On Cobaloximes with Cobalt–Sulfur Bonds and Some Model Studies Related to Cobamide-Dependent Methyl-Group-Transfer Reactions

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Abstract: Preparation and properties of several mercaptocobaloximes (bis(dimethylglyoximato)cobalt complexes with Co–S bonds) are reported. Cobaloximes(II) catalyze the oxidation of thiols and reduction of disulfides. Alkylcobaloximes undergo nucleophilic Co–C bond cleavage with mercaptide ions. It is shown that the *in vitro* synthesis of methionine from homocysteine and methylcobaloxime is not light induced, but rather a nucleophilic displacement reaction. Reduced cobaloximes are alkylated by adenosylmethionine to produce methylcobaloxime. The selectivity of the methyl-group transfer in this reaction is demonstrated and explained on the basis of model experiments with diethylmethylsulfonium iodide as the alkylating agent. In relation to the methyl-group transfer from 5-methyltetrahydrofolic acid to homocysteine in certain methionine synthetases, methyl-transfer reactions from nitrogen to cobalt and nitrogen to sulfur have been studied. Whereas all attempts to achieve alkyl transfer from nitrogen to cobalt were unsuccessful, examples for nonenzymatic nitrogen–sulfur methyl transfer from a number of N-alkyl compounds are reported which indicate that this process can occur without the participation of the B_{12} component. In the B_{12}-dependent methionine synthetase of *E. coli* the vitamin is postulated to function as the catalyst in the maintenance of certain thiol groups of the enzyme protein in the reduced form. Recent observations suggest that the vitamin also controls the *de novo* synthesis of 5-methyltetrahydrofolic acid from 5,10-methylenetetrahydrofolic acid, which may in fact be its most important function in this enzyme.

The role of the cobamide cofactor in the enzymatic methionine synthesis and related methyl-transfer reactions is not yet understood.[2] Enzyme fractions of strains of *E. coli* have been isolated[3] which synthesize methionine from 5-methyltetrahydrofolic acid (5-Me-THF) both with and without the specific requirement of the cobamide cofactor. Although the methyl group of methylcobalamin was shown to be transferred to homocysteine both nonenzymatically[4] and with the appropriate apoenzyme fractions,[5] the organocobalt derivative cannot be regarded the intermediate carrier of the methyl group. It was shown, in fact, to be a less efficient methylating agent than 5-Me-THF.[6] From this it was concluded that the cobamide cofactor participates only indirectly, perhaps by maintaining the delicate balance of folate and sulfur compounds in the tissues.[7–9]

Before any mechanism can be proposed, the various affinity relationships between the essential constituents of the methionine synthetase system must be established. We have therefore carried out a series of model experiments to be described in the present paper.

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(2) For detailed literature references see S. K. Shapiro and F. Schlenk, Ed., "Transmethylation and Methionine Biosynthesis," The University of Chicago Press, Chicago, Ill., 1965.

(3) D. D. Woods, M. A. Foster, and J. R. Guest, ref 2, p 138.

(4) A. W. Johnson, N. Shaw, and F. Wagner, *Biochem. Biophys. Acta*, 72, 107 (1963).

(5) J. R. Guest, S. Friedman, M. J. Dilworth, and D. D. Woods, *Ann. N. Y. Acad. Sci.*, **112**, 744 (1964).

(6) S. S. Kerwar, J. H. Mangum, K. G. Scrimgeour, and F. M. Huennekens, *Biochem. Biophys. Res. Commun.*, **15**, 377 (1964).

(7) J. M. Buchanan, H. L. Elford, R. E. Loughlin, B. M. McDougall, and S. Rosenthal, *Ann. N. Y. Acad. Sci.*, **112**, 757 (1964).

(8) R. L. Kisliuk, *J. Biol. Chem.*, **236**, 817 (1961).

(9) L. Jaenicke and C. Kutzbach, *Fortschr. Chem. Org. Naturstoffe*, **21**, 183 (1963).

Reactions of Cobalamins and Cobaloximes with Sulfur Compounds

Little is known on the properties of cobalamins with Co–S bonds, although they seem to play an important part in biological systems. It was observed[10] that the treatment of hydroxyaquocobalamin with thiols reduces it to vitamin B_{12r}. In some enzymes dihydrolipoic acid is most probably the *in vivo* reducing agent.[11] Reaction of vitamin B_{12a} with glutathione at pH 1–10 yields Co–S glutathionylcobalamin.[12] This was found to react with methyl iodide to produce methylcobalamin if excess of glutathione was present.[12a,13] Hydroxyaquocobalamin *in vitro* also catalyzes the oxidation of thiols to disulfides[14] and the reduction of disulfides to thiols.[15] The affinity of the cobalt atom to sulfur appears to be independent on the special structure of the corrin ligand, which follows from our study of the reactions of cobaloximes with thiols, dialkyl disulfides, and sulfonium compounds. In the absence of added base, chloro or cyanocobaloximes, X–(Co)–B (X = Cl or CN, (Co) = abbreviation for the bis(dimethylglyoximato)cobalt systems, B = a base, *e.g.*, pyridine), react with mercaptans slowly, if at all. Addition of a stoichiometric amount of base converts the chlorocobaloxime into the hydroxy derivative, which in turn combines with the mercaptan to produce the mercaptocobaloxime.

