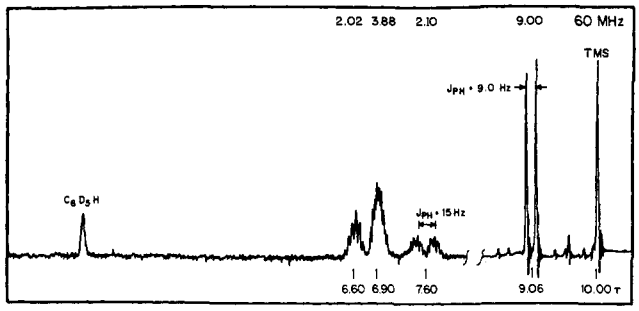


Figure 1. Proton nmr spectrum of IIb in $C_6D_6$. Intensities are listed above the peaks. The amplification is decreased above $\tau$ 8.

have thus prepared the first stable pentaalkylphosphorane.

The methylphosphonium salt IIIc in ether with phenyllithium[12] gives the corresponding phosphorane IId (50% yield), a solid, mp 124–125°, which can be crystallized from ether.[13,14] The dimethylphosphonium salt IIIb with phenyllithium similarly gives IIc, a liquid isolable by distillation (60° ($10^{-6}$ mm),[13] 55% yield), while with methyllithium[15] it gives the trimethylphosphorane IIb, also a distillable liquid (room temperature ($10^{-6}$ mm), 20% yield).[13]

Alternatively, the diphenylmethylphosphorane IId can be prepared by treating the diphenyl salt IIIa with methyllithium (75% yield), but the analogous procedure, the reaction of the phenylmethyl salt IIIc with methyllithium, does not give the simple product IIc cleanly; it gives a mixture of the three phosphoranes IIb, IIc, and IId in the ratio 17:63:20.[16,17] In air IIc decomposed slowly, but IIb and IId were unchanged after a few days. The proton nmr spectrum of the trimethylphosphorane IIb is shown in Figure 1.

To prepare the methylphosphine oxide IVb, the phenyl group in IVa was replaced. Reaction of IVa with methyllithium in ether–benzene at 0° gave IVb directly (66% yield, mp, after crystallization from cyclohexane and sublimation at 90° (0.1 mm), 98.5–100°).[13,14,18] The phosphine oxides IVa and IVb were reduced with $Si_2Cl_6$ in benzene[19] and quaternized with methyl iodide to give the salts IIIc (95%, mp 195–196°)[20] and IIIb (80%, mp 308–309°).[20]

Like the triphenylphosphorane IIa, the methylphosphoranes IIb, IIc, and IId upon heating fragment to the corresponding phosphines and *syn*-tricyclo[4.2.0.$0^{2,5}$]-octa-3,7-diene (which rearranges thermally to cyclooctatetraene),[6,21] and the half-lives at 75° decrease with increasing substitution by phenyls from 108 hr for the trimethylphosphorane to 36 hr for the dimethylphenyl-, 23 hr for the methyldiphenyl-, and 7 hr for the triphenylphosphorane, as should be expected if the phenyls conjugatively delocalize electrons freed from two-center bonds.

The trimethylphosphorane IIb absorbs strongly in the ultraviolet, exhibiting a shoulder at 230 nm (log $\epsilon$ 3.45) and strong absorption extending to 300 nm. Photolysis of benzene or cyclohexane solutions through Pyrex fragments the molecule to trimethylphosphine and *syn*-tricyclo[4.2.0.$0^{2,5}$]octa-3,7-diene.

The phosphorane IIb in $C_6D_6$ shows a $^{31}P$ resonance at +90 ppm higher magnetic field than 85% $H_3PO_4$.[22,23] It has a pleasant odor reminiscent of camphor.

**Acknowledgments.** We are grateful to the National Institutes of Health (MH-08912) for its support and Badische Anilin und Sodafabrik, A.G. for gifts of cyclooctatetraene.

(12) Phenyllithium in 70:30 benzene–ether from Alfa Inorganics, Inc., Beverly, Mass.

