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**Structure and Methylation of Coenzyme M (HSCH₂CH₂SO₃)**

C. D. Taylor and R. S. Wolfe

The first description of the structure of a novel coenzyme from a methanogen, this work reinvigorated the search for new coenzymes in procaryotes. Since then, the structures of five more novel coenzymes have been described in methanogens. These include the deazaflavin coenzyme F₄₂₀, the nickel tetapyrrole coenzyme F₄₃₀, tetrahydromethanopterin, methanofuran, and 7-mercaptoheptanoyl threonine phosphate (component B). Two of these, coenzyme F₄₂₀ and tetrahydrodromethanopterin, were subsequently shown to be more widely distributed among the procaryotes. Similarly, novel coenzymes have been discovered in methanotrophic and other bacteria.

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Structure and Methylation of Coenzyme M (HSCH₂CH₂SO₃)∗

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SUMMARY

Coenzyme M is a recently discovered cofactor which is involved in methyl transfer reactions in Methanobacterium. Information derived from infrared, proton NMR, and ultraviolet spectroscopy as well as from chemical tests and quantitative elemental analysis reveals that the coenzyme is 2,2'-dithiodiethanesulfonic acid. Verification of this structure resides in the comparison of authentic with chemically synthesized 2,2'-dithiodiethanesulfonic acid. Evidence indicates that an active form of this cofactor is 2-mercaptoethanesulfonic acid which is methylated producing 2-(methylthio)ethanesulfonic acid; this derivative is subsequently reductively demethylated, yielding methane.

Recent studies of methane formation have been devoted to methyltransfer reactions in Methanobacterium (1-3) and Methanosarcina (4-6). When cell extracts of Methanobacterium strain M. o. H. were subjected to anaerobic dialysis, they were resolved for a heat-stable organic cofactor, coenzyme M (CoM), which was required for methane formation from methylcobalamin (1). Two enzyme reactions were defined:

\[
\begin{align*}
\text{anaerobic conditions} & \quad \text{electron donor} & \quad \text{methyltransferase} & \quad \text{methyl acceptor} \\
\text{CH₃-BH₃ + CoM} & \quad \text{H₃} & \quad \text{CH₃-CoM + BH₃} \quad \text{(1)} \\
\text{anaerobic conditions} & \quad \text{H₂, ATP, Mg²⁺} & \quad \text{methylreductase} & \quad \text{CH₄ + CoM} \\
\end{align*}
\]

Reaction 2, catalyzed by methylreductase, was specifically inhibited by triphosphopyridine and measurement of the radioactivity trapped in [methyl-¹⁴C]CoM served as an assay for CoM (1). Subsequently, sodium borohydride was found to be an excellent electron donor for Reaction 1, and use of 100-fold purified methyltransferase eliminated methyl reductase activity (7). We present here the purification, structural identification, and chemical synthesis of three biologically active forms of CoM. A preliminary report has appeared (8).

EXPERIMENTAL PROCEDURE

Materials—Methylcobalamin was chemically synthesized by the method of Müller and Müller (9); salts were removed by phenol extraction (10). The preparation was eluted from a water-equilibrated column of SP-Sephadex C-25 (ammonium form) with a 0 to 0.1 m linear ammonium acetate gradient. Ammonium acetate was removed by lyophilization. [methyl-¹⁴C]Methylcobalamin was prepared as described by Wood et al. (11) and the preparation was purified by cation exchange chromatography as outlined above. The concentration of each preparation was determined spectrophotometrically (12). Methyl iodide and sodium 2-bromothi-ethanesulfonate were purchased from Aldrich Chemical Co.; [¹⁴C]Methyl iodide from Amersham-Searle; cyanocobalamin and sodium borohydride from Sigma Chemical Co.; deuterium oxide (99.8% isotopic purity) from Diaprep, Inc.; and sodium 3-tri-methylsilylpropionate-2,2,3,3-d₄ (TSP) from Merck, Sharp, and Dohme of Canada, Ltd. Methylmercaptan and argon were obtained from Union Carbide Corp.; QA (quaternary aminoa)-Sephadex A-25, SP-Sephadex C-25, and Sephadex G-10 from Pharmacia Fine Chemicals, Inc.; and AG 50W-X4 (200 to 400 mesh) cation exchange resin (H⁺ form) from Bio-Rad Laboratories. Methanobacterium strain M. o. H. was mass cultured and cells were stored as previously described (1,7).

