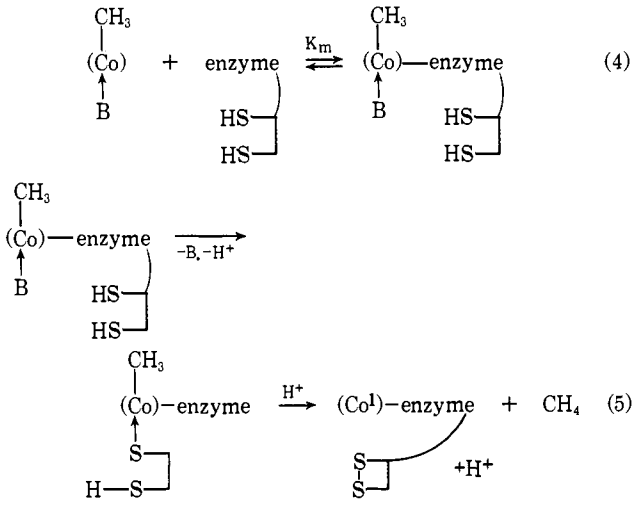
The ability of thiols such as dithioerythritol (DTE) to reductively cleave the Co–C bond in methylcobalamin and alkylcobaloximes (eq 3)[9] nonenzymatically provides a simple means of testing the validity of mechanism II.

The relative rates of methane evolution in aqueous solution at pH 4.6 with DTE as the sole reducing agent present are given in Table I, column II, revealing a definite correlation with the specific activities of the cobaloximes in the enzymatic process. This convincingly supports mechanism II and suggests that a thioprotein SH group provides the active site for the reductive demethylation of both methylcobalamin and methylcobaloximes in this enzyme system. Figure 1 shows that the substrates fall into two groups. Group A substrates are reactive enzymatically as well as nonenzymatically. Group B substrates are only active nonenzymatically. Group A substrates are all derived from unmodified bis(dimethylglyoximato)- or bis(diphenylglyoximato)cobalt complexes. The specific enzymatic activity appears to converge to a maximum rate equal to two-thirds of that of methylcobalamin, suggesting a smaller value of K_m for the cobaloxime model compounds. The relative rates of methane evolution increase in the order of axial bases CNC_6H_{11} < $P(C_6H_5)_3$ < benzimidazole < pyridine ≃ H_2O. This is a sequence of decreasing stability of the axial base adducts,[10] suggesting that the axial base is displaced on interaction with the enzyme. Group B substrates are derived from modified bis(dimethylglyoximato) ligands (*e.g.*, by substituting the oxime protons with BF_2 groups) or from unrelated ligands (*e.g.*, of cobalt bis(salicylaldehyde)ethylenediimine). They are in most cases inactive presumably because of their weak binding to the active site of the enzyme. The principal steps in the enzymatic demethylation of methylcobalamin or of methylcobaloximes thus are described by eq 4 and 5.



The available evidence suggests that cobaloxime model compounds compete with corrins for the same enzyme binding site. The demethylation leaves the enzyme in the oxidized state; its conversion to the original reduced form requires the reduction of a disulfide group. Reduced corrins are much more efficient catalysts of thiol–disulfide oxidation–reduction than cobaloximes[11] and may be the catalysts in the reactivation of the active site. The methylcobaloximes are only demethylated and thus cannot completely replace the corrin cofactor. Methyl(aquo)rhodoxime, the rhodium analog of methyl(aquo)cobaloxime,[12] is a weak inhibitor of the enzymatic methane production with methylcobalamin as the substrate. Its nonenzymatic reductive demethylation with DTE proceeds at only one-fifth of the rate of methyl(aquo)cobaloxime under identical conditions. The different behavior of the rhodoxime is primarily a consequence of the greater thermodynamic stability of the Rh–C bond.

