BIOSYNTHETIC STUDIES WITH ^{13}C: A NOVEL FAVORSKY REARRANGEMENT ROUTE TO THE FUNGAL METABOLITE ASPYRONE

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DEDICATED TO DR. KENICHI TAKEDA ON HIS 70TH BIRTHDAY

The ^{13}C-nmr investigation of the biosynthesis of the pyrone metabolite 2-(1',2'-epoxypropyl)-5,6-dihydro-5-hydroxy-6-methylpyran-2-one, aspyrone, isolated from *A. melleus* is described. Labeling distribution and incorporation level data from 1-^{13}C-acetate, 2-^{13}C-acetate and 1,2-^{13}C-acetate supports a proposed Favorsky rearrangement route from a linear pentaketide unit for the biosynthesis of this fungal metabolite. ^{13}C-nmr chemical shift assignments and carbon-carbon coupling data are presented. A Favorsky route is also proposed to account for bisfuran ring formation in aflatoxin and sterigmatocystin biosynthesis.

The lactone metabolite 2-(1',2'-epoxypropyl)-5,6-dihydro-5-hydroxy-6-methylpyran-2-one (1) has been isolated from laboratory cultures of several *Aspergillus* species including *melleus*[1] and *ochraceus*.[2] Although *A. ochraceus* also produces the mycotoxin ochratoxin,[3] the possible significance of the lactone pyrone metabolite as a mycotoxin remains unexplored.

We report results of our ^{13}C-nmr investigation of the biosynthesis of the pyrone metabolite for which we propose the trivial name aspyrone. Earlier 1-^{14}C-acetate and 2-^{14}C-acetate labeling studies established a pentaketide pathway to aspyrone and yielded the labeling pattern shown in (2) of Figure 1. Thus any biosynthetic proposal must account for the label distribution having the adjacent C-2 and C-7 carbons labeled by the methyl group of acetate. Carbon branching at C-2 also indicates aspyrone biosynthesis results from carbon skeleton rearrangement of a linear pentaketide unit.

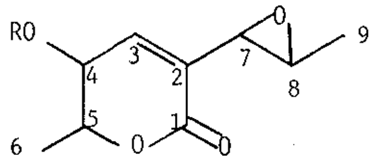
^{13}C-nmr chemical shift assignments of aspyrone were obtained from known chemical shifts correlations and from off resonance decoupling for C-1, C-2 and C-3. From the known proton chemical shift assignments of aspyrone,[1] the carbon shifts of the four chemically similar oxygenated carbons at C-4, C-5, C-7 and C-8 were obtained by specific single frequency proton decoupling. The two methyl signals for C-6 and C-9 could not be distinguished from each other in an unlabeled sample of aspyrone. The chemical shift assignments of aspyrone are given in Table 1.

The ^{13}C-nmr spectra of 1-^{13}C-acetate and 2-^{13}C-acetate enriched aspyrone, produced by cultures of *A. melleus* are shown in (2) and (3) of Figure 1. Strong signal enhancement over natural abundance is observed for the C-1, C-3, C-5 and C-8 signals (2) of Figure 1, showing they are

Table 1

^{13}C-NMR DATA FOR ASPYRONE (1)

	Aspryone (1)		Mesylate (2)	
Carbon	δc	J_{Hz}	δc	J_{Hz}
1	163.6	6.0	161.3	6.2
2	128.7	67.8	132.8	*
3	142.3	67.2	133.8	*
4	67.4	40.5	72.7	41.0
5	79.5	40.5	76.5	41.1
6	18.0	--	18.1	--
7	54.7	6.1	54.4	5.9
8	59.0	44.1	59.2	44.3
9	17.5	44.0	17.6	44.2
$-SO_2CH_3$	--	--	39.0	--



(1) R = H

(2) R = $-SO_2CH_3$

RELATIVE ENRICHMENT OF MESYLATE (2)

Carbon	
1	1.87
2	1.87[1]
3	1.87[1]
4	1.64
5	--[3]
6	1.80[2]
7	1.74
8	2.09
9	1.80[2]
$-SO_2CH_3$	1.00

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* Since the chemical shifts of these signals are very close, the extreme AB coupling pattern prevents detection of these outer satellites.

