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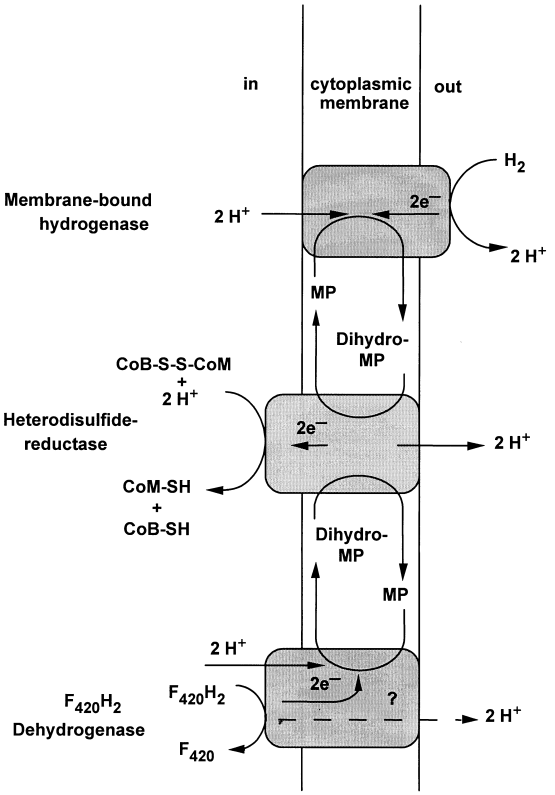
[15] Crystallography: The intensity data were collected on a Bruker CCD diffractometer with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) at room temperature. All calculations were performed by using the SHELXTL-PL version 5.10 package on an HP computer. The structure was solved by direct methods and refined by full-matrix least-squares methods. Crystal data for **1** with two water molecules per formula unit: colorless needle, dimensions $0.24 \times 0.18 \times 0.16$ mm, $C_{90}H_{90}N_{31}O_{27}Ag_7S_{12}Cl_2$, $M_r = 3248.64$, monoclinic, space group $C2/c$; $a = 28.1019(20)$, $b = 16.8556(11)$, $c = 27.9278(21)$ Å, $\beta = 117.9154(15)^\circ$, $V = 11\,689.3(25)$ Å$^3$; $\rho_{calcd} = 1.846$ g cm$^{-3}$, $Z = 4$; $F(000) = 6472$; $\mu(Mo_{K\alpha}) = 1.493$ mm$^{-1}$; 38 967 reflections were collected, of which 7252 with $|F_o| \geq 2.0\,\sigma(|F_o|)$ were observed; $R_1 = 0.057$; $wR_2 = 0.184$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139799. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

## Methanophenazine: Structure, Total Synthesis, and Function of a New Cofactor from Methanogenic Archaea**

Uwe Beifuss,* Mario Tietze, Sebastian Bäumer, and Uwe Deppenmeier

*Dedicated to Professor Sir Alan R. Battersby on the occasion of his 75th birthday*

Methanogenic organisms belong to the kingdom of archaea and differ significantly from eukarya and bacteria.[1] Methanogenic archaea are widespread in anaerobic environments such as the sediments of lakes and rivers as well as the intestinal tract of ruminants. They form the end of the anaerobic food chain and transform simple substrates such as hydrogen/carbon dioxide, formic acid, methanol, methylamines, and acetic acid into methane. The latter subsequently is oxidized under aerobic conditions and is thus able to reenter the carbon cycle. Owing to the influences of civilization the amount of methane, one of the greenhouse gases, in the atmosphere has continuously increased during the last century.[2] A variety of unique enzymes and unusual cofactors contribute to its formation by methanogenic archaea. The central intermediate of all metabolic pathways of methanogens is methyl-S-CoM, which is reductively demethylated to methane under the catalytic influence of methyl-CoM-reductase.[3] The two electrons required in this process are derived from CoB-SH and lead to the formation of a heterodisulfide (CoB-S-S-CoM) from CoB-SH and CoM-SH.[4] The reduction of CoB-S-S-CoM is an energy-conserving step in the metabolism of methylotrophic methanogens (Scheme 1).[5] Two