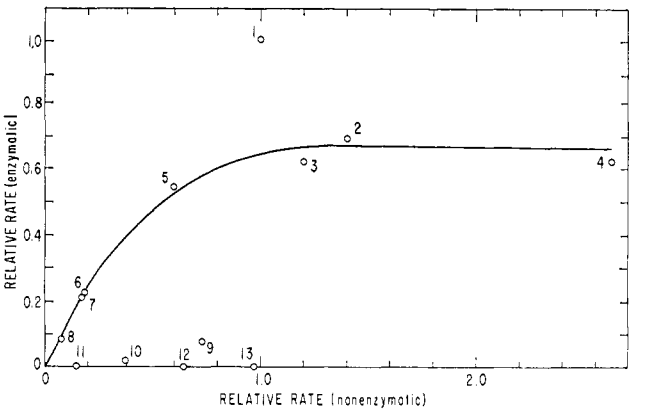


Figure 1. Relative rates of the nonenzymatic methane evolution *vs.* relative rates of the enzymatic methane evolution by cell extracts of *M. omelianskii* in the presence of ATP (10 μmol): TES buffer (100 μmol, pH 7.0), gas phase H_2, incubation temperature 40°. The numbers refer to the complexes listed in Table I.

The present work has led to the postulate of a mechanism of reductive Co–C bond cleavage in a methane-producing enzyme which may be the prototype of other thioredoxine-B_{12} dependent reductases (*i.e.*, of ribonucleosides). It also strikingly demonstrates the usefulness of cobaloximes in the study of vitamin B_{12} dependent enzymatic processes.

Acknowledgment. We thank Drs. J. M. Wood and B. C. McBride (Urbana) for determining the enzymatic activity of the cobaloxime substrates.

(9) G. N. Schrauzer and J. W. Sibert, submitted for publication. For initial work on the reductive cleavage of Co–C bonds by thiols, see G. N. Schrauzer and R. J. Windgassen, *J. Amer. Chem. Soc.*, **89**, 1999, 3607 (1967).

(10) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **88**, 3738 (1966).

(11) G. N. Schrauzer and J. W. Sibert, *Arch. Biochem. Biophys.*, **130**, 257 (1969).

(12) J. H. Weber and G. N. Schrauzer, *J. Amer. Chem. Soc.*, **92**, 726 (1970).

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Conformation of Metalloporphyrins in Solution

Sir:

In recent years there have been a number of X-ray crystallographic studies carried out on metalloporphyrins. The most interesting result from the studies completed to date is the variation of planar or nonplanar conformation of the metalloporphyrin depending on the metal involved and the nature of the extra planar ligand or ligands involved. We wish to report here a method, based on the high-field shift of the coordinated extra planar ligand in the proton magnetic resonance

Table I. Metalloporphyrin–Pyridine Proton Magnetic Resonance Chemical Shifts

No.	Compd	α H[a]	Shift[b]	β H	Shift	γ H	Shift
1	py	1.42		2.79		2.38	
2	py$_2$(meso IX)DME[c] Co(III) acetate	10.00	8.58	5.30	2.51	4.10	1.72
3	py(etio(II))Mg[11d]	7.15	5.73	4.60	1.81	3.89	1.51
4	py(TPP)Mg[11d]	6.99	5.57	4.29	1.50	3.53	1.15
5	py(TPP)Zn[11e]	7.34	5.92	4.50	1.71	3.66	1.28

[a] Chemical shifts reported as τ values relative to tetramethylsilane internal standard; $\tau = 10$. [b] Difference in parts per million from free ligand. [c] C. B. Storm and E. W. Baker, unpublished observations. [d] Reference 4. [e] C. H. Kirksey, W. P. Hambright, and C. B. Storm, *Inorg. Chem.*, **8**, 2141 (1969).

(prnr) spectrum, of calculating the conformation of a number of metalloporphyrins in solution.

The results previously reported in the literature may be summarized briefly. Five-coordinate metalloporphyrins [Mg(II), Zn(II), high-spin Fe(III)] have the metal displaced 0.2–0.5 Å out of the plane of the porphyrin ring toward the one extraplanar ligand.[1] Six-coordinate low-spin Fe(III) has the metal atom nearly in the plane (0.009 Å) of the porphyrin ring.[2] The four-coordinate Cu(II) and Ni(II) porphyrins have the metal atom in or nearly in the plane of the porphyrin ring.[3] In any case where one is interested in the conformation of a molecule the possibility exists that in the solid state the dominant forces in determining this conformation will arise from the packing constraints in the crystal.

