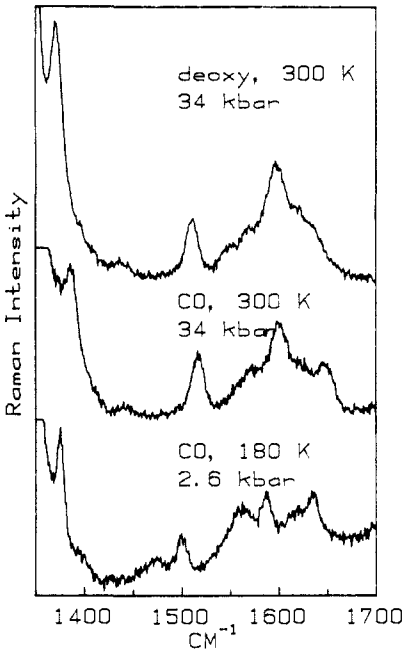

Figure 2. Resonance Raman spectra of deoxy-Hb prepared as in Figure 1 and CO-Hb, prepared by concentrating Hb solution with a stream of CO, at the pressures and temperatures noted. The apparatus has been described previously.[2,7] The 4579-Å line of a Spectra Physics 171 Ar ion laser operating at 50–100 mW was used as the excitation source. The spectral resolution was 10 cm^{-1} and 25–50 scans at 2 cm^{-1}/s were averaged. The large feature at 1335 cm^{-1} is the first-order Raman mode of diamond.

coordination of the distal histidine residue in deoxy-Hb at elevated pressures acts to prevent structural relaxation of the distal heme pocket when the pressure is released. Surprisingly, earlier studies of oxidized heme proteins showed no evidence of precipitation.

Acknowledgment. We are indebted to W. H. Woodruff for helpful comments. This work was performed under the auspices of the U.S. Department of Energy. The support of the NIH (GM33330) and Research Corporation to M.R.O. are also acknowledged.

Synthetic Approaches to Molecules with Sterically Hidden Functional Groups. 2. Bicyclo[8.8.2]eicosa-1(19),10(20),19-triene: The First Bicyclic Cumulatriene[1]

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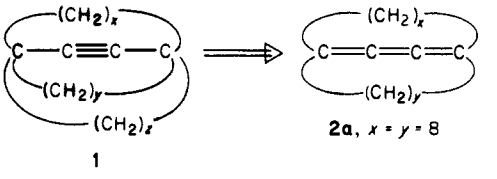
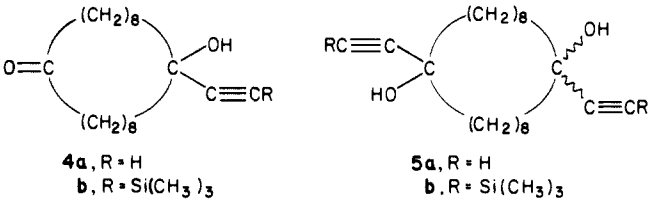
Recently we described a general method for the preparation of cumulatrienes from allenic phosphonates through a variant of the Horner–Emmons–Wittig reaction.[2] For some time, we have been exploring possible synthetic routes to tricyclic alkynes of structure **1**,[3] and retrosynthetic analysis suggested that $[\pi 6_s + \pi 4_s]$ cycloaddition[4] of (*Z*)-1,3,5-hexatriene to bicyclic cumulatriene **2** might lead to the desired ring system. We now wish to report the successful synthesis of the title compound, **2a**.

