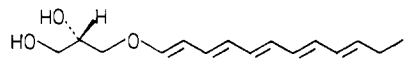
These data indicate conclusively that the hydrogenated mutagen has the structure **1**. Since the mutagen itself has five double bonds, it must either have the the structure **2** (excluding stereochemistry)



2

or an isomer in which the conjugated system is located at the 2,4,6,8,10- or 3,5,7,9,11-positions. Support for the assigned structure **2** is obtained from the compound's ^{1}H NMR spectrum. This shows signals at 6.56 (1 H, d, J = 12 Hz, OCH=), 5.5-5.8, and 5.9-6.3 (~8 H, C*H*=C*H*), 5.2 (1 H, m, OCH=C*H*), 3.5-3.9 (~5 H, m, CH_2O and CH(OH)), 2.05 (2 H, m, CH_2—CH=C), and 0.94 (3 H, t, J = 8 Hz, CH_3CH_2). Confirmation of the structural assignment was obtained by microozonolysis[16] of the mutagen, which yielded inter alia propionaldehyde, identified by comparison of its 2,4-dinitrophenylhydrazone with an authentic sample.[17] In addition, the formation of glycerol[18] on mild acid hydrolysis of the mutagen confirmed the presence of an enol ether linkage.

These data establish the structure of the mutagen as **2**, excluding stereochemistry. The enolic double bond must have the E configuration on the basis of the coupling constant of 12 Hz observed for one of its protons, and it seems probable that the remaining double bonds also have the stable E configuration.

The configuration of the glyceryl moiety was determined by derivatization with (+)-α-methoxy-α-(trifluoromethyl)-α-phenylacetyl chloride ((+)MTPA chloride).[19] Acylation of synthetic racemic **1** with (+)MTPA chloride yielded a mixture of two diastereomeric bis(+)MTPA esters that could be resolved by HPLC.[20] The bis(+)MTPA ester of the hydrogenated mutagen gave a single peak on HPLC corresponding to the faster eluting peak of the diastereomeric mixture, and the bis(+)MTPA ester of (*S*)-**1**[13,21] gave a single peak which also coincided with the first eluting peak on HPLC. The natural mutagen thus has the *S* configuration, as do naturally occurring ether lipids such as batyl alchol and chimyl alcohol,[22] and is hereby defined as (*S*)-3-(1,3,5,7,9-dodecapentaenyloxy)-1,2-propanediol (**2**).

The observation that a simple glyceryl ether lipid has strongly mutagenic properties has potentially important implications for the etiology of colon cancer. We are currently in the process of synthesizing **2** in sufficient quantity to enable us to evaluate its biological and particularly its carcinogenic activity. We have previously shown that **2** is produced by colonic bacteria[5] and that there is a correlation between the excretion of this mutagen and populations at risk for colon cancer.[3] If it proves to be carcinogenic and to be involved in the initial lesion in colon cancer, then colon cancer may well turn out to be preventable by interfering with the biosynthesis of this compound by chemotherapy or by dietary methods.

Acknowledgment. This work was supported by Grant CA 23857 from the National Cancer Institute. Initial ^{1}H NMR spectra were obtained on a Bruker WH-400 spectrometer; we acknowledge the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723). We acknowledge mass spectrometric assistance from Dr. J. D. Henion, Dr. R. D. Macfarlane, Dr. W. Garland, and K. Harich and ^{1}H NMR assistance from T. Glass.

Registry No. 2, 83248-46-8.

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(19) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543-2549. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512-519.
(20) μPorasil column, 3.9 mm × 30 cm, with elution by 1:1 dichloromethane:hexane.
(21) Prepared from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (Fluka) by standard methods.[12]
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Cobalt-Catalyzed Carbalkoxylation of Olefins: A New Mechanism

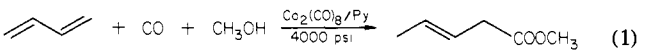
David Milstein* and John L. Huckaby

Contribution No. 3088 from
Central Research and Development Department
E. I. du Pont de Nemours and Company
Experimental Station, Wilmington, Delaware 19898

Received July 12, 1982

Transition-metal-catalyzed carbalkoxylation of olefins to esters, a synthetically very important reaction,[1] can, in principle, proceed by two main mechanisms, involving M-H or M-COOR addition to the double bond. Evidence for each one of these mechanisms has been presented for palladium-catalyzed olefin carbalkoxylations,[1-3] whereas only the hydrido route has been proposed for Co-catalyzed carbalkoxylation reaction.[1,4] We report here evidence that supports a mechanism involving both carbalkoxycobalt and hydridocobalt complexes synergistically in which addition of the carbalkoxy complex to the olefin is preferred.