Storm and Corwin[4] have shown that the high-field shift for the protons in the center of the porphyrin ring in N-methyl- and N-ethyletioporphyrin(II) may be described by the function $\Delta = 66.6/r^2$ (eq 1) where Δ is the difference in chemical shift between the N-alkylporphyrin and the corresponding N-alkylpyrrole in parts per million and r is the distance of the proton in the alkyl group from the porphyrin current loop of 2.2-Å radius (R).[5] This relationship permits the calculation of the position of a proton lying on the symmetry axis perpendicular to the porphyrin ring and passing through the center of the molecule. The γ hydrogen of a pyridine ligand lies on this line. The distance between the γ hydrogen and the pyridine nitrogen is known and representative metal–nitrogen bond lengths may be obtained from the literature. It is an easy matter to then determine how much the metal must be displaced from the mean plane of the porphyrin ring to maintain these bond lengths at reasonable values.

The chemical shifts for pyridine coordinated to several metalloporphyrins are given in Table I.[6] One can calculate $1/r^2$ values from eq 1. If the distance from the mean plane of the porphyrin ring to the γ-pyridine hydrogen is S, then $S^2 = r^2 - R^2$. The distance from the γ-pyridine hydrogen to the pyridine nitrogen is 3.90 Å. The results of this calculation for the compounds in Table I are given in Table II. The distance of the pyridine nitrogen from the mean plane of the porphyrin ring is given in the column headed P–N and is obtained from $S - 3.90$. One can then obtain typical metal–nitrogen covalent bond lengths and determine the distance the metal is displaced from the mean plane of the porphyrin ring.

Direct application of this scheme to the two TPP derivatives in Table I leads to the conclusion that both metals are out of the plane of the porphyrin ring, magnesium 1.24–1.33 Å and zinc 0.73–0.93 Å. One of the referees suggested that the shielding effect of the four phenyl groups should be considered in determining the true high-field shift of the γ-pyridine hydrogen. A magnetic dipole calculation[7] indicates that a proton in the plane of the phenyl ring and 9.95 Å away from the center of the phenyl ring would be shielded by 0.04 ppm. Since we have four phenyl rings acting on the pyridine ligand this would mean that the observed shift to high field is 0.16 ppm less than it should be for the pyridine γ hydrogen. This quantity is thus added to the values in the last column of Table I, lines 4 and 5, before making the calculation given in Table II.

Table II. Calculated Values for Porphyrin–Pyridine Distances

No.	r^2	S^2	S	P–N[b]
2[a]	38.80	33.96	5.82	1.92
3	44.10	39.96	6.26	2.36
4	50.90	46.06	6.79	2.89
5	46.30	41.46	6.44	2.54

[a] Refers to compound number in Table I. [b] Distance of the pyridine ntirogen from the mean plane of the porphyrin ring.

Cobalt(III)–nitrogen bond lengths vary from 1.92 to 1.98 Å.[8] This would place the cobalt in the plane of the porphyrin ring. Since only one peak is observed for the coordinated pyridines the cobalt must be in the plane of the porphyrin ring or, if slightly displaced, inverting rapidly through the center and averaging the pyridine signals.

Magnesium–nitrogen bond lengths vary from 2.07 to 2.16 Å.[1a,9] This would place the magnesium 0.20–0.30 Å out of the plane of the porphyrin ring in py(etio(II))Mg^{II} and 0.72–0.81 Å out of the plane in py(TPP)Mg^{II}. The buttressing effect of the eight alkyl groups in the etio(II) case could prevent a larger distor-

(1) (a) R. Timkovich and A. Tulinsky, *J. Amer. Chem.* Soc., **91**, 4430 (1969); (b) J. L. Hoard, G. H. Cohen, and M. D. Glick, *ibid.*, **89**, 1992 (1967); (c) M. D. Glick, G. H. Cohen, and J. L. Hoard, *ibid.*, **89**, 1996 (1967).

(2) R. Countryman, D. M. Collins, and J. L. Hoard, *ibid.*, **91**, 5166 (1969).

(3) (a) E. B. Fleischer, *ibid.*, **85**, 1353 (1963); (b) T. A. Hamor, W. S. Caughey, and J. L. Hoard, *ibid.*, **87**, 2305 (1965).

(4) C. B. Storm and A. H. Corwin, *J. Org. Chem.*, **29**, 3700 (1964).

(5) R. J. Abraham, *Mol. Phys.*, **4** (1961).

