The overall stereochemical outcome of going from chiral [^{1}H,^{2}H,^{3}H]-N_5-methyl tetrahydrofolate to acetate has also recently been determined to be retention,[23] supporting the above sequence.

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Stereochemistry of Acetic Acid Formation from 5-Methyltetrahydrofolate by *Clostridium thermoaceticum*

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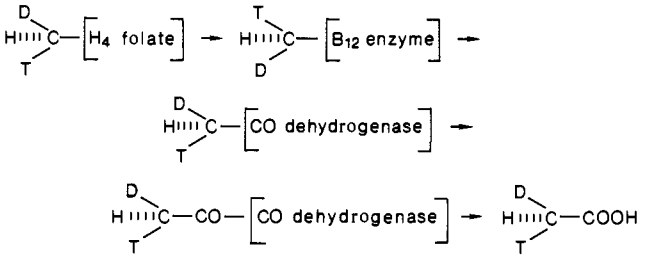
Some acetogenic bacteria ferment hexoses almost stoichiometrically to 3 mol of acetic acid per 1 mol of sugar.[1,2] Two of the three moles of acetic acid arise by decarboxylation of pyruvate formed via the Embden-Meyerhof pathway, and the third mole, remarkably, is produced reductively from 2 mol of CO_2.[3,4] Thus, an organism like *Clostridium thermoaceticum* can synthesize acetic acid entirely from CO or from $CO_2 + H_2$.[5] One mole of CO_2 is reduced via formate, 5,10-methenyltetrahydrofolate, and 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate,[6,7] and another mole is reduced to CO.[8] These two products condense in the presence of coenzyme A to produce acetyl-CoA, which then gives acetate with formation of 1 mol of ATP. The formation of acetyl-CoA from methyltetrahydrofolate requires several enzymes, including CO dehydrogenase,[9,10] a B_{12} enzyme,[11-13] and a methyltetrahydrofolate: B_{12} methyltransferase.[13,14]

The role of CO dehydrogenase was originally thought to be limited to the reduction of CO_2 to CO,[13] and it was assumed that the methyl-B_{12} species might give rise directly to the acetyl group,[8,11-14] by either a carboxylation or a carbonylation of the methyl-cobalamin. Recent studies by Wood and co-workers, however, point to a far more central role of CO dehydrogenase in this process, as the enzyme on which the actual assembly of acetyl-CoA from CoASH, CO, and a methyl group takes place.[5] The most critical piece of evidence is the finding that purified CO dehydrogenase alone is capable of catalyzing exchange between CO and the carbonyl group of acetyl-CoA.[15] This exchange requires that the enzyme can bind the methyl, the carbonyl, and the CoA group of acetyl-CoA. Binding of CO to CO dehydrogenase generates a paramagnetic nickel-iron-carbon center,[16] and EPR studies have shown that acetyl-CoA and CoA bind near this center.[15] This suggests the possibility that the acetyl group may be formed by a "carbonyl insertion" reaction on nickel or iron.

Scheme I. Stereochemical Fate of the Methyl Group in the Conversion of Methyltetrahydrofolate to Acetic Acid by *C. thermoaceticum*



It should be possible to test further the mechanism proposed by Wood and co-workers based on stereochemistry. Intermolecular methyl transfers proceed with inversion of configuration.[17,18] Chemically catalyzed carbonyl insertion, more properly migratory insertion,[19] reactions proceed with retention of alkyl group configuration.[20-23] Wood's mechanism involves two methyl transfers, one from methyltetrahydrofolate to B_{12} and another from methyl-B_{12} to CO dehydrogenase, and an insertion reaction; the predicted overall stereochemical outcome would be net retention of methyl group configuration, if retention of configuration is assumed for the CO dehydrogenase catalyzed insertion reaction, too. A process involving carbonylation on the cobalt of B_{12}, on the other hand, would only include one methyl transfer and the insertion reaction, and should thus proceed with net inversion of methyl group configuration.[24]

