Study of the Biosynthesis of Sterigmatocystin and Reassignment of ^{13}C Nuclear Magnetic Resonance Spectrum

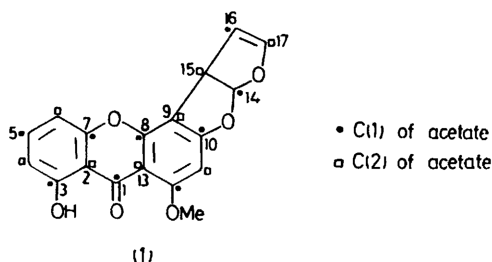
By Klaus G. R. Pachler, Pieter S. Steyn,* Robert Vleggaar, and Philippus L. Wessels

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

Summary New ^{13}C n.m.r. assignments of sterigmatocystin and their important implications on its biosynthesis are reported.

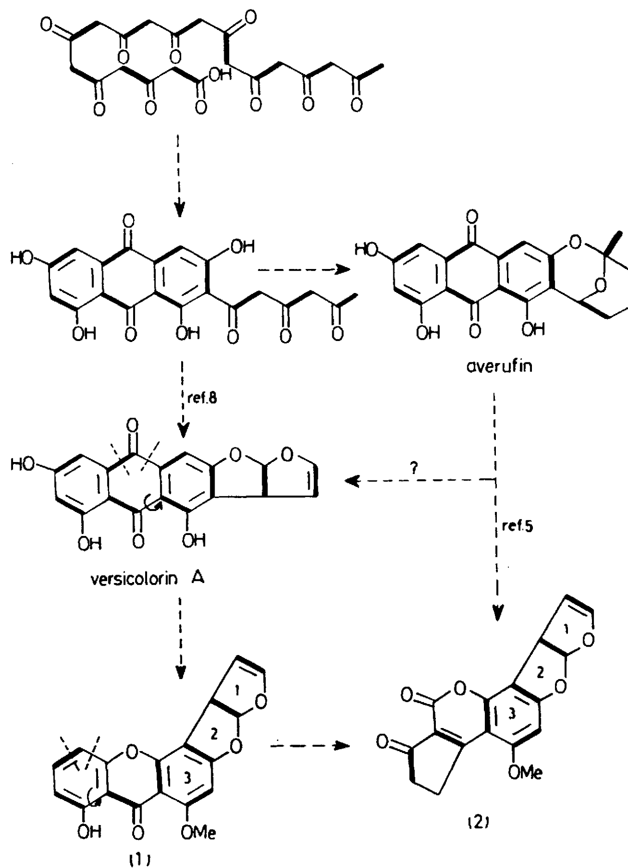
In view of the recent communication by Seto *et al.*[1] we now present our results on the ^{13}C n.m.r. assignment of sterigmatocystin (**1**), together with the subsequent biogenetic implications.

The ^{13}C n.m.r. spectral data of (**1**) derived from coupled, proton-noise decoupled, and off-resonance decoupled spectra are given in the Table. Methoxy and tertiary carbon atoms have been assigned by correlating residual splittings in off-resonance decoupled spectra with proton chemical shifts. Directly bonded C–H couplings support the assignments of aromatic, olefinic and aliphatic carbon atoms. These assignments agree largely with those of Seto *et al.*[1] except for the crucial interchange of the C(4) and C(6) resonances. The resonance centred around $\delta = 111{\cdot}0$ shows two long-range couplings of 7 Hz, one of which is removed by exchanging the OH proton with deuterium. This clearly distinguishes C(4) from C(6), which is five bonds away from the hydroxy-proton.



(1)

Quarternary carbons have been assigned from long-range couplings, assuming that three-bond C–H couplings in aromatic systems are larger than two-bond couplings and from comparisons with chemical shifts in related compounds [dihydrosterigmatocystin, *O*-acetyldihydrosterigmatocystin and aflatoxin B_1 (**2**)[2]]. The following evidence supports the assignments different from those of Seto *et al.*, [C(7), C(8), C(10) and C(12)]. Selectively decoupling the proton on C(5) changes the resonances at $\delta = 162{\cdot}1$ and $154{\cdot}7$ to a doublet of doublets (*J* 5 and 2 Hz) and a broad unresolved singlet, respectively. Deuterium exchange of the hydroxy-proton removes the 5 Hz coupling from the resonance at $\delta = 162{\cdot}1$. This confirms the assignments of C(3) (at $\delta = 162{\cdot}1$) and C(7) (at $\delta = 154{\cdot}7$). The complex pattern at $\delta = 163{\cdot}0$ is assigned to C(12) since decoupling of the methoxy-proton changes the signal to a simple doublet (*J* 2 Hz). This assignment is also supported by $Eu(fod)_3$ shift experiments, the methoxy carbon atom experiences the biggest shift indicating that complexation occurs preferentially at the methoxy-group. The second biggest shift is shown by the resonance of C(12). The assignment of C(8) ($\delta = 153{\cdot}7$) and C(10) ($\delta = 164{\cdot}3$) follows from a comparison with the corresponding carbon atoms in aflatoxin B_1 (**2**) which resonate at $\delta = 153{\cdot}0$ and $165{\cdot}8$, respectively.[2]



Scheme

Assignments of the three quaternary carbon atoms [C(2), C(9), and C(13)], previously not assigned,[1] were made as follows. The resonance at $\delta = 106{\cdot}4$ is shifted least by $Eu(fod)_3$ and is therefore assigned to C(9). Acetylation of the C(3) hydroxy-group in dihydrosterigmatocystin caused the resonance at $\delta = 108{\cdot}8$ C(2) to shift to lower field ($\delta = 115{\cdot}9$), thus identifying C(2). The remaining resonance at $\delta = 105{\cdot}7$ in (**1**) was, therefore, assigned to C(13).