Synthesis of Ammonium S-Mercaptoethanesulfonate (HS-CoM)—Sodium 2-bromothiethanesulfonate monohydrate (4.1 g) was dissolved in a 40-mole excess of concentrated ammonium hydroxide, and the solution was rapidly saturated with hydrogen sulfide gas. The mixture was stirred for 3 hours with a slow stream of hydrogen sulfide bubbling through the mixture. Precautions were taken to prevent entrance of air into the reaction mixture. The following steps were performed as quickly as possible to prevent autooxidation of the thiol. The ammonium hydroxide and volatile ammonium sulfide were removed by flash evaporation. The solid residue was dissolved in water and applied to a QAE-Sephadex A-25 (acetate form) column (5 x 20 cm) that had been equilibrated with water. HS-CoM was separated from the bromide salts by elution with a linear 0 to 3 m ammonium acetate gradient. The fractions which contained the thiol were located by reacting small samples with nitrous acid; HS-CoM was detected by the formation of the red S-nitroso derivative. The desired fractions were pooled and flash-evaporated to remove ammonium acetate. HS-CoM which may have oxidized to the corresponding disulfide was removed by precipitation with aqueous acetone. HS-CoM remained in solution. The acetone solution was removed by flash evaporation. HS-CoM was crystallized from methanol upon addition of diethyl ether and was recrystallized two times from the same solvent mixture. A 30% yield was obtained. Data from quantitative elemental analysis are provided in Table I.

Synthesis of Ammonium S, S'-Dithiodiethanesulfonate—HS-CoM (0.5 g) was dissolved in 30 ml of 30% aqueous ammonium hydroxide, and the solution was bubbled with oxygen until the thiol could no longer be detected with nitrous acid. (S-CoM) was crystallized from water upon addition of acetone. The compound was recrystallized two times from the same solvents. A 20% yield
was obtained. Data from quantitative elemental analysis are presented in Table I. For certain studies the ammonium ions of synthetic (S-CoM), were replaced by sodium ions by passage of an aqueous solution of the coenzyme through an SP-Sephadex C-25 column (sodium form) that had been equilibrated with water. Sodium (S-CoM) was crystallized twice from aqueous acetone. 

**Synthesis of Ammonium 2-(Methylthio)ethanesulfonate**—The procedure for the synthesis of CH₂-S-CoM was identical with the synthesis of HS-CoM except that methylmercaptan was used instead of hydrogen sulfide. CH₂-S-CoM was recrystallized three times from aqueous acetone. A 20% yield was obtained. Results of quantitative elemental analysis are shown in Table I.

A large scale enzymic methylation of HS-CoM was performed in a 125-ml double sidearm Erlenmeyer flask. The reaction mixture (25 ml) contained 1.25 mmoles of potassium phosphate buffer at pH 7.1, 154 mmoles of HS-CoM, 31 mmoles of [methyl-¹⁴C]methylcobalamin (specific activity, 1020 cpm per mmole), 415 mmoles of unlabeled methylcobalamin, and 100-fold purified membrane methyltransferase (1.6 mg of protein). The reaction flask was made anaerobic, and the reaction was initiated by tipping in [methyl-¹⁴C]methylcobalamin from one of the sidearms. The reaction was allowed to proceed until all of the [methyl-¹⁴C]methylcobalamin had reacted.

The excess nonradioactive methylcobalamin was tipped in from the other sidearm, and the reaction was allowed to proceed until all of the HS-CoM was methylated. The reaction mixture was passed through a water-equilibrated Bio-Rad AG 50W-X4 (H+ form) column (2.5 X 10 cm) to remove methylcobalamin and aqua-
ocirco;cobalamin. The radioactive effluent was concentrated by flash evaporation and eluted with water through a Sephadex G-10 column (2.5 X 20 cm). Ninety-eight percent of the recovered radioactivity was contained within a single peak. A Beckman DU spectrophotometer was used to follow the absorption of fractions at 260 and 400 nm. A Perkin-Elmer 252 infrared spectrometer, Varian HA-100 and A-60 NMR spectrometers, a Nuclear-Chicago Mark I liquid scintillation system, a Deagro-Brinkmann TLE system, and a Packard gas chromatograph were used for appropriate analyses.