(10) D. H. Dolphin and A. W. Johnson, *Proc. Chem. Soc.*, 311 (1963).

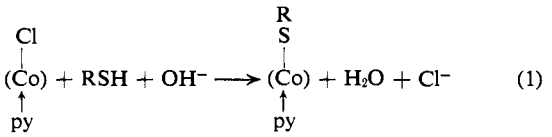
(11) E. Vitols, G. Walker, and F. M. Huennekens, *Biochem. Biophys. Res. Commun.*, **15**, 372 (1964).

(12) (a) F. Wagner and P. Renz, *Tetrahedron Letters*, 259 (1963); (b) N. Adler, T. Medwick, and T. J. Poznanski, *J. Am. Chem. Soc.*, **88**, 5018 (1966).

(13) F. Wagner and K. Bernhauer, *Ann. N. Y. Acad. Sci.*, **112**, 580 (1964).

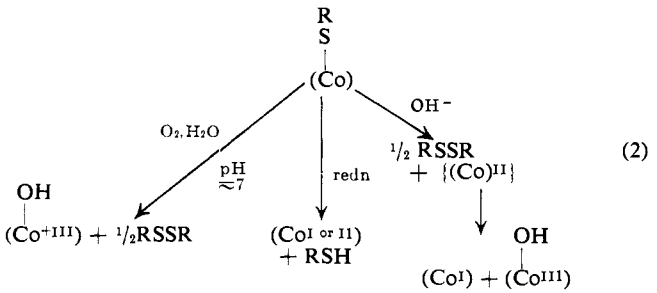
(14) J. L. Peel, *J. Biol. Chem.*, **237**, PC263 (1962).

(15) J. Arnovitch and N. Grossowicz, *Biochem. Biophys. Res. Commun.*, **8**, 416 (1962).



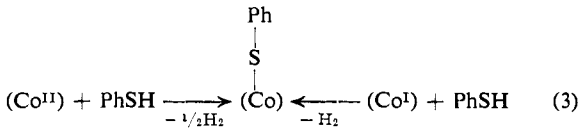
(1)

The cyanocobaloximes, *e.g.*, NC-Co(D_2H_2)py, are more stable and do not react under the same conditions. Several mercaptocobaloximes have been prepared and are described in the Experimental Section. The complexes are somewhat sensitive to air, particularly in solution, forming hydroxycobaloxime(III) and dialkyl disulfide. In alkaline medium they decompose into dialkyl disulfide and a mixture of cobaloxime(I) and hydroxycobaloxime (eq 2, axial base components are not shown). The Co–S bond is easily cleaved by a variety



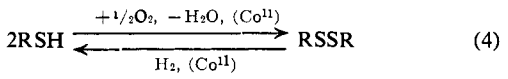
(2)

of reducing agents, *e.g.*, molecular hydrogen, in mildly acidic, alkaline, or neutral solution. The products are reduced cobaloxime (cobaloxime$_r$ or cobaloxime$_s$, depending on the pH) and the respective thiol (eq 2). Thiophenol is more reactive and combines with cobaloxime$_r$ and cobaloxime$_s$ to produce the Co–S thiophenolatocobaloximes under evolution of *the equivalent amount of* hydrogen.



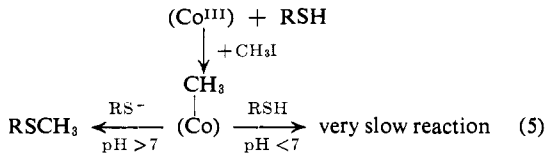
(3)

With molecular hydrogen, dialkyl disulfides in alkaline or neutral solution are reduced to thiols in the presence of catalytic amounts of cobaloximes(II). With molecular oxygen, thiols are catalytically oxidized to disulfides (eq 4).



(4)

Alkylcobaloximes are readily attacked by mercaptide ions to produce cobaloxime$_s$ (or -$_r$)[16] plus the respective dialkyl sulfide[17] (eq 5). On the other hand, the reaction



(5)

of *alkyl*cobaloximes or methylcobalamin in neutral or mildly acidic solution is extremely slow. Just as in the reaction of Co–S glutathionylcobalamin with CH_3I, it is possible to use thiols as the *in situ* reducing agents in the synthesis of alkylcobaloximes from cobaloximes(III) and alkylating agents. Thus, addition of excess methyl iodide to a solution of hydroxocobaloxime(III) in the presence of methyl mercaptan affords methylcobaloxime *if the pH of the solution is adjusted to remain close to neutrality during the reaction* (eq 5). With a stoichiometric amount of methyl iodide in a neutral solution of methylmercaptocobaloxime, dimethyl sulfide plus iodocobaloxime are formed. Substituted alkylcobaloximes are more reactive than alkylcobaloximes inasmuch as the Co–C bond is reductively cleaved by thiols under mild conditions. For example, carboxymethylcobaloxime is decomposed by methylmercaptan, producing acetic acid plus methylmercaptocobaloxime.[18] This reactivity difference has biochemical implications as it prevents methyl groups bound to cobalt from being lost as methane.[19]

Thioethers were found to be stable to cobaloximes and cobalamins in all oxidation states. Dimethyl sulfide, for example, forms a labile adduct with methylcobaloxime which decomposes in hot water to produce methylaquocobaloxime.[17] Methionine was recovered unchanged after prolonged standing in aqueous methanolic solution in the presence of cobaloxime(II) (or vitamin B_{12r}) and 1 atm of hydrogen. Preliminary experiments were also carried out with **thioesters.** With ethyl thioacetate at pH 7, ethylmercaptocobaloxime was isolated owing to the rapid saponification of the thioester under these conditions.