To examine this question we incubated (*methyl-R*)- and (*methyl-S*)-[methyl-^{2}H$_1$,^{3}H]methyltetrahydrofolate (1.0×10^6 and 1.15×10^5 dpm of ^{3}H, respectively, in less than 0.1 μmol), prepared by sequential reduction of 5,10-methenyltetrahydrofolate with deuteriated and tritiated sodium borohydride,[26] anaerobically under a CO atmosphere for 30 min at 55 °C with a cell-free extract of *C. thermoaceticum* in the presence of 0.1 mM CoASH, 4 mM ATP, 1.4 mM Fe^{2+} 16 mM dithiothreitol, and 90 mM potassium phosphate, pH 6.0.[14] Following denaturation with $HClO_4$, acetic acid was isolated, with carrier dilution, in 17.4% and 23.5% yield, respectively, by passage of the reaction mixture through a column of 8 mL of Dowex 50 H^+, neutralization and

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evaporation of the effluent, and steam distillation of the acidified residue. The acetic acid samples were subjected to chirality analysis by the method of Cornforth *et al.*[27] and Arigoni and co-workers,[28] using the procedure described by Floss and Tsai.[29]

The samples of chiral methyl methyltetrahydrofolate had been degraded in earlier work[26] and found to contain 44% ee (*R*)-methyl groups and 37% ee (*S*)-methyl groups, respectively. The acetic acid obtained from the (*R*)-methyltetrahydrofolate gave an *F* value[17] of 59.5, corresponding to 33% ee *R* configuration of the methyl group. Analysis of a sample from a second incubation with the same substrate gave *F* equals 58.3% or 29% ee *R* configuration. The acetic acid generated from (*S*)-methyltetrahydrofolate in two independent analyses gave *F* values of 37.2 and 37.2, corresponding to 44% ee *S* configuration of the methyl group.[30] It follows that the methyl group of methyltetrahydrofolate is converted by *C. thermoaceticum* into the methyl group of acetic acid with overall retention of configuration. This result argues against acetyl group formation directly from the B_{12} enzyme but is consistent with the mechanism proposed by Wood and collaborators[5,15] involving transfer of the methyl group from methyltetrahydrofolate to B_{12} and then to CO dehydrogenase followed by carbonylation on the latter (Scheme I).

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(30) *F* values in our assay are accurate to ±2; the *F* values of precursor and product are the same within the limits of accuracy of the assay.

Synthesis of [4.4.4]Propellahexaene

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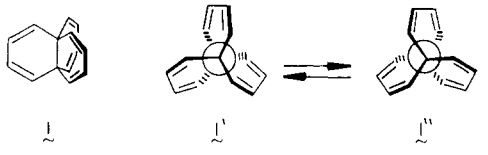
Two limitations have severely restricted synthetic access to [4.4.4]propellahexaene (**1**) and its derivatives. Due to proximity

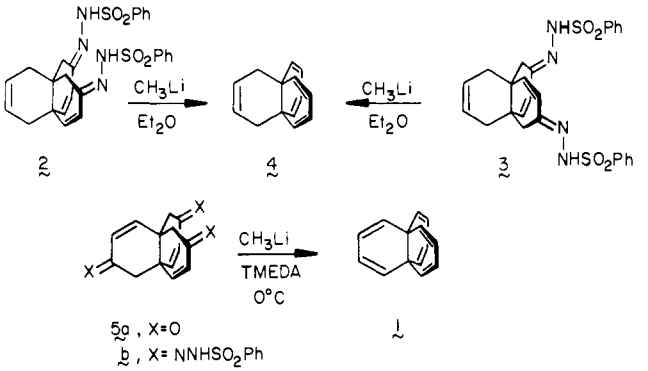


1 **1′** **1″**

factors, intraring reactions are not easily inhibited.[1] Neither are cationic rearrangements capable of profoundly reorganizing the carbocyclic framework readily circumvented.[2] One fundamental property of the maximally unsaturated D_3-symmetric hydrocarbon **1** and its derivatives relates to the unusual arrangement of the constituent six-membered rings, which radiate as blades from a common axis (see **1′** ⇄ **1″**). Extensive theoretical[3] and experimental[4] discussion attests to the general interest attached to this dynamical conformation question.

