Mechanism of 3-Acetyldeoxynivalenol Biosynthesis

Lolita O. Zamir,*[a,b] Yves Nadeau,[a] Cong-Danh Nguyen,[a] Kenneth Devor,[a] and Françoise Sauriol[b]

[a] *Université du Québec, Institut Armand-Frappier, 531, boul. des Prairies, C.P. 100, Laval-des-Rapides, Laval, Québec H7V 1B7, Canada*

[b] *McGill University, 801 Sherbrooke St. West, Montréal, Québec H3H 2K6, Canada*

The distribution of label of the trichothecene, 3-acetyldeoxynivalenol (3-ADN) derived from mevalonate and three of the major steps in its biosynthesis (the folding of farnesyl pyrophosphate, the methyl shifts, and the stereochemistry of oxygenation) were rigorously proven.

The biological activity of Fusarium metabolites and the publicity of the 'yellow rain' controversy have generated considerable interest in research on various aspects of mycotoxins. Deoxynivalenol (also known as vomitoxin) has been identified as the main cause for mycotoxicoses of farm animals. The only published work on the biosynthesis of deoxynivalenol (vomitoxin) or its derivatives is the acetate distribution in 15-acetyl deoxynivalenol which is a metabolite of *Fusarium graminearum*.[1] 3-Acetyldeoxynivalenol (3-ADN) is the major secondary metabolite formed by *Fusarium culmorum* and its structure has been established unambiguously by *X*-ray crystallography.[2] In this study, we report† the mevalonate distribution of label of 3-acetyldeoxynivalenol and rigorous proofs for the folding of farnesyl pyrophosphate, the methyl shifts, and the stereochemistry of oxygenation.

† The first report on the folding of farnesyl pyrophosphate, the methyl shifts, and the stereochemistry of the oxygenations in the biosynthesis of 3-acetyldeoxynivalenol was given by one of us (L. O. Z.) at the I.U.P.A.C. conference on Mycotoxins and Phycotoxins, Pretoria, South Africa, July 1985.

Table 1. ^{13}C N.m.r. data for 3-ADN derived from the feeding of $[3,4\text{-}^{13}C_2]$mevalonolactone.

Enriched atom	δ —	Multiplicity	*J*/Hz (coupled atoms)
C(2)	79.0	d	47.3 (2,12)
C(5)	45.8	d	32.2 (5,6)
C(5)	45.8	dd	— —
C(6)	51.95	d	32.2 (6,5)
C(9)	135.9	d	68.5 (9,10)
C(10)	138.4	d	68.5 (10,9)
C(12)	65.1	d	48.3 (12,2)
C(12)	65.1	dd	40.3 (12,5)

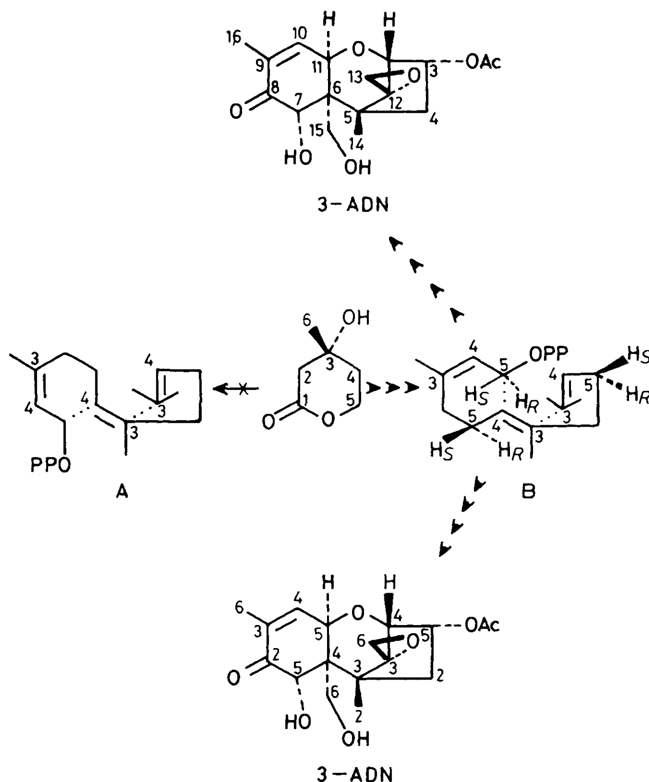


Figure 1. Origin of carbon atoms of 3-ADN, derived from mevalonolactone. The upper structure of 3-ADN shows the conventional numbering of 3-ADN whereas the lower structure shows the derived numbering from mevalonolactone. The label distribution of (3*RS*)-[3,4-$^{13}C_2$]mevalonolactone-derived 3-ADN proved conclusively the B folding of farnesyl pyrophosphate.