(13) The proton nmr spectrum and the parent peak in the mass spectrum are in accord with the structure.

(14) The analyses for carbon, hydrogen, and phosphorus are satisfactory.

(15) Methyllithium in ether containing 0.4% LiCl from Foote Mineral Co.

(16) The composition of this mixture is indicated by identifying the chemical shifts and the phosphorus coupling constants of the methyl proton nmr resonances with those of pure samples of IIb, IIc, and IId.

(17) The exchange of phenyllithium for methyllithium[11] would account for these products.

(18) For analogies see D. Seyferth, D. E. Welch, and J. K. Heeren, *J. Amer. Chem. Soc.*, **86**, 1100 (1964), and L. Horner and P. Beck, *Chem. Ber.*, **93**, 1371 (1960).

(19) K. Naumann, G. Zon, and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 2788 (1969).

(20) Satisfactory proton nmr spectra and analyses for carbon, hydrogen, phosphorus, and iodine were obtained.

(21) The final products are the phosphines and cyclooctatetraene, but that tricyclooctadiene is formed initially is suggested by the observation of its proton nmr absorption early in the reaction. The half-life for the conversion of the diene to cyclooctatetraene at 75° is 7.5 hr.[6]

(22) V. Mark, C. H. Dungan, M. M. Crutchfield, and J. R. van Wazer, *Top. Phosphorus Chem.*, **5**, 227 (1967).

(23) The spectrum was determined by Bruker Physik A.G., Karlsruhe, Germany.

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## Optical Activity of Flow-Oriented Deoxyribonucleic Acid[1]

*Sir:*

We wish to report measurements of the circular dichroism (CD) and absorption spectra of deoxyribonucleic acid (DNA) oriented by flow. The measurements show that the ellipticity $\theta_{33}$ of light propagating down the axis of DNA in the B form is more than six times greater than $\theta_{11}$ and $\theta_{22}$, the ellipticities for light propagating perpendicular to the helix axis. These results provide new support for the theory of polynucleotide optical activity advanced by Tinoco and coworkers.[2-4]

Salmon sperm DNA (Worthington, MW $\sim 6 \times 10^6$) is oriented by flow through a parallel array of capillaries. Light for the optical measurements propagates parallel to the capillary axes. The circular dichroism $\theta$ and absorbance $A$ are determined in the same cell in Cary 6001 and Cary 15 instruments. We verified that residual linear dichroism had negligible effect on the measured CD.[5]

The experimental circular dichroism with and without flow is shown in Figure 1. Under our conditions the 275-nm peak increases with flow by 30 ± 5% and the magnitude of the 245-nm trough increases by 40 ± 5%,

(1) Supported by U. S. Public Health Service Grant No. NS-07286; S-Y. W. acknowledges support from U. S. Training Grant No. GM 780.

(2) (a) W. C. Johnson and I. Tinoco, Jr., *Biopolymers*, 7, 727 (1969); (b) I. Tinoco, Jr., *Advan. Chem. Phys.*, **4**, 133 (1962).

(3) I. Tinoco, Jr., *J. Chim. Phys. Physicochim. Biol.*, **65**, 91 (1968).

(4) I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **86**, 297 (1964).

(5) R. Mandel and G. Holzwarth, *Rev. Sci. Instrum.*, **41**, 755 (1970).

Table I. Measured and Calculated Circular Dichroism Components[a]

| | Wavelength, nm | $(\epsilon_L - \epsilon_R)_{11}$ | $(\epsilon_L - \epsilon_R)_{22}$ | $(\epsilon_L - \epsilon_R)_{33}$ | $\overline{\epsilon_L - \epsilon_R}$ |
|---|---|---|---|---|---|
| Peak, expt | 275 | $-0.2 \mp 0.8$ | $-0.2 \mp 0.8$ | $7.7 \pm 1.6$ | 2.46 |
| Peak, theory[b] | 280 | 0 | 0 | 5.7 (7.8) | 1.9 (2.6) |
| Trough, expt | 245 | $1.2 \mp 1.1$ | $1.2 \mp 1.1$ | $-11.0 \pm 2.2$ | $-2.84$ |
| Trough, theory[b] | 238 | 0 | 0 | $-3.9$ ($-4.5$) | $-1.3$ ($-1.5$) |