We studied the $Co_2(CO)_8$/pyridine-catalyzed carbomethoxylation of butadiene to methyl 3-pentenoate (eq 1), a potential precursor for dimethyl adipate and nylon 66.[5]



(1)

To probe the plausibility of a route based on a carbomethoxycobalt complex in this reaction, we prepared the complex **1**[6] from methyl oxalyl chloride (eq 2). Methyl chloroformate does

(2)

1

not react with $NaCo(CO)_4$ or $TlCo(CO)_4$ because of insufficent electrophilicity of the carbonyl in this compounds.[7] **1** is a volatile liquid and can be isolated pure from the solvent dimethyl ether. **1** decomposes slowly at 25 °C ($\tau_{1/2} \approx 1$ h). When condensed into an ether solution of PPh_3, the complex $MeOC(O)Co(CO)_3PPh_3$ (**2**) is obtained, the crystal structure of which is presented in Figure 1.[8] The complex is trigonal bipyramid with the three carbonyls in the equatorial plane. All bond parameters appear reasonable;

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(5) Matsuda, A. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 524.
(6) This compound was unambiguously characterized on the basis of NMR and IR spectra. Satisfactory C, H analysis was obtained.
(7) Reaction of methyl oxalyl chloride with $NaCo(CO)_3PPh_3$ yields the first stable α,β-dicarbonylcobalt complex, $MeOC(O)C(O)Co(CO)_3PPh_3$: D. Milstein, to be submitted for publication.
(8) Crystal structure information: triclinic, space group $P\bar{1}$; at −100 °C, a = 10.349 (1), b = 21.095 (3), c = 10.271 (1) Å; α = 91.20 (1), β = 91.91 (1), γ = 76.72(1)°; V = 2180.9 Å^3; Z = 4. Syntex P3 diffractomer, graphite monochrometer, Mo Kα radiation, λ = 0.710 69 Å, ω scans of 1.0°, $4 < 2\theta < 50°$, 7695 reflections. An empirical absorption correction was applied; the "transmission factors" ranged from 0.958 to 0.999. The structure was refined by full-matrix least-squares techniques: 5280 reflections with $I > 3\sigma(I)$, 661 variables (non-hydrogen atoms with anisotropic thermal parameters, hydrogen atoms with isotropic parameters), R = 0.036, R_w = 0.038. The final difference Fourier map showed only residues, the largest having a magnitude of 0.33 e Å^{-3}. The mathematical and computational details may be found in the following: Nugent, W. A.; Harlow, R. L. *Inorg. Chem.* **1979**, *18*, 2030-2032.

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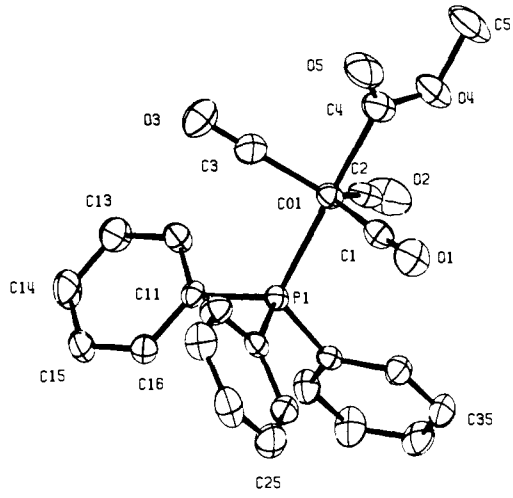
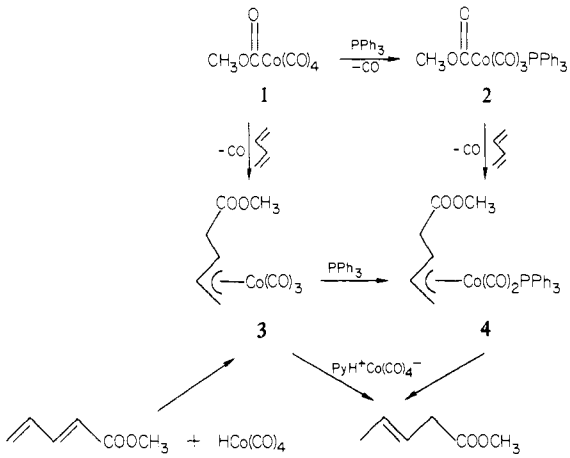


