan derivative[6] facilitated complete carbon and proton assignments for C-2 to C-10.

The presence of a benzyloxy type precursor also was apparent from high resolution mass spectral analysis of intense fragments (EI) at *m/z* 107 ($C_7H_7O$) and *m/z* 89 ($C_7H_5$). Regiochemical assignment of the hydroxy substituent to the *meta* ring position was based on observed $^1H$ and $^{13}C$ n.m.r. chemical shifts for *m*-hydroxyphenylacetic acid and $^1H$ homonuclear shift correlation spectroscopy (COSY).

Based on the unassigned $^1H$ and $^{13}C$ n.m.r. signals the remaining subunit $C_6H_8N_2O_3$ had to accommodate: two *N*-methyl groups ($^1H$ n.m.r. absorptions at δ 2.81 and 3.03), two carbonyl moieties ($^{13}C$ n.m.r. absorptions at δ 168.33 and 166.81), a one proton quartet centred at δ 3.86 and coupled to the 4-nitroindol-3-yl moiety, plus an $sp^3$ carbon atom ($^{13}C$ n.m.r. absorption at δ 88.03) compatible with the presence of a tertiary hydroxy group. This information together with i.r. data which displayed disubstituted amide bands at 1650 $cm^{-1}$ was rationalized as indicative of a biosynthetically feasible *N,N*-dimethyl-2,5-dioxo-3-hydroxypiperazine unit coupling the 4-nitroindol-3-yl and *m*-hydroxybenzyl groups.

For the purposes of structural confirmation, determination of relative stereochemistry (at C-11 and C-14) and investigation of structure–activity relations, cyclo-(L-4-nitrotryptophyl-L-phenylalanyl) and cyclo-(D-4-nitrotryptophyl-L-phenylalanyl) were prepared. Significantly, only the former compound exhibited thaxtomin-like activity. Appropriately, it also displayed the most notably comparable $^1H$ n.m.r. spectrum. Other synthetic analogues, *i.e.*, cyclo-(L-tryptophyl-L-phenylalanyl)[7] and cyclo(D-tryptophyl-L-phenylalanyl) proved inactive.

The minor product, thaxtomin B (**2**) crystallized from methanol–acetone as pale orange needles, m.p. 238 °C (decomp.), $M^+$ 422.1753, and was assigned structure (**2**) based on deductions from comparison of its spectral properties with those of thaxtomin (**A**) (**1**).

We thank Pierre Lafontaine, C.B.R.I., Ottawa for the mass spectral determinations.

*Received, 9th January 1989, Com. 9/00165D*

† *N.m.r. data* for thaxtomin A (**1**): $^1H$ n.m.r. ($CD_3OD$, 200 MHz) δ 1.62 (dd, 1H, *J* 14.2, 8.9 Hz, H-$10_b$), 2.60 (dd, 1H, *J* 14.1, 6.2, H-$10_a$), 2.81 (s, 3H, N-$CH_3$), 3.03 (s, 3H, N-$CH_3$), 3.22 (AB, 2H, *J* 13.6, H-17), 3.86 (dd, 1H, *J* 8.9, 6.3, H-11), 6.71 (m, 3H, H-19, H-21, H-23), 6.95 (s, 1H, H-2), 7.19 (t, 1H, *J* 8.0, H-6), 7.23 (t, 1H, *J* 8.1, H-22), 7.68 (dd, 1H, *J* 8.1, 1.0, H-7), 7.84 (dd, 1H, J 7.9, 1.0, H-5); $^{13}C$ n.m.r. ($CD_3OD$, 50 MHz): 28.45 (q, N-Me), 33.51 (t, C-10), 34.20 (q, N-Me), 43.53 (t, C-17), 64.56 (d, C-11), 88.03 (s, C-14), 110.52 (s, C-3), 115.84 (d, C-21), 118.40 (d, C-19), 118.56 (d, C-7), 119.23 (d, C-5), 119.78 (s, C-8), 120.98 (d, C-6), 122.73 (d, C-23), 131.19 (d, C-22), 132.51 (d, C-2), 137.37 (s, C-18), 141.15 (s, C-4), 143.64 (s, C-9), 159.11 (s, C-20), 166.81 (s, C=O, C-12), 168.33 (s, C=O, C-15).

## References

1 C. H. Lawrence, M. C. Clark, and R. R. King, *Phytopathology*, 1989, in the press.
2 R. Sakai, H. Kawamura, Y. Mino, R. Emami-Saravi, and A. Tanii, *Ann. Phytopath. Soc., Jpn.*, 1984, **50**, 646.
3 J. E. Saxton, *Nat. Prod. Reports*, 1987, **4**(**6**), 591.
4 S. M. Parmerter, A. G. Cook, and W. B. Dixon, *J. Am. Chem. Soc.*, 1958, **80**, 4621.
5 M. Somei, S. Inoue, S. Tokutake, F. Yamata, and C. Kaneko, *Chem. Pharm. Bull.*, 1981, **29**, 726.
6 Y. Endo, K. Shudo, A. Itai, M. Hasegawa, and S. Sakai, *Tetrahedron*, 1986, **42**, 5905.
7 C. M. Maes, M. Potqieter, and P. S. Steyn, *J. Chem. Soc., Perkin Trans. 1*, 1986, 861.