Methyl Transfer from Cobalt to Sulfur. Synthesis of Methionine

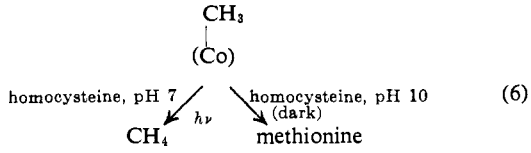
The enzymatic formation of methionine from methylcobalamin and homocysteine was observed by Woods, *et al.*[5] *In vitro*, Johnson, *et al.*,[4] found that methionine is formed on light irradiation of a near-to-neutral mixture of methylcobalamin and homocysteine; the reaction was slow in the dark. Since light irradiation suggests that methyl radicals were the alkylating species, we have performed a similar experiment with methylcobaloxime. Although we could detect methionine by paper chromatographic identification, the yield was very low and most of the methyl groups released as radicals on photolysis were lost as methane, just as it occurs in the absence of homocysteine.[17] The expected methylation took place in the dark in *mildly alkaline solution* (pH ~10), affording methionine in amounts permitting preparative isolation. Analogous transmethylations were also verified with the ions CH_3S^-, n-$C_4H_9S^-$, and $C_6H_5S^-$ as the alkyl acceptors. When methylcobalamin was allowed to react under identical conditions (pH ~10, 25°, solvent ethanol) with CH_3S^-, it was found to react about 1000 times faster than methylaquocobaloxime, producing dimethyl sulfide and vitamin B_{12s} (eq 6). The greater reactivity of the

(16) (a) Whether cobaloxime$_s$ or cobaloxime$_r$ is formed depends on the pH of the solution. In view of the existing equilibria between the Co^I, Co^{III}, and Co^{II} complexes, in general, it is not always possible to differentiate between the reduced forms. The same also applies for the corresponding reduced cobalamins; (b) see, *e.g.*, G. N. Schrauzer and R. J. Windgassen, *Chem. Ber.*, **99**, 602 (1966).

(17) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **88**, 3738 (1966).

(18) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **89**, 143 (1967).

(19) The final step in the acetic acid synthesis of *Clostridium thermoaceticum* may be the reductive cleavage of carboxymethylcobalamin, which could take place with a thiol as the intrinsic reducing agent.



(6)

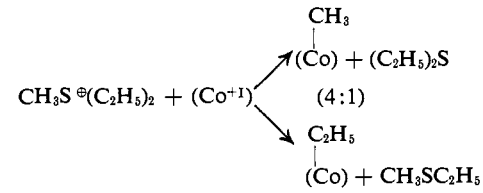
alkylcobalamin may be caused by slight differences in the effective ligand-field strength between cobalamins and cobaloximes.

Attempted Methyl Transfer from Nitrogen to Cobalt

If the cobalt atom would be the intermediate carrier of the methyl group, 5-Me-THF should act as the alkylating agent of vitamin B_{12s}. *In vitro*, no reaction was observed between 5-Me-THF and vitamin B_{12s}.[5] We have treated vitamin B_{12s}, vitamin B_{12r}, as well as the reduced cobaloximes with 5-Me-THF and also could not observe cobalt methylation under a variety of reaction conditions. In view of the comparative stability of N–C bonds, it would seem that transalkylation from nitrogen to cobalt would not be energetically favored. In the absence of any data on the stability of Co–C bonds in cobalamins or cobaloximes, it appeared nevertheless necessary to test this question experimentally. The N-alkyl nitrogen compounds employed as potential alkylating agents apart from 5-Me-THF included tertiary, quaternary aliphatic, as well as mixed aliphatic–aromatic amines. Since all failed to undergo alkyl transfer, it thus seems that 5-Me-THF cannot alkylate the cobalt atom of the corrin directly. An indirect pathway will be mentioned in a later chapter.

