Biosynthesis of Asteltoxin by Cultures of *Emericella variecolor*. The Role of Propionate in the Biosynthesis and Evidence for a 1,2-Bond Migration in the Formation of the Bistetrahydrofuran Moiety

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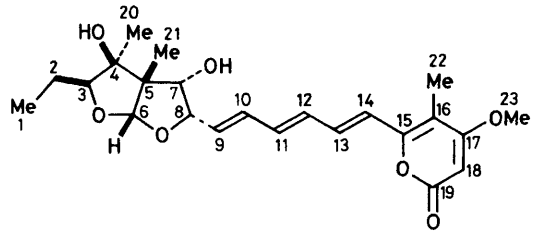
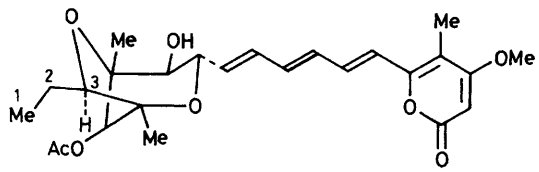
The incorporation of [1-^{13}C]propionate, (2*S*)-[*methyl*-^{13}C]methionine, and [1,2-$^{13}C_2$]acetate into asteltoxin by cultures of *Emericella variecolor* point to the operation of two biosynthetic pathways; the arrangement of intact acetate units in asteltoxin derived from [1,2-$^{13}C_2$]acetate proves that a 1,2-bond migration occurs during the biosynthesis.

Investigations of toxic maize cultures of *Aspergillus stellatus* Curzi,† strain MRC 641 led to the isolation of asteltoxin (**1**),[2] a potent inhibitor of bacterial ATPase,[3] structurally related to citreoviridin[4] and aurovertin B (**2**).[5] Extensive incorporation studies with ^{13}C-labelled precursors have revealed the simultaneous operation of two apparently independent pathways (A and B), distinguishable by the different origins of C-1—C-3, in the biosynthesis of aurovertin B (**2**).[6] Pathway A involves the *C*-methylation of a C_{20}-polyketide precursor at C-18, followed by the loss of the chain-initiating acetate unit, C-19–C-20, and C-1 in aurovertin B is thus derived from methionine and C-2 and C-3 from malonate. In contrast, pathway B involves a C_{19}-precursor, formed from a propionate chain-initiating unit and eight malonate units; C-1—C-3 are thus derived from propionate.[6] These results prompted us to investigate the biosynthesis of asteltoxin (**1**) as, in addition to the above two possible biosynthetic pathways, a rearrangement of the polyketide chain must be invoked to explain the formation of the bistetrahydrofuran moiety.



(1)



(2)

The ^{1}H and ^{13}C n.m.r. data for asteltoxin (**1**) are collated in Table 1. First-order analysis of the multiplets in the ^{1}H n.m.r. spectrum of asteltoxin yielded the values of the proton chemical shifts and proton–proton coupling constants. From the value of the coupling constants as corroborated by ^{1}H{^{1}H} homonuclear decoupling experiments the proton–proton connectivity pattern could be constituted. The residual (C,H) splittings observed in a series of off-resonance proton-decoupled ^{13}C n.m.r. experiments enabled us to correlate the signals of the proton-bearing carbon atoms with specific proton resonances.[7] In the assignment of the different ^{13}C resonances use was made of chemical shift values of the related compounds, aurovertin[6] and citreoviridin,[8] (C,H) coupling constants, and heteronuclear ^{13}C{^{1}H} selective population inversion[9] experiments. The detailed ^{1}H and ^{13}C n.m.r. study will be described in a subsequent publication.

Cultures of *Emericella variecolor*, strain NHL 2881 were grown on a malt extract medium (15%). Studies on the course of fermentation indicated that asteltoxin production commenced on day 8 and reached a level of 60—80 mg l^{-1} after 25

† *Aspergillus stellatus* Curzi is synonymous with *Aspergillus variecolor* (Berk. and Br.) Thom and Raper, the imperfect state of *Emericella variecolor* Berk. and Br. (ref. 1).