**RESULTS**

**Purification of CoM**—Whole cells were suspended in distilled water (1:1, v/v) and stirred for 30 min at 80–90°C. The cell residue from this extraction was subjected to a second extraction by the same procedure. The supernatant solution was lyophilized, ground into a fine powder, and stored in a sealed bottle at room temperature. CoM was extracted by stirring the powder in anhydrous methanol (1:10, v/v) for 30 to 40 min. The insoluble residue was removed by filtration. Two additional extractions were performed by this procedure. The clear red-orange supernatant solution was flash-evaporated to yield a red-brown oil-like residue. This fraction, obtained from the extraction of 1.5 to 2 kg of wet cells, was taken up in 100 ml of water and applied to a QAE-Sephadex A-25 column (5 X 30 cm) (acetate form) that had been equilibrated with water. The sample was eluted with 3 liters of a 0 to 3 M ammonium acetate linear gradient (Fig. 1). CoM activity was always located at two positions, Peak A and Peak B. Peak B co-eluted with coenzyme F₉₅, a fluorescent electron carrier found in high amounts.
in Methanobacterium (13). From preparation to preparation, the per cent of the total activity found in Peak A varied in a reciprocal manner to that found in Peak B. Both derivatives possessed a strong negative charge, requiring 1.8 and 2.5 m ammonium acetate to elute Peaks A and B, respectively, from the column. Since Peak B contained from 70 to 88% of the total activity, this fraction was used for further purification. Ammonium acetate was removed by flash evaporation at 40°. The CoM-containing eluate from the equivalent of 4 kg of wet cells was dissolved in 8 to 10 ml of water and fractionated on a Sephadex G-10 column (2.5 × 90 cm) that had been equilibrated with water. The elution fluid was water. As shown in Fig. 2, CoM eluted in a single peak and was separated from much contaminating material that absorbed at 260 nm, the bulk of which was coenzyme F₆₈₄. The pooled active fractions from the G-10 eluate were reduced to 10 ml by flash evaporation. The sample was applied to an continuous electrophoresis apparatus, and separation was effected on a paper cuvet at 400 volts (8 to 10 ma) in a pH 4, 0.02 m ammonium acetate buffer. CoM migrated 10 to 13 cm toward the positive pole while descending 7 cm and was separated from fluorescent compounds. The active fractions were flash-evaporated at 40° to remove the ammonium acetate buffer. The solid residue was taken up in a small amount of water and recrystallized three times from aqueous acetone, yielding colorless platelets with a melting point greater than 250°.

The recovery of CoM from this purification scheme was 65 mg of crystalline CoM per kg of wet packed cells. Accurate determination of the recovery from this scheme was not possible as inhibitors of the methyltransferase reaction were present in both the initial, lyophilized water extract and the methanol-soluble fraction. For example, the total activity found in Peak B of the anion exchange eluate (Table I) increased nearly 3-fold over that observed in the methanol-soluble residue.

Structure of CoM—Evidence obtained from infrared, proton NMR, and ultraviolet spectroscopy as well as from chemical tests and quantitative elemental analyses revealed that CoM as isolated is the ammonium salt of 2,2'-dithiodiethanesulfonic acid (S-CoM). This compound possesses the following structure:

\[ \text{+H}_2\text{N}-\text{O}_2\text{SCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{SO}_2\text{NH}^+ \]

Fig. 3 shows that the infrared spectra of authentic and synthetic (S-CoM) are identical. Each spectrum is largely dominated by the absorptions of the sulfonate and ammonium ions. The strong absorptions at Peaks d and e (1170 and 1035 cm⁻¹) represent, respectively, the asymmetric and symmetric SO₂ stretching modes characteristic of sulfonic acid salts. The shoulders on the left of Peak d are probably the wag motion of the methylene groups attached to sulfur. The ammonium ion stretch and deformation modes appear at a and c, respectively (3200, 3060, and 1435 cm⁻¹); these absorptions disappear revealing the methylene stretch (2970 and 2930 cm⁻¹) and deformation (1420 cm⁻¹) modes when the ammonium ion is replaced by sodium ion as shown in the inset of Fig. 3. The fact that untreated aqueous solutions of both authentic and synthetic (S-CoM₂) gave a positive reaction with Nessler's reagent provides additional chemical evidence for the presence of the ammonium ion. Evidence suggests that the peaks located at g (590 and 530 cm⁻¹) may involve the sulfonate group; Palmer (14), working with potassium dithionate, assigned the sharp peaks observed at 577 and 516 cm⁻¹ to be fundamental absorptions of the SO₂ deformation modes.

