Biosynthesis of Viridicatumtoxin, a Mycotoxin from *Penicillium expansum*†

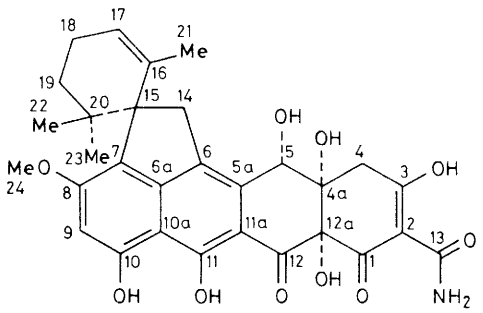
Amelia E. de Jesus,[a] William E. Hull,[b] Pieter S. Steyn,[a] Fanie R. van Heerden,[a] and Robert Vleggaar*[a]

[a] *National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa*

[b] *Bruker Analytische Messtechnik, Silberstreifen, D–7512 Rheinstetten-Fo, Federal Republic of Germany*

Incorporation of sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, and [1-^{13}C,^{18}O]-acetate, sodium [1,2,3-$^{13}C_3$]-malonate, and (2*S*)-[*Me*-^{13}C]methionine as well as sodium hydrogen[^{14}C]carbonate and sodium [1-^{14}C]pyruvate into viridicatumtoxin, a metabolite from *Penicillium expansum*, indicates its formation by a mixed polyketide–terpenoid biosynthetic pathway.

Investigation of a toxigenic strain of *Penicillium expansum* (MRC 97) resulted in the isolation and structure elucidation of a new toxin, viridicatumtoxin (**1**), $C_{30}H_{31}NO_{10}$.[1,2] The new mycotoxin is structurally related to the tetracyclines, *e.g.* tetracycline (**2**), a group of broad-spectrum antibiotics produced by various *Streptomyces* spp. The formation of a tetracycline-type compound by micro-organisms other than the *Streptomyces* prompted us to investigate the biosynthesis of viridicatumtoxin.



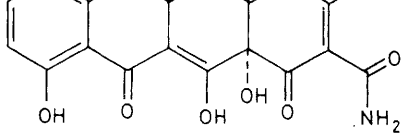
(1)

† This strain (MRC 97) of *Penicillium expansum* was identified as such by Dr. J. I. Pitt, CSIRO, Australia and was known previously as *Penicillium viridicatum* Westling.

Table 1. ^{13}C N.m.r. (100.62 MHz) data for viridicatumtoxin (**1**).

Carbon atom	δ_C[a]/p.p.m.	$^1J_{CH}$/Hz	$^1J_{CC}$/Hz
1	190.47s		42.9
2	99.56s		65.0
3	192.85s		—
4	40.40t	130	40.1
4a	71.54s		40[b]
5	71.65d	149	46.3
5a	123.88s		45.9
6	137.07s		50[b]
6a	147.19s		50.1
7	122.72s		73.6
8	160.76s		73.8
9	99.89d	157.7	76.3
10	157.98s		76.2
10a	105.52s		66.7
11	166.01s		67.3
11a	105.09s		57.7
12	195.22s		57.6
12a	80.20s		43.0
13	172.77s		64.2
14	41.17t	133	33.8
15	60.15s		33.7
16	136.58s		45.4
17	121.40d	152.6	—
18	22.87t	124.5	33.6
19	33.91t	126.7	33.6
20	38.54s		36.0
21	23.97q[c]	126	35.9
22	25.48q[c]	125	—
23	20.96q	125.3	45.0
24	55.53q	144.7	—

[a] Recorded on a Bruker WM-400 spectrometer for solutions in $CDCl_3$. Chemical shifts relative to internal Me_4Si. Letters refer to the pattern resulting from directly-bonded (C–H) couplings ($^1J_{CH}$), with s = singlet, d = doublet, t = triplet, and q = quartet. [b] One transition obscured. [c] May be interchanged.



(**2**)

A novel feature of (**1**) compared with tetracycline (**2**) is the lack of the 4-dimethylamino-group and the spiro-arrangement of the geranyl moiety to form the two additional rings. A hypothetical biosynthetic pathway for the remaining tetracyclic ring structure is based on the biosynthesis of the tetracyclines[3] and utilises malonamoyl-CoA, formed from malonyl-CoA by amidation, for the carbon atoms C-1, C-2, and C-13 of the chain-initiation unit. The C-2 carboxamide group is thus derived from carbon dioxide. The intact incorporation of a malonate unit has been demonstrated unambiguously for the biosynthesis of cycloheximide, an antibiotic produced by *Streptomyces griseus* and *S. naraensis*.[4]

The assignment of the natural abundance 100.62 MHz ^{13}C n.m.r. spectrum of viridicatumtoxin (see Table 1) is derived from coupled, proton-noise-decoupled (p.n.d.), single frequency off-resonance proton-decoupled, and selective proton-decoupled spectra. The detailed basis of the assignments will be given in our full paper.