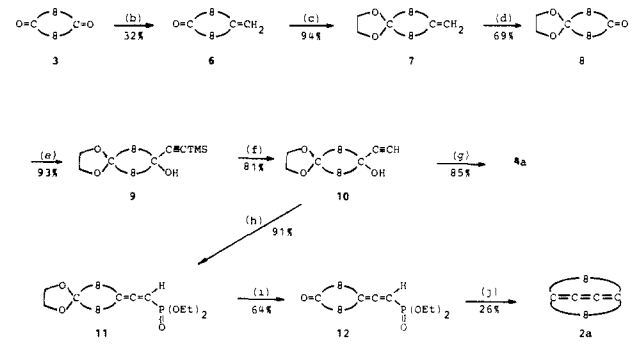


Our approach, which utilized 1,10-cyclooctadecanedione (**3**)[5] as starting material, is outlined in Scheme I. Initial attempts to prepare monoadduct **4** by the addition of one mole equivalent of acetylide to **3** led instead to diadduct **5**, with 41% of **3** recovered. Similar results were obtained during attempts to prepare the monoketal of **3**,[5] even though a 1:2:1 ratio of **3**/mono adduct/diadduct is statistically predicted in both cases.[6] Through some type of intramolecular interaction (dipole–dipole?), the reactivity of one carbonyl is *retarded* by the presence of the second.[7] However, once one of the groups suffered attack, the second is left "unprotected".



Considerable exploratory work revealed that a Wittig reaction with **3** gave the highest yields of a monoadduct.[8] The resulting enone **6** was subjected to ketalization then ozonolysis to provide the desired monoketal **8**. Addition of trimethylsilyl acetylide and removal of the Me_4Si group gave **10**, which incidently provided **4a** upon hydrolysis. Treatment of propargyl alcohol **10** with chloro diethyl phosphite,[9] followed by gentle hydrolysis, provided allenic

Scheme I[a]



[a] (a) 8 = $(CH_2)_8$; (b) 1.4 mol equiv of $Ph_3P{=}CH_2$ in THF, reflux 20 h; (c) 4.0 mol equiv of glycol, catalytic *p*-TsOH in benzene, reflux 105 min; (d) 1. CH_2Cl_2 solution, excess O_3 at −78 °C; 2. $(PhO)_3P$ workup; (e) 2.0 mol equiv of $Me_3SiC{\equiv}CLi$ (from $Me_3SiC{\equiv}CH$ and *n*-BuLi) in THF, 0 °C, 4 days; (f) 3.5 mol equiv of $AgNO_3$, 1.3 equiv of KCN in EtOH/H_2O (2/1 v/v), 1 h at 25 °C; (g) THF solution, 9% (v/v) 1 N HCl, 2 days at 25 °C; (h) 1.3 mol equiv of $(EtO)_2PCl$, 2.0 mol equiv of pyridine in CH_2Cl_2, 48 h at 25 °C; (i) THF/H_2O (8/1 v/v), 1 equiv of HCl, 60 h at 25 °C; (j) 1.0 equiv of LDA, THF, 60 °C, 4.5 days.

(1) Abstracted from: Hemling, T. C. Ph.D. Dissertation, University of Cincinnati, 1985. Parts of this work have been described: Joint Great Lakes and Central Regional Meeting of the American Chemical Society, Kalamazoo, MI, May 24, 1985. Abstr. 309. Hemling, T. C.; Macomber, R. S. *Abstr. Pap.—Am. Chem. Soc.* **1985**, *189th*, ORGN180.

(2) Macomber, R. S.; Hemling, T. C. *Isr. J. Chem.* **1985**, *26*, 136.

(3) Macomber, R. S.; Bauer, D. P. *J. Chem. Res.* Synop. **1978**, 194; *J. Chem. Res. Miniprint* **1978**, 2469.

(4) Houk, K. N.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 4145.

(5) Blomquist, A. T.; Spencer, R. D. *J. Am. Chem. Soc.* **1948**, *70*, 30. Blomquist, A. T.; Prager, J.; Wolensky, J. *J. Am. Chem. Soc.* **1955**, *77*, 1804.

(6) Macomber, R. S.; Bopp, T. T. *Synth. Commun.* **1980**, *10*, 767.

(7) However, the ^{13}C spectrum of **3** in CD_2Cl_2 solution at −90 °C showed no increase in multiplicity beyond the original five lines: δ 213.6, 42.3, 29.5, 28.2, 24.3.

(8) Unless otherwise noted, all compounds were purified by flash chromatography (Still, C. W.; Kahn, M.; Miltra, A. *J. Org. Chem.* **1978**, *43*, 2923) on 230–400 silica gel, providing products that were homogeneous by TLC and exhibited satisfactory spectral data (see supplementary material).

(9) As a leading reference, see: Macomber, R. S.; Krudy, G. A.; Seff, K.; Diaz-Miron, L. E. R. *J. Org. Chem.* **1983**, *48*, 1425.