Alkyl Transfer from Sulfur to Cobalt. Reactions with S-Adenosylmethionine and Trialkylsulfonium Ions

Addition of S-adenosylmethionine (SAM) and $FADH_2$ in catalytic amounts greatly stimulates the methionine synthetase activity.[3,20] The observed requirement for SAM may be a result of deactivating changes in the apoenzyme protein during its isolation and purification. In addition, the vitamin probably becomes attached to the enzyme protein and therefore must be reactivated by methylation. A mechanism in which SAM is a stoichiometric participant or carrier of the methyl group has been rejected previously in view of the inability of S-adenosylhomocysteine to replace it.[21] SAM reacts with vitamin B_{12s} to form methylcobalamin.[22–25] The two other possible alkylation products (adenosylcobalamin and α-amino-γ-(Co–C)cobalaminbutyric acid) are apparently not formed. Although this selective methyl transfer is suggestive of a "corrin effect," it is nonetheless to be expected for any SN2 reaction involving SAM. To demonstrate this we have treated SAM with cobaloxime$_s$ and also obtained only methylcobaloxime. With methyldiethylsulfonium ion (which was employed as the iodide), a mixture of methyl- and ethylcobaloxime in the ratio of 4:1 was formed (eq 7). This is about the normal ratio between

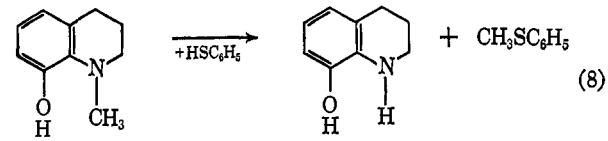


(7)

methyl and ethyl in SN2 group-transfer reactions of trialkylsulfonium ions.

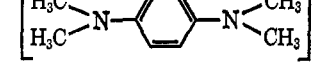
Methyl Transfer from Nitrogen to Sulfur

It has been established[3] that the *cobalamin-independent* methyl transferase enzyme of *E. coli* is specific for 5-N-methyltetrahydropteroyltriglutamic acid as the methyl donor. Thus the cobalamin is in fact not required for the actual transfer of a methyl group from nitrogen to sulfur. We have carried out alkyl-transfer reactions, at first by employing quaternary N-alkyl nitrogen compounds as the donors of the methyl group. Tetraalkylammonium ions did not react with thiols in neutral or weakly alkaline solution up to 60°, although this reaction is energetically feasible.[21] N-Methylpyridinium ion reacted with thiophenolate quite rapidly at room temperature; N-methyl-8-hydroxyquinolinium ion was less effective but still more reactive than methylaquocobaloxime (relative rates at 25° in ethanol, pH 7–8, N-methylpyridinium:N-methyl-8-hydroxyquinolinium:methylaquocobaloxime = 3600:1:0.1. It is important to point out, however, that the 5-N atom in tetrahydrofolic acid is *tertiary* and is not quaternary in the normal sense during the methyl transfer. At least a polarization of the $N–CH_3$ bond is nevertheless deemed necessary[9] to allow the transalkylation to whichever methyl acceptor might be involved. We have therefore also tried to achieve transalkylation reactions from nitrogen to sulfur using tertiary amines as the methyl "donors." Simple trialkylamines did not react with thiols, at least not up to 100°. *When 5-Me-THF was heated with thiophenol, the formation of phenylmethyl thioether was observed*, although the conversion was low. The N-methyl derivative of tetrahydro-8-hydroxyquinoline, which was selected in view of its formal resemblance to the folate cofactor, *reacted with thiophenol slowly at room temperature and gave a significant conversion* on heating to reflux (170°) (eq 8).



(8)

Dimethylaniline failed to transfer. The monohydrochloride of N,N,N′,N′-tetramethyl-*p*-phenylenediamine, however, methylated thiophenol slowly at 100° in ethanol, at pH 5 (eq 9). The dihydrochloride was

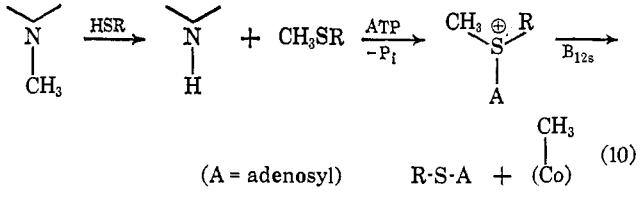


$$CH_3SC_6H_5 + [(CH_3)_2NC_6H_4NH(CH_3)]\cdot HCl \quad (9)$$

(20) J. H. Mangum and K. G. Scrimgeour, *Federation Proc.*, 21, 242 (1962).
(21) G. L. Cantoni, ref 2, p 21.
(22) M. A. Foster, M. J. Dilworth, and D. D. Woods, *Nature*, 201, 39 (1964).
(23) W. Friedrich and E. Königk, *Biochem. Z.*, **336**, 444 (1962).
(24) O. Müller and G. Müller, *ibid.*, **337**, 179 (1963).
(25) S. S. Kerwar, J. H. Mangum, K. G. Scrimgeour, J. D. Brodie, and F. M. Huennekens, *Arch. Biochem. Biophys.*, **116**, 305 (1966).

less effective but still transferred a methyl group at about one-tenth the rate of the monohydrochloride. The monohydrochloride also methylated methyl mercaptan, but no transmethylation reaction was observed with the free base in alkaline medium.

