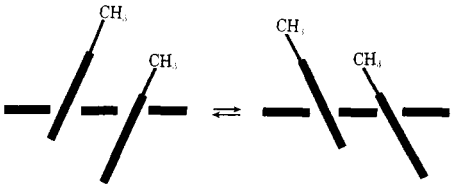
ring-current effects. However, for the trans isomer, the ring-flip forms are expected to be of unequal energy and, as a result, the meta proton adjacent to the methyl group will spend a different fraction of the time over the plane of the adjacent phenyl ring than for the meta protons opposite the methyl group. Consequently, these meta protons are expected to experience different diamagnetic ring current shifts. The resulting differences in chemical shifts are expected to rise to a multiplet proton spectrum. We therefore assign the trans configuration to isomer **1b** and the cis configuration to isomer **1a.** This assignment is consistent with the observation that **1a** is the less favorable isomer. With rapid flipping of the phenyl rings at room temperature, the trans isomer would be a racemic mixture, while the cis isomer would be achiral. We have not attempted to identify the isomers by resolution.

References and Notes

(1) Supported by the National Science Foundation.
(2) H. O. House and R. W. Bashe, III, *J. Org. Chem.*, **30**, 2942 (1965).
(3) (a) H. O. House, W. J. Campbell, and M. Gall, *J. Org. Chem.*, **35**, 1815 (1970); (b) R. L. Clough and J. D. Roberts, unpublished results.
(4) (a) R. J. P. Corriu and J. P. Masse, *J. Chem. Soc., Chem. Commun.*, **144** (1972); (b) R. L. Clough, P. Mison, and J. D. Roberts, submitted to *J. Org. Chem.*
(5) (a) P. G. Evrard, P. Piret, and M. van Meerssche, *Acta Crystallogr. Sect. B*, **28**, 497 (1972); (b) R. A. Ogilvie, PhD Thesis, Massachusetts Institute of Technology, 1971; (c) R. L. Clough, W. J. Kung, R. E. Marsh, and J. D. Roberts, submitted to *J. Am. Chem. Soc.*
(6) We have no reason to suspect that there is a substantial barrier to a 30–60° twist of the phenyl rings about their bonds to the naphthalene nucleus. Thus, the following equilibrium should be fast.



(7) J. E. Anderson, R. Franck, and W. Mandella, *J. Am. Chem. Soc.*, **94**, 4608 (1972).
(8) National Defense Education Act Fellow, 1971–1973.

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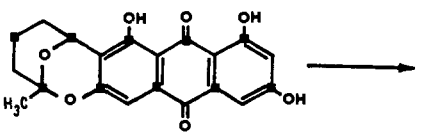
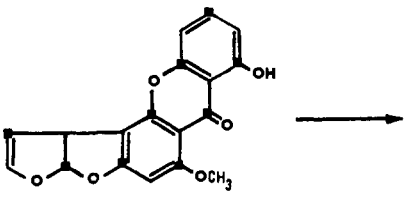
Received October 17, 1975



III



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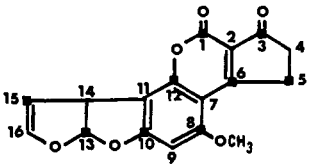

I

Table I. Comparison of the Signal Intensities of Aflatoxin B_1 and Aflatoxin B_1 Derived From ^{13}C-Labeled Averufin

Carbon no.	δ^9	Peak height (cm) Unlabeled B_1	Labeled B_1	Rel intens
1	155.2	0.6	0.8	1.3
3	201.3	2.5	2.4	1.0
5	29.0	2.7	3.6	1.3
6	177.1	3.8	2.5	0.7
8	161.6	3.5	3.8	1.1
10	165.8	2.8	3.0	1.1
12	153.0	1.4	0.9	0.6
13	113.6	5.0	7.5	1.5
15	102.7	4.6	9.0	2.0
2	117.4	1.5	0.6	0.4
4	35.1	2.8	1.3	0.5
7	104.0	1.4	0.7	0.5
9	90.9	5.0	2.4	0.5
11	107.9	1.7	0.9	0.5
14	47.9	4.4	2.3	0.5
16	145.4	5.9	3.2	0.5
OCH_3	56.6	3.6	1.9	0.5

Origin of the Bisfuran Ring Structure in Aflatoxin Biosynthesis

Sir:

