Biosynthetic Studies with Carbon-13: ^{13}C Nuclear Magnetic Resonance Spectra of the Metabolite Sterigmatocystin

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Summary The ^{13}C n.m.r. spectra of ^{13}C labelled sterigmatocystin defines the biogenetic origin of all carbons in the metabolite.

WE report on the biosynthesis of sterigmatocystin obtained from growing cultures of *Aspergillus versicolor* supplemented with either of the precursors sodium [1-^{13}C]acetate (56%) or sodium [2-^{13}C]acetate (61%).

Location and identification of the ^{13}C-labelled sites in the sterigmatocystins was accomplished by ^{13}C n.m.r. at 25·15 MHz. Each carbon appears as a singlet in the spectrum (Figure) obtained in dioxan solution as the homonuclear lock signal with simultaneous proton noise decoupling.[1] The spectra were obtained on a Varian HA-100 spectrometer in 8 mm spinning tubes using a V-3530 RF/AF sweep unit with a Spectro System 100 for multiscan averaging.

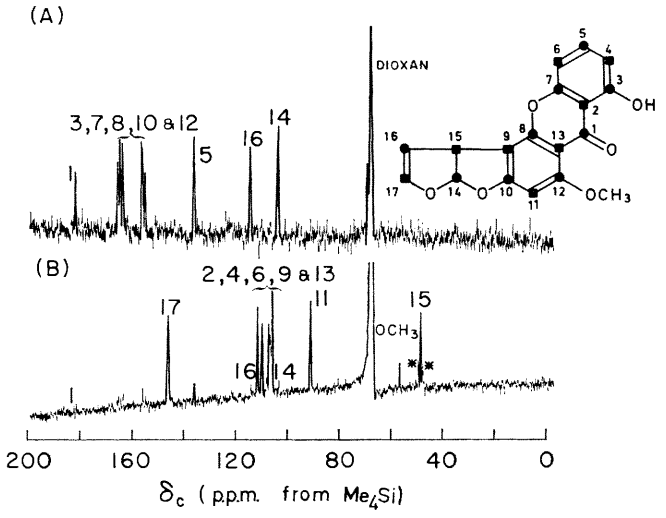
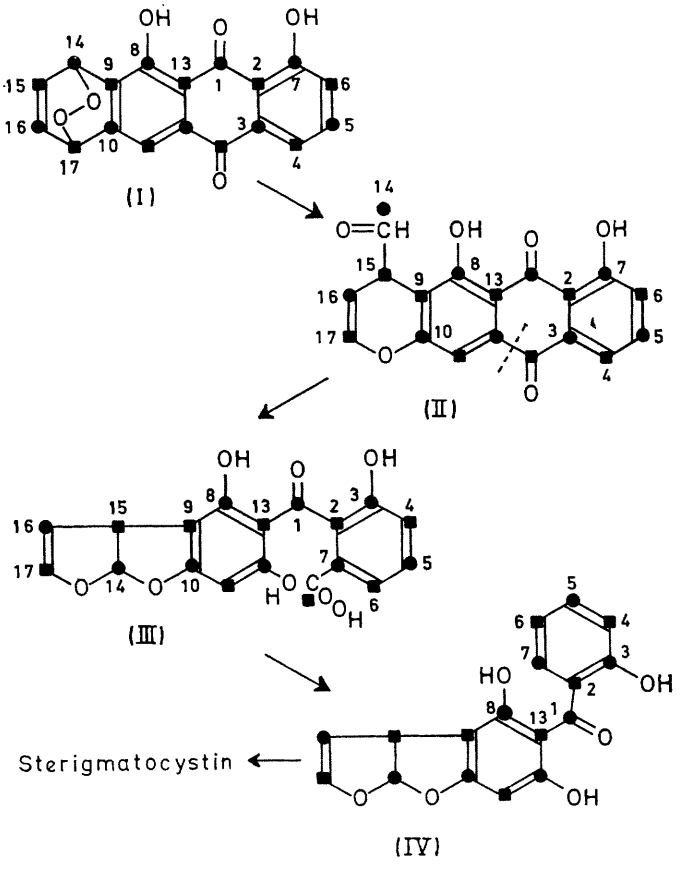


FIGURE. ^{13}C *N.m.r. spectrum of sterigmatocystin in dioxan*: (A) *from* $CH_3{}^{13}CO_2Na$, 30 *mg*/1·0 *ml with* 63 *scans of* 5030 *Hz at* 200 *s*/*scan*; (B) *from* $^{13}CH_3CO_2Na$, < 27 *mg*/1·0 *ml* (*saturated solution*) *with* 1055 *scans of* 5030 *Hz at* 200 *s*/*scan*. *The labelling pattern in the structure of sterigmatocystin indicates the precursors as* ● *from* [1-^{13}C]*acetate and* ■ *from* [2-^{13}C]*acetate*. * *Indicates* C–C *coupling between* C-9 *and* C-15.