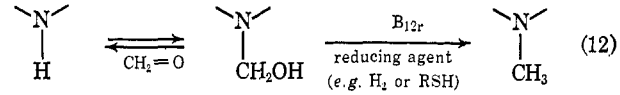
The detailed reasons for the observed lability of the methyl groups in certain N-alkyl compounds and the mechanism of their transfer to sulfur are not yet known and are currently under active investigation. None of the N → S transmethylation reactions could be accelerated or affected in any way by adding varying amounts of cobaloximes (with Co^{+3}, Co^{+2}, and Co^{+}), vitamin B_{12a}, or vitamin B_{12r}. We have mentioned above that the direct transfer of the methyl group of THF to cobalt is unlikely. With the demonstration of methyl transfer from nitrogen to sulfur and from sulfur to cobalt the *indirect* transfer from nitrogen to cobalt is possible and may be part of the feed-back mechanism of methionine biosynthesis to be discussed in the next section. In other enzyme systems (*e.g.*, those of *Methanosarcina barkeri*), this indirect transport of methyl group from nitrogen (tetrahydrofolic acid) to cobalt may be part of the final steps in the methane production (eq 10).



(10)

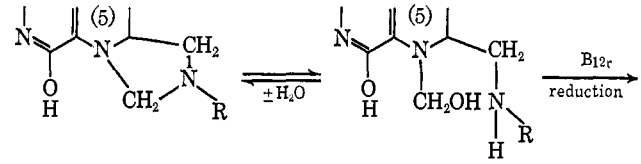
On the Possible Role of the Cobamide Cofactor

In view of the ease with which methylcobalamin reacts with mercaptide ions to produce vitamin B_{12r}, it must be concluded that *it cannot persist in the presence of an excess of homocysteine if the pH in the enzyme–substrate mixture is >7.* On the other hand, if methionine plus ATP are present in excess, the concentration of SAM is expected to also increase. This in turn leads to the conversion of vitamin B_{12r} back to methylcobalamin and causes the observed[2] product repression of enzyme activity (eq 11). The activation–deactivation of the cobinamide coenzyme *via* demethylation–methylation of the cobalt atom (eq 11) suggests that the reactive coenzyme form (vitamin B_{12r} derivative) is *only released at the optimal homocysteine/methionine concentration. It hence would exert a sensitive controlling function* of methionine production. It has been observed recently[26] that methyl transfer from 5-Me-THF is inhibited by propylated cobamides as well as by deoxyadenosyl B_{12}. This could be due to the slower rate with which these substituted cobamides are converted into the active coenzyme form. In view of the high affinity of the cobalt atom for sulfur, it is possible that the cobalamin controls a disulfide–thiol equilibrium of the enzyme which (at least *in vitro*) is crucial for enzyme activity. Since it does not seem to be involved more directly in the actual process of transmethylation, it is difficult to see why the enzyme should utilize B_{12} for such an apparently nonspecific process. Two recent observations suggest, however, that the cobalamin has in fact a more specific function in *catalyzing the de novo synthesis of 5-Me-THF* from 5,10-methylene-THF. We have recently shown that vitamin B_{12r} as well as cobaloximes(II) *in vitro* catalyze the reductive methylation of amines with formaldehyde (eq 12.)[27] Since the reduction of



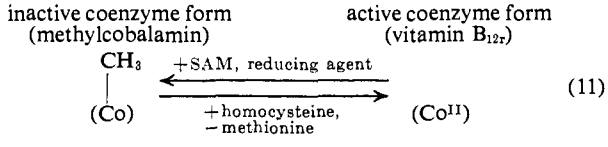
(12)

5,10-methylene-THF should be possible *via* the same mechanism (eq 13) a B_{12} requirement for the 5,10-methylene-THF reductase was postulated.[27]



5-Me-THF (13)

Katzen and Buchanan[28a] and Taylor, Dickerman, and Weissbach[28b] have shown that 5,10-methylene-THF reductase activity of a strain of *E. coli* is *repressed at high concentrations of* L-*methionine* in the system, which indirectly proves the B_{12} requirement suggested by us. As was outlined above, methionine in the presence of ATP will react to form SAM, which in turn will convert the vitamin into its inactive methylated form (eq 11). This would necessarily lead to the inhibition of reaction 13 at other than optimal methionine/homocysteine concentrations. *Control of reaction 13 may thus be the vitamin's key role in this enzyme system.* It is in complete accord with this proposal that extracts of an *E. coli* mutant could utilize an equimolar mixture of formaldehyde and tetrahydropteroylglutamate for the synthesis of the methyl group of methionine, and that vitamin B_{12} stimulated this synthesis six- to eightfold.[29] In animal metabolism, vitamin B_{12} has also been implicated to be the catalyst of the tetrahydrofolate-dependent synthesis of methyl groups from the formaldehyde level.[30] The experiments reported in this paper thus provide the first direct supporting evidence for this function of vitamin B_{12}.



(11)

Experimental Section

Phenylthiolatopyridinatocobaloxime, $C_6H_5SCo(D_2H_2)py$. To a stirred suspension of 36.8 g (0.1 equiv) of $pyCo(D_2H_2)$-Co-

(26) N. Brot, R. Taylor, and H. Weissbach, *Arch. Biochem. Biophys.*, 114, 256 (1966).

(27) G. N. Schrauzer and R. J. Windgassen, *Nature*, 214, 492 (1967).

(28) (a) H. M. Katzen and J. M. Buchanan, *J. Biol. Chem.*, 240, 825 (1965); (b) R. T. Taylor, H. Dickerman, and H. Weissbach, *Arch. Biochem. Biophys.*, 117, 405 (1966).