[a] Concentrations derived by assuming $\epsilon$ 6600 at the 257-nm absorbance maximum. All molar absorptivities are expressed in liters per mole per centimeter. [b] Based upon the calculations of Johnson and Tinoco[2a] for the planar B form. The numbers in parentheses are for the normal B form.

but the bands do not shift significantly from their normal spectral positions. The effects of identical flow gradients on the absorption spectrum were also measured. The broad 260-nm band of DNA increases in intensity by a uniform factor, $7 \pm 1\%$, between 290 and 240 nm; this is consistent with the previous linear dichroism work.[6,7] Similar results were obtained with calf thymus DNA (Worthington). Previous studies[8] of the CD of flow-oriented DNA have not reported the degree of orientation. The results differ somewhat from ours, and no quantitative interpretation was made.

Molecular quantities may be extracted from our measured CD data by using the absorption data to estimate the degree of orientation, as follows.[9,10] Assume that the molecule is a rigid cylindrical rod with coordinate system $\{1, 2, 3\}$; the 3 axis is the cylinder axis. Similarly, $\{\hat{x}, \hat{y}, \hat{z}\}$ is a space-fixed coordinate system with $\hat{z}$ parallel to the capillary axis. The orientation of the molecule in space is then defined by angles $\alpha$ and $\phi$, the spherical coordinates of the 3 axis in the $xyz$ frame. Here $\alpha$ is the angle between the 3 axis and $\hat{z}$; $\phi$ is the angle between $\hat{x}$ and the projection of the 3 axis onto the $xy$ plane. Now the distribution of molecular orientations is given by a function $f(\alpha, \phi)$, which depends upon the velocity gradient and the molecular geometry. The measured ellipticity $\theta_f$ of the flow-oriented molecules is then given by

$$\theta_f = (1 - \bar{b})\theta + \bar{b}\theta_{33} \qquad (1)$$

where $\bar{b}$ has the form

$$\bar{b} = \frac{1}{4\pi}\int_0^{2\pi}\int_0^{\pi}\frac{1}{2}(3\cos^2\alpha - 1)f(\alpha, \phi)\sin\alpha\, d\alpha d\phi$$

Here $\theta$ is the ellipticity of the unoriented solution, which is equal to $(\theta_{11} + \theta_{22} + \theta_{33})/3$. The quantity $\theta_{ii}$ is the ellipticity when all the molecules are aligned parallel to the $i$ axis.

A similar analysis applies to the absorbance $A_f$ of molecules oriented by flow

$$A_f = (1 - \bar{b})A + \bar{b}A_\perp \qquad (2)$$

where $A$ equals $(2A_\perp + A_{||})/3$; $A_\perp$ and $A_{||}$ are the absorbances for light with electric field perpendicular and parallel to the rod axis, respectively.

Now, it is known that the $\pi \rightarrow \pi^*$ transitions of the bases are polarized in the plane. Therefore, we assume that $A_{||} = 0$ and $A_\perp = {}^3/_2A$ for the B form of DNA. From eq 1 and 2, and the path length and molar concentration of the solute, one may evaluate the components of the molar circular dichroism $(\epsilon_L - \epsilon_R)_{ii}$ along the three molecular principal axes; values thus obtained are shown in Table I, together with the predictions of theory. It is found that $(\epsilon_L - \epsilon_R)_{33}$ is at least six times larger than $(\epsilon_L - \epsilon_R)_{11}$ and $(\epsilon_L - \epsilon_R)_{22}$.