[1] Average intensity of the combined C-2 and C-3 signals.

[2] Average intensity of the combined C-6 and C-9 signals.

[3] Obscured next to the $CDCl_3$ solvent peak.

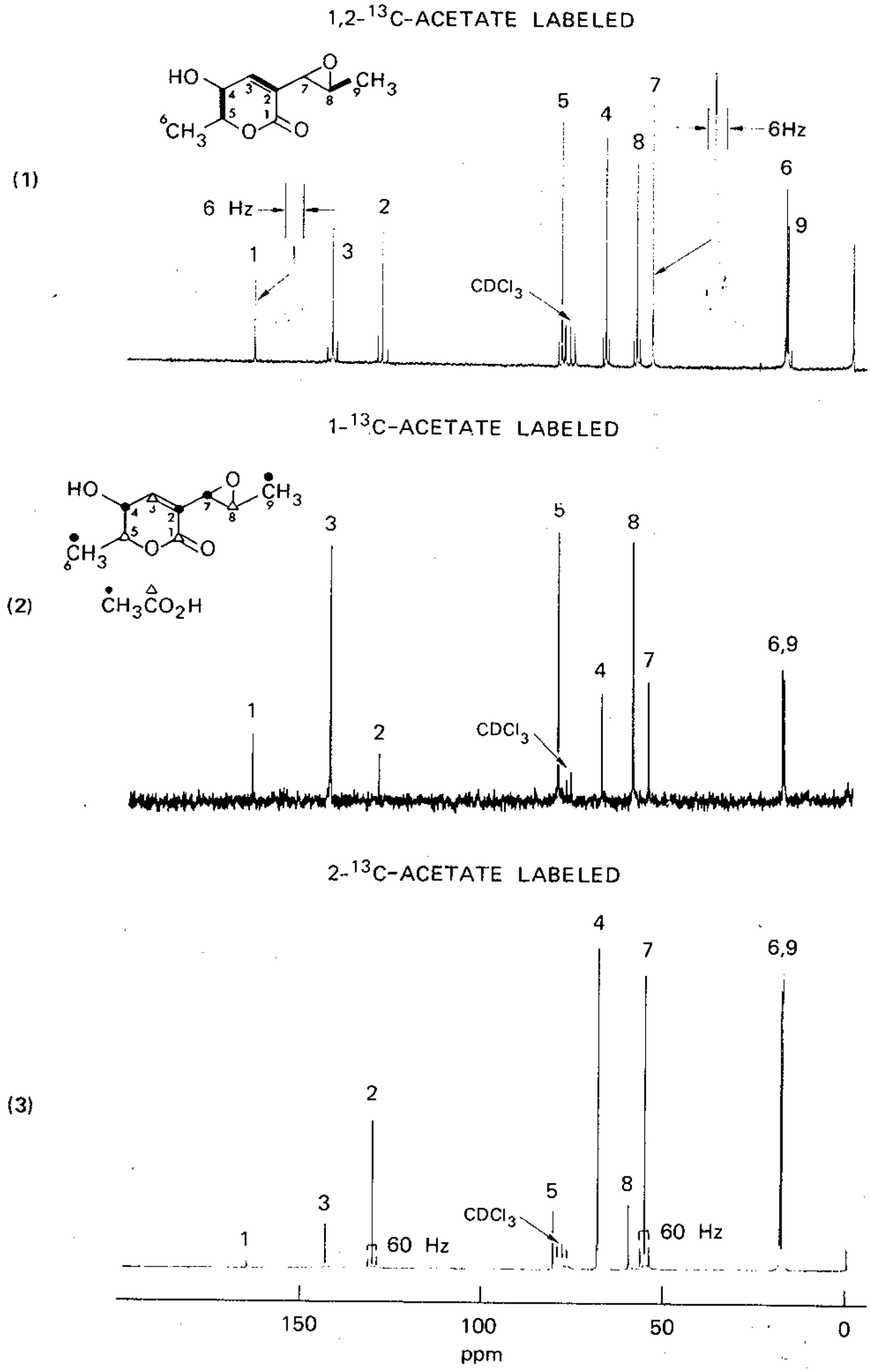

1,2-13C-ACETATE LABELED
(1)
HO
CH3
O
6 Hz
6Hz
1
2
3
4
5
6
7
8
9
CDCl3
1-13C-ACETATE LABELED
(2)
CH3CO2H
1
2
3
4
5
7
8
6,9
CDCl3
2-13C-ACETATE LABELED
(3)
1
2
3
4
5
7
8
6,9
CDCl3
60 Hz
60 Hz
150
100
50
0
ppm

FIGURE 1

labeled by the carboxyl of acetate. The enhanced C-2, C-4, C-6, C-7 and C-9 signals (3) of Figure 1 establishes these carbons are labeled by 2-^{13}C-acetate. These results confirm the previous ^{14}C-labeling study,[4] as well as showing that adjacent labeling of C-2 and C-7 by the methyl group of acetate occurs.

The use of doubly labeled 90% enriched 1,2-^{13}C-acetate as a precursor afforded a clearer insight into the biosynthetic pathway to aspyrone. The 1,2-^{13}C-acetate enriched spectrum of aspyrone (1) of Figure 1 showed vicinal 1,2 coupling between C-2 and C-3 (67 Hz), C-4 and C-5 (40 Hz) and C-8 and C-9 (44 Hz). The magnitudes of these observed coupling constants are appropriate for the degree of hybridization of the respective carbons and electronegative substitution pattern. Coupling between adjacent incorporated acetic acid units was minimized and are not visible in the spectrum since the 90% 1,2-^{13}C-acetate was initially diluted with 1.5 parts of unlabeled acetate in the feeding experiment.