The absorptions observed with a variety of CoM derivatives which possess an intact sulfonate group as well as with sodium thanesulfonate suggest that the peaks located at f and g involve the sulfonate moiety. All absorptions in this region as well as in the SO₂ stretch region were lost in the spectrum of ammonium 2,3'-dithiodipropionate where the sulfonate group is replaced by a carboxyl group. Data provided by Bellamy (15) and Colthup et al. (16) also were used to aid in peak assignments.

The proton NMR spectra of deuterium oxide solutions of authentic and synthetic (S-CoM) are identical in all respects as shown in Fig. 4. There are no additional resonances downfield.
of 5 ppm. The observed symmetrical resonances at 3.28 and 3.11 ppm arise from a 4-proton system of the type 3\(\text{H}_2\) characteristic of an aliphatic compound of the configuration \(\text{XCH}_2\text{CH}_2\text{Y}\). The possibility of having an AA'BB' system of the configuration of \(\text{X'(CH}_2\text{CH}_2)_2\text{Y}\) is eliminated on the basis of quantitative elemental analysis, and particularly the synthesis of \(\text{(S-CoM)}_2\). The assignments made were based on observing the chemical shifts of the ethylene resonances of a variety of aliphatic sulfonate, sulfinyl, and disulfide compounds (17, 18). The presence of both a sulfonate and disulfide group in \(\text{(S-CoM)}_2\) places the resonances of both methylene groups approximately 0.4 ppm downfield relative to the resonances observed in aliphatic compounds which contain only one of the functional groups of interest. Resonances of methylene groups next to the sulfonate functional group were in general located downfield of those methylene groups next to sulfinyl or disulfide moieties.

Ultraviolet spectra of authentic and synthetic \(\text{(S-CoM)}_2\) exhibit absorption maxima at 191 to 193 and 245 nm. The extinction coefficients which were determined for the short wavelength band for both preparations ranged between 5800 and 6400 liters mole\(^{-1}\) cm\(^{-1}\). Because these measurements were made at the extreme lower end of the useful wavelength range of the Cary model 14 instrument, attention should be drawn only to the order of magnitude. The extinction coefficient at 245 nm, however, may be determined with accuracy and for synthetic \(\text{(S-CoM)}_2\) was found to be 380 liters mole\(^{-1}\) cm\(^{-1}\). The absorption maxima and extinction coefficients for \(\text{(S-CoM)}_2\) are close to those obtained for straight chain aliphatic disulfides. For example, diethylidisulfide possesses absorption maxima at 194 and 230 nm with extinction coefficients of 5500 and 380 liters mole\(^{-1}\) cm\(^{-1}\), respectively (19). The elemental compositions of authentic and synthetic \(\text{(S-CoM)}_2\) are shown in Table 1; neither differs from that calculated by greater than 0.46%.

Authentic and synthetic \(\text{(S-CoM)}_2\) were identical in their biological behavior. When 1.5 \(\mu\)moles of synthetic \(\text{(S-CoM)}_2\) were added to a standard reaction mixture which contained 4.9 \(\mu\)moles of \([\text{methyl-}^{14}\text{C}]\text{methylenobalamine}\) and 100-fold purified methylenobalamine-CoM methyltransferase (8 \(\mu\)g of protein), a perfectly linear reaction rate was produced, with 400 nmoles of \(\text{CH}_3\text{-S-CoM}\) being produced in 10 min. The reaction rate of authentic \(\text{(S-CoM)}_2\) was identical. In a separate experiment where the reaction was allowed to proceed to completion, the reaction mixture contained 52 \(\mu\)g of 100-fold purified methyltransferase and 2.5 \(\mu\)moles of \([\text{methyl-}^{14}\text{C}]\text{methylenobalamine}\). Two levels of synthetic \(\text{(S-CoM)}_2\), 152 and 304 nmoles, and two levels of authentic \(\text{(S-CoM)}_2\), 148 and 296 nmoles, were tested. For each mole of \(\text{(S-CoM)}_2\) added 1.98 ± 0.10 moles of methyl groups were bound. The ratio remained within the above described limits when either 100-fold purified methyltransferase was used with sodium borohydride as the electron donor or when crude extracts were used with sodium borohydride or hydrogen as the electron source.