(6) Abbreviations used in this paper: pyridine = py; α,β,γ,δ-tetraphenylporphin = TPP; etioporphyrin(II) = etio(II); mesoporphyrin IX dimethyl ester = meso IX.

(7) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p 182.

(8) (a) U. Thewalt and R. Marsh, *J. Amer. Chem. Soc.*, **89**, 6364 (1967); (b) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1962, p 728.

(9) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, p 228, 256.

tion and it would not be surprising if the TPP system were more flexible.

Zinc–nitrogen bond lengths vary from 2.03 to 2.23 Å.[1c,9,10] This would place the zinc 0.30–0.50 Å out of the plane of the porphyrin ring.

Our results for the conformation of metalloporphyrins in solution agree qualitatively with results obtained by X-ray crystallographic methods. It appears that the conformation of the metalloporphyrin is determined by the number of extra planar ligands; no ligands, essentially planar; one ligand, metal out of the plane toward the ligand; two ligands, essentially planar.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(10) P. C. Tain, E. C. Lingafetter, and P. Paoletti, *J. Amer. Chem. Soc.*, 90, 519 (1968).

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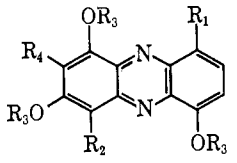
Lomofungin. I. Degradative Studies of a New Phenazine Antibiotic

Sir:

The recently described antibiotic lomofungin[1] has been reported to inhibit the growth of gram-positive and gram-negative bacteria as well as fungi. We assign here structure **1** (1-carbomethoxy-5-formyl-4,6,8-trihydroxyphenazine) to the antibiotic.

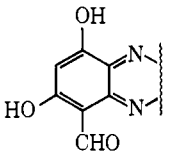


1, R_1 = $COOCH_3$; R_2 = CHO; R_3 = R_4 = H
2, R_1 = $COOCH_3$; R_2 = $CH(OCOCH_3)_2$; R_3 = $COCH_3$; R_4 = H
3, R_1 = $COOCH_3$; R_2 = CHO; R_3 = CH_3; R_4 = H
4, R_1 = $COOCH_3$; R_2 = CH_2OH; R_3 = CH_3; R_4 = H
5, R_1 = $COOCH_3$; R_2 = CH_2OCH_3; R_3 = CH_3; R_4 = H
6, R_1 = $COOCH_3$; R_2 = H; R_3 = CH_3; R_4 = H
7, R_1 = $COOCH_3$; R_2 = CHO; R_3 = R_4 = CH_3
8, R_1 = COOH; R_2 = CHO; R_3 = CH_3; R_4 = H
9, R_1 = COOH; R_2 = CH_2OCH_3; R_3 = CH_3; R_4 = H
10, R_1 = H; R_2 = CH_2OCH_3; R_3 = CH_3; R_4 = H
11, R_1 = R_2 = R_3 = CH_3; R_4 = H

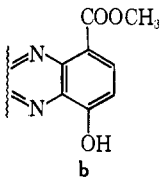
Lomofungin is indicated to be highly aromatic by its molecular formula ($C_{15}H_{10}N_2O_6$;[2a,b] confirmed by high-resolution mass spectrometry), is limited to a linear three-ring system[3] by the electronic spectrum ($\lambda\lambda_{max}$ 257, 364 mμ, $\epsilon\epsilon_{max}$ 72,500, 19,000, respectively) of its acetylation product (**2**; $C_{25}H_{22}N_2O_{12}$;[2a] mp 166–168°), formed at room temperature in pyridine–acetic anhydride or in acetic anhydride–sulfuric acid, and is defined as a phenazine by oxidation of the antibiotic with refluxing concentrated nitric acid to 2,3,5,6-pyrazinetetracarboxylic acid, characterized as its tetramethyl ester [$C_{12}H_{12}N_2O_8$;[2a,b] single nmr peak in $CDCl_3$ at δ 4.05 s; mp 180–181° (lit. 181–182°)[4]].

Methylation of lomofungin with methyl iodide and silver oxide in chloroform at 40° gave its trimethyl ether **3** ($C_{18}H_{16}N_2O_6$;[2a,b] mp 215–217°), whose nmr spectrum[5] contains three new $ArOCH_3$ singlets at δ 4.14, 4.17, and 4.23. In addition to these three phenolic hydroxyl groups lomofungin contains as functional groups an aldehyde and a methyl ester.

