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.

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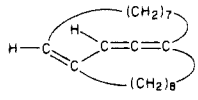
Supplementary Material Available: Spectroscopic data (^1H and ^{13}C NMR, IR, and mass spectral) are provided for compounds **4a**, **5b**, **6–12** (2 pages). Ordering information is given on any current masthead page.

(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



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

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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

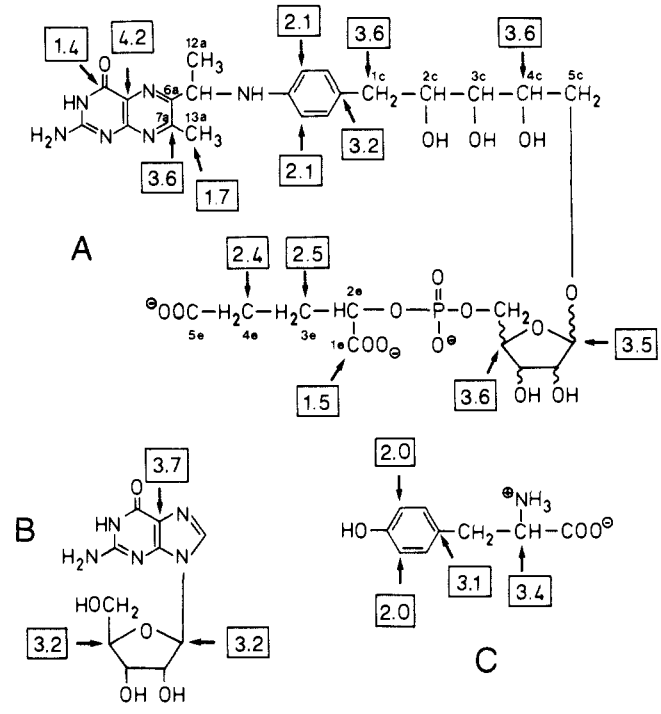

Figure 1. Relative ^{13}C abundances (numbers in boxes) in methanopterin (A), guanosine (B), and tyrosine (C). Carbon atoms without numbers had relative abundances of 0.8–1.2.

degradation. The structural similarity of methanopterin with folic acid is apparent and corresponds to the close functional homology of the two compounds.

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

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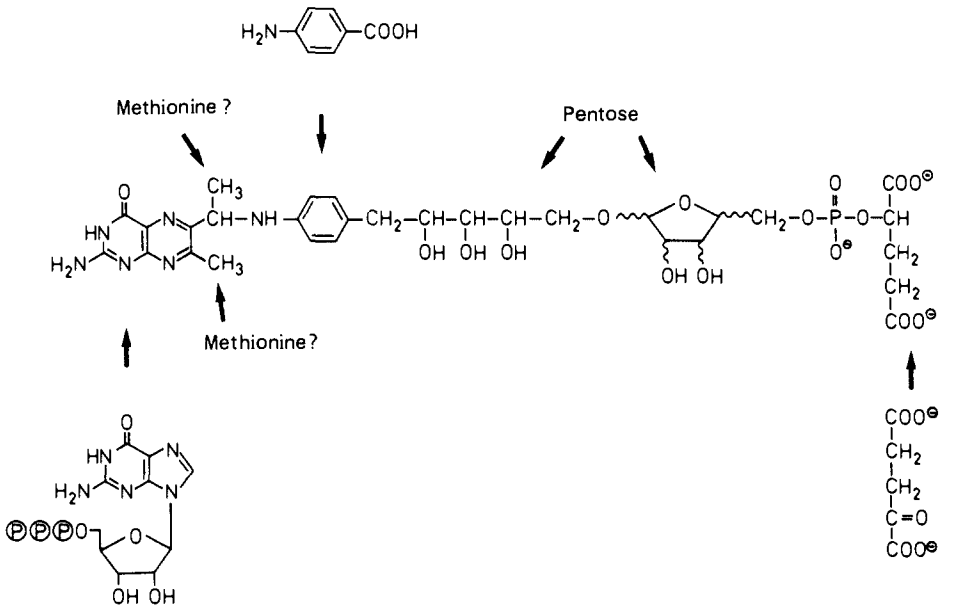

