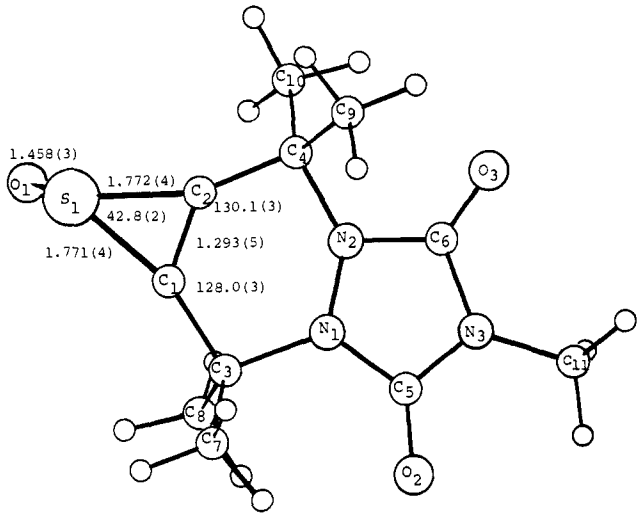


Figure 1. Molecular crystal structure of thiirene sulfoxide **5a**. Numerical values written out are bond lengths (Å) and angles (deg).

In the ^{13}C NMR spectra of **5a** and **5b**, the olefinic carbon resonances are assigned to the signals at 153.2 and 153.5 ppm, respectively, by comparison with those found for dipropylcyclopropenone (160.9 ppm), diphenylcyclopropenone (148.7 ppm),[12] and cyclopropenone **3b** (169.0 ppm).[5] Thiiranoradialene sulfoxide (**2**) showed an olefinic ring carbon resonance at 131.2 ppm.[1] A characteristic feature of both **5a** and **5b** is seen in the IR spectra, where the stretching vibrational absorption due to the S–O bond appears at the unusually high frequency of 1115 cm^{-1}.[13] The value indicates a surprisingly short S–O bond length, the shortest found for any type of sulfoxide.

The structure of **5a** was determined by X-ray analysis. The crystal has monoclinic space group $p2/n$ with a = 15.208 (2) Å, b = 6.083 (1) Å, c = 16.139 (2) Å, and β = 117.06 (1)° with Z = 4. Intensity data were collected on a four-circle diffractometer with graphite monochromated Cu Kα radiation. Of 2457 reflections obtained with $2\theta \leq 158°$, 1691 had intensities greater than $3\sigma|F_o|$ and were used for structure analysis. The structure was refined to a value of 0.058.

The molecular structure of **5a** is revealing (Figure 1). In the crystalline state the S–O bond length (1.458 Å) is substantially shorter than that of the diphenyl analogue **6** (1.467 Å),[5] as expected from the infrared spectra (1115 and 1061 cm^{-1} for **5a** and **6**, respectively), but not shorter than those in thiirene sulfones.[14]

The short S–O bond length has repercussions on the length of the C_1=C_2 bond (1.293 Å) and the C–S bond (1.771 Å), which are both shorter than those in **6** (1.305 and 1.784 Å). Consequently, this is a considerable increase in π-bond character over the three-membered ring and the attached S–O bond, which may be attributed to the combined effects of ring fusion and alkyl substitution.[15] Although the bond angle of C_1–S_1–C_2 of **5a** (42.8°) is comparable to that of **6** (42.9°), the C_4–C_1–C_2 and C_1–C_2–C_3 angles (128.0 and 130.1°) are ~20° smaller than those in **6** (151.9 and 152.7°). Once again ring fusion accounts for this angular compression. The angle of deviation of the sulfoxide oxygen from the thiirene ring is 63.9°. Thus, the thiirene ring of our thiirene sulfoxide **5a** is significantly shortened in all bonds compared to that in **6**.

We hope the data reported here will stimulate theoretical studies on thiirenes. Furthermore, since [2 + 4] cycloadditions of [3] radialenes are uncommon, the successful [2 + 4] cycloaddition of hetero [3] radialene is correspondingly noteworthy.[16] The termini of the diene fragment of [3] radialene are far apart; therefore the dienophile has difficulty in spanning this distance in the transition state.

Success in the present instance is undoubtedly due to the unique structure of thiiranoradialene sulfoxide **2**.

Acknowledgment. We are indebted to Professor C. W. Jefford, University of Geneva, for his useful comments.

Registry No. 2, 81355-46-6; **4a**, 13274-43-6; **4b**, 4233-33-4; **5a**, 82613-75-0; **5b**, 82613-76-1.