In a high resolution 32K Fourier transform, proton noise decoupled ^{13}C spectrum of the 1,2-^{13}C-acetate enriched aspyrone, carbon-carbon coupling for the C-1 and C-7 signals was observed with a coupling constant of $^{3}J_{C-C}$ = 6 Hz (see (1) of Figure 1). This value is consistent with a 1,3-coupling of these carbons. This result establishes that C-1 and C-7 of aspyrone are derived from the same acetate molecule that was initially incorporated into the linear pentaketide precursor. Our finding is clearly inconsistent with a previous biosynthetic scheme for aspyrone which proposes that C-1 and C-6 are derived from a common acetic acid unit and C-7 is derived from another acetate unit.[5]

Relative enrichment levels of the carbons in the 1,2-^{13}C-acetate labeled aspyrone were determined by comparison of relative signal intensities. Since no unlabeled carbon in the ^{13}C-enriched aspyrone is present to serve as a reference standard, it was converted to the 5-methanesulfonate ester by treatment with methanesulfonyl chloride [mp 113-115°, IR^{nujol} 1725, 1345, 1175, $1170^{cm^{-1}}$; NMR^{CDCl_3} 1.44 (3H, d, H-9), 1.48 (3H, d, H-6), 2.85 (1H, m, H-8), 3.14 (3H, $-SO_2CH_3$), 3.57 (1H, d, H-7), 4.69 (1H, m, H-5), 5.18 (1H, dd, H-4), 6.72 δ (1H, d, H-3)]. The methyl signal of the mesylate was a convenient unlabeled reference standard.