Reduction of \(\text{(S-CoM)}_2\)—The function of the electron requirement in the methyltransferase reaction was found to be that of reducing the disulfide bond of \(\text{(S-CoM)}_2\) prior to methylation. HS-CoM functions as a methyl acceptor in the absence of an electron donor as shown by the open and closed triangles in Fig. 5. Comparison of the open and closed circles shows that methylation of \(\text{(S-CoM)}_2\) requires an electron donor. There was a negligible chemical methylation of HS-CoM from methyl-
cobalamin. HS-CoM was stoichiometrically methylated when the methyltransferase-catalyzed reaction was allowed to proceed to completion.

*Ethylthio)ethanesulfonic Acid*—The results presented above indicate that two methyl groups are bound for each mole of (S-CoM)₄. The methyl derivative of the coenzyme retained a strong negative charge, suggesting that methyl-CoM could be 2-(ethylthio)ethanesulfonic acid.

Results presented in Fig. 6 show that the methylated coenzyme which was isolated from a large scale reaction mixture (see "Experimental Procedure") possesses an NMR spectrum identical with that of ammonium 2-(ethylthio)ethanesulfonate which was chemically synthesized. The resonances of protons a and b appear, respectively, at 3.15 ppm (relative intensity, 1.8) and 2.88 ppm (relative intensity, 2.0). The large singlet labeled e at 2.17 ppm (relative intensity, 3.1) is typical of the proton resonances of a methyl group attached to sulfide. The small singlet at 2.24 ppm resulted from a contaminant in the solvent used for crystallization. The presence of the methyl group shifts resonances a and b upfield, with that of b being shifted to a greater extent. This lends additional support to the proton assignments made. No evidence was obtained to support the possibility that the methylated derivative of CoM is the methylsulfonate ester. Such a CoM derivative would be a neutral molecule and possess physical properties considerably

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**Fig. 4.** NMR spectra of authentic and synthetic (S-CoM)₄. The NMR spectra of (S-CoM)₂ in deuterium oxide (99.8% isotopic purity) were obtained with a Varian HA-100 NMR spectrometer. A NMR tube (2-mm inside diameter × 5-mm outside diameter × 17.7 mm) was used. The spectra were obtained under the following conditions (where the conditions are different, the value for authentic (S-CoM)₄, precedes that for synthetic (S-CoM)₄): concentration, 9 mg per 0.07 ml and 9 mg per 0.06 ml; temperature, 28°C; frequency response, 2 and 20 Hz; radio frequency attenuator, 20 db; sweep time, 500 s; sweep width, 500 Hz (inset, 250 Hz); sweep offset, 0 and 472 Hz; spectrum amplitude, 10,000; lock signal, sodium 3-trimethylsilyl-propionate-2,2,3,3-d₄ (TSP) and hydrogen-deuterium oxide (HOD); field milligauss (manual oscillator frequency), 0.1 mG; field milligauss (sweep frequency), 0.04 and 0.1 mG; reference compound, TSP.

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**Fig. 5.** Comparison of the reductive requirement of the methylcobalamin-CoM methyltransferase reaction when HS-CoM or (S-CoM)₂ was used as the methyl acceptor. The reaction mixtures (0.25 ml) contained 12.5 µmoles of potassium phosphate buffer at pH 7.1; where indicated, 1.50 µmoles of (S-CoM)₂ or 3.01 µmoles of HS-CoM; where indicated, 550 µmoles of sodium borohydride; 3.7 µmoles of [methyl-³¹C]methylocobalamin (specific activity, 1720 cpm per nmole); and 100-fold purified methylreductase (15 µg of protein).