Supplementary Material Available: Listings of atomic positional parameters, bond lengths, and bond angles for compound **5a** (5 pages). Ordering information is given on any current masthead page.

(12) Stang, P. J.; Maas, G.; Smith, D. L.; McCloskey, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 4837-45.

(13) Infrared spectrum of **5a** in $CDCl_3$ also showed ν_{S-O} at 1115 cm^{-1}.

(14) Ammon, H. L.; Fallon, L.; Plastas, L. A. *Acta Crystallogr., Sect. B* **1976**, *B32*, 2171-9.

(15) The influence of alkyl and aryl substituents on the dimensions of the three-membered ring has been noted in thiirene sulfone. X-ray analyses show that all bond lengths of the thiirene ring and the S–O bond of 2,3-dimethylthiirene sulfone are shorter than those in 2,3-diphenylthiirene sulfone.[11] This effect may be accounted for by the π-electron delocalization of the thiirene double bond with the benzene ring and the magnitude of the C–S bond π character.

(16) To determine whether the cycloaddition proceeds concertedly (in Diels–Alder type) or stepwise is usually difficult in a [2 + 4] cycloaddition of TAD with dienes.[10] In our result on solvent effect, which is consistent with the previous report,[10] the rate of the [2 + 4] cycloaddition of **2** with 4a is decreased in polar solvent; the rate constants are 110 M^{-1} s^{-1} in benzene, 54 M^{-1} s^{-1} in dichloromethane, and 9.5 M^{-1} s^{-1} in acetonitrile (at 23.2 °C).

Preparation and Study of a Low-Potential Flavin Analogue

Edward B. Skibo and Thomas C. Bruice*

Department of Chemistry
University of California at Santa Barbara
Santa Barbara, California 93106

Received May 20, 1982

Flavins (7,8-dimethylisoalloxazines) serve as cofactors in numerous biochemical redox reactions. The basic flavin structure is present in certain enzymes in modified forms (substitution upon the methyl group at the 8-position, substitution at the 6-position, replacement of the 8-methyl group by hydroxyl or dimethylamino functional groups, replacement of the N^5 of the isoalloxazine ring by carbon, etc.).[1] The effect of these modifications may be to bind flavin to enzyme, to modify the potential, or to alter the chemistry to fit the catalytic role selected for the enzyme in question. Alteration in redox potentials of the 7,8-dimethylisoalloxazines is also brought about by the nature of the catalytic site of the apoprotein and by the preferential binding of the oxidized, reduced, and radical species. Notably absent among the known isoalloxazines are molecules with low reduction potentials comparable to that of the *N*-alkylnicotinamides. Our interest in low-potential flavin analogues is their possible great utility in the study of reaction mechanisms.

We report here preliminary results of our studies of 3,7,9,10-tetrahydro-3,7,10-trimethylpyrimido[5,4-*g*]pteridine-2,4,6,8-tetrone (PPT_{ox})[2] and 1,3,5,7,9,10-hexahydro-3,7,10-trimethylpyrimido[5,4-g]pteridine-2,4,6,8-tetrone ($PPTH_2$)[3] as mimics of flavin and 1,5-dihydroflavin, respectively. The $E^{0\prime}$ for the two-electron reduction of $PPT_{ox} \rightarrow PPTH_2$ (25 °C, H_2O, carbon paste electrode) has been determined as −346 mV vs. the NHE. This

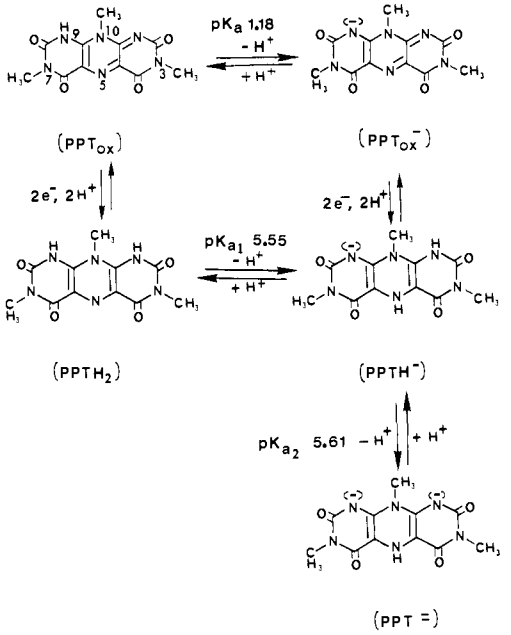
(1) Walsh, C. *Acc. Chem. Res.* **1980**, 13, 148. Merrill, A. H., Jr.; Lambeth, J. D.; Edmondson, D. E.; McCormick, D. B. *Annu. Rev. Nutr.* **1981**, *1*, 281.

