Table II. Labeling of Amino Acids in *Methylobacterium* AM1 by [1-^{13}C]Ethanol

amino acid	^{13}C enrichments from [1-^{13}C]ethanol[a] (atom % ^{13}C)				
	C-1	C-2	C-3	C-4	C-5
alanine	58	14	15		
aspartate	68	17	17	68	
glutamate	52	13	13	2	72

amino acid	^{13}C enrichments from [1-^{13}C]ethanol[a] (atom % ^{13}C)						
	C-1	C-2	C-3	C-1′	C-2′ (C-6′)	C-3′ (C-5′)	C-4′
tyrosine	73	17	17	17	18 (18)	61 (17)	61

[a] [2-^{13}C]Ethanol labeled the alternate positions with the exception that C-3′ (C-5′) of tyrosine was labeled by both C-1 and C-2 of ethanol. ^{13}C NMR spectra were obtained with the ^{1}H decoupler gated off (60–300 s, 45° pulse). ^{13}C Enrichments were determined as in Table I and normalized to the enrichment at the α-carbons as determined by ^{1}H NMR.

[1-^{13}C]Ethanol labels the phenol ring of tyrosine at C-3′ and C-4′ yielding an NMR spectrum that exhibits $^{1}J_{C-C}$ coupling (Figure 1C); this labeling pattern is identical with that observed in tyrosine isolated from *E. coli* cultured on [1-^{13}C]lactate.[14] The adjacent labeling of C-3′ and C-4′ of tyrosine is diagnostic of compounds that arise from the shikimate pathway.

The ^{13}C NMR spectrum of PQQ isolated from *Methylobacterium* AM1 cultures containing [1-^{13}C]ethanol is shown in Figure 1A; the relative peak intensities are a clear indication that incubation with [1-^{13}C]ethanol selectively labels PQQ. The ^{13}C enrichments in PQQ based on analysis of these NMR intensities are summarized in Table I. C-1 of ethanol labels predominantly the three carboxylates (C-2′, -7′, and -9′) and carbons 5, 5a, and 9a. Obviously, the biosynthesis of PQQ does not involve the "head-to-tail" joining of acetate units characteristic of fatty acids or polyketides.[15] The predominantly singlet character of the carboxylates indicates that they are incorporated into positions in which their neighbors arise from C-2 of ethanol. Carbons 5, 5a, and 9a each yield three resonances which are the combination of a singlet from singly labeled species and doublet ($^{1}J_{C-C}$ = 60 Hz) from species labeled at C-5 and C-5a or C-9a and C-5a. The [1-^{13}C]ethanol labeling experiment coupled with the obvious structural homologies provide a working hypothesis for the biosynthetic origins of PQQ (Figure 2). We propose that glutamate provides N-6 and carbons 7′, 7, 8, 9, and 9′, while the remaining nine carbons and N-1 are donated by an amino acid from the shikimate pathway.

The precursors were identified by comparing the selective ^{13}C-labeling patterns in PQQ with those observed in amino acids. In PQQ, C-1 of ethanol significantly labels C-7′ (59%) and C-9′ (>99%) but not C-9 (<2%); similarly, C-2 of ethanol labels PQQ at C-7 (64%), C-8 (61%), and C-9 (76%) but not C-9′. These labeling patterns are essentially identical with those observed in glutamate (Table II). The incorporation of C-1 of ethanol into C-2, 5, 5a, and 9 of PQQ is equivalent to its incorporation into C-1, 3′, and 4′ of tyrosine. The adjacent labeling evident from the high degree of ^{13}C coupling at C-4′ and C-3′ in tyrosine is also observed in the orthoquinone-containing ring in PQQ. Tyrosine C-3′ and C-5′ are biosynthetically inequivalent because the aromatic ring is a product of asymmetric synthesis via the shikimate pathway;[16] C-3′ arises from ethanol C-1, whereas C-5′ arises from ethanol C-2. PQQ derived from [1-^{13}C]ethanol has adjacent ^{13}C labeling (doublets) at C-5a and C-5 or C-5a and C-9a. This labeling implies that the orthoquinone-containing ring arises from a symmetric compound (C_2 axis through C-1′ and C-4′) and predicts that C-5 and C-9a will be labeled equivalently and to an intermediate extent by both C-1 and C-2 of ethanol. Indeed, [2-^{13}C]ethanol labels C-5 and C-9a but not C-5a. This symmetric labeling pattern rules out indole as a precursor for that portion of PQQ containing the orthoquinone and pyrrole rings.