Figure 1. ORTEP drawing of a molecule of **2**. Hydrogens have been left out for clarity. Selected bond lengths (Å) and angles (deg): Co–P, 2.232; Co–C (ax), 1.976; Co–C (eq), 1.786; P–C, 1.823; C≡O (carbonyl), 1.138; C=O (carbomethoxy), 1.196; C–O (carbomethoxy), 1.342; CH_3–O, 1.484 Å; P_1–Co–C_4, 174.7°; Co–C_4–O_4, 112.9°; C_4–O_4–C_5, 113.1°.

Scheme I

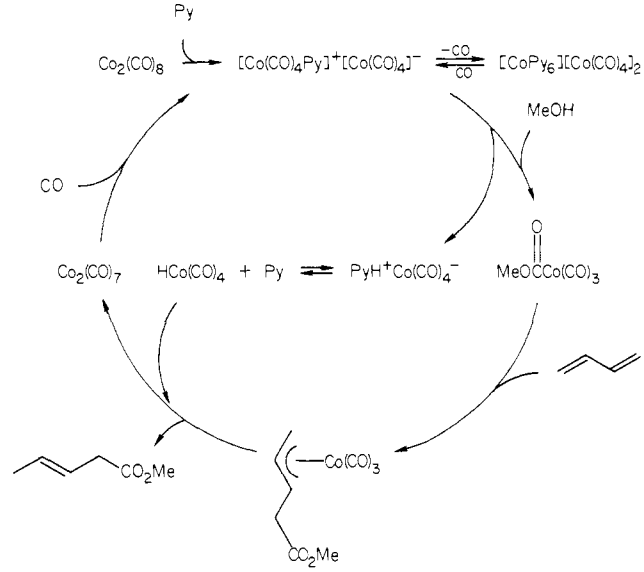


the equatorial atoms are nearly planar, and the axial atoms are within 6° of the equatorial planes. There do not appear to be any specific intermolecular interactions.

Compound **1** smoothly adds to butadiene at 25 °C to give the π-allylic complex **3**[6,9] quantitatively (Scheme II). **3** reacts with an equivalent amount of PPh_3 to yield complex **4** that can be directly obtained by adding **2** to butadiene which is, however, much slower than adding **1**. Complex **3** was also independently prepared by adding $HCo(CO)_4$ to methyl 2,4-pentadienoate. To our knowledge, these experiments constitute the first direct demonstration of an addition of a well-characterized carbalkoxymetal complex to an olefin.[10-12]

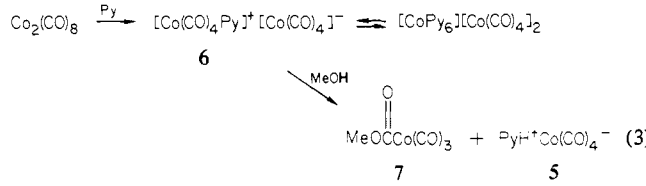
The π-allylic complexes **3** and **4** react rapidly at 25 °C wtih the salt $PyH^+Co(CO)_4^-$ (**5**, Py = pyridine) or $HCo(CO)_4$[13] to yield methyl 3-pentenoate (Scheme I) with only minor amounts (ca. 5%) of methyl 4-pentenoate formed, in full agreement with the catalytic reaction (eq 1). When this reaction is carried out under

Scheme II



1 atm of CO, $Co_2(CO)_8$ is also quantitatively obtained.