(29) C. W. Helleiner, R. L. Kisliuk, and D. D. Woods, *J. Gen. Microbiol.*, 18, 23 (1957).

(30) See, *e.g.*, H. R. V. Arnstein in "Proceedings of the Fourth International Congress of Biochemistry, Vienna, 1958," O. Hoffmann-Ostenhof, Ed., Pergamon Press Inc., New York, N. Y., 1960, p 286.

(D_2H_2)py[16b] in 500 ml of methanol under nitrogen, there was added 12.1 g (0.11 mole) of benzenethiol. Hydrogen (1.1 l., 0.05 mole) evolved and after 30 min the black crystals (40.2 g, 84%) were collected by filtration, washed with water and methanol, and air-dried, dec pt 200°. *Anal.* Calcd for $C_{19}H_{24}N_5O_4SCo$ (mol wt, 477.43): C, 47.79; H, 5.07; N, 14.67. Found: C, 48.01; H, 5.03; N, 14.87.

Methylthiolatopyridinatocobaloxime, $CH_3SCo(D_2H_2)$py. A suspension of 23.8 g (0.1 mole) of $CoCl_2 \cdot 6H_2O$ and 23.2 g (0.2 mole) of dimethylglyoxime in 400 ml of methanol was stirred under nitrogen until all cobalt chloride had dissolved. Subsequently, 8.0 g of pyridine and 8.0 g (0.2 mole) of NaOH (dissolved in 50 ml of water) was added. Stirring was continued for 15 min when 5.0 g (0.053 mole) of dimethyl disulfide was added. After 0.5 hr the mixture was diluted with water and the olive crystals (36.0 g, 87%) were collected by filtration, washed with water, and dried at 25° (0.1 mm), dec pt 180°. *Anal.* Calcd for $C_{14}H_{22}N_5O_4SCo$ (mol wt, 415.36): C, 40.48; H, 5.34; N, 16.86; S, 7.72. Found: C, 40.22; H, 3.65; N, 16.67; S, 7.80. Using different bases and disulfides the following thiolatocobaloximes were prepared.

Ethylthiolatopyridinatocobaloxime, $C_2H_5SCo(D_2H_2)$py. *Anal.* Calcd for $C_{15}H_{24}N_5O_4SCo$: C, 41.95; H, 5.64; N, 16.31. Found: C, 42.07; H, 5.59; N, 16.50.

Methylthiolato(N,N-dimethyl-*p*-phenylendiaminato)cobaloxime, $CH_3SCo(D_2H_2) \cdot (CH_3)_2N$-*p*-$C_6H_4NH_2$. *Anal.* Calcd for $C_{17}H_{29}N_6O_4SCo$ (mol wt, 472.48): C, 43.21; H, 6.19; N, 17.79. Found: C, 43.01; H, 6.28; N, 17.96; mp 159°.

Phenylthiolato(N,N-dimethyl-*p*-phenylenediaminato)cobaloxime, $C_6H_5SCo(D_2H_2) \cdot (CH_3)_2N$-*p*-$C_6H_4NH_2$. *Anal.* Calcd for $C_{22}H_{31}N_6O_4SCo$ (mol wt, 534.55): C, 49.42; H, 5.85; N, 15.72. Found: C, 49.28; H, 5.98; N, 16.01.

Catalytic Oxidation of Thiol and Reduction of Disulfide. A suspension of 1.0 g of $CH_3SCo(D_2H_2)$py and 6.0 g (0.125 mole) of methyl mercaptan in 200 ml of methanol was stirred under oxygen; reaction was complete in 3 min with absorption of 700 ml (0.031 mole) of oxygen. Addition of a further 6.0 g of methyl mercaptan caused a gain in absorption of 700 ml of oxygen. Replacing the atmosphere with hydrogen did not lead to reduction of the dimethyl disulfide formed. However, the addition of 1.2 g (0.025 mole) of methyl mercaptan led to a gradually increasing rate of hydrogen absorption, complete after 0.5 hr with 2800 ml (0.125 mole) absorbed. According to glpc analysis all transformations were quantitative.

Preparation of Methylcobaloxime Using CH_3SH as Reducing Agent. A suspension of 32.7 g (0.1 mole) of diaquocobaloxime(II) in 200 ml of methanol was oxidized to hydroxyaquocobaloxime(III) by stirring under oxygen. Under nitrogen this solution was then mixed with 15 ml of methyl iodide and 10 ml of methyl mercaptan and allowed to stand for 48 hr. The solution was then concentrated to 75 ml with a stream of air, diluted with 300 ml of water, and extracted with benzene containing pyridine. The organic extract was concentrated and the residue, on recrystallization from methanol–water, afforded 4.1 g (11%) of methylpyridinatocobaloxime.