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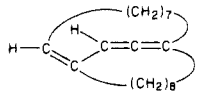
ketone **12**. Treatment of **12** with 1 equiv of LDA gave the title compound **2a** in modest yield.

The high symmetry of **2a** is obvious from its six-line ^{13}C spectrum,[10] while its other spectra were consistent with typical acyclic cumulatrienes.[2] The reaction is quite sensitive to adventitious oxygen and can be conveniently monitored by the product's UV band at 268 nm. Because varying amounts of **12** could be reisolated from the product mixture, we expect that the reaction can be pushed further toward completion. The fact that the cyclization takes place at all indicates that enolate formation from **12**, if it occurs, does not preclude the competing intramolecular cyclization. Although a few monocyclic cumulatrienes are known,[11] **2a** represents the first bicyclic one.

Acknowledgment. We thank the Research Council of the University of Cincinnati for a grant which partially supported this work. T.C.H. further acknowledges a fellowship from the Research Associates Program of the Department of Chemistry.

Supplementary Material Available: Spectroscopic data (^1H and ^{13}C NMR, IR, and mass spectral) are provided for compounds **4a**, **5b**, **6–12** (2 pages). Ordering information is given on any current masthead page.

(10) Analytical data for **2a**: semisolid, mp 82–92 °C; ^{13}C NMR δ 159.59, 115.29, 34.25, 25.24, 24.85, 23.57; ^1H NMR δ 2.4–2.0 (m, 8 H), 1.8–1.0 (m, 24 H); IR 2930, 2860, 2340, 1460, 1445, 1260, 1050, 810, 660 cm^{-1}; UV λ_{max} 268 nm (log ϵ 4.2); MS 272.2517 (calcd for $C_{20}H_{32}$ 272.2550, 55%), 175 (70%), 161 (32%), 147 (49%), 133 (64%), 119 (76%), 105 (92%), 91 (100%). Samples of **2a** prepared by this route contained about 10% of a more polar unidentified side product, which was apparent from a shoulder on the otherwise homogeneous HPLC peak for **2a**. Differential scanning UV detection indicated that the shoulder has a λ_{max} at 236 nm and is nearly transparant at 268 nm. One possible structure for this compound would be enallene i; an



analogous product has been formed from an acyclic cumulatriene.[2] No conditions were found to completely separate **2a** from this side product.

(11) Angus, R. O., Jr.; Johnson, R. P. *J. Org. Chem.* **1984**, *49*, 2880. Kaneda, T.; Minumi, S.; Negi, T.; Sakata, Y. *Chem. Lett.* **1972**, 703. Moore, W. R.; Ozretich, T. M. *Tetrahedron Lett.* **1967**, 3205.

Biosynthesis of Methanopterin in *Methanobacterium thermoautotrophicum*

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Methanogenic bacteria generate cell mass by the reductive assimilation of CO_2 and energy by the reduction of CO_2 to methane. Consequently, the transformation of one-carbon intermediates is a dominant aspect of their metabolism. For this purpose, the organisms use a variety of unusual cofactors such as methanoproterin, methanofuran, and the coenzymes designated F_{420}, F_{430}, and CoM.[1]

The structure of methanopterin (Figure 1)[2,3] has been determined recently by spectroscopic techniques and by chemical degradation. The structural similarity of methanopterin with folic acid is apparent and corresponds to the close functional homology of the two compounds.