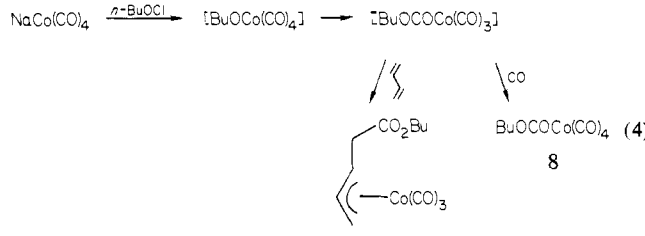
Although still to be demonstrated, generation of a carbomethoxycobalt complex in reaction 1 could very likely take place by methanolysis of the ion pair **6**, the intermediacy of which has



(3)

been invoked in the pyridine-promoted disproportionation of $Co_2(CO)_8$.[14] The salt $PyH^+Co(CO)_4^-$ would also be generated by this reaction (eq 3). An analogous reaction of $[Co(CO)_3(PPh_3)_2]^+$ has been reported.[15]

For a rough comparison of the rate of addition of an *unsaturated* carbalkoxycobalt complex of the type **7** to butadiene with that of $PyH^+Co(CO)_4^-$, *n*-BuOCl was reacted with $NaCo(CO)_4$ at −40 °C. Under CO, the carbobutoxy complex **8** is formed, whereas in the presence of butadiene and absence of CO, instantaneous addition to form complex **9**[6] takes place at −40 °C (eq 4). On the other hand, reacting **5** with butadiene to form



(4)

π-crotylcobalt tricarbonyl was incomplete even after 2 h at 25 °C. Thus, under competitive conditions, it is expected that addition of **7** to butadiene will prevail. From a comparison of the rates of addition of the carbalkoxy complexes to butadiene, the following trend is observed: $[BuOCOCo(CO)_3] > MeOCOCo(CO)_4 \sim BuOCOCo(CO)_4 > MeOCOCo(CO)_3PPh_3$. This suggests that ligand dissociation to generate a coordinatively unsaturated complex is probably rate determining in the addition sequence and provides a possible explanation to the observed rate retardation of reaction 1 by added phosphines (the rate of reaction 1 with

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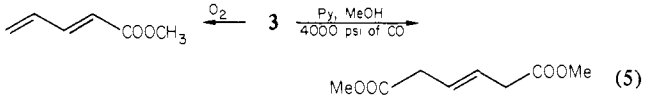
added ligands follows as trend $PPh_3 < P(OEt)_3 \ll PF_3 < (n\text{-octyl})_3PO < CO$).

Thus, a plausible comprehensive mechanism for reaction 1 is presented in Scheme II. Based on this mechanism, scavenging of $HCo(CO)_4$ from the reaction mixture should stop the catalytic cycle and result mostly in dicarbomethoxylation to dimethyl 3-hexenedioate, since we have observed that complex **3** undergoes carbomethoxylation to yield this diester (eq 5). Indeed, when



(5)

sodium carbonate is added to reaction 1, a stoichiometric reaction takes place, yielding mainly dimethyl hexenedioate esters. When reaction 1 is carried out in the presence of traces of oxygen, methyl 2,4-pentadienoate is also formed. This can also be explained by the intermediacy of **3**, which readily undergoes this oxidation process (eq 5). Studies aimed at direct observation of the methanolysis of the ion-pair **6** are now in progress.

Acknowledgment. Discussion with Drs. C. A. Tolman, S. S. Wreford, and R. L. Harlow of this department and with Professors J. K. Stille, J. K. Kochi, and B. M. Trost are greatly appreciated. We thank M. A. Cushing, Jr., and L. F. Lardear for excellent technical assistance.

Registry No. 1, 83269-56-1; **2**, 83269-57-2; **3**, 83269-58-3; **4**, 83269-59-4; **5**, 64537-27-5; $Co_2(CO)_8$, 10210-68-1; py, 110-86-1; butadiene, 106-99-0; methyl 3-pentenoate, 818-58-6.

Supplementary Material Available: Tables of positional and thermal parameters for compound **2** (7 pages). Ordering information is given on any current masthead page.