The biogenetic origin of the bisfuran ring structure in the molecule of aflatoxin B_1 (I) has been a subject of disagreement among investigators. Based upon the apparent difference in labeling density between the bisfuran ring and the xanthone moiety in sterigmatocystin (II) derived from ^{14}C-labeled acetate,[1] it was proposed that the two moieties have separate biogenetic origins and II is formed through the fusion of a C_4 and C_{14} unit. II was shown to be convertible into I by *Aspergillus parasiticus*[2] and was recognized as a precursor of I. Specific chemical degradations of I[3] synthesized from [1-^{14}C]- and [2-^{14}C]acetate, however, revealed a uniform label distribution throughout the molecule I. Thus a biosynthetic scheme was advanced in which I is derived from a single C_{18} polyketomethylene unit and the bisfuran ring system was proposed to be formed through endoperoxidation of the terminal phenyl group of a polyhydroxynaphthacene intermediate. However, the recent finding that averufin (III), a C_{20} polyketide, can be readily converted into I by *A. parasiticus*[4,5] indicates the biosynthesis of I involves a C_{20} rather than a C_{18} intermediate. This implies (1) the C_6 side chain of averufin is converted into the bisfuran ring system or (2) the C_6 side chain is removed and replaced by a C_4 acetoacetate unit which is converted into the bisfuran ring system.

In the present study we subjected ^{13}C-labeled I derived from ^{13}C-labeled III to ^{13}C NMR analysis in order to show that nine of the ten carbon atoms in III originating from [1-^{13}C]acetate are incorporated into I.

The ^{13}C-labeled III was synthesized by cultures of *A. parasiticus* ATCC 24551 supplemented with [1-^{13}C]acetate.[6] The purified ^{13}C-labeled III was then converted into I by the mycelium of *A. parasiticus*.[5] Previous ^{13}C NMR analysis of ^{13}C-labeled III has revealed that III is biosynthesized through a head-to-tail assembly of ten acetate units,[7] with the labels occupying alternating positions throughout the molecule.

Comparison of the relative signal intensities of I and I derived from ^{13}C-labeled III, as shown in Table I, indicates that the ratios of the intensities (last column) for the ex-

pected labeling sites are consistently higher than those expected not to be labeled. This result confirms that when ^{13}C-labeled III synthesized from [1-^{13}C]acetate is converted into I, the latter is labeled in the same pattern as that directly derived from [1-^{13}C]acetate.[8,9]

Despite the considerable variation in the enrichment of various carbons as shown by relative intensities, which warrants further investigation, our data does clearly indicate that C-13 and -15 of I are enriched. A strong evidence thus is provided that III is the only carbon source for the biosynthesis of I, implying the bisfuran ring system is derived from the C_6 side chain of III. A mechanism for the C_6 to C_4 conversion has been proposed by Thomas,[10] but experimental evidence is still not available. The typical alternating labeling pattern of III from [1-^{13}C]acetate is indicative of the involvement of a single polyketomethylene unit rather than two preformed units in the biosynthesis of III, II, and I, although little has been reported of the pathway leading to III.

Acknowledgment. This study is supported by Public Health Service Grant ES00612 and Western Regional Research Project W-122.

References and Notes

(1) J. S. E. Holker and L. J. Mulheirn, *Chem. Commun.*, 1576 (1968).
(2) D. P. H. Hsieh, M. T. Lin, and R. C. Yao, *Biochem. Biophys. Res. Commun.*, **52**, 922 (1973).
(3) M. Biollaz, G. Büchi, and G. Milne, *J. Am. Chem. Soc.*, **92**, 1035 (1970).
(4) M. T. Lin and D. P. H. Hsieh, *J. Am. Chem. Soc.*, **95**, 1668 (1973).
(5) M. T. Lin, D. P. H. Hsieh, R. C. Yao, and J. A. Donkersloot, *Biochemistry*, **12**, 5167 (1973).
(6) For fermentation technique see D. P. H. Hsieh and R. I. Mateles, *Appl. Microbiol.*, **22**, 79 (1971).
(7) D. L. Fitzell, D. P. H. Hsieh, R. C. Yao, and G. N. La Mar, *J. Agric. Food Chem.*, **23**, 442 (1975).
(8) D. P. H. Hsieh, J. N. Seiber, C. A. Reece, D. L. Fitzell, S. L. Yang, J. I. Dalezios, G. N. La Mar, D. L. Budd, and E. Motell, *Tetrahedron*, **31**, 661 (1975).
(9) P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Chem. Commun.*, 193 (1975).
(10) R. Thomas, personal communication to M. O. Moss, "Aflatoxin and Related Mycotoxins, Phytochemical Ecology", J. B. Harborne, Ed., Academic Press, London, 1972, p 140.