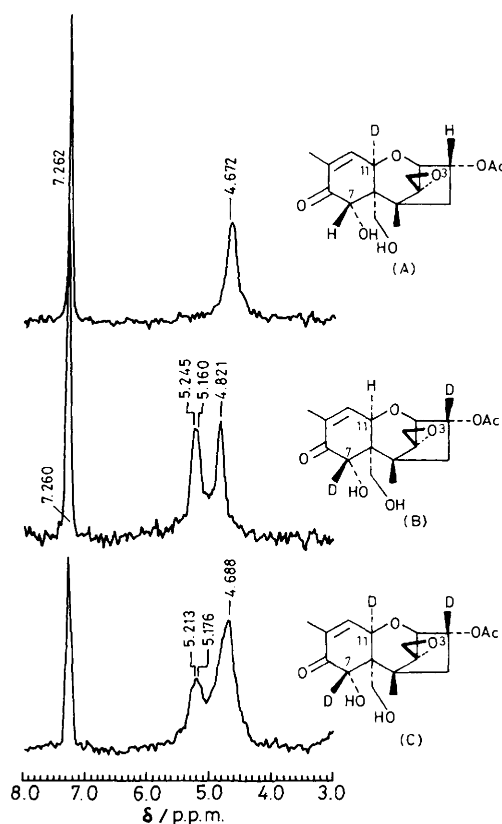


Figure 2. N.m.r. spectra of 3-ADN obtained from deuteriated mevalonolactones (MVL). The structures are of 3-ADN obtained from cultures of *F. culmorum* supplemented with (A) (5*R*)-[5-^{2}H]MVL, (B) (5*S*)-[5-^{2}H]MVL, and (C) [5-$^{2}H_2$]MVL, respectively.

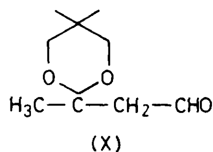
There has been considerable work[3] on the biosynthesis of trichothecenes [albeit most of them bearing an oxygen function at C(4) and not at C(3) and C(7)]. [2-^{13}C]-Mevalonate-derived trichothecolone[4] indicated a preference for the 'B' folding of farnesyl pyrophosphate. In order to ensure that in the biosynthesis of 3-ADN this is indeed the case (Figure 1), (3*RS*)-[3,4-$^{13}C_2$]mevalonate was synthesized[5] and fed to *F. culmorum*.‡ The intact 3,4 bond of mevalonate could be followed in the derived 3-ADN by ^{13}C n.m.r. spectroscopy. The 'A' folding of farnesyl pyrophosphate would result in 3-ADN with ^{13}C–^{13}C couplings between the C(2) and C(12); C(5) and C(6); C(8) and C(9). Table 1 shows ^{13}C–^{13}C couplings between the carbons C(2) and C(12); C(5) and C(6), C(9) and C(10).

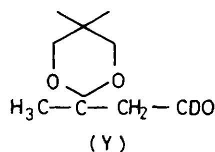
These data prove rigorously that the 'B' folding orientation of farnesyl pyrophosphate (Figure 1) is involved. Owing to the high incorporation of (3*RS*)-[3,4-$^{13}C_2$]mevalonolactone into 3-ADN (10%) we could also observe (Table 1) an additional coupling between C(12) and C(5). The methyl shifts were always implied in the biosynthesis of trichothecenes and the only proof derives from the incorporation of 1,2-[$^{13}C_2$]acetate into trichothecin.[6] In order to demonstrate that one of the methyl shifts originates from C(6) of mevalonate and the transfer is from its attachment on C(3) to C(4), mevalonolactone was synthesized with two simultaneous ^{13}C labels on the alternate carbons C(4) and C(6).[5] The 3-ADN derived from the feeding of (3*RS*)-[4,6-$^{13}C_2$]mevalonolactone showed ^{13}C–^{13}C direct coupling between C(6) and C(15) (38.5 Hz), demonstrating conclusively that there was a 1,2-methyl shift involving the transfer of C(6) to C(4) of mevalonolactone. Mevalonolactone doubly labelled with ^{13}C at C(2) and C(4) was prepared§ to confirm the label distribution in 3-ADN and to ensure that C(2) and C(6) of mevalonolactone retain their individuality. Indeed, (3*RS*)-[2,4-$^{13}C_2$]mevalonolactone-derived 3-ADN showed no direct ^{13}C–^{13}C coupling and the enrichment sites fit the expected pattern shown in Figure 1.

The pattern of oxygenation of deoxynivalenol derivatives seems to have a direct relationship to their toxicity. In this context, it is important to verify that in 3-ADN, microbial hydroxylations at positions 3, 7, and 11 occur with retention of configuration. Stereospecifically deuteriated (3*RS*)(5*R*)-[5-^{2}H]-, (3*RS*)(5*S*)-[5-^{2}H]-, and (3*RS*)-[5-$^{2}H_2$]-mevalonolactones (MVL) were synthesized. The correct stereochemistry of mevalonolactone at position 5 was obtained[7] by enzymic (horse liver alcohol dehydrogenase) reduction of an appro-

‡ A representative feeding experiment is as follows. A culture of *F. culmorum* (C.M. 1-16764, HLX-1503) (kindly supplied by R. Greenhalgh, and D. Miller, Agriculture Canada) was grown in a shaker in a rich medium for 3.5 days then transferred to a production medium for 48 h. At this stage, 12 ml of enriched mevalonic acid (200 mg) was added to four 50 ml cultures containing the production medium. After 5 days of incubation on a shaker at 25 °C, 3-ADN was isolated and purified by high performance liquid chromatography.