Scheme 1. Model of the membrane-bound electron transfer of *Methanosarcina mazei* Gö1. CoM-SH = coenzyme M; CoB-SH = coenzyme B; $F_{420}$ = coenzyme $F_{420}$; $F_{420}H_2$ = reduced form of the coenzyme $F_{420}$; MP = methanophenazine; dihydro-MP = reduced form of methanophenazine.

recently detected proton-translocating enzyme systems, the $H_2$:heterodisulfide oxidoreductase and the $F_{420}H_2$:heterodisulfide oxidoreductase, are involved in the membrane-bound electron transfer of *Methanosarcina mazei* Gö1.[6] The electron transport of $F_{420}H_2$ to CoB-S-S-CoM is catalyzed by an $F_{420}H_2$ dehydrogenase transferring the electrons to the heterodisulfide reductase. In the presence of molecular hydrogen a membrane-bound hydrogenase serves as an electron-feeding component to the heterodisulfide reductase (Scheme 1).

The structure of the electron carrier was not known. Recently, we have been able to isolate a phenazine ether from membranes of *Methanosarcina mazei* Gö1.[7] Detailed

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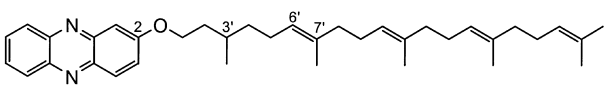
NMR analysis of the sensitive natural product, which could only be obtained in small amounts, indicated that its lipophilic side chain, which we assume to be responsible for the anchorage in the membrane, consists of five isoprenoid units linked to each other in a head-to-tail manner. Unlike the saturated $C_5$ unit, which is directly linked to the 2-phenazinyl residue, the remaining four units are unsaturated. Three of them exhibit *E*-configurated double bonds. The redox-active natural product referred to as methanophenazine (MP) **1** is the first phenazine whatsoever isolated from archaea.

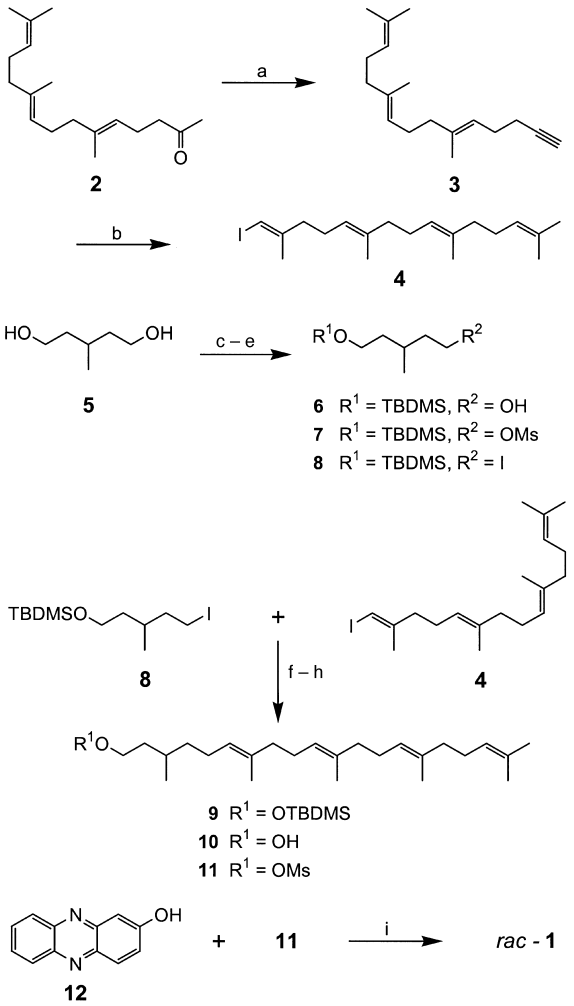



**1**

Sincemethanogens lack the usual quinones it was assumed that **1** functions as an electron carrier in the cytoplasmic membrane and unlike other natural phenazines takes part in the energy-conserving electron transport. Larger amounts of the natural product are necessary to determine the biological function of **1** and to elucidate the absolute configuration at C-3′. As its isolation from *Methanosarcina mazei* Gö1 is quite time-consuming the only way possible was to synthesize the natural product. For this purpose **1** was to be convergently built from the three building blocks **4**, **8**, and **12** (Scheme 2). Diastereoselective linking of **4** and **8** to give **9** and the subsequent etherification of **11** and **12** were regarded key steps. While the latter process seemed to be completely unproblematic it was unclear whether the reactivity of the alkyl metal derivative would be sufficient for the transition metal catalyzed linking to the vinyl iodide.