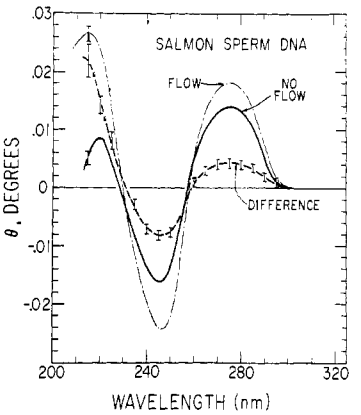


Figure 1. Circular dichroism of flow-oriented DNA at pH 7 in 0.01 $M$ KF and 0.01 $M$ phosphate buffer. Average flow gradient, $5 \times 10^3$ sec$^{-1}$; cell path length 3 cm.

The theoretical values in Table I are based upon the studies by Johnson and Tinoco.[2a] Their theory assigns the optical activity of polynucleotides largely to exciton interaction among the $\pi \rightarrow \pi^*$ transitions of the bases. It has already been shown[2a] that the theory reproduces quite successfully the CD band shapes of many polynucleotides in unoriented solution.[11,12] In particular, Johnson and Tinoco calculated the solution CD, $\overline{\epsilon_L - \epsilon_R}$, for unoriented DNA in the B form[13] and in a planar B form in which the base planes are orthogonal to the helix axis. For the planar B form, $(\epsilon_L - \epsilon_R)_{11}$ and $(\epsilon_L - \epsilon_R)_{22}$ are predicted to be zero; $(\epsilon_L - \epsilon_R)_{33}$ then equals $3\overline{(\epsilon_L - \epsilon_R)}$. We have used the identical procedure to obtain the theoretical values of $(\epsilon_L - \epsilon_R)_{33}$ entered in Table I for the nonplanar B form (in parentheses), although this procedure is not strictly correct. A comparison between theory and experiment (Table I) shows excellent agreement in both signs and magnitudes for all three components of $\epsilon_L - \epsilon_R$ at 275

(6) D. M. Gray and I. Rubenstein, *Biopolymers*, **6**, 1605 (1968).
(7) A. Wada, *ibid.*, **2**, 236 (1964); *J. Polym. Sci., Part A*, **2**, 853 (1964).
(8) S. F. Mason and A. J. McCaffery, *Nature (London)*, **204**, 468 (1964).
(9) N. Go, *J. Phys. Soc. Jap.*, **23**, 88 (1967); *J. Chem. Phys.*, **43**, 1275 (1965).
(10) I. Tinoco, Jr., and W. G. Hammerle, *J. Phys. Chem.*, **60**, 1619 (1956).
(11) J. Brahms and S. Brahms in "Fine Structure of Proteins and Nucleic Acids," Vol. IV, G. D. Fasman and S. N. Timasheff, Ed., Marcel Dekker, New York, N. Y., 1970, p 191.
(12) J. T. Yang and T. Samejima, *Progr. Nucl. Acid Res. Mol. Biol.*, **9**, 223 (1969).
(13) R. Langridge, D. A. Marvin, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, **2**, 38 (1960).

nm. The agreement at 245 nm is less satisfactory, but observed relative magnitudes of the three components of $\epsilon_L - \epsilon_R$ are in good accord with theory.

The fact that the value of $\Delta\theta/\theta$ at the 275-nm band is slightly less than that at the 245-nm band suggests the existence of a weak n → π* transition[11,14] with positive rotational strength in the 275-nm band region; this would contribute to $\theta_{11}$ and $\theta_{22}$.

These studies are being continued with other polynucleotide systems.

**Acknowledgment.** We are grateful to I. Tinoco, Jr., for helpful comments.

(14) K. Kasha in "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins University Press, Baltimore, Md., 1960, p 31.