Figure 2. Hypothetical building blocks of methanopterin.

pentityl side chain of the aniline moiety. With these assignments at hand, we obtained the relative ^{13}C abundances shown in Figure 1 (numbers in boxes). Carbon atoms without designation had relative abundances of 0.8–1.2. It is immediately apparent that the metabolism of acetate in *M. thermoautotrophicum* proceeds almost without randomization of the isotope in agreement with our earlier findings.[5]

Comparison of guanosine and methanopterin shows that, as expected, the pentose moieties have exactly the same labeling patterns in both molecules. The labeling patterns of the heterocyclic moieties confirm that the pyrimidine ring of methanopterin is derived from a purine precursor in analogy with the biosynthesis of folic acid and unconjugated pteridines.

The origin of the pyrazine ring in methanopterin is less obvious. A ring expansion in analogy to folate biosynthesis would correctly predict the labeling of position 7a which should be derived from the labeled C-1′ of a purine nucleotide, presumably GTP. This would imply that the methyl group at C-7a is introduced into the pteridine system by secondary methylation, e.g., from methionine as donor.

We can rule out the possibility that both carbon atoms of the alkyl side chain at C-6a are derived from the ribose moiety of the purine nucleotide, with removal of the terminal carbon, because C-12a, which would then originate from the labeled C-4 of a pentose, is not enriched. We propose that the pyrazine moiety is formed by the classical ring expansion, and the C-6 substituent is subsequently either shortened by two carbon atoms or removed entirely, with subsequent introduction of one or two carbon atoms from a hitherto unknown source. This question requires additional experimentation.

A comparison of the aniline moiety of methanopterin with tyrosine shows a very similar labeling pattern clearly suggesting that the aromatic ring is derived from the shikimate pathway. It is also apparent that the ^{13}C-enriched side-chain atom C-1c is not derived from either the carboxyl group of shikimic acid or C-3 of pyruvate, via chorismate, since both should be unlabeled. On the other hand, the labeling pattern of the pentityl side chain is in full agreement with that of a pentose. We therefore suggest that the pentitylaniline moiety arises from the ring carbon atoms of a shikimate derivative, e.g., 4-aminobenzoate, and a pentose chain incorporated in its entirety. Mechanistic precedent for such an origin exists, e.g., in the formation of indoleglycerol phosphate from phosphoribosylanthranilate in tryptophan biosynthesis. The suggested pathway again requires confirmation by additional experiments.

Finally, the labeling pattern of the hydroxyglutarate moiety is exactly as expected if this moiety is formed by reduction of 2-ketoglutarate. Ketoglutarate should originate from acetate by three steps of reductive carboxylation via pyruvate, oxaloacetate, and succinate. The symmetry of succinate would lead to the equal distribution of the isotope between positions 3e and 4e.

The above results establish most of the building blocks of methanopterin as summarized in Figure 2.

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Nucleophilic Catalysis of the Migratory Insertion of Carbon Monoxide. Evidence for a Dissociative Trapping Mechanism

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Migratory insertion of carbon monoxide into transition-metal alkyl bonds constitutes one of the most fundamental and extensively investigated reactions in organometallic chemistry and homogeneous catalysis.[1] Several earlier studies have described marked enhancements of the rates of such migratory insertion reactions by nucleophilic solvents or catalysts (S) and have interpreted these enhancements in terms of the mechanistic scheme of eq 1 and 2.[2,3] This interpretation ascribes the catalytic influence

$$L_nM(CO)R + S \rightleftarrows L_nMC(=O)R(S) \quad (1)$$

$$L_nMC(=O)R(S) + L' \rightarrow L_nL'MC(=O)R + S \quad (2)$$

$$L_nM(CO)R + L' \xrightarrow{S} L_nL'MC(=O)R \quad (3)$$

of the nucleophile S to stabilization of the coordinatively unsaturated intermediate that is generated by the migratory insertion

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