Biosynthetic studies on sterigmatocystin using ^{14}C- and ^{13}C-labelled acetate precursors[3] have revealed the distribution of labels in the acetate derived skeleton shown in (**1**). Our ^{13}C assignments (Table), together with the reported appearance of only one singlet[1] (δ_C 154·7, which we assigned to C(7) in the doubly labelled sterigmatocystin, as well as the reassigned reported coupling constants *J* (δ 135·4–105·7)

TABLE

The ^{13}C chemical shifts, directly bonded (^{1}J) and long-range ($>{}^{1}J$) ^{13}C–^{1}H coupling constants of sterigmatocystin (**1**) in $CDCl_3$.

Carbon	δ p.p.m.[a]	^{1}J/Hz	$>{}^{1}J$/Hz
1	180·9 S	—	—
2	108·8 S	—	—
3	162·1 Sddd	—	10; 5; 2
4	111·0 Dt	165	7
5	135·4 D	164	—
6	105·7 Dd	167	8
7	154·7 Sdd	—	12; 3
8	153·7 S	—	—
9	106·4 S	—	—
10	164·3 Sdd	—	8; 4
11	90·4 D	165	—
12	163·0 Sqd	—	7;3
13	105·7 ± 0·3 S[b]	—	—
14	113·1 Dddd	187	9; 6; 3
15	47·9 Dq	146	6
16	102·4 Dddd	181	13; 5; 2
17	145·1 Ddt	197	12; 5
OMe	56·6 Q	146	—

[a] Chemical shift relative to internal Me_4Si. Capital letters refer to the pattern resulting from directly bonded protons and small letters to long-range ^{13}C–^{1}H coupling. S = singlet, D = doublet, T = triplet, Q = quartet.

[b] This resonance overlaps with that of C(6). The position was backextrapolated from a $Eu(fod)_3$ experiment.

58 Hz; J (δ 113·1–47·9) 34 Hz and J (δ 102·4—145·1) 76 Hz, allow only one arrangement of intact acetate units in sterigmatocystin. The ^{13}C n.m.r. assignment of sterigmatocystin (**1**) by Seto *et al.*[1] leads to the incorrect arrangement of intact acetate units, *e.g.* the location of only two intact acetate units in ring 3 and furthermore to a different folding of the original polyketide (Scheme).

The above results and our recent findings[2] on aflatoxin B_1 (**2**) show that ring 3 in (**1**) and (**2**) contains three intact acetate units. A C_{18} naphthacene precursor[4] is thus no longer tenable. Our data are in agreement with the biosynthesis from a single C_{20} polyketide precursor which is folded only in one mode leading to averufin and sterigmatocystin (Scheme). However, the intermediacy of a formal 'C_4-unit' which is linked in a head-to-tail fashion to a C_{14} precursor, in the biosynthesis of (**1**) cannot be completely excluded. The proposed C_{20} pathway (Scheme) is supported by recent experiments which established that averufin[5] and sterigmatocystin[6] can be converted very efficiently by cultures of *Aspergillus parasiticus* into aflatoxin B_1. In addition feeding experiments by Heathcote *et al.*[7] have proved that 'C_4-units' were very poorly incorporated into aflatoxin B_1. The unique head-to-head linkage of the two acetate units in (**1**) and (**2**) for the coupling of the dihydrofuran ring and the aromatic system can be adequately accommodated by a mechanism proposed by Thomas.[8]

(*Received*, 19*th February* 1975; *Com.* 209.)

[1] H. Seto, L. W. Cary, and M. Tanabe, *Tetrahedron Letters*, 1974, 4491.
[2] P. S. Steyn, R. Vleggaar, P. L. Wessels, and D. B. Scott, *J.C.S. Chem. Comm.*, 1975, 193.
[3] J. S. E. Holker and L. J. Mulheirn, *J.C.S. Chem. Comm.*, 1968, 1576; M. Tanabe, T. Hamasaki, and H. Seto, *ibid.*, 1970, 1539.
[4] M. Biollaz, G. Büchi, and G. Milne, *J. Amer. Chem. Soc.*, 1970, **92**, 1035.
[5] M. T. Lin and D. P. H. Hsieh, *J. Amer. Chem. Soc.*, 1973, **95**, 1668.
[6] D. P. H. Hsieh, M. T. Lin, and R. C. Yao, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 992.
[7] J. G. Heathcote, M. F. Dutton, and J. R. Hibbert, *Chem. and Ind.*, 1973, 1027.
[8] R. Thomas, personal communication to M. O. Moss, in 'Phytochemical Ecology,' ed. J. B. Harborne, Academic Press, London, 1972, p. 140.