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Solvated Nickel Atoms and Their Free Cluster Formation in Organic Media

Sir:

We recently reported that codeposition of metal vapors (atoms) with certain solvents allows the formation of very reactive high surface area metal slurries.[1,2] Further work showed that significantly different clustered forms of each metal were observed when different solvents were employed.[3] It seemed to be of importance to study these clustering processes in more detail for two reasons: (1) we have observed differing reactivity for these metal particles when formed in different solvents, and so anticipated the possibility of tailoring metal particles for specific purposes, and (2) to gain some understanding of the metal atom clustering processes in an organic matrix, which has not been studied previously even though there has been interest expressed in such studies.[4,5] We report here some of our work with nickel.[6]

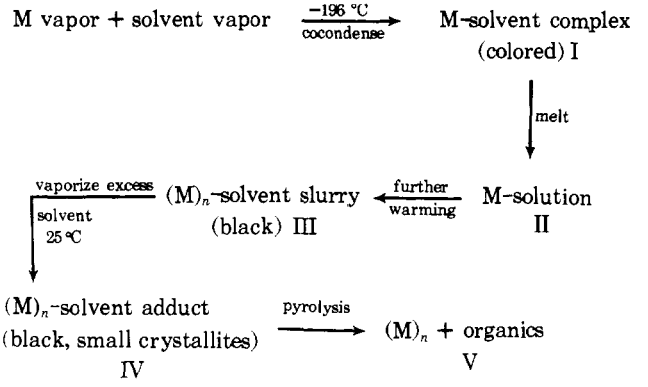
The clustering of nickel atoms in organic media is a process that has discrete stages (Scheme I). Stage I is a weak σ or π complex formed when excess (>30:1) solvent is codeposited with the metal vapor. Stage II forms with some solvents upon low temperature melt down of the matrix. If well diluted, a homogeneous metal atom (or metal telomer) solution forms. On further warming small metal particles form, and the final size and shape of these particles is dependent on the solvent employed. Evaporation of excess solvent yields stage IV with little apparent sintering in going from III to IV. Examination of the stage IV particles has been carried out in some detail employing scanning electron microscopy, x-ray powder methods, and chemical methods. Description of three solvent systems follows (all solvents were freshly distilled from benzophenone ketal under purified nitrogen before use).

Table I. Physical Data on Metal Particles (Stage IV)

M (solvent)	Solvent:metal ratio[a]	Surface area (m^2/g)[b]	Particle Size[e] (μ)
Ni(hexane)	1:25	45	5 × 20 rough pieces[c]
Ni(toluene)	1:40	100	0.5–0.8 spheres
Ni(THF)	1:3	400	0.5–1.5 spheres
Raney Ni[d]	- - -	80–100	90% 2–40

[a] Determined by desorption of organics upon pyrolysis. [b] BET methods used except with Ni-THF where solvent and gas desorption was employed to calculate surface area. [c] Very rough edges and an average size (see SEM photos). [d] Grace #28. [e] Crystallite sizes could not be determined quantitatively due to the large line broadenings observed. These wide lines indicate crystallite sizes <100 Å, a region where the Scherrner equation does not apply. Relative crystallite sizes were determined by comparing the broad line coincident with the 111 reflection (d-spacing 2.01 Å) for commercial Ni powder utilizing Cu Kα radiation: Raney Ni (2θ broadening of 2°) > Ni-hexane (3° broadening) > Ni-toluene (4° broadening) > Ni-THF (8° broadening); commercial Ni powder (0.5° broadening). More detailed analysis of the Ni-toluene showed only the one broad line coincident with the 111 reflection, whereas Ni-THF showed two very broad lines, one corresponding to the 111 reflection and the other at lower angle with d spacing of about 4 Å.

Scheme I



Ni-Hexane. A black matrix forms during codeposition due to metal cluster formation at −196 °C. Upon melt down small particles form along with some larger flakes (that readily break up to smaller particles). These metal particles are pyrophoric and quite reactive with alkyl halides, even after removal of excess solvent under vacuum at room temperature (several hours pumping down to 5×10^{-3} Torr). Solvent retention ratio, surface area, particle size, and qualitative information on crystallite size are recorded in Table I. This Ni-hexane powder is an extremely active hydrogenation catalyst for both benzene and norbornene, more active than Raney Ni (Grace #28-W-2).

Ni-Toluene. A red-brown matrix forms during codeposition due to the formation of a toluene-Ni π-complex (−196 °C).[7] Deposition of triethyl phosphite on top of this matrix followed by melt down results in the formation of Ni-