The aldehyde group is indicated by reduction of **3** with sodium borohydride in methanol to **4** ($C_{18}H_{18}N_2O_6$;[2a,b] mp 222–225°; $-CH_2O-$ singlet at δ 5.39), accompanied by its aliphatic methyl ether **5** ($C_{19}H_{20}N_2O_6$;[2a,b] mp 177–178°; $ROCH_3$ singlet at δ 3.52). The substitution pattern of the ring bearing the formyl group is defined as shown in **1** by decarbonylation of **3**, employing chlorotris(triphenylphosphine)rhodium(I),[6] to give **6** ($C_{17}H_{16}N_2O_5$;[2a,b] mp 180–182°), whose nmr spectrum contains an aromatic *meta*-AB quartet (δ 6.87 and 7.32; J = 2.5 Hz) in place of the one-proton singlet (δ 6.91) of **3**. Since the aromatic proton generated on decarbonylation is that at lower field (δ 7.32) the formyl group is placed as shown (α) rather than between the two methoxyl groups (β). Isolation of a fully aromatic C-methylated side reaction product **7** ($C_{19}H_{18}N_2O_6$;[2a,b] mp 240–243°; $ArCH_3$ singlet at δ 2.44) from the above methylation of lomofungin also indicates a vacant position between the hydroxyl groups of **1**. These results define unit **a** in the antibiotic.



a



b

The methyl ester function is defined by saponification of **3** to give **8** and of **5** to give **9** ($C_{18}H_{18}N_2O_6$;[2a,b] mp 231–234°), and is located by decarboxylation of **9** over copper powder in pyridine at 220° to give **10** ($C_{17}H_{18}N_2O_4$).[2b] The nmr spectrum of the latter compound (**10**) shows the presence of three aromatic protons on adjacent carbons (δ 7.03, J = 7.96, 1.22 Hz; δ 7.64, J = 7.96, 8.56 Hz; δ 7.95, J = 8.56, 1.22 Hz), while its precursor **9** shows an *ortho*-substitution pattern (δ 7.16, 8.67; J = 8.0 Hz). Since H-1 in the spectrum of **10** must be the proton at δ 7.95, the expected deshielding effect of the carboxyl group is only consistent with its placement at C-1 in **9** (H-2 at δ 8.67 in **9** *vs.* δ 7.64 in **10**; H-3 at δ 7.16 in **9** *vs.* δ 7.03 in **10**) and this placement is consistent with the similar J_{23} coupling constants in the two compounds (8.0 Hz in **9**, 7.96 Hz in **10**). The ring containing the carbomethoxy group is thus unit **b**.

The substitution patterns of the terminal rings are supported by the nmr spectrum of **11** ($C_{17}H_{18}N_2O_3$,[2a] mp 238°), obtained by prolonged lithium aluminum hydride reduction of **3** in refluxing tetrahydrofuran. In the

(1) M. E. Bergy and L. E. Johnson, U. S. Patent 3,359,165 (1967); *Chem. Abstr.*, 68, 38164y (1968). In the patent the name lomondomycin is used for lomofungin.

(2) (a) Mass spectra, obtained on an Atlas CH4 mass spectrometer by the direct inlet technique, employing an oven inlet lock, were in agreement with the formula cited. (b) Elemental analyses agree with the formula given.

(3) *Cf.*, *i.a.*, L. Birkofer, *Chem. Ber.*, 85, 1023 (1952); J. A. Van Allan, G. A. Reynolds, and R. E. Adel, *J. Org. Chem.*, 27, 1659 (1962); M. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), 6, 401 (1958); K. L. Rinehart, Jr., and H. B. Renfroe, *J. Amer. Chem. Soc.*, 83, 3729 (1961).

(4) T. Asao, *Bull. Chem. Soc. Jap.*, 34, 151 (1961).

(5) Nmr spectra were determined on deuteriochloroform solutions; chemical shifts are expressed in parts per million relative to internal tetramethylsilane (δ = 0).

(6) J. Tsuji and K. Ohno, *Tetrahedron Lett.*, 2173 (1967).