Methylation of Homocysteine with Methylpyridinatocobaloxime. A. In the Dark. To a solution of 13.4 g (0.05 mole) of homocystine in 350 ml of anhydrous ammonia, small pieces of sodium were added until a permanent blue color resulted. The color was then discharged by the addition of a trace of methanol, and the solvent was evaporated under a stream of nitrogen. A solution of 38.3 g (0.1 mole) of methylpyridinatocobaloxime in 400 ml of methanol was then added, and the solution was allowed to stand for 12 days in the dark. The reaction mixture was filtered from pyridinatocobaloxime(II) (under nitrogen) and the filtrate concentrated to 100 ml and subsequently diluted with 200 ml of water. After 6 hr of standing 12.3 g of methylpyridinatocobaloxime precipitated. The filtrate was acidified to pH 6 with HCl and concentrated to 75 ml. On further standing for 24 hr in the presence of air 4.6 g of homocystine precipitated; the methionine in the filtrate was isolated by the formation of the mercuric chloride complex, followed by decomposition of the complex with H_2S and purification of the crude methionine by precipitation in water–methanol. The yield was 1.15 g (7.7%). *Anal.* Calcd for $C_5H_{11}NO_2S$ (mol wt 149.2: C, 40.25; H, 7.43; N, 9.39; S, 21.49. Found: C, 40.32; H, 7.58; N, 9.31; S, 21.26.

B. On Light Irradiation. The experiment described under part A was repeated identically except that the methylpyridinatocobaloxime–homocysteine mixture was light irradiated in neutral solution for 12 days, using a 200-w GE visible light source. Under these conditions formation of methane was observed (identified by mass spectrography). On work-up only traces of methionine could be detected (identification was possible by comparison of the R_f values using thin layer chromatography).

Nucleophilic Displacement Reactions. A solution of 3.22 g (0.01 mole) of methylaquocobaloxime, $CH_3Co(D_2H_2) \cdot H_2O$, containing 1.9 g (0.04 mole) of CH_3SH and 0.8 g (0.02 mole) of NaOH in 50 ml of methanol was allowed to stand under nitrogen at 25° for 48 hr. Analysis of the reaction solution by glpc indicated the formation of 0.004 mole of $(CH_3)_2S$; ethylaquocobaloxime, $C_2H_5Co(D_2H_2) \cdot H_2O$, under the same conditions gave only 0.001 mole of $CH_3SC_2H_5$. This corresponds to 40 and 10% conversion based on the amount of the alkylcobaloxime present in the solution. The displacement reactions involving thiophenolate ion or methylcobalamin as the reaction participants were carried out analogously. Assuming pseudo-first-order kinetics, the calculated approximate rate constants are given in Table I.

Table I. Approximate Pseudo-First-Order Rate Constants for Four Nucleophilic Displacement Reactions, at 25° and pH 10

Reaction	k, sec^{-1}
$CH_3Co(D_2H_2) \cdot H_2O + SCH_3^-$	3×10^{-6}
Methylcobalamin + SCH_3^-	3×10^{-3}
$C_2H_5Co(D_2H_2) \cdot H_2O + SCH_3^-$	6×10^{-7}
$CH_3Co(D_2H_2) \cdot H_2O + SC_6H_5^-$	2×10^{-4}

Methyl Transfer from 5-Me-THF to Thiophenol. A slurry of 2 g of 5-Me-THF in 10 ml of ethanol was heated with 4 ml of thiophenol in a sealed tube under nitrogen for 24 hr at 100°. Glpc analysis of the concentrated reaction solution indicated the presence of a small amount of $CH_3SC_6H_5$ (yield ~2%).

Methyl-Transfer Experiments with Other Donors and Acceptors. The following are examples of typical experiments. Product concentrations were determined by glpc.

1. Methyl Transfer from N-Methyl-8-hydroxytetrahydroquinoline. A solution of 5.6 g (0.04 mole) of N-methyl-8-hydroxytetrahydroquinoline in 50 ml of benzenethiol was refluxed under nitrogen for 24 hr, resulting in the formation of 0.0028 mole (7%) of phenyl methyl sulfide.

2. Methyl Transfer from the Monohydrochloride of N,N,N′,N′-Tetramethyl-*p*-phenylenediamine to CH_3SH, C_2H_5SH, and C_6H_5SH. A solution of 2.0 g (10 mmoles) of N,N,N′,N′-tetramethyl-*p*-phenylenediamine monohydrochloride and 4.8 g of methyl mercaptan in 50 ml of ethanol was kept at 100° for 48 hr. Analysis by glpc indicated the formation of 0.1 mmole (1%) of dimethyl sulfide. In parallel experiments with ethyl mercaptan and benzenethiol, methylation occurred to the extent of 0.5 and 2.5%, respectively.

Reaction of Diethylmethylsulfonium Iodide with Cobaloxime. To a suspension of 32.7 g (0.1 mole) of diaquocobaloxime(II) in 500 ml of methanol under hydrogen there was slowly added with stirring 20.5 g (0.1 mole) of tributylphosphine. To this was added 0.1 mole of aqueous NaOH. After 2 hr of stirring 1.1 l. (0.05 mole) of hydrogen was consumed, yielding a deep blue solution of the tributylphosphinatocobaloxime anion. At room temperature a solution of 24.4 g (0.105 mole) of diethylmethylsulfonium iodide in 150 ml of water was added. Reaction was complete in 7 min as evidenced by the disappearance of the blue color; the ratio of methyl ethyl sulfide to diethyl sulfide in the solution was 1:4 as determined by glpc. When the reaction mixture was poured into 2 l. of water, a yellow oil formed which slowly crystallized affording 50 g of yellow solid. When 2 g of this product was heated to 230° and the gases analyzed, methane and ethylene were present in a ratio of about 4:1.