Cultures of *P. expansum* (MRC 97) were grown at 23 °C in stationary culture on a chemically defined medium, CSM[5] at pH 6.2. The optimum time and yields (*ca.* 70 mg l^{-1}) for incorporation studies with the fungus were determined.

Table 2. ^{13}C N.m.r. data (125.76 MHz) for viridicatumtoxin derived from [1-^{13}C,^{18}O]acetate.

Carbon atom	$\Delta\delta$[a]	$^{13}C^{18}O$: $^{13}C^{16}O$[b]
1	0.036	52:48
3	not labelled	
4a	0.019	59:41
8	0.017	42:58
10	[c]	
11	[c]	
12	0.036	59:41
13	0.019	15:85

[a] $^{13}C^{18}O$ upfield shift in p.p.m. [b] Approximate values obtained from peak heights of ^{13}C–^{16}O and ^{13}C–^{18}O signals and corrected for contribution of natural abundance ^{13}C to the ^{13}C–^{16}O signal. [c] Unresolved signal.

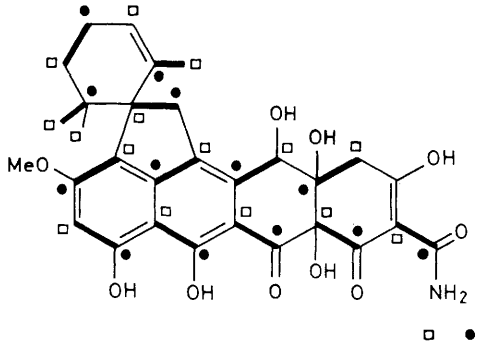


Figure 1. Arrangement of intact acetate units (CH_3CO_2H) in viridicatumtoxin.

Preliminary experiments with [1-^{14}C]acetate and (2*S*)-[*Me*-^{14}C]methionine indicated that both precursors were efficiently incorporated (0.3 and 0.4%, respectively) and that satisfactory dilution values[6] of 39.8 (assuming 13 labelled positions) and 8.0 (assuming one labelled position), respectively were obtained by feeding the labelled precursors every 12 h from day 3 to day 9.

The p.n.d. ^{13}C n.m.r. spectrum of viridicatumtoxin derived from (2*S*)-[*Me*-^{13}C]methionine showed enhancement (enrichment factor[6] 11.1) only of the signal attributed to C-24 (δ 55.53 p.p.m.).

The p.n.d. ^{13}C n.m.r. spectrum of [1-^{13}C]acetate-derived viridicatumtoxin showed enhancement of the signals of 13 carbon atoms (average enrichment factor 2.7) *viz.* C-1, C-4a, C-5a, C-6a, C-8, C-10, C-11, C-12, C-13, C-14, C-16, C-18, and C-20 whereas that of viridicatumtoxin derived from [2-^{13}C]acetate showed 15 enhanced signals (average enrichment factor 2.0) representative of C-2, C-4, C-5, C-6, C-7, C-9, C-10a, C-11a, C-12a, C-15, C-17, C-19, C-21, C-22, and C-23. In the latter spectrum the resonances due to C-7 (δ 122.71) and C-15 (δ 60.15 p.p.m.) exhibit a one-bond (C–C) coupling of 44.2 Hz as a result of multiple labelling. It is of interest to note that C-3 does not originate from either C-1 or C-2 of acetate. This finding is corroborated by the p.n.d. ^{13}C n.m.r. spectrum of viridicatumtoxin derived from [1,2-$^{13}C_2$]acetate which showed that all the signals with the exception of those for C-3, C-17, C-22, and C-24, exhibited one-bond (C–C) couplings. The measured $^1J_{CC}$ values are given in Table 1 and indicate the arrangement of intact acetate units as shown in Figure 1.

The above results indicate that the C-2 carboxamide group in viridicatumtoxin is derived from C-1 of acetate as opposed to carbon dioxide in the tetracyclines.[3] The involvement of malonyl-CoA in the biosynthesis of viridicatumtoxin would imply that either C-3, C-4, and C-4a or C-3, C-2, and C-13

originate from such an intact unit and that C-3 is thus derived from carbon dioxide. An alternative origin for the three-carbon unit could be oxaloacetate, one of the intermediates in the Krebs cycle. This postulate can, however be discounted as formation of oxaloacetate from [1,2-$^{13}C_2$]-acetate proceeds *via* a symmetrical intermediate, succinate, and incorporation into viridicatumtoxin would yield an isotopomer in which C-3 is labelled.