Spectrum (A) for sterigmatocystin derived from [1-^{13}C] acetate (Figure) shows nine resonances for carbons between 100 and 182 p.p.m. downfield from Me_4Si. Similarly, spectrum (B) shows signals of enhanced intensity for eight carbons of sterigmatocystin derived from [2-^{13}C]acetate. These labelling studies define the specific acetate precursor of each carbon of the metabolite.



Assignment of the resonances in (A) and (B) was aided by recognized correlations in carbon chemical shifts with carbonyl groups appearing at low field and saturated carbons at high field.[2] In spectrum (A) the lowest field signal is assigned to the C-1 xanthone carbonyl.[3] The cluster of resonances between 154 and 164 p.p.m. represents the aromatic carbons at positions 3,7, 8, 10, and 12 directly bound to oxygen, in agreement with the analogous carbons in 1,3-dimethoxybenzene,[4] and similarly the higher field C-5 signal is in agreement with substitution *meta* to two oxygens. The β-carbon of furan at 110 p.p.m.[5] nearly coincides with C-16, and the C-14 shift position is close to the reported values for anomeric carbons in carbohydrates.[6] In spectrum (B), C-17 agrees with the α-carbon shift of furan, 144 p.p.m.,[5] and the carbons at positions 2, 4, 6, 7, 11,

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^{13}C *N.m.r. data for* ^{13}C*-labelled sterigmatocystin*

Sterigmatocystin carbon	Source[a]	Shift δ_c (p.p.m.)	Reference compound	Carbon	Shift δ_c (p.p.m.)[c]	Ref.
C-1	A	181	aromatic ketones	Carbonyl	190—200	3
C-5	A	135	1,3-dimethoxybenzene	C-5	131	4
C-3, -7, -8, -10, and -12	A	154—164	"	C-1 and -3	162	4
C-2, -4, -6, -9, and -13	B	104—112	"	C-4 and -6	107	4
C-11	B	91	"	C-2	102	4
C-14	A	103	glucose, carbohydrates	C-1	94—104	6
C-15	B	49	isopropylbenzene	α	33	7
C-16	A	115	furan	C-3 and -4	110	5
C-17	B	146	"	C-2 and -5	144	5

[a] A: from sodium [1-^{13}C]acetate [spectrum (A)]; B: from sodium [2-^{13}C]acetate [spectrum (B)].
[b] δ_c p.p.m. downfield from dissolved Me_4Si calculated from the lock signal dioxan using $\delta_c(Me_4Si) = \delta_c$ dioxan) + 67 p.p.m.
[c] δ_c p.p.m. downfield from Me_4Si calculated from reference data.

and 13 show upfield aromatic resonances situated *ortho* or *para* to oxygen functions.[4] The resonance for the aliphatic C-15 stands far upfield, similar to the methylene resonances of substituted benzenes (*ca.* 30 p.p.m.).[7]

The labelling pattern shown in the Figure is consonant with the novel biogenetic hypothesis[8] that sterigmatocystin, as an early precursor of the aflatoxins, arises *via* a nona-acetyl naphthacene *endo*-peroxide (I). Rearrangement of (I) generates the elements of the difuran rings in (II) with adjacent carbons (C-9 and C-15) derived from the Me of $MeCO_2$. The biological equivalent of a Baeyer–Villiger cleavage at the quinone carbonyl derived from the Me of $MeCO_2$ yields (III) which decarboxylates, accounting for the loss of this acetate methyl label before cyclization to the xanthone, sterigmatocystin.

Although nuclear Overhauser effects can enhance signal intensities,[9] comparison of intensities of the aromatic and difuran carbon signals show nearly equal labelling, indicating a common carbon pool for these atoms as in (I). The earlier radiocarbon study detected an insignificant difference in labelling of these two moieties.[10]

In spectrum (B) most of the carbons at natural abundance are visible and comparison of peak heights shows a ^{13}C-enrichment of about 5%, assuming a constant nuclear Overhauser enhancement factor.

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