Bisfuran Formation in Aflatoxin Biosynthesis: The Fate of the Averufin Side Chain

Craig A. Townsend,*† Siegfried B. Christensen, and Steven G. Davis

Department of Chemistry, The Johns Hopkins University Baltimore, Maryland 21218

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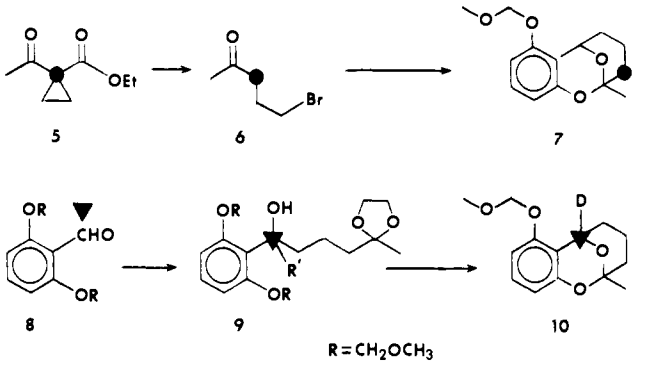
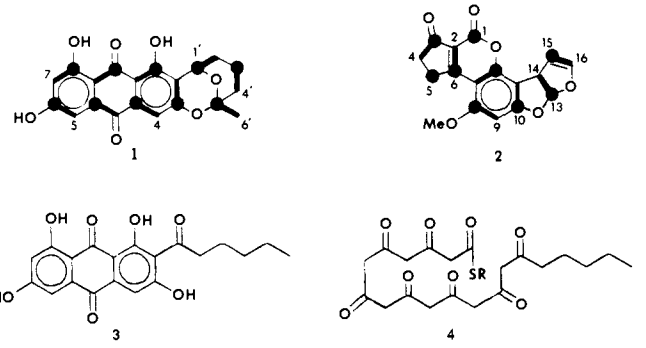
Biollaz, Büchi, and Milne in classic studies of aflatoxin B_1 (**2**) biosynthesis determined the origin of 13 of its 17 carbon atoms from acetate and methionine, results that indicated derivation of this toxin from a single, albeit highly rearranged, polyketide chain.[1] In the intervening years a chemically plausible series of intermediates has been advanced from extensive experiments using blocked mutants of *Aspergillus parasiticus* and metabolic inhibitors of the wild-type strain.[2-5] A second line of evidence has established[6] a common polyketide folding pattern through these intermediates as revealed in [1,2-$^{13}C_2$]acetate incorporation experiments (so indicated in **1** and **2** by heavy lines, the dot signifying C-1). However, the mechanistic details of the remarkable transformations required to connect the proposed intermediates of this pathway have been the object only of speculation for lack of discriminating experimental evidence. Described in this communication and its companion are experiments that specifically address the issue of bisfuran formation, the unique structural feature of this family of mycotoxins, and the seat of their lethal biological activity.[7]

Scheme I





Averufin (**1**) has emerged as the pivotal anthraquinone intermediate in the pathway to aflatoxin.[3,6] It is apparently derived by way of norsolorinic acid (**3**),[3,4] which may in turn be formulated in conventional fashion from a C_{20} polyketide, e.g., **4**.[8] As revealed in earlier work,[9,10] the linear C_6 ketal side chain of averufin (**1**), containing three intact acetate units, becomes branched in the C_4 bisfuran of aflatoxin B_1 (**2**), and one of the three acetate units is lost. Outlined in Scheme I are total syntheses of specifically labeled specimens of racemic averufin, (**11**) and (**13**), and below the results of studies that demonstrate their intact incorporation into aflatoxin B_1.

[2-^{13}C]Ethyl acetate (90% enriched) was homologated[11] to [2-^{13}C]ethyl acetoacetate, which was treated with 1,2-dibromoethane (acetone/K_2CO_3) to afford cyclopropane derivative **5**.[12] Hydrobromination 48% HBr) gave [3-^{13}C]-5-bromo-2-pentanone (**6**)[13] in 45% overall yield. Protection of **6**, formation of the corresponding lithium reagent (2% Na/Li) followed by reaction with 2,6-bis(methoxymethyl)benzaldehyde and partial deprotection as previously described,[14] gave the 4′-labeled tricyclic ketal **7** in

† Research Fellow of the Alfred P. Sloan Foundation 1982-1984.

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