As demonstrated by Gould and co-workers,[17] the quinoline system of streptonigrin is biosynthesized by condensing three carbons of D-erythrose with 4-aminoanthranilate, a novel product of the shikimate pathway. Our data indicate that the quinoline portion of PQQ is formed by a novel condensation of N-1, C-2, -3, and -4 of glutamate with a symmetrical six-carbon ring derived from the shikimate pathway. It is most likely that tyrosine is the shikimate-derived precursor, since the pyrrole could be formed by the internal cyclization of the amino acid backbone. This is analogous to the cyclization of dopaquinone to form dopachrome.[18] Dopaquinone is a product of the oxidation of tyrosine (via dopa) in reactions catalyzed by monophenol monooxygenase (EC 1.14.18.1).

Acknowledgment. This work was supported by the DOE, OBES, Division of Biological Energy Research and by the National Stable Isotopes Resource NIH (RR 02231).

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Synthesis of a Remarkably Stable Bicyclo[7.3.1]diynene Esperamicin A_1/Calicheamicin γ System. Structural Requirements for Facile Formation of a 1,4-Diyl

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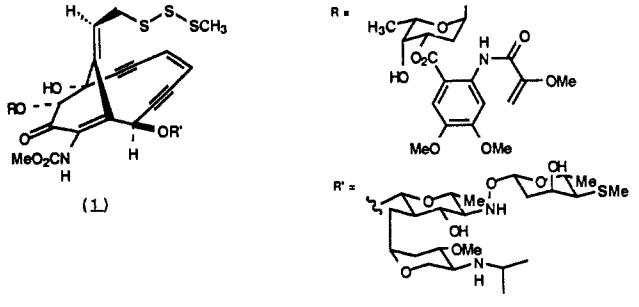
Received June 23, 1988

In our previous paper which described a model for the proposed mechanism of action of the potent antitumor agents esperamicin A_2/calicheamicin γ_1 **1**[1] we showed that oxidative decomplexation



[†] Department of Chemistry.
[‡] Molecular Structure Center.
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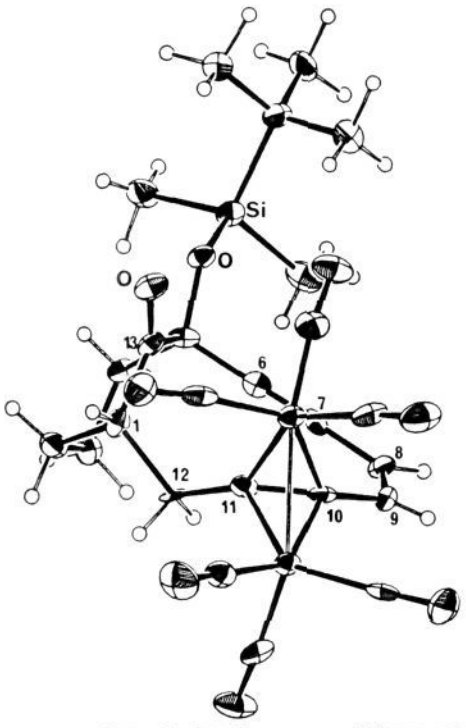


Figure 1. ORTEP of **15**. C6–C11 distance 3.39 Å[10] N.B. The C1–C12 bond is axial in **15**, whereas in **5** it is equatorial.

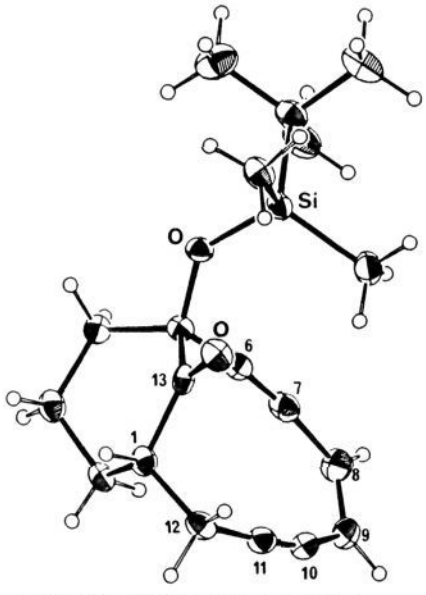
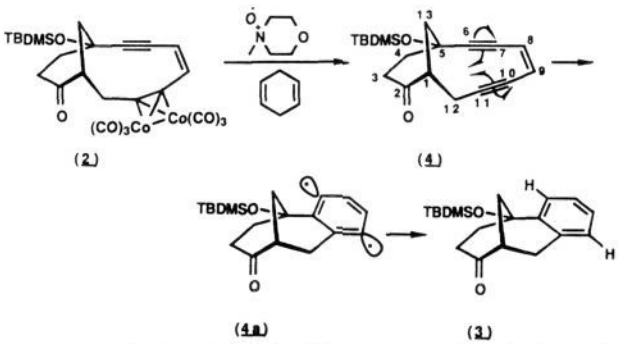
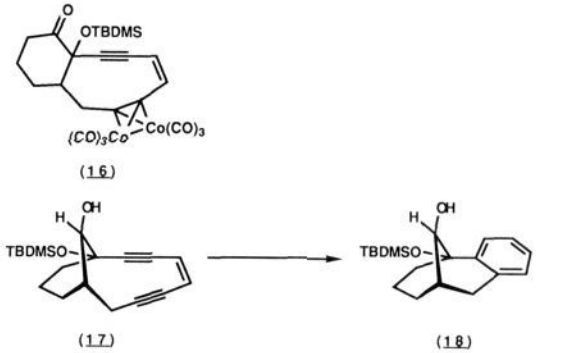