To prepare **4** (*E*,*E*)-farnesyl acetone (**2**) was first transformed into the terminal alkyne **3**,[8] subsequently providing the (*E*)-vinyl iodide **4** in diastereomerically pure form and 74% yield by the Zr-catalyzed carboalumination with trimethylaluminum and quenching with iodine[9] (Scheme 2). The $C_5$ building block *rac*-**8** required for the linking with **4** was made available by selective monofunctionalization[10] of 3-methyl-pentane-1,5-diol **5** in a few steps. The advantage of this prochiral compound is the chance to form the enantiomerically pure compounds (*R*)-**8** and (*S*)-**8** that are necessary to determine the absolute configuration of methanophenazine **1** by differentiating the enantiotopic $(CH_2)_2$-OH groups.

The coupling between the alkyl metal compounds released in situ from *rac*-**8** and the vinyl metal derivatives resulting from **4** caused considerable difficulties thus indicating that the reactivity of the $sp^3$ component was too low. The (*E*)-selective construction of the C-6′, C-7′ double bond[11] of the sesterterpene building block **9** could only be achieved in 65% yield when the $Pd^0$-catalyzed coupling of the organozinc compound derived in situ from *rac*-**8**, *tert*-butyllithium, and zinc chloride was performed with vinyl iodide **4**. Cleavage of the *tert*-butyldimethylsilyl ester and activation of the resulting alcohol **10** with methanesulfonic acid chloride provided mesylate **11**, which was etherified with 2-hydroxyphenazine **12**[12] to give *rac*-**1**.[13] When the synthesis was performed without isolation and purification of various intermediates, *rac*-**1** was produced from farnesyl acetone **2** in a total yield of more than 30%. Spectroscopic data of the synthetic methanophenazine *rac*-**1** corresponded to those of the natural product in every respect.[14] Starting from the enantiomerically pure $C_5$ building blocks, **1** and *ent*-**1** will be accessible as well. And in this way it will be possible to determine the absolute configuration of the natural product.




Scheme 2. a) LiTMP, THF, −78 °C; ClP=O(OEt)$_2$, −78 °C→RT; LiTMP, THF, −78 °C→RT, $H_2O$, 75%; b) cat. [$Cp_2ZrCl_2$], $Me_3Al$, $CH_2Cl_2$; $I_2$, THF, −30 °C→RT; $K_2CO_3$, $H_2O$, 0 °C, 74%; c) NaH, TBDMSCl, THF, 83%; d) $NEt_3$, MsCl, $CH_2Cl_2$, 0 °C; e) NaI, acetone, reflux, 93% for two steps; f) **8** + $ZnCl_2$, $Et_2O$, RT → −100 °C, *t*BuLi; **4** + [$Pd(PPh_3)_4$],(0.05 equiv), −70 °C→RT, 65%; g) TBAF, THF, RT; h) $NEt_3$, MsCl, $CH_2Cl_2$, 0 °C; i) KOH, aliquot, THF 90% for three steps. Aliquot = methyltrioctylammonium chloride, Ms = methanesulfonyl, TBAF = tetrabutylammonium fluoride, TBDMS = *tert*-butyldimethylsilyl, TMP = 2,2,6,6-tetramethylpiperidine.