(2) Prepared by the condensation of *N*-methylalloxan with 3-methyl-5-amino-6-(methylamino)uracil in refluxing acetic acid;[1] NMR (TFA) δ 3.61 [s, 3 H, N^{10}-methyl], 3.20 [s, 6 H, N^3- and N^7-methyls]. Anal. Calcd. for $C_{11}H_{10}N_6O_4 \cdot 1.5H_2O$: C, 41.63; H, 4.12; N, 26.49. Found: C, 41.61; H, 4.16; N, 26.53.

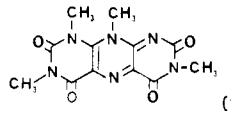
(3) By photochemical reduction with EDTA.

0002-7863/82/1504-4982$01.25/0

Scheme I



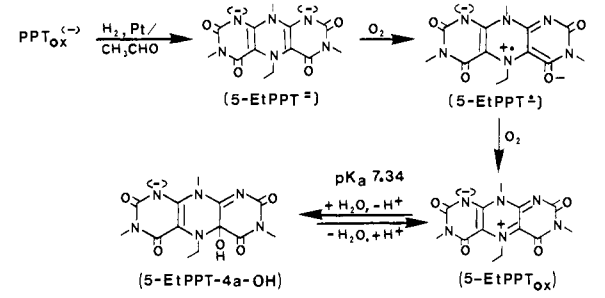
potential is 148 mV more negative than that of lumiflavin[4] and comparable to that for NAD^+ ($E^{0\prime} = -320$ mV).[5] The structures and acid–base properties (H_2O, 30 °C) of PPT_{ox} and $PPTH_2$ are shown in Scheme I. The low pK_a for $PPT_{ox} \rightleftarrows PPT_{ox}^-$ reflects the great degree of conjugation in PPT_{ox}^- wherein the negative charge may be delocalized over 15 atoms. Also noteworthy is the identity of the pK_{a1} and pK_{a2} values for $PPTH_2 \rightarrow PPTH^- \rightarrow PPT^{2-}$. The two enamine anion functions of PPT^{2-} contribute to the reducing power of this species. The N^1-methyl derivative, 1-MePPT_{ox}, possesses an $E^{0\prime}$ of −127 mV. Thus, removal of the



negative charge of PPT_{ox}^- by alkylation increases $E^{0\prime}$ by 219 mV. Both PPT_{ox}^- and PPT^{2-} are quite stable in aqueous solution. The rate constant for HO^- hydrolysis of 1-MePPT_{ox} exceeds that for PPT_{ox}^- by a factor of 10^6 ($\Delta\Delta G^{\ddagger} = 35$ kJ M^{-1}). It is most likely that no more than ~6 kJ M^{-1} of the rate enhancement can be attributed to an electrostatic effect[6] due to the cancellation of the negative charge of PPT_{ox}^- accompanying its methylation. The rapid rate of hydrolysis of 1-MePPT_{ox} when compared to PPT_{ox}^- is due mostly to the release of peri-strain accompanying HO^- addition to the 10a-position of 1-MePPT_{ox} and the resonance stabilization of PPT_{ox}^- due to charge delocalization.

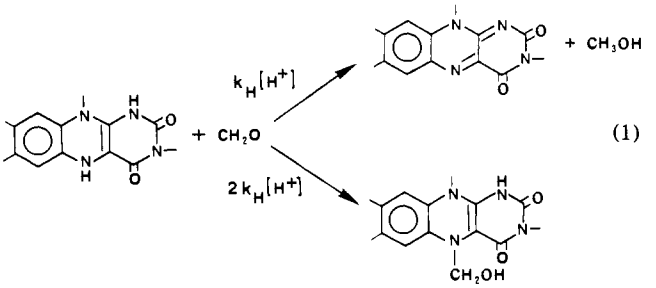
Reductive alkylation[7] of PPT_{ox}^- may be employed to obtain 5-EtPPT^{2-}, which on careful treatment with O_2 yields the iminium cation radical 5-Et$PPT^{-\cdot}$. Further oxidation of 5-Et$PPT^{-\cdot}$ provides the iminium zwitterion 5-EtPPT_{ox}[8] (Scheme II). In water (μ 1.0, 30 °C), 5-EtPPT_{ox} yields the 4a-pseudobase 5-EtPPT-4a-OH. The reactions of Scheme II are analogous to those shown by flavins. Comparison of the pK_a for pseudobase formation with 5-EtPPT_{ox} to that for pseudobase formation with N^5-ethyl-3,7,8,10-tetramethylisoalloxazine (pK_a 7.34 vs. pK_a 4.0[9]) points out the lessened