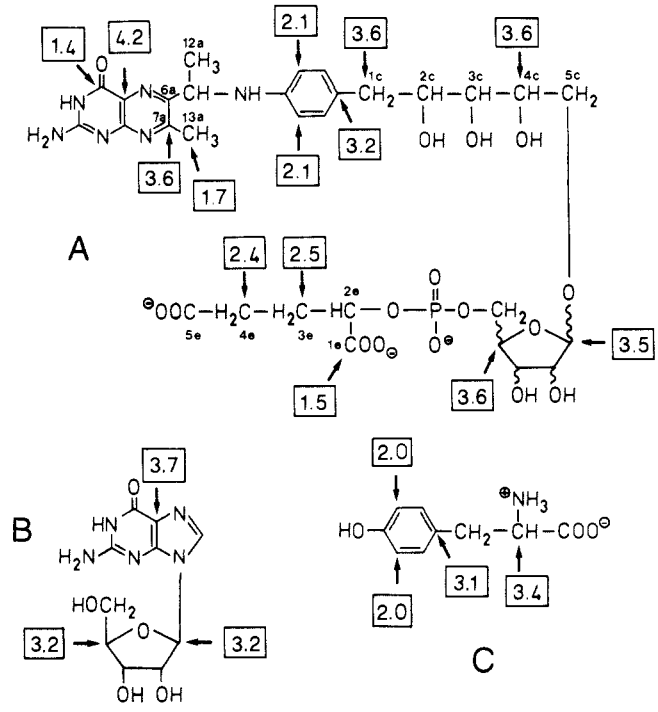

Figure 1. Relative ^{13}C abundances (numbers in boxes) in methanopterin (A), guanosine (B), and tyrosine (C). Carbon atoms without numbers had relative abundances of 0.8–1.2.

The biosynthesis of folic acid has been studied in close detail.[4] Starting from GTP, a ring expansion with inclusion of side chain carbon atoms 1′ and 2′ into the newly formed pyrazine ring leads to dihydroneopterin triphosphate. An aldolase type cleavage of the side chain and the introduction of 4-aminobenzoyl glutamate yields dihydrofolate.

The biosynthesis of methanopterin has not been studied to date. We have found that ^{13}C-labeled acetate can serve as a precusor for biosynthetic studies in *Methanobacterium thermoautotrophicum*.[5] The organism was cultured in 14 L of minimal medium containing 5 mM [1-^{13}C]acetate under an atmosphere of H_2/CO_2 (80:20, v/v). The cells (99 g wet weight) were extracted with 50% aqueous acetone. Methanopterin was isolated from the cell extract by anion exchange chromatography (QAE Sephadex) followed by preparative HPLC yielding approximately 230 μmol of product. In addition, tyrosine and guanosine were isolated as described earlier.[5]

^{13}C NMR spectra of the isolated compounds were recorded at 7.1 T with a Bruker WM300 spectrometer. Methanopterin was measured in D_2O at an apparent pH of 10.4 (uncorrected glass electrode reading). Tyrosine was measured in Me_2SO-d_6 and guanosine in D_2O at neutral pH. Relative ^{13}C abundances were calculated for each carbon atom of the compounds studied by comparison with natural abundance spectra.

^1H and ^{13}C NMR assignments for methanopterin have been reported.[2] These assignments were based on two-dimensional ^1H–^1H and ^1H–^{13}C J-correlation spectra. We have reinvestigated the crowded region between 3.6 and 4.1 ppm in the ^1H NMR spectrum and the correlation of these signals to the ^{13}C NMR resonances. From COSY, ^1H–^{13}C-relayed coherence, ^1H–^{13}C J correlation, and DEPT spectra it was concluded that the assignments for H-4c and one of the H-5c protons need to be interchanged. This revision of the ^1H NMR assignments leads to a reversal of the ^{13}C NMR assignments of C-2c and C-4c of the

(1) Daniels, L.; Sparling, R.; Sprott, G. D. *Biochim. Biophys. Acta* **1984**, *768*, 113.

(2) Van Beelen, P.; Stassen, A. P. M.; Bosch, J. W. G.; Vogels, G. D.; Guijt, W.; Haasnoot, C. A. G. *Eur. J. Biochem.* **1984**, *138*, 563.

(3) Van Beelen, P.; Labro, J. F. A.; Keltjens, J. T.; Geerts, W. J.; Vogels, G. D.; Laarhoven, W. H.; Guijt, W.; Haasnoot, C. A. G. *Eur. J. Biochem.* **1984**, *139*, 359.

(4) Brown, G. M.; Williamson, M. *Adv. Enzymol.* **1982**, *53*, 345.

(5) Le Van, Q.; Schwarzkopf, B.; Bacher, A.; Keller, P. J.; Lee, S.; Floss, H. G. *J. Am. Chem. Soc.*, in press.

(6) Stupperich, E.; Fuchs, G. *Arch. Microbiol.* **1984**, *139*, 8.

0002-7863/86/1508-0344$01.50/0