Figure 2. ORTEP of **5**. C6–C11 distance is 3.39 Å.

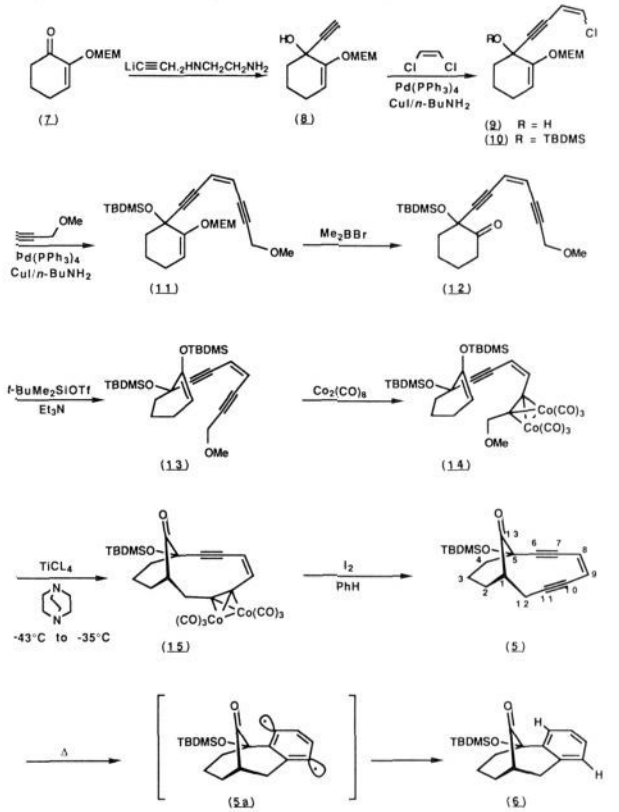
of the dicobalt hexacarbonyl adduct **2** at 20 °C in 1,4-cyclohexadiene gave the benzenoid adduct **3**.[2] We could not detect the presumed intermediate bicyclo[7.3.1]diynene **4** *nor* unambiguously exclude that **3** is formed by a cobalt-mediated process,[3]



rather than via the 1,4-diyl **4a**. Here we report that the isomeric bicyclo[7.3.1]diynene **5** is an isolable crystalline compound and is converted into the benzenoid adduct **6** in 72% yield by heating in 1,4-cyclohexadiene at 80 °C for 48 h. In contrast, the alcohol **17** rapidly (0.5 h at 20 °C) cyclized to the aromatic adduct **18**.



Treatment of cyclohexane-1,2-dione with NaH(−10 °C)/MEMCl gave **7** (80%), which was exposed to lithium acetylide ethylenediamine complex in dioxane to give **8** (74%). Coupling



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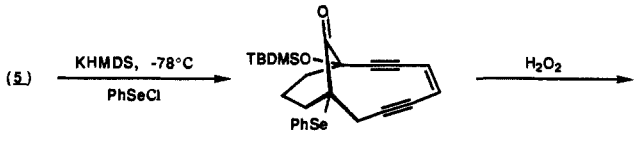
(3) The oxidative decomplexation of **2** in the presence of 1,4-cyclohexadiene gave the benzenoid adduct **3**. We could not detect the diynene **4** at 0 °C, although it was not possible to further lower the temperature, since oxidative decomplexation became too slow.