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**Fig. 6.** Comparison of the NMR spectra of authentic and synthetic CH₃-S-CoM. The NMR spectra of CH₃-S-CoM in deuterium oxide (99.8% isotopic purity) were obtained with a Varian HA-100 NMR spectrometer. A NMR tube (2-mm inside diameter × 5-mm outside diameter × 17.7 mm) was used. The spectra were obtained under the following conditions (where the conditions are different, the value for the enzymatically synthesized, authentic CH₃-S-CoM precedes that for synthetic CH₃-S-CoM): concentration, 3 mg per 0.05 ml and 10 mg per 0.07 ml; temperature, 28°C; frequency response 1 and 3 Hz; radio frequency attenuator, 20 db; sweep time, 1000 s and 5000 s; sweep width, 500 Hz; sweep offset, -473 and 0 Hz; spectrum amplitude, 10,000 (inset, 10 × gain), and 6000; lock signal, sodium 3-trimethylsilyl-propionate-2,2,3,3-d₄ (TSP) and hydrogen deuterium oxide (HOD); field milligauss (manual oscillator frequency), 0.1 and 0.05 mG; field milligauss (sweep frequency), 0.05 and 0.1 mG; reference compound, TSP.
different than those observed. The methyl proton resonances of an aliphatic methylsulfonylamine ester lie very close to 3.9 ppm, a position very easily distinguishable from the resonances observed.

We examined the possibility that (S-CoM)₂ was methylated to yield 2-(dimethylsulfonyl)ethanesulfonate ((CH₃)₂S-CoM) which decomposed to CH₃S-CoM prior to analysis. The NMR spectrum of chemically synthesized (CH₃)₂S-CoM is presented in Fig. 7. The spectrum in deuterium oxide revealed symmetrical ethylene proton resonances of the type AB₂ at 3.65 and 3.46 ppm (cumulative relative intensity, 4.0). A singlet corresponding to the methyl proton resonances was located at 3.00 ppm (relative intensity, 5.5). CH₃S-CoM and (CH₃)₂S-CoM were readily separable by anion exchange chromatography and thin layer electrophoresis. (CH₃)₂S-CoM forms an internal salt between the sulfonium and sulfonate moieties. This imparts a charge considerably different from that of CH₃S-CoM. (CH₃)₂S-CoM was found to be completely stable under the conditions used for analysis. Reaction mixtures which contained methyltransferases were directly fractionated by anion exchange chromatography (Fig. 8A). When limiting amounts of [methyl-³H]methylcobalamin were allowed to react to completion, 99% of the radioactivity added was found in a single peak which possessed electrophoretic properties identical with that of chemically synthesized CH₃S-CoM (Fig. 8B). No radioactivity above background was found in the void volume (V₀) where (CH₃)₂S-CoM and methylcobalamin elute. Incubation of (CH₃)₂S-CoM in a methyltransferase reaction for 30 min at room temperature and for 30 min at 40°C did not result in decomposition to CH₃S-CoM.

**Biological Activity of CH₃S-CoM**—Chemically synthesized and authentic CH₃S-CoM exhibited identical biological activity. When 2.9 μmoles of authentic or synthetic CH₃S-CoM were added to a reaction mixture (see "Experimental Procedure") which contained crude cell extract (3.3 mg of protein), a perfectly linear rate of methane formation was observed in each reaction vessel, the rates being identical; 22 μmoles of methane were produced in 25 min. In a separate experiment, the reaction

was allowed to proceed to completion with the methyl group from 101 and 202 μmoles of synthetic CH₃S-CoM being completely converted to methane. From 101 and 202 μmoles of authentic CH₃S-CoM, 92 and 190 μmoles of methane, respectively, were detected.

As shown in Table II, (CH₃)₂S-CoM was found to be completely inert as a methyl donor in the methyldiuretase-catalyzed reaction in incubation periods up to 70 min. No methane production was observed when (CH₃)₂S-CoM and HS-CoM were present together in the reaction mixture; (CH₃)₂S-CoM would not methylate HS-CoM to yield biologically active CH₃S-CoM. Results from a separate experiment showed that CH₃S-CoM could not be further methylated by methyltransferase.