Scheme II



electronegativity of the 4a-position in 5-EtPPT_{ox} as compared with the cation 5-EtFl_{ox}^+.[10]

The close similarity of PPT^{2-} to dihydroflavin is shown in its reaction with formaldehyde (30 °C, H_2O, μ 1.0). 1,5-Dihydro-3-methyllumiflavin anion (FlH^-) reacts with CH_2O to provide the N^5-carbinolamine plus oxidized flavin (Fl_{ox}) in a ratio of ~2:1 (eq 1).[11] The carbinolamine of eq 1 has been trapped by $CNBH_3^-$



(1)

reduction to yield 5-MeFlH. The reaction of PPT^{2-} with formaldehyde yields PPT_{ox} and N^5-carbinolamine in the ratio of 3:1. As in the case of the flavin reaction, the carbinolamine could be trapped by $CNBH_3^-$ to yield 5-MePPT^-. The rate constant for the dihydroflavin reduction of CH_2O is 2.3×10^4 M^{-2} s^{-1}, while the like constant for PPT^{2-} was determined as 3.3×10^6 M^{-2} s^{-1}. The logarithmic difference (Δ log k_i) in these rate constants amount to 2.15. The difference in the $2e^-$ reduction potentials of Fl_{ox} and PPT_{ox}^- is 146 mV. It follows that the increase in the rate of reduction of formaldehyde obtained by exchanging FlH^- by PPT^{2-} follows the relationship 67 mV/Δ log k_i. From the Nernst equation, a plot of the log of the rate constants vs. $E^{0\prime}$ for a two-electron reduction by a series of 1,5-dihydroflavins should be linear and of slope 60 mV/Δ log k_N. Thus, the second-order rate constant for reduction of CH_2O by PPT^{2-} is that expected of a 1,5-dihydroflavin possessing the redox potential of PPT^{2-}. 1,5-Reduced 1-MePPT_{ox} (1-Me$PPTH_2$) does not react with formaldehyde, and 1-MePPT_{ox} is reduced by $CNBH_3^-$ to yield 1-Me$PPTH_2$. 1,5-Reduced flavin and PPT^{2-} obviously do react with formaldehyde, and both PPT_{ox} and Fl_{ox} are not reduced by $CNBH_3^-$. PPT_{ox}, like Fl_{ox}, is photoreduced by EDTA.

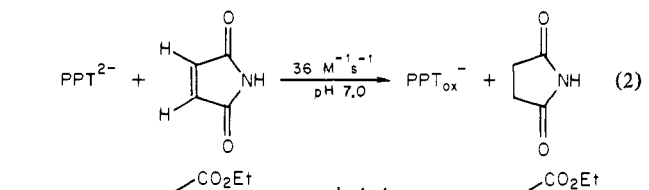
Due to its negative potential, PPT^{2-} reduces N-alkylnicotinamides. When following the formation of PPT_{ox}^- (423 nm) under the pseudo-first-order conditions of [N-alkylnicotinamide] (2×10^{-4} to 3×10^{-3} M) >> [PPT^{2-}] (1×10^{-5} M), the reactions follow the first-order rate law to ~$8t_{1/2}$. Plots of k_{obsd} vs. [N^1-alkylnicotinamide] provide as slopes the second-order rate constants k_2 ($k_2 = 0.33$ M^{-1} s^{-1} for NAD^+ and 0.26 M^{-1} s^{-1} for N-benzylnicotinamide; H_2O, pH 7.0, μ 1.0). For substrate product