of **8** to (*Z*)-dichloroethylene to give **9** (80%) was accomplished with $Pd(PPh_3)_4/CuI/n\text{-}BuNH_2$.[4] Protection of **9** ($t\text{-}BuMe_2SiOTf/NEt_3/CH_2Cl_2$) gave **10** (70%), which was coupled to methyl propargyl ether [$Pd(PPh_3)_4/CuI/n\text{-}BuNH_2$] to give **11** (88%). Selective removal of the MEM ether from **11** using Me_2BBr[5] at -35 °C gave **12** (99%), from which the derived $t\text{-}BuMe_2Si$-ether **13** (94%) ($t\text{-}BuMe_2SiOTf/NEt_3$) was prepared. When **13** was treated with $Co_2(CO)_8$/heptane, the adduct **14** was isolated in 90% yield. Exposure of **14** to $TiCl_4$ (3.0 equiv)/DABCO (1.0 equiv)/-43 °C to -35 °C gave the bicyclo[7.3.1]ynene-10,11-dicobalt hexacarbonyl adduct **15** (50%) as a crystalline material. Figure 1 shows an ORTEP representation of **15**[6] and a small amount (ca. 10%) of the α-ketol shift isomer **16**.[7] Decomplexation of **15** using conditions (I_2/PhH) that aromatize **2** gave the 13-ketobicyclo[7.3.1]diyene **5** (70%) as a reasonably stable crystalline compound, Figure 2.[6] In going from the cobalt adduct **15** to the diynene **5** the conformation of the cyclohexanone ring changes from a chair to a boat. The bond angles C-6,7,8 and C-9,10,11 in **5** are substantially bent, 168.7° and 165.7°, respectively. In contrast, the double bond angles are 118.95° and 119.13°, which indicates that the strain in **5** is accommodated by the weak bending modes of the triple bonds.[8] When **5** was heated in 1,4-cyclohexadiene at reflux (82 °C) for 48 h, the benzenoid derivative **6** was isolated in 72% yield. This should be contrasted with its carbonyl regioisomer **4**, which could not be detected at 0 °C. Clearly, an unexpected parameter in controlling the rate of diynene cyclization to the diyl appears to be the hybridization of the bridged carbon (C-13). Reduction of the ketone **5** using DIBAL in toluene containing 1,4-cyclohexadiene at -78 °C gave the alcohol **17**, which upon standing at 20 °C for 0.5 h cyclized to the corresponding benzenoid adduct **18**. It is evident that changing C-13 from trigonal to tetrahedral geometry considerably lowers the activation barrier leading to diyl formation.

Is it possible to introduce a bridgehead double bond (C-1,2) and thus prevent diyl formation? Treatment of **5** with potassium



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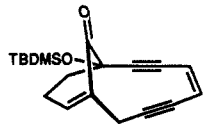

(20)

hexamethyldisilazide/THF/-78 °C, followed by phenylselenenyl chloride gave **19**. Oxidation of **19** with H_2O_2 gave **20** contaminated with **5**. Though they could not be separated by chromatography,[9] merely heating the mixture of **20** and **5** at 80 °C 1,4-cyclohexadiene converted **5** into the less polar benzenoid adduct **6** while **20** was recovered unchanged.

This study reveals that changes in hybridization at the bridging carbon (C-13) dramatically change the rate of diyl formation. We are continuing studies on the functionalization of C-12 and C-13, the role of the trisulfide trigger, and quantitative rate measurement of benzenoid formation.

Acknowledgment. The National Institutes of Health and the National Science Foundation are thanked for their financial support Dr. Jason Elliott is thanked for helpful discussions. Dr. Witold Danikiewicz is thanked for his assistance with obtaining high field NMR data.

Supplementary Material Available: Spectroscopic data (IR, ^1H and ^{13}C NMR, and HRMS) on compounds **5**, **6**, **15**, and **20** and X-ray crystallographic data on compounds **5** and **15** (11 pages). Ordering information is given on any current masthead page.

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Oxygen-17 and Molybdenum-95 Coupling in Spectroscopic Models of Molybdoenzymes

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Received May 9, 1988

Recent reports[2-4] of the generation of *cis*-[$Mo^VO(OH)$] centers in solution support the presence of such sites in the ESR-active[5] low pH forms of sulfite oxidase[6] and nitrate reductase[7] and in the inactive "slow" form of xanthine oxidase.[5,8] In addition, assignment of [Mo^VOS] and *cis*-[$Mo^VO(SH)$] centers in active xanthine oxidase (very rapid and rapid ESR signals)[5,8] is supported by generation[4] of those species in solution.

The most direct evidence for the structural assignments of the synthetic species is the observation of ligand hyperfine coupling to (a) a single proton in each species,[2-4], (b) a single oxygen atom ($a(^{17}O)$, 2.0×10^{-4} cm^{-1}) in *cis*-[$MoO(SH)L^a$] (L^aH_2 = (*o*-$HS\text{-}C_6H_4\text{-}NMe\text{-}CH_2\text{-})_2$),[4] and (c) two inequivalent oxygen atoms ($a(^{17}O)$, 7.5 and 2.3×10^{-4} cm^{-1}) in *cis*-[$MoO(OH)L^a$][4]. The reactive synthetic species have yet to be isolated in substance, and it is essential to corroborate the structural assignments.

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