**DISCUSSION**

The fact that CoM was isolated as the disulfide possibly reflects an artifact of isolation. Thiol derivatives such as HS-CoM are, under neutral or alkaline conditions, easily oxidized to disulfides. Anaerobic precautions were not taken when CoM was isolated. Early work on the isolation of CoM revealed that much of it was in the form of a disulfide (30). Mild hydrolysis of partially purified CoA released 2,2'-dithiodiethylenamine. Disulfide formation may likely explain the presence of more than one active derivative of CoM in crude preparations. The component of Peak A in the QAE-Sephadex A-25 eluate may be a heterodisulfide possessing only one sulfonate moiety RSHCH₂CH₂SO₄⁻ (R-S-S-CoM). The sulfonate group will significantly govern the behavior of such a CoM derivative on an anion exchange column. Thus, (S-CoM)₂, possessing two sulfonate moieties would be expected to elute at a higher ionic strength than a derivative possessing only one sulfonate group. This is in fact the case. (S-CoM)₂ elutes from a QAE-Sephadex A-25 column at approximately 2.5 m ammonium acetate whereas CH₃S-CoM and HS-CoM both elute at a concentration less than 2 m. The observation that the ratios of CoM activity in Peaks A and B vary in a reciprocal manner from preparation to preparation may simply reflect the proportions of (S-CoM)₂ and R-S-S-CoM present.

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**Fig. 7.** NMR spectrum of (CH₃)₂S-CoM. The spectrum was obtained in deuterium oxide (99.8% isotopic purity) with a Varian A-60 NMR spectrometer. An NMR sample tube (2-mm inside diameter × 5-mm outside diameter × 17.7 mm) was used. The spectrum was obtained under the following conditions: concentration of (CH₃)₂S-CoM. 9 mg per 0.07 ml; temperature, 4°C; filter band width 4 Hz; radio frequency field, 0.15 mG; sweep time 500 s; sweep width, 500 Hz; sweep offset, 0 Hz; spectrum amplitude, 32; integral amplitude, 50; reference compound, sodium 3-trimethylsilyl-propionate-2,2,3,3-d₄ (TSP). (a,b) indicates only that the cluster of resonances is from protons a and b; assignments have not been made. HOD, hydrogen deuterium oxide.
Comparison of biological activity of CH$_2$-S-CoM and (CH$_2$)$_2$-S-CoM

Each reaction mixture (2.0 ml) contained 100 μmoles of potassium phosphate buffer at pH 7.1, 10 μmoles of magnesium sulfate (in the appropriate flask 4.9 μmoles of CH$_3$-S-CoM, 5.0 μmoles of HS-CoM, and 5.0 μmoles of (CH$_2$)$_2$-S-CoM), 10 μmoles of ATP, and dialyzed crude extract (33.8 mg of protein). The reaction rates of the positive control reactions were linear for 20 min.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Reaction rate (CH$_3$ produced per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CoM derivative</td>
<td>2</td>
</tr>
<tr>
<td>HS-CoM</td>
<td>2</td>
</tr>
<tr>
<td>(CH$_2$)$_2$-S-CoM</td>
<td>2</td>
</tr>
<tr>
<td>HS-CoM + (CH$_2$)$_2$-S-CoM</td>
<td>2</td>
</tr>
<tr>
<td>CH$_3$-S-CoM</td>
<td>125</td>
</tr>
<tr>
<td>CH$_3$-S-CoM + (CH$_2$)$_2$-S-CoM</td>
<td>80</td>
</tr>
</tbody>
</table>

will be interesting to see if the coenzyme can handle a C$_1$ moiety more oxidized than a methyl group.

**Acknowledgments**—We thank Kenneth L. Rinehart for suggesting the use of 2-bromoethanesulfonic acid in the chemical synthesis of CoM and for helpful discussions on the structure of derivatives. We appreciate the interest of Robert L. Switzer in this investigation. The assistance of Victor Gabriel and Jerry Althaus in the mass culture of Methanobacterium is gratefully acknowledged. We thank the School of Chemical Sciences for use of their facilities in obtaining the NMR spectra, infrared spectra, and quantitative elemental analyses.

**REFERENCES**