First experiments on the biological function were performed by using the model compound 2-hydroxyphenazine **12** and its reduced form (dihydro-**12**). It was demonstrated that all key enzymes react with the artificial electron carrier.[7] After completion of the total synthesis similar tests were also performed with *rac*-**1** (Table 1). Here, washed cytoplasmic membranes of *Methanosarcina mazei* Gö1 were combined with *rac*-**1** and the activities of the respective enzymes determined. The results clearly indicated that methanophenazine **1** serves as an electron acceptor to both the membrane-bound hydrogenase and the $F_{420}H_2$ dehydrogenase if $H_2$ and $F_{420}$ were added, respectively. In addition, the heterodisulfide

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Table 1. Specific activities of the enzymes of the $F_{420}H_2$:heterodisulfide oxidoreductase and $H_2$:heterodisulfide oxidoreductase systems.

| Enzyme | Electron donor | Electron acceptor | Spec. activity[a] [U mg protein$^{-1}$] |
|---|---|---|---|
| $F_{420}H_2$ dehydrogenase | $F_{420}H_2$ | **12** | 0.20 |
| $F_{420}H_2$ dehydrogenase | $F_{420}H_2$ | MP | 0.15 |
| membrane-bound hydrogenase | $H_2$ | **12** | 2.2 |
| membrane-bound hydrogenase | $H_2$ | MP | 3.2 |
| heterodisulfide reductase | dihydro-**12** | CoB-S-S-CoM | 2.3 |
| heterodisulfide reductase | dihydro-MP | CoB-S-S-CoM | 2.6 |

[a] 1 U = 1 μmol substrate converted per minute.

reductase uses the reduced form of methanophenazine (dihydro MP) as an electron donor for the heterodisulfide reduction. Therefore methanophenazine is able to mediate the electron transport between the membrane-bound enzymes, so that the conversion by the proton-translocating electron transport systems[6] can be subdivided in two partial reactions each (Scheme 1). In this way methanophenazine **1** was characterized as the first phenazine derivative involved in the electron transport of biological systems. The experiments reported here suggest that its role in the energy metabolism of methanogens is similar to that of ubiquinone in mitochondria and bacteria.

### *Experimental Section*

The growth of *Methanosarcina mazei* Gö1 and the preparation of cytoplasmic membranes was performed as previously described.[7] Photometrical analysis to determine enzymatic activities were carried out at room temperature in glass cuvettes (1.7 mL) that were gassed with $N_2$ or $H_2$ and closed with rubber stoppers. The optical-enzymatic determination of the $F_{420}H_2$-dependent reduction of MP and the dihydro-MP dependent heterodisulfide reduction was performed under a nitrogen atmosphere. The cuvette was flushed with hydrogen to determine the hydrogen-dependent reduction of MP. The reactions were initiated by adding the respective electron acceptors. Final concentrations of the reactants were: $F_{420}$: 25 μM; **12**: 25 μM; MP: 24 μM (stock solution in dimethylformamide), and CoB-S-S-CoM: 38 μM. Protein concentration was 7.5 μg membrane protein per mL assay. Extinction coefficients: $F_{420}$: $\varepsilon_{420} = 40\,mM^{-1}cm^{-1}$; MP: $\varepsilon_{414} = 3.17\,mM^{-1}cm^{-1}$; **12** $\varepsilon_{425} = 4.5\,mM^{-1}cm^{-1}$.