Repetition of the above reaction with trimethylsulfonium iodide afforded 46 g (90%) of tributylphosphinatomethylcobaloxime.

Reaction of S-Adenosylmethionine with Cobaloximes. To a solution of tributylphosphinatocobaloxime(I), prepared from 1.0 g of tributylphosphinatochlorocobaloxime in 5 ml of methanol with sodium borohydride, there was added a solution of 1.0 g (0.002 mole) of adenosylmethionine (as the iodide) in 10 ml of water. After 10 min the solution was shaken with air, then with water and benzene. The benzene solution was concentrated and adsorbed on 15 ml of alumina (Woelm, alkaline) and eluted further with benzene. A yellow band was eluted, and the solid from this eluate on recrystallization from ethanol–water afforded 0.2 g of tributylphos-

phinatomethylcobaloxime, identical with authentic material by melting point, mixture melting point, and infrared comparison. No other product could be detected.

Methylation of Methylaniline Catalyzed by Vitamin B_{12r}. Details of the catalytic action of vitamin B_{12r} and the cobaloximes in the *de novo* synthesis of N-methyl groups have been described.[27] For the sake of completeness the procedure used in a specific example will be reported. To a solution of 0.5 g of cyanocobalamin in 50 ml of methanol under a stream of hydrogen there was added a trace of previously formed vitamin B_{12r} in methanol. The mixture was kept in the stream of hydrogen for 2 hr, reducing the volume of methanol to about one-half of the original. During this time the cyanocobalamin was reduced completely. To this solution there was added 0.5 g of N-methylaniline, and 0.5 g of 40% formaldehyde solution. This solution absorbed 18 ml (0.8 mmole) of hydrogen in 24 hr. The solution was diluted with 200 ml of water and extracted with benzene. Glpc analysis of the benzene layer indicated the presence of 0.5 mmole of N,N-dimethylaniline (10% yield, based on methylaniline).

Acknowledgment. We are indebted to Professor F. M. Huennekens, Scripps Clinic and Research Foundation, for a sample of 5-methyltetrahydrofolic acid.

Interaction and Association of Bases and Nucleosides in Aqueous Solutions. V. Studies of the Association of Purine Nucleosides by Vapor Pressure Osmometry and by Proton Magnetic Resonance[1a,b]

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Abstract: The physical properties of 14 purine nucleosides in aqueous solutions have been studied by vapor pressure osmometry and pmr. These compounds were shown to associate extensively in solution, not by hydrogen bonding, but through the formation of vertical stacks. The concentration dependence of the chemical shifts of various protons provided some detailed information about the nature and the orientation of the nucleosides in these partially overlapping stacks. Two plausible models were proposed. The tendencies of self-association among various nucleosides do not correlate with the dipole moment values of the corresponding bases but correlate reasonably well with the calculated polarizability. The spectral position of the chemical shifts of these nucleosides at infinite dilution provides much valuable information, for example, the indication of intramolecular hydrogen bonding of the 2′-OH group of the ribose to N-3 of the base in adenosine. Based upon consideration of π-charge density distribution, ring currents, and the effect of nitrogen magnetic anisotropy, the spectral positions of H-2 and H-8 for several nucleosides were calculated. The agreement between the theoretical calculations and experimental measurement is substantial.

Previous work from our laboratories has established that purine, 6-methylpurine, and pyrimidine nucleosides associate extensively in aqueous solution by a mechanism involving vertical stacking of bases.[2-5] The self-association of the purines was shown to be much greater than that of the pyrimidine nucleosides and the cross-interaction of purine with pyrimidine nucleosides was shown also to be substantial by measurements of solubilities and by pmr.[2,5] Recent investigation on the cooperative interaction of adenosine with polyuridylic acid demonstrates experimentally that the stacking energy involved in the interaction of the neighboring bases is the major force contributing to the stability of nucleic acid helices.[6]

In this communication, we wish to report the physical properties of 14 purine nucleosides in aqueous and neutral solutions as studied by vapor pressure osmometry and pmr. The major difficulty in this study is the low solubilities of these purine nucleosides, and this is why the properties of these compounds in solution have not been studied before.

Vapor pressure measurements and pmr results both show that, in general, the self-association of these purine nucleosides is even more extensive than that of purine. The effect of methylation clearly indicates that the mechanism of association is not by hydrogen bonding. For example, 1-methylinosine and N-6-dimethyladenosine, in spite of the fact the purine base hydrogen-bond donor sites of these two compounds have been completely removed by methylation, do associate substantially more than inosine or adenosine, respectively.

Studies by pmr on the concentration dependence of the chemical shifts indicate that the base protons (es-

(1) (a) Paper presented in part at the 150th National Meeting of American Chemical Society, Atlantic City, N. J., Sept 1965. (b) This work was supported in part by a Program Project Grant, National Institutes of Health (GM 10802-03), and by a grant from the National Science Foundation (GB-767).

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