X-Ray Crystal Structure of Oxalicine A, a Novel Alkaloid from *Penicillium oxalicum*

Rosa Ubillas,[a] Charles L. Barnes,[a] Hanna Gracz,[a] George E. Rottinghaus,[b] and Michael S. Tempesta*[a]

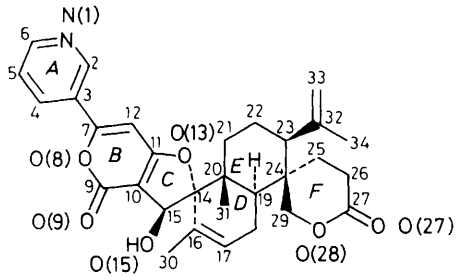
[a] *Department of Chemistry and* [b] *Veterinary Medical Diagnostic Laboratory, University of Misssouri, Columbia, Missouri, 65211, U.S.A.*

The stereostructure of oxalicine A, an alkaloidal meroterpene with a new carbon skeleton isolated from *Penicillium oxalicum*, has been established from spectral data and single crystal *X*-ray analysis.

Penicillium oxalicum is a ubiquitous toxigenic fungus found in soil, as well as in maize and other cereals.[1] Previous investigations of this fungus led to the isolation of oxaline,[2,3] secalonic acid D,[4] as well as an uncharacterized 'alkaloid B' of molecular formula $C_{30}H_{33}NO_6$[5] as major metabolites. We now report on the isolation and structural characterization of a novel hexacyclic alkaloidal meroterpene, oxalicine A (**1**) from *P. oxalicum*, which is the first member of a new skeletal class.

Oxalicine A (**1**) [$C_{30}H_{33}NO_6$, *m/z* 503 (M^+), m.p. 193—198 °C (decomp.), UV (MeOH) λ_{max} 232 (log ε 4.25) and 329 (3.84); IR (film) 3409(OH), 1736(C=O) cm^{-1}] was isolated in 0.012% yield from the CH_2Cl_2 extract of 12 day cultures[6] of *P. oxalicum* by repeated chromatography over silica gel. The ^1H NMR spectrum (300 MHz, $CDCl_3$) exhibited signals appropriate for a sharp quaternary (δ 1.34) and two broad vinylic (δ 1.61, 1.81) methyl singlets, an isolated methylene next to oxygen (δ 4.43, 4.59, J_{AB} 12 Hz), a 1,1′-disubstituted ene (δ 4.78, 4.96, both br. s), a methine singlet next to oxygen (δ 5.54), two broad vinyl (δ 5.81, 6.72) singlets, as well as a 3-substituted pyridine (δ 7.45, dd, *J* 4, 8 Hz; δ 8.15, d, *J* 8 Hz; δ 8.71, br. s; δ 9.04, br. s). The ^{13}C NMR data† supported the above observations, and yielded little further information.

† ^{13}C NMR (75.5 MHz, $CDCl_3$) for (**1**): δ 16.0 (q, C-31), 19.4 (q, C-30), 21.6 (q, C-34), 23.5 (t, C-21), 24.8 (t, C-22), 30.5 (t, C-18), 30.6 (t, C-25), 35.9 (t, C-26), 40.3 (s, C-24), 42.0 (s, C-20), 48.1 (d, C-19), 54.9 (d, C-23), 67.7 (t, C-29), 74.0 (d, C-15), 94.0 (d, C-12), 100.7 (s, C-14), 105.9 (s, C-10), 116.0 (t, C-33), 123.7 (d, C-5), 127.4 (s, C-3), 130.0 (d, C-17), 130.7 (s, C-16), 133.4 (d, C-4), 146.0 (s, C-32), 146.9 (d, C-2), 151.7 (d, C-6), 160.3 (s, C-11), 161.6 (s, C-7), 170.5 (s, C-9), 173.8 (s, C-27).