(4) Draper, R. D.; Ingraham, L. L. *Arch. Biochem. Biophys.* **1968**, *125*, 802.
(5) "Handbook of Biochemistry"; CRC Press: Cleveland, OH, 1970; p J-33.
(6) Holmquist, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1967**, *89*, 4028; *Ibid.* **1969**, *91*, 2982, 2985.
(7) By the general method of Ball and Bruice (Ball, S. S.; Bruice, T. C. *J. Am. Chem. Soc.* **1981**, *103*, 5494).
(8) Analysis for 5-EtPPT_{ox}. Calcd. for $C_{13}H_{14}N_6O_4{}^1/_4H_2O$: C, 48.36, H, 4.52; N, 26.03. Found: C, 48.48; H, 4.61; N, 25.30.
(9) Ghisla, S.; Hartmann, U.; Hemmerich, P.; Muller, F. *Justus Liebigs. Ann. Chem.* **1973**, 1388. Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 3977. Ball, S.; Bruice, T. C. *Ibid.* **1981**, *103*, 5494.
(10) The spectral characteristics of the compounds reported herein follow: PPT_{ox}, 407 (2.36×10^4), 274 nm (1.54×10^4); PPT_{ox}^-, 423 (3.81×10^4), 288, (9.62×10^3), 242 nm (4.65×10^4); $PPTH_2$, 292 nm (1.03×10^4); $PPTH^-$, 292 nm; PPT^{2-}, 292 nm (1.68×10^4); 5-Et$PPT^{-\cdot}$, 580 nm; 5-EtPPT_{ox} (in DMF), 454 (1.2×10^4), 481 nm (1.4×10^4); 5-EtPPT-4a-OH, 365 nm.
(11) Williams, R. F.; Shinkai, S. S.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 921. See also: Williams, R. F.; Bruice, T. C. *Ibid.* **1976**, *98*, 7752.

analysis, the hydrolytically stable[12] *N*-alkylnicotinamide mimic, *N*-benzylquinoline-3-carboxamide, was employed. This compound (3×10^{-4} M) in aqueous solution is reduced by PPT^{2-} (1×10^{-5} M) on mixing. The percentage yield of PPT_{ox}^- was determined spectrophotometrically (423 nm) as 100%. The identity of *N*-benzyl-1,4-dihydroquinoline-3-carboxamide was obtained in a preparative experiment and shown to be formed in 100% yield. Flavins do not reduce *N*-alkylnicotinamides but are themselves rapidly reduced by *N*-alkyldihydronicotinamides.

The oxidation of mercaptans by flavins has been studied in detail.[13] The mechanism is thought to be well understood. By use of PPT^{2-} this reaction may be examined in the retrodirection and by this means verification of the accepted mechanism may be sought. Also, disulfide bond reduction by 1,5-dihydroflavin cofactor is an important part of the mechanism of glutathoine reductase, dihydrolipoamide dehydrogenase, and thioredoxin reductase.[14] In studies in aqueous solution, thioglycolic acid disulfide is readily reduced ($k = 2 \times 10^{-2}$ M^{-1} s^{-1}, 30 °C, pH 7) by PPT^{2-} to provide PPT_{ox} (90% yield) plus thioglycolic acid (in ~100% yield based on spectrophotometric assay with 5,5′-dithiobis[2-nitrobenzoic acid]). Reduction of cystine by PPT^{2-} provided PPT_{ox}^- (~100%) and cysteine (90%).

The oxidation of succinic acid by succinic acid dehydrogenase to yield fumaric acid represents one of a class of reversible flavoenzyme oxidations that yield C–C double bonds through the intermediacy of a substrate carbanion species.[1,15] There is great current interest in the mode of two-electron transfer from carbanion to enzyme-bound flavin.[16] Are the electrons transferred one by one to provide radical intermediates, is hydride transfer involved, or does a two-electron transfer occur through the formation of a covalent intermediate followed by an elimination reaction? PPT^{2-} reacts with *N*-methylmaleimide to provide PPT_{ox} in 100% yield. The *N*-methylmaleimide is converted to *N*-methylsuccinimide. At least two intermediates can be detected (pH 7.0) by kinetic measurements. In the reduction of maleimide (4×10^{-4} to 7×10^{-4} M) and diethyl fumarate (2×10^{-3} to 2×10^{-4} M) by PPT^{2-} (10^{-5} M), the reactions followed the first-order rate law to $7t_{1/2}$ or $8t_{1/2}$ and provided PPT_{ox} in 100% of theory (eq 2 and 3). Plots of k_{obsd} vs. [substrate] are linear. With



these substrates only carbon–carbon double bond reduction can be seen, and the reactions are simply first order in PPT^{2-} and substrate. No intermediate can be noted.

The various reactions alluded to herein will be described in detail in forthcoming publications. The results provided at this time serve to indicate the utility of PPT^{2-} as a low-potential flavin analogue.