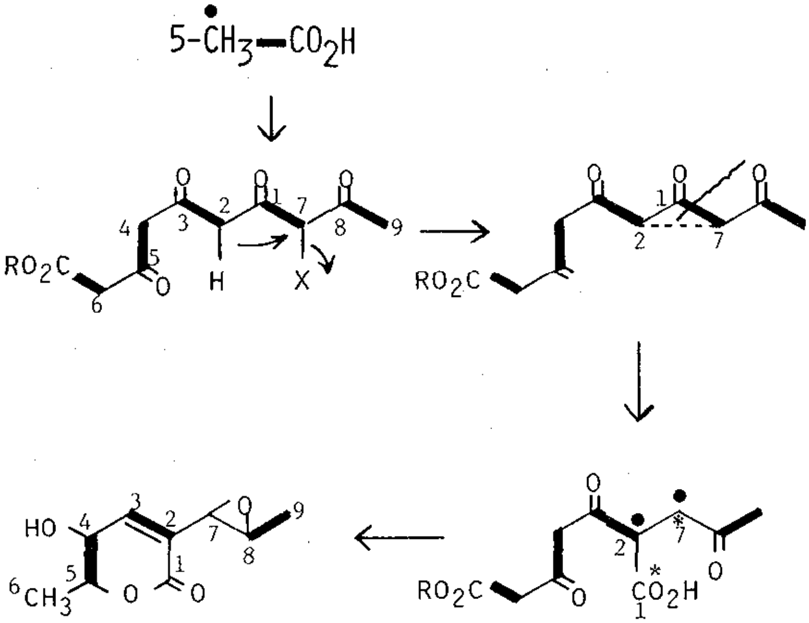
For the intensity measurements, the mesylate sample was doped with paramagnetic *tris*(acetylacetonate)chromium [$Cr(acac)_3$] in order to minimize variations in signal intensities due to the variable NOE effects and T_1 relaxation times of the individual carbons.[6] The relative intensities obtained in this way show all nine aspyrone carbons uniformly enriched to about twice natural abundance. The data are summarized in Table 1. This result establishes that the five acetic acid units that form the linear pentaketide precursor of aspyrone are incorporated at the same stage of metabolism.

In Scheme 1 a biosynthetic mechanism is given which is consistent with all the labeling distribution and incorporation level data. Initial condensation of five acetyl co-enzyme A units forms a uniformly labeled linear pentaketide. Biological introduction of a leaving group at C-7 (x) is followed by intramolecular displacement by a C-2 carbanion to form a cyclopropanone analogous to the Favorsky rearrangement intermediate. Specific opening of the C-1—C-7 cyclopropanone bond can be facilitated by its 1,3 dicarbonyl relationship with C-8. This specific cyclopropanone C-1—C-7 ring opening process accounts for the 1,3 coupling observed between C-1* and C-7* in the 1,2-^{13}C-acetate labeled aspyrone. The cyclopropanone intermediate also accounts for the 1,2 coupling observed between

C-2 (●) and C-7 (●) in the 2-^{13}C-acetate labeled aspyrone. Further elaboration of the carboxylic acid intermediate to aspyrone is well precedented. This proposed Favorsky rearrangement route to aspyrone appears to be one of many examples of this process occurring in fungal metabolite biosynthesis.

In this connection, the biosynthesis of two other fungal metabolites sterigmatocystin and aflatoxin B_1 conceivably can proceed through similar Favorsky type intermediates. Hsieh[7] has elegantly demonstrated with a blocked mutant of *A. parasiticus* the obligatory intermediacy of averufin