§ [2,4-$^{13}C_2$]Mevalonolactone was synthesized from pyruvic aldehyde dimethyl acetal in 6 steps in an overall yield of 40%. The ^{13}C label was introduced as [2-^{13}C]ethyl acetate.





priately labelled aldehyde.¶ The ^1H n.m.r. spectra of 3-ADN derived from (5*R*)-[5-^2H]-, (5*S*)-[5-^2H]-, and [5-$^2H_2$]-mevalonolactones are identical to the one published.[8] Integration of the signals where deuterium is incorporated did not give a clear result. ^2H N.m.r. spectroscopy was found to be the best technique for locating the deuterium labels unambiguously, despite its wider line-width. Figure 2 shows the ^2H n.m.r. spectra|| in chloroform of the various 3-ADN compounds resulting from the feeding of the three deuteriated mevalonolactones. They show unambiguously that deuterium from (5*R*)-mevalonolactone is incorporated only at C(11) (δ_D 4.67 p.p.m.) and from (5*S*)-mevalonolactone at C(3) (δ_D 5.20) and C(7) (δ_D 4.82). The spectrum of 3-ADN derived from [5-$^2H_2$]mevalonolactone shows two signals (δ_D 5.19 and 4.69), with an intensity ratio 1:2, the latter arising from overlap of the broad signals from 7- and 11-^2H. These results show that the hydroxylation of 3-ADN at C(3) and C(7) replaced a 5-*pro-R* mevalonoid hydrogen atom whereas the hydroxylation at C(11) replaced a 5-*pro-S* mevalonoid hydrogen atom. The folding of farnesyl pyrophosphate (Figure 1) shows that in all three positions the new carbon–oxygen bonds possess the same relative stereochemical orientation as the carbon–hydrogen bond replaced, *i.e.*, there is retention of configuration during oxygenation.

We thank Agriculture Canada and the National Science and Engineering Research Council of Canada for support of this work. We thank the Armand-Frappier Foundation for the Jean-Louis Lévesque Scholarship to Y. N.

Received, 18th August 1986; Com. 1195

¶ The aldehydes (X) and (Y) were used as substrates for horse liver alcohol dehydrogenase in the presence of NAD^+ and [$^2H_6$]ethanol or unlabelled ethanol respectively. The resulting alcohols were converted into the desired deuteriomevalonates by common chemical reactions. These aldehydes were preferred to the isopentenals previously used,[7] since they are less volatile and do not have a double bond which is prone to isomerize to the more stable α, β position.

|| Spectrometer operating at 46.05 MHz on deuterium. The samples were dissolved (10 mg) in chloroform. The chloroform signal was assigned to be δ 7.26 p.p.m.; spectral window, 0—1000 Hz; acquisition time, 1 s; pulse width 60°; number of transients 1000—4000 scans depending on the sample.

References

1 B. A. Blackwell, J. D. Miller, and R. Greenhalgh, *J. Biol. Chem.*, 1985, **260**, 4243.
2 R. Greenhalgh, A. W. Hanson, J. D. Miller, and A. Taylor, *J. Agric. Food Chem.*, 1984, **32**, 945.
3 Three recent reviews summarize this work: Ch. Tamm and W. Breitenstein, in 'The Biosynthesis of Mycotoxins. A Study in Secondary Metabolism,' ed. P. S. Steyn, Academic Press, New York, 1980, p. 69; W. B. Turner and D. C. Aldridge in 'Fungal Metabolites, II,' Academic Press, New York, 1983, p. 225; M. O. Moss, 'The Applied Mycology of Fusarium,' eds. M. O. Moss and J. E. Smith, Cambridge University Press, 1984, p. 195.
4 J. R. Hanson, T. Marten, and M. Siverns, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1033.
5 D. E. Cane and R. H. Levin, *J. Am. Chem. Soc.*, 1976, **98**, 1183.
6 B. Dockerill, J. R. Hanson, and M. Siverns, *Phytochemistry*, 1978, **17**, 427.
7 D. E. Cane and P. P. Murthy, *J. Am. Chem. Soc.*, 1977, **99**, 8327; J. W. Cornforth, F. P. Ross, and C. Wakselman, *J. Chem. Soc., Perkin Trans. 1.*, 1974, 429.
8 R. J. Cole and R. H. Cox in 'Handbook of Toxic Fungal Metabolites,' Academic Press, New York, 1981, p. 209; B. A. Blackwell, R. Greenhalgh, and A. D. Bain, *J. Agric. Food Chem.*, 1984, **32**, 1978.