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation.

(12) Shinkai, S.; Hamada, H.; Ide, T.; Manabe, O. *Chem. Lett.* **1978**, 685.
(13) Yokoe, I.; Bruice, T. C. *J. Am. Chem. Soc.* **1975**, *97*, 450. Loechler, E. L.; Hollacher, T. *Ibid.* **1975**, *97*, 3235. Sayer, J. M.; Conlon, P.; Hupp, J.; Faucher, J.; Belanger, R.; White, E. J. *Ibid.* **1979**, *101*, 1890. Loechler, E. L.; Hollacher, T. *Ibid.* **1980**, *102*, 7312, 7322, 7328.
(14) Williams, C. H. In "The Enzymes", 3rd Ed.; Academic Press: New York, 1976; Vol. 13, p 90.
(15) Vik, S. B.; Hatefi, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981** *78*, 6749. Gomes, B.; Fenrich, G.; Abeles, R. H. *Biochemistry* **1981**, *20*, 1481.
(16) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256. Bruice, T. C. *Prog. Bioorgan. Chem.* **1976**, *4*, 1.

A Carbonate Receptor Model by Macromonocyclic Polyamines and Its Physiological Implications

Eiichi Kimura* and Atsuko Sakonaka

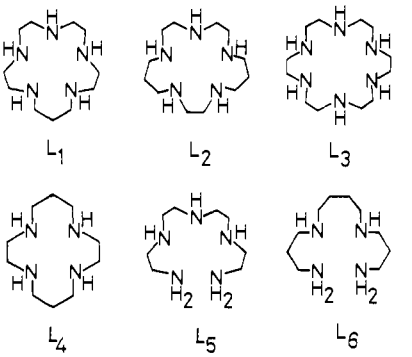
Department of Medicinal Chemistry, Hiroshima University School of Medicine, Kasumi, Hiroshima 734, Japan

Mutsuo Kodama

Department of Chemistry, College of General Education Hirosaki University, Bunkyo, Hirosaki 036, Japan

Received December 29, 1981

We recently discovered that macromonocyclic pentaamines L_1



and L_2 and hexaamine L_3 form stable 1:1 complexes at neutral pH with polycarboxylates (e.g., citrate, succinate, etc.)[1] and phosphates (e.g., inorganic phosphate, AMP, ADP, ATP, etc.).[2] The 3+ charges by three protons contained in the macrocycles and the favorable orientations of the amine protons for hydrogen bondings make these polyamine ligands suitable model receptors to the biochemically important polyoxy anions. Herein we communicate that these polyamines can take up another polyanion, physiologically essential carbonate, CO_3^{2-}. The results obtained not only add a further step to an emerging new field of anion coordination chemistry but also may provide a possible chemical model of membrane recognition of carbonate that is a key step of respiratory regulation of acid–base balance in our body.[3] Our discovery also may imply that the hemoglobin-coordinated carbonate anion constitutes an considerable part of the CO_2 transported in the blood.

The interaction of carbonate anions with macrocyclic polyamines L_1–L_3 and linear polyamines L_5 and L_6 (spermine) was first demonstrated in paper electrophoresis: in the absence of carbonate ions, the polyamines L_1–L_6 moved to the negative electrode as normal polycations in pH 7 Tris buffer (Figure 1a); when bicarbonate ion was added to the Tris buffer, the speed of the negative shift slowed down except for macrocyclic tetraamine L_4 (Figure 1b); in carbonate buffers (pH 9.3) they moved to the reverse, positive electrode (Figure 1c). A similar peculiar behavior at electrophoresis of L_1–L_3 was earlier observed in polycarboxylate (e.g., citrate) buffers, which led to the discovery of the polyamine complexes with polycarboxylates.[1]

A quantitative measurement of the polyamine–carbonate interaction has been made with an anodic wave polarography in Tris (0.05 M) and borate (0.03–0.075 M) buffers, where diffusion-controlled two-step waves were observed with L_1–L_3 and L_5 (Figure 2).[4] The potentials of the first waves were identical with

(1) Kimura, E.; Sakonaka, A.; Yatsunami, T.; Kodama, M. *J. Am. Chem. Soc.* **1981**, *103*, 3041.
(2) Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182.
(3) Harper, H. A.; Rodwell, V. W.; Mayes, P. A. "Physiological Chemistry"; Lange Medical Publications: Los Altos, CA, 1979; pp 222–227, 245–246, 634–640.

0002-7863/82/1504-4984$01.25/0