(1)

The stereostructure of oxalicine A (**1**) was determined by single crystal *X*-ray analysis‡ (Figure 1). Scarcity of data and the disorder of one of two $CHCl_3$ molecules of crystallization resulted in relatively large standard deviations in bond lengths and angles of 0.01 Å and 0.8°, respectively. Given these error limits, individual bond parameters are consistent with the structure proposed. Ring *D* has a distorted half-chair conformation, while the saturated ring *E* has a chair form and lactone ring *F* assumes a twist-boat conformation. The crystal packing includes a hydrogen bonding interaction between N-1 and the hydroxy O-15 of the molecule translated one unit cell along *a* [N · · · O = 2.74(1) Å]. Complete assignment of the ^{13}C chemical shifts of (**1**)† was carried out by the use of the heteronuclear techniques INAPT[7] and HMQC.[8]

‡ *Crystal data* for $C_{30}H_{33}NO_6 \cdot 2CHCl_3$ (**1**): M = 742.35, yellow prism, 0.25 × 0.3 × 0.4 mm, orthorhombic, space group $P2_12_12_1$, a = 10.239(3), b = 11.876(3), c = 28.65(1) Å (from 25 orientation reflections: 10° < θ < 15°), U = 3484 Å^3, Z = 4, D_c = 2.830 g cm^{-3}. Diffraction data: Enraf-Nonius CAD4 automated κ-axis diffractometer, graphite-monochromated Mo radiation [$\lambda(K_\alpha)$ = 0.71069 Å], range 0 < 2θ < 46°, 3193 reflections [3067 unique, R_{inf} = 0.038, 1646 observed, $I > 2.0\sigma(I)$]; corrected for Lorentz and polarization effects, no absorption correction (μ = 10.74 cm^{-1}). Solution: direct methods (SHELXS-86)[9] and difference Fourier syntheses. Refinement: anisotropic thermal coefficients for non-hydrogen atoms; one $CHCl_3$ found to be rotationally disordered included with one full and four partial sites of Cl occupancies; hydroxy H atom located in difference Fourier and included at fixed position, all other H atoms included at calculated positions (SDP).[10] Final: no significant features in the difference Fourier map (range −0.30 < e/Å^3 < +0.30); agreement factors, R = 0.054, R_w = 0.061 and S = 2.04 (Figure 1). Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

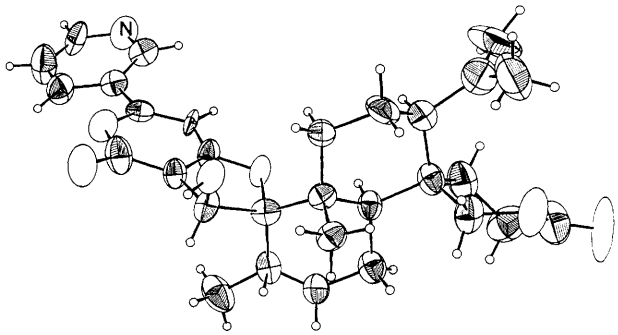


Figure 1. Structure and solid-state conformation of oxalicine A (**1**) with small circles representing hydrogen atoms, and no implied absolute stereochemistry. Thermal ellipsoids are drawn at the 50% probability level. Heteroatoms are given as plain ellipsoids.

Biosynthetically, oxalicine A (**1**) appears to derive from an unprecedented pathway involving nicotinic acid (ring *A*), polyketide (ring *B*), and diterpenoid (rings *C*, *D*, *E*, and *F*) moieties.

M. S. T. is grateful for partial financial support from the National Science Foundation for the NMR (PCM-8115599) and MS (PCM-8117116) Facilities, as well as for a University of Missouri Institutional Biomedical Research Grant (RR07053) from the NIH.

Received, 30th May 1989; Com. 9/02235J

References

1 D. B. Scott, *Mycopathol. Mycol. Appl.*, 1965, **25**, 213.
2 D. W. Nagel, K. G. R. Pachler, P. S. Steyn, P. L. Wessels, G. Gafner, and G. J. Kruger, *J. Chem. Soc., Chem. Commun.*, 1974, 1021.
3 D. W. Nagel, K. G. R. Pachler, P. S. Steyn, R. Vleggaar, and P. L. Wessels, *Tetrahedron*, 1976, **32**, 2625.
4 P. S. Steyn, *Tetrahedron*, 1970, **26**, 51.
5 P. S. Steyn, *J. S. Afr. Chem. Inst.*, 1969, **22**, S20.
6 A. Ciegler, A. W. Hayes, and R. F. Vesponder, *Appl. Environ. Microbiol.*, 1980, **39**, 285.
7 A. Bax, J. A. Ferretti, N. Nashad, and D. M. Jerina, *J. Org. Chem.*, 1985, **50**, 3029.
8 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.
9 G. M. Sheldrick, in 'Crystallographic Computing,' eds. G. M. Sheldrick, C. Kruger, and R. Godard, Oxford University Press, 1985, p. 175.
10 Enraf-Nonius, 'Structure Determination Package,' Delft, The Netherlands, 1982.