The present purpose is to detail the first synthesis of **1** and to call attention to unprecedented reactions that can materialize upon the attempted preparation of **1** substituted with methoxyl groups. To construct **1**, the pitfalls alluded to above had to be consciously avoided and reactions promising little or no risk of intramolecularity implemented.

In order to evaluate the possible merits of the Shapiro reaction,[5] a mixture of the bis(phenylsulfonyl)hydrazones **2** and **3** was prepared and treated with methyllithium in ether (0 °C → room temperature). Following the identification of **4** as product, the previously described [4.4.4]propellatrienetrione **5a**[4a] was similarly transformed almost quantitatively into **5b** (mp 195 °C). Base-induced elimination of benzenesulfinic acid and nitrogen from **5b** (CH_3Li, TMEDA, 0 °C) expediently gave the desired **1** (colorless needles, mp 48 °C, 15% isolated). To our knowledge, this reaction sequence represents only the second time a triple-Shapiro degradation has been deployed in a synthetic strategem.[6]



The NMR spectra of **1** reflect its D_3 symmetry. In $CDCl_3$ at 500 MHz, the vinyl protons appear as an AA′XX′ pattern with A centered at δ 5.91 and X at δ 5.27. Its three carbon types resonate in $CDCl_3$ at 129.44, 122.65, and 34.35 ppm. The electronic spectrum (in isooctane) is characterized by several maxima: 234 (ε 25 730), 242 (24 100), 251 (27 780), 271 (9560), 279 (7615), and 291 nm (6975).

The hexaene is remarkably stable in air. After heating bromobenzene-d_5 solutions of **1** at 105 °C for 4.5 h, the ^1H NMR spectrum remained unaltered. An increase in temperature to 150 °C caused gradual decomposition to unidentified *nonaromatic* products ($t_{1/2} \approx 5$ h). We cannot exclude the possibility that this destruction was catalyzed by traces of acid.

In an attempt to produce **9**, triketone **6**[4a] was treated sequentially with excess potassium *tert*-butoxide in dry DMF and dimethyl sulfate,[7] all at 0 °C. Chromatography of the resulting mixture on basic alumina afforded the dimethoxy pentaenone **7** (31%),[8] 2,7-dimethoxynaphthalene (**8**, 3.4%), and trace quantities of **9**, which were visible only in the ^1H NMR spectrum of the unpurified product. Complex mixtures were obtained when **7** was resubmitted to the original reaction conditions or to the action of $KN(SiMe_3)_2/THF-Me_2SO_4$ at low temperatures. In contrast, the combination of KH in anhydrous DMF (0 °C) and Me_2SO_4 gave rise to **8** and **9** in a 17:1 ratio (^1H NMR analysis).[9] Since

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(8) ^1H NMR (300 MHz, $CDCl_3$) δ 6.27 (d, *J* = 10 Hz, 1 H), 6.02 (d, *J* = 10 Hz, 1 H), 5.92 (dd, *J* = 9.7, 2.1 Hz, 2 H), 5.51 (d, *J* = 9.7 Hz, 2 H), 4.29 (d, *J* = 2.1 Hz, 2 H), 3.51 (s, 6 H), 2.48 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 199.23, 152.33, 144.32, 128.85, 127.64, 124.50, 99.45, 54.38, 46.87, 42.13, 41.08; MS, *m/z* (M^+) calcd 256.1099, obsd 256.1109.
(9) (a) The IR and ^1H NMR spectra of **8** (mp 137-139 °C) were identical with those of a commercial sample (Aldrich). (b) For **9**: ^1H NMR (300 MHz, C_6D_6) δ 5.99 (dd, *J* = 9.8, 2.1 Hz, 3 H), 5.19 (d, *J* = 9.8 Hz, 3 H), 4.35 (d, *J* = 2.1 Hz, 3 H), 3.23 (s, 9 H); MS, *m/z* (M^+) calcd 270.1256, obsd 270.1235.

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