Table 1. ^{1}H (500.13 MHz) and ^{13}C (125.76 MHz) n.m.r. data for asteltoxin.[a]

Carbon atom	δ_C[b]	^{1}J(CH)/Hz	^{1}J(CC)/Hz[c]	δ_H[d]	^{1}J(HH)/Hz
1	11.71 Q △	122.6		0.956 t	7.5
2	22.47 T	126.0	40.9	1.542 ddq	13.8, 9.1, 7.5
				1.480 ddq	13.8, 3.5, 7.5
3	90.10 D ●	141.6	40.7	4.312 dd	9.1, 3.6
4	80.93 S		—		
5	62.60 S		37.0		
6	112.98 D ●	176.7	—	5.152 s	
7	80.19 D ●	151.6	37.0	3.815 dd	5.6, 3.1
8	84.80 D	145.3	51.8	4.634 ddd	6.6, 3.2, 1.3
9	133.84 D ●	157	51.7	6.035 dd	15.2, 6.6
10	133.20 D	156	56.2	6.460 ddd	15.3, 10.8, 1.3
11	137.95 D ●	156	56.4	6.644 dd br	14.8, 10.8
12	132.58 D	155	57.4	6.499 dd br	14.7, 11.0
13	135.68 D ●	155	57.1	7.063 dd	15.0, 11.0
14	120.93 D	159.1	69.5	6.614 d	15.0
15	154.80 S ●		69.5		
16	108.72 S		61.3		
17	171.06 S ●		61.7		
18	89.42 D	168.5	78.4	5.603 s	
19	162.79 S ●		78.3		
20	18.38 Q △	127.6		1.350 s	
21	16.55 Q △	126.2		1.165 s	
22	8.97 Q △	128.9		1.970 s	
23	56.91 Q △	146.6		3.896 s	

[a] Recorded on a Bruker WM-500 spectrometer; solvent $(CD_3)_2CO$. [b] Relative to Me_4Si. Capital letters refer to the pattern resulting from directly bonded (C,H) couplings. S = singlet, D = doublet, T = triplet, and Q = quartet. ● = enriched by [1-^{13}C]acetate; △ = enriched by (2*S*)-[*methyl*-^{13}C]methionine. [c] Value obtained from the broad-band proton-decoupled spectrum of asteltoxin derived from [1,2-$^{13}C_2$]acetate. [d] Relative to internal Me_4Si. s = singlet, d = doublet, t = triplet, q = quartet, and br = broad. The proton of the C-7 hydroxy group appears as a doublet (*J* 5.6 Hz) at δ 3.937.

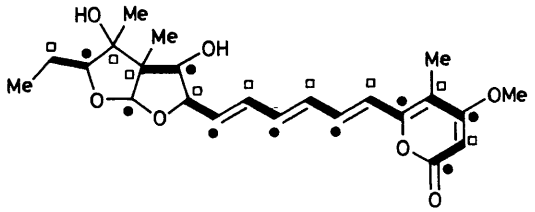


Figure 1. Arrangement of intact acetate units in asteltoxin (**1**) (● corresponds to C-1 and □ to C-2 of acetate).

days. Preliminary feeding experiments with [1-^{14}C]acetate as precursor established that high but satisfactory dilution values[6] (200.5, assuming nine labelled positions) were obtained by feeding cultures of *E. variecolor* every 24 h from day 6 to day 24 with sodium acetate to a total amount of 2.5 g l^{-1}.