Preliminary experiments with [2-^{14}C]malonic acid and diethyl [2-^{14}C]malonate indicated that both precursors were efficiently incorporated (0.15%) but the high dilution value of 692 (assuming 15 labelled positions) for malonic acid proved unacceptable for ^{13}C labelling studies. Although the dilution value of 162 found for diethyl malonate is an improvement, the p.n.d. ^{13}C n.m.r. spectrum of viridicatumtoxin derived from dimethyl [1,2,3-$^{13}C_3$]malonate indicated an extremely low level of enrichment and the spectrum was similar to that of viridicatumtoxin derived from [1,2-$^{13}C_2$]-acetate as a result of an initial conversion of malonate into acetate. Significantly no one-bond (C–C) coupling was observed for the C-3 signal.

The possible derivation of C-3 from carbon dioxide was also studied by addition in separate experiments of sodium hydrogen[^{14}C]carbonate (absolute incorporation 0.002%, dilution 140) and sodium [1-^{14}C]pyruvate (absolute incorporation 0.016%, dilution 69.5) to cultures of *P. expansum*.

Further insight into the biosynthetic pathway comes from the incorporation of [1-^{13}C,^{18}O]acetate (57.9% $^{13}C^{18}O_2$, 25.1% $^{13}C^{18}O$, 4.7% $^{13}C^{16}O$) into viridicatumtoxin. The sites of ^{18}O enrichment were determined by ^{13}C n.m.r. spectroscopy taking advantage of the ^{18}O isotope-induced upfield shifts for the signals of directly attached ^{13}C carbon atoms.[7–9] The presence of [^{18}O]acetate-derived oxygen atoms in viridicatumtoxin could thus be detected at C-1, C-4a, C-8, C-12, and C-13 (see Table 2). Theoretically the maximum (^{13}C, ^{18}O)-labelling probability of C-13 should be equal to that of the other (^{13}C, ^{18}O)-enriched positions if cleavage of a C-13 thioester bond occurs *via* an amidation reaction. The lower extent of labelling at this position can be rationalized by the introduction of $H_2{}^{16}O$ through hydrolysis of the C-13 thioester bond and subsequent amidation of the formed carboxylic acid at some stage of the biosynthetic pathway.[10]

Received, 6th May 1982; Com. 507

References

1 R. D. Hutchison, P. S. Steyn, and S. J. van Rensburg, *Toxicol. Appl. Pharmacol.*, 1973, **24**, 507.
2 C. Kabuto, J. V. Silverton, T. Akiyama, U. Sankawa, R. D. Hutchison, P. S. Steyn, and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1976, 728.
3 R. H. Turley and J. F. Snell in 'Biosynthesis of Antibiotics,' ed. J. F. Snell, Academic Press, New York, 1966, Vol. 1, p. 95.
4 P. W. Jeffs and D. McWilliams, *J. Am. Chem. Soc.*, 1981, **103**, 6185; H. Shimada, H. Noguchi, Y. Iitaka, and U. Sankawa, *Heterocycles*, 1981, **15**, 1141.
5 D. C. Neethling and R. M. McGrath, *Can. J. Microbiol.*, 1977, **23**, 856.
6 P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Perkin Trans.* 1, 1981, 1298.
7 J. M. Risley and R. L. van Etten, *J. Am. Chem. Soc.*, 1979, **101**, 252; *ibid.*, 1980, **102**, 4609, 6699; *ibid.*, 1981, **103**, 4389.
8 J. C. Vederas, *J. Am. Chem. Soc.*, 1980, **102**, 374.
9 J. C. Vederas and T. T. Nakashima, *J. Chem. Soc., Chem. Commun.*, 1980, 183; T. T. Nakashima and J. C. Vederas, *ibid.*, 1982, 206; M. P. Lane, T. T. Nakashima, and J. C. Vederas, *J. Am. Chem. Soc.*, 1982, **104**, 913; J. G. Hill, T. T. Nakashima, and J. C. Vederas, *J. Am. Chem. Soc.*, 1982, **104**, 1745.
10 S. Gatenbeck and K. Mosbach, *Acta Chem. Scand.*, 1959, **13**, 1561; A. I. Scott and K. J. Wiesner, *J. Chem. Soc., Chem. Commun.*, 1972, 1075; C. T. Mabuni, L. Garlaschelli, R. A. Ellison, and C. R. Hutchinson, *J. Am. Chem. Soc.*, 1979, **101**, 707.