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[14] $R_f = 0.51$ (diethyl ether/hexane = 1/1); $^1$H NMR (500 MHz, $C_6D_6$, TMS): $\delta = 0.83$ (d, $^3J = 6.6$ Hz, 3H; 3′-$CH_3$), 1.14 – 1.23 (m, 1H; 4′-$H_A$), 1.32 – 1.39 (m, 1H; 4′-$H_B$), 1.44 (dt, $^2J = 13.5$ Hz, $^3J = 6.4$ Hz, 1H; 2′-$H_A$), 1.56 (s, 3H; 19′-$CH_3$), 1.60 – 1.65 (m, 1H; 3′-H) superimposed by 1.61, 1.62, 1.64 (3s, 3H each; 7′-$CH_3$, 11′-$CH_3$, 15′-$CH_3$), 1.68 (d, $^4J = 0.8$ Hz, 3H; 20′-$H_3$), 1.72 (ddt, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz, $^3J = 6.8$ Hz, 1H; 2′-$H_B$), 1.97 – 2.27 (m, 14H; 5′-$H_2$, 8′$H_2$, 9′$H_2$, 12′-$H_2$, 13′-$H_2$, 16′-$H_2$, 17′-$H_2$), 3.79 (dt, $^2J = 10.7$ Hz, $^3J = 6.8$ Hz, 1H; 1′-$H_A$), 3.81 (dt, $^2J = 10.7$ Hz, $^3J = 6.4$ Hz, 1H; 1′-$H_B$), 5.25 (tt, $^3J = 6.9$ Hz, $^4J = 1.4$ Hz, 1H; 18′-H), 5.28 – 5.38 (m, 3H; 6′-H, 10′-H, 14′-H), 7.29 (ddd, $^3J = 8.6$ Hz, $^3J = 6.8$ Hz, $^4J = 1.6$ Hz, 1H; 7-H or 8-H), 7.32 (dd, $^3J = 9.6$ Hz, $^4J = 2.8$ Hz, 1H; 3-H), 7.35 (ddd, $^3J = 8.6$ Hz, $^3J = 6.8$ Hz, $^4J = 1.6$ Hz, 1H; 8-H or 7-H), 7.53 (d, $^4J = 2.8$ Hz, 1H; 1-H), 8.12 (d, $^3J = 9.6$ Hz, 1H; 4-H), 8.30 (dd, $^3J = 8.6$ Hz, $^4J = 1.6$ Hz, 1H; 6-H or 9-H), 8.31 (dd, $^3J = 8.6$ Hz, $^4J = 1.6$ Hz, 1H; 6-H or 9-H); $^{13}$C NMR (125 MHz, $C_6D_6$): $\delta = 16.12$, 16.13, 16.15 (7′-$CH_3$, 11′-$CH_3$, 15′-$CH_3$), 17.73 (19′-$CH_3$), 19.56 (3′-$CH_3$), 25.78 (C-20′), 25.83 (C-5′), 27.11, 27.12, 27.23 (C-9′, C-13′, C-17′), 29.80 (C-3′), 36.01 (C-2′), 37.45 (C-4′), 40.20, 40.23 (C-8′, C-12′, C-16′), 66.91 (C-1′), 105.80 (C-1), 124.73, 124.77, 124.93, (C-10′, C-14′, C-18′), 125.04 (C-6′), 126.29 (C-3), 128.29 (C-7 or C-8), 129.58 (C-8 or C-7), 130.15 (C-6 or C-9), 130.27 (C-9 or C-6), 131.08 (C-19′), 131.30 (C-4), 134.99, 135.04, 135.09 (C-7′, C-11′, C-15′), 141.36 (C-4a), 142.63 (C-5a or C-9a), 144.12 (C-9a or C-5a), 145.89 (C-10a), 160.78 (C-2); IR (film): $\tilde{\nu} = 3059\ cm^{-1}$ (CH, olefinic), 2954, 2924, 2853 (CH, aliphat.), 1632, 1605, 1560, 1518 (C=C), 1483, 1445 ($CH_2$, $CH_3$), 1380, 1360 ($CH_3$), 1196 (C-O-C), 828, 757 (C=C, arom.); UV($CH_3CN$): $\lambda_{max}$(lg $\varepsilon$) = 388 (3.72), 355 (3.78), 256 nm (4.71); MS(70 eV): *m*/*z* (%): 538 (100) [$M^+$], 470 (6) [$M^+ - C_5H_8$], 402 (8) [$470 - C_5H_8$], 334 (20) [$402 - C_5H_8$], 265 (4) [$334 - C_5H_9$], 196 (82) [$C_{12}H_8N_2O^+$], 168 (7) [196 – CO]; HR-MS: calcd for $C_{37}H_{50}N_2O$: 538.3923; found: 538.3923.


## First Artificial Receptor for Caffeine—A New Concept for the Complexation of Alkylated Oxopurines**

Siegfried R. Waldvogel,* Roland Fröhlich, and Christoph A. Schalley

*Dedicated to Professor R. R. Schmidt on the occasion of his 65th birthday*

Molecular recognition and reactions with signaling ability are of potential use for the development of novel sensors.[1] The detection of small, biorelevant molecules is of particular interest because of their omnipresence in everyday life. Alky-


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