Scheme 1



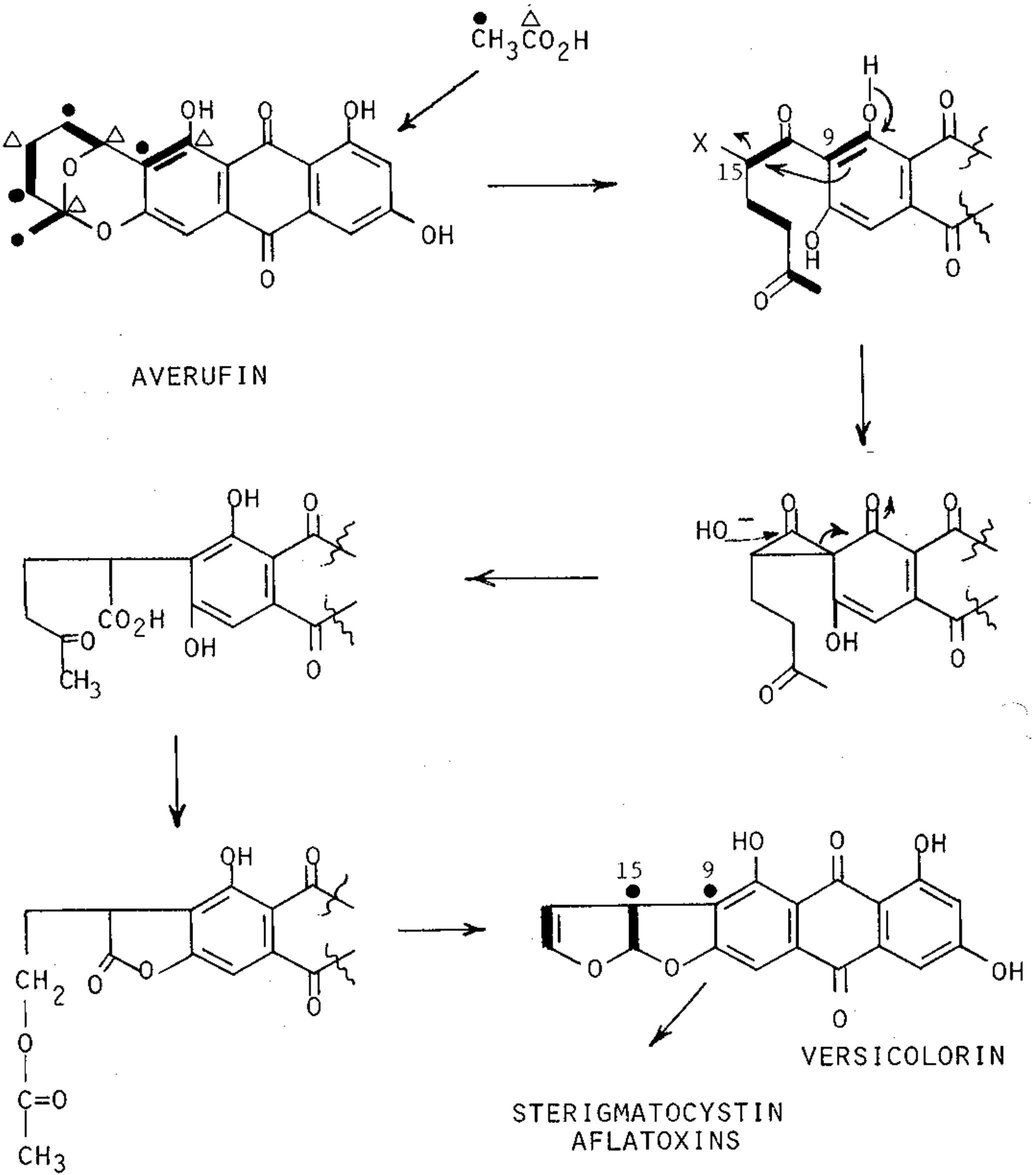
in aflatoxin B_1 biosynthesis. Another mutant oxidatively converts the xanthone, sterigmatocystin, to the coumarone aflatoxin B_1. Feeding experiments have also established that the adjacent carbons C-9 and C-15 in both of these metabolites are labeled by the methyl carbon of acetate.[8,9]

A mechanistically acceptable biotransformation route from averufin's six carbon side chain to sterigmatocystin's and aflatoxin B_1's characteristic branched difurano four carbon side chain is through a cyclopropanone derivative. Such a biosynthetic route is compatible with the observed label distribution.[10]

This proposed route is outlined in Scheme 2. Introduction of a biological leaving group at C-15 (x = pyrophosphate) in averufin is followed by cyclopropanone formation assisted by $Ar_{\overset{\ominus}{1}}$—3 participation from the ortho phenolic hydroxyl group.[11] Opening of the cyclopropanone next affords a branched carboxylic acid and its conversion to the characteristic branched four carbon sidechain found in the fungal metabolites can proceed by the equivalent of a Baeyer-Villager oxidation of the terminal acetyl unit as shown. Ultimate transformation to the bisfurano ring can proceed as indicated with reduction of the carboxylic acid to the aldehyde level occurring at some stage of the transformation, to give the bisfurano-anthraquinone derivative versicolorin. An alternate biotransformation route from averufin to sterigmatocystin and aflatoxin B_1 through a Baeyer-Villager reaction on an acetyl-furan has also been proposed.[12]