The broad-band proton-decoupled ^{13}C n.m.r. spectrum of asteltoxin derived from [1-^{13}C]acetate (91.6 atom % ^{13}C) showed nine enhanced signals (average enrichment factor[6] 1.5) attributed to C-3, C-6, C-7, C-9, C-11, C-13, C-15, C-17, and C-19 and pointed to the involvement of nine acetate units in the formation of the metabolite. The arrangement of intact acetate units in asteltoxin was studied by addition of [1,2-$^{13}C_2$]acetate to cultures of *E. variecolor*. The broad-band proton-decoupled ^{13}C n.m.r. spectrum of the enriched asteltoxin exhibited, as a result of multiple labelling, one bond (C,C) coupling between carbon atoms derived from adjacent acetate units (interacetate coupling), in addition to the expected spin–spin coupling between carbon atoms derived from intact acetate units (intra-acetate coupling). The intra-acetate (C,C) couplings could be distinguished readily by their greater (5-fold) intensities. The measured ^{1}J(CC) values of these couplings are given in Table 1 and prove the presence of eight intact acetate units arranged as shown in Figure 1: C-2–C-3, C-5–C-7, C-8–C-9, C-10–C-11, C-12–C-13, C-14–C-15, C-16–C-17, and C-18–C-19. The additional much lower intensity one-bond (C,C) couplings observed for the C-3 (38.0 Hz), C-7 (37.0 Hz), and C-6 (35.2 Hz) resonances are ascribed to interacetate coupling with C-4, C-8, and C-5, respectively. The results indicate that a 1,2-shift of the C-16 carbon atom from C-15 to C-14 of a C_{20}- or C_{18}-polyketide precursor occurs in the course of the biosynthesis. In this 1,2-bond migration an intact acetate unit is cleaved in a pinacol or epoxide rearrangement to generate a branched aldehyde which is subsequently utilised in the formation of the bistetrahydrofuran moiety.

The above results, obtained from feeding experiments using ^{13}C-labelled acetate, account for the origin of 18 of the 23 carbon atoms of asteltoxin. On feeding (2*S*)-[*methyl*-^{13}C]methionine (420 mg, 90 atom % ^{13}C), containing (2*S*)-[*methyl*-^{14}C]methionine (50 μCi) as a tracer, asteltoxin with a specific activity of 3.23 μCi $mmol^{-1}$ was obtained. This result indicates a dilution value of 27.2 (assuming the presence of 5 labels) and thus an enrichment factor of 3.9. The broad-band proton-decoupled ^{13}C n.m.r. spectrum of the metabolite showed enhancement of the signals attributed to C-1, C-20, C-21, C-22, and C-23.

The incorporation of [2-^{13}C]acetate (91.0 atom % ^{13}C) into asteltoxin was subject to a too high dilution value as no reliable enhancement factors[6] were obtained from the proton-decoupled ^{13}C n.m.r. spectrum of the enriched asteltoxin. However, a number of resonances exhibited low intensity satellite signals due to one-bond (C,C) coupling. Analysis of the one-bond (C,C) coupling constants indicated the presence of eight intact acetate units with an arrangement identical to that observed in [1,2-$^{13}C_2$]acetate-derived asteltoxin. In addition the spectrum revealed one-bond interacetate (C,C)

couplings for C-4 (38.3 Hz) and C-6 (35.4 Hz) which probably arise from coupling in each case with C-5. It is evident that some [1,2-$^{13}C_2$]acetate is formed during the fermentation by the frequent recycling of [2-^{13}C]acetate in the Krebs citric acid cycle. A similar phenomenon was observed in the biosynthesis of penitrem A.[10]

The origin of C-1–C-3, as in the case of aurovertin B (**2**),[6] from acetate and methionine, indicates that asteltoxin is formed by pathway A outlined above. The possible involvement of propionate in the biosynthesis of asteltoxin (pathway B) was investigated by administration of [1-^{14}C]propionate (430 mg, specific activity 11.29 μCi mmol^{-1}) to growing cultures of *E. variecolor*, to give asteltoxin (26 mg, specific activity 0.76 μCi mmol^{-1}). The low dilution value of 14.9 (assuming one labelled position) indicates that high enrichment can be obtained in studies with ^{13}C-labelled propionate. In the broad-band proton-decoupled ^{13}C n.m.r. spectrum of asteltoxin derived from [1-^{13}C]propionate (430 mg, 93.2 atom % ^{13}C) only the signal assigned to C-3 (δ 90.10) was enhanced (enrichment factor 5.4).

The results indicate that asteltoxin can be formed *via* two biosynthetic pathways which are distinguishable by the different origins of C-1–C-3. An alternative explanation, for which there is no precedent, and which involves the formation of propionyl-CoA by methylation at C-2 of malonyl-CoA and subsequent decarboxylation, is under investigation.

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