Since the completion of our work on aspyrone biosynthesis, similar results and conclusions have appeared.[13]

Scheme 2


CH3CO2H
AVERUFIN
X
15
9
HO
CO2H
CH3
CH2
C=O
VERSICOLORIN
STERIGMATOCYSTIN
AFLATOXINS

Acknowledgement - We thank Professor D.H.R. Barton, Dr. Haruo Seto, Dr. Yoshiyuki Suzuki and Dr. Kazuo T. Suzuki for helpful discussions and the U.S. Public Health Service for support of this work under NIH Grant No. AI07987.

REFERENCES

† Visiting Scientist (1974-76) from the Institute of Physical and Chemical Research, Saitama, Japan.

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(1) S. D. Mills and W. B. Turner. *J. Chem. Soc. (C)*, 1967, 2242.

(2) (a) S. Natori, S. Sakaki, H. Kurata, S. Udagawa, M. Ichinoe, M. Saito, and M. Umeda. *Chem. Pharm. Bull. (Tokyo)*, 1970, 18, 2259.

(b) W. Rosenbrook, Jr. and R. E. Carney. *Tetrahedron Letters*, 1970, 1867.

(c) J. H. Moore, T. P. Murray and M. E. Marks. *J. Agr. Food Chem.* 1974, 22, 697.

(3) C. W. Hesseltine, E. E. Vandergraft, E. E. Fennell, D. I. Smith, and M. L. Shotwell. *Mycologia*, 1972, 64, 539.

(4) W. B. Turner. 'Fungal Metabolites,' Academic Press, London and New York, 1972, p. 193.

(5) T. J. Simpson. *Tetrahedron Letters*, 1975, 175.

(6) M. Tanabe, K. T. Suzuki and W. C. Jankowski, *Tetrahedron Letters*, 1973, 4723.

(7) (a) J. A. Donkersloot, R. I. Mateles, and S. S. Yang. *Biochem. Biophys. Res. Commun.*, 1972, **47**, 1051.

(b) M. T. Lin, D.P.H. Hsieh, R. C. Yao and J. A. Donkersloot. *Biochemistry*, 1973, **12**, 5167.

(c) R. C. Yao and D.P.H. Hsieh. *Appl. Microbiol.*, 1974, **28**, 52.

(d) M. T. Lin and D.P.H. Hsieh. *J. Am. Chem. Soc.*, 1973, **95**, 1668.

(e) D.P.H. Hsieh, R. C. Yao, D. L. Fitzell and C. A. Reece. *J. Am. Chem. Soc.*, 1976, **98**, 1020.

(8) M. Biollaz, G. Buchi and G. Milne. *J. Am. Chem. Soc.*, 1970, **92**, 1035.

(9) M. Tanabe, T. Hamasaki and H. Seto. *Chem. Commun.*, 1970, 1539.

(10) (a) P. S. Steyn, R. Vleggar, P. L. Wessels and D. B. Scott. *Chem. Comm.*, 1975, 193.

(b) K.G.R. Dachler, P. Steyn, R. Vleggar, and P. L. Wessels. *Chem. Comm.* 1975, 355.

(c) H. Seto, L. W. Cary and M. Tanabe. *Tetrahedron Letters*, 1974, 4491.

(11) R. Baird and S. Winstein. *J. Am. Chem. Soc.*, 1963, **85**, 567.

(12) R. Thomas. Personal communication to M. O. Moss In 'Phytochemical Ecology,' ed., J. B. Harborne, Academic Press, London, 1972, p. 10.

(13) T. J. Simpson and J.S.E. Holker. *Tetrahedron Letters*, 1975, 4693.

Received, 10th August, 1976