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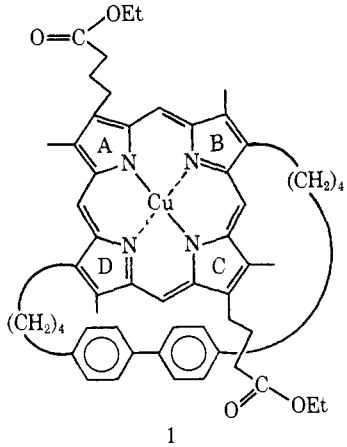
## Cyclophane Porphyrin[1]

*Sir:*

We report the synthesis of copper 1,3,5,7-tetramethyl-2,6-di-γ-carbethoxypropyl-4,8-(biphenyl-4,4′-ditetramethyleno)porphyrin (**1**), a cyclophane metallo-

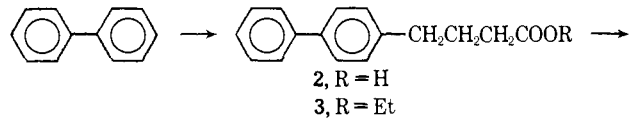



1

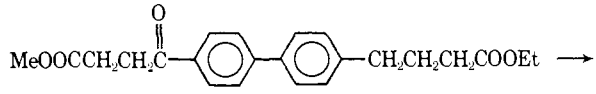
porphyrin. This compound was prepared as part of an effort to synthesize the active site in myoglobin or hemoglobin, without the accompanying protein. We conjectured that if the proper environment such as a histidine molecule on one side and a hydrophobic environment on the other side of the ferroheme[2] were assembled, the molecule might reversibly bind oxygen. These two features would be incorporated by converting **1** into a histidyl derivative of the corresponding ferroporphyrin. A successful implemention of this idea would provide much information concerning the function of the large proteins in enzymatic catalysis and also allow a detailed study of the mechanism of reversible oxygen binding.

After unsuccessful attempts to synthesize the cyclophane porphyrin by building a cyclophane ring system, $-(CH_2)_n$-aromatic-$(CH_2)_n-$, onto a porphyrin, we adopted the more tactical approach of constructing the porphyrin through dipyrromethenes which were attached to both ends of an aromatic system. The intramolecular porphyrin condensation takes advantage of copper(II) ion chelation to fix the dipyrromethene ends in a proper position for bond formation between the dipyrromethene containing rings A and D and that containing rings B and C in **1**.

Biphenyl was converted into γ-(4-biphenyl)butyric acid (**2**) (mp 118°) by Friedel–Crafts reaction with succinic anhydride,[3,4] followed by a Wolff–Kishner reduction. Esterification gave the ethyl ester **3**. Friedel–




2, R = H
3, R = Et

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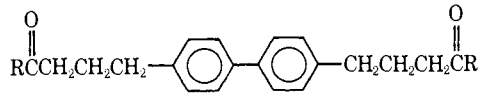


5, R = OH
6, R = Cl

Crafts reaction of **3** and β-carbomethoxypropionyl chloride[5] gave 4-(γ-carbethoxypropyl)-4′-(β-carbomethoxypropionyl)biphenyl (**4**) (mp 68°) in 70% yield. Hydrolysis of the ester and reduction of the carbonyl group by Wolff–Kishner reaction carried out in triethylene glycol gave biphenyl-4,4′-dibutyric acid (**5**) (mp 185°). The overall yield to this step was 22% based on biphenyl. Treating the acid **5** with thionyl chloride gave biphenyl-4,4′-dibutyric acid chloride (**6**), mp 72–73°.

Formation of bis[*p*-phenylenetrimethylenecarbonyl-(2,4-dimethyl-5-carbethoxypyrrol-3-yl)] (**8**) was accomplished by Friedel–Crafts acylation of 2,4-dimethyl-5-carbethoxypyrrole (**7**)[6,7] with the acid chloride (**6**) in the presence of 4 equiv (based on pyrrole) of anhydrous aluminum chloride in nitrobenzene.[8] After work-up and recrystallization from acetone, **8**, mp 192°, was obtained in 59% yield.[9] Reduction of **8** with diborane[10] in tetrahydrofuran gave bis[*p*-phenylenetetramethylene-(2,4-dimethyl-5-carbethoxypyrrol-3-yl)] (**9**), mp 164–165°. The benzyl ester **10** was prepared by transesterification of **9** in the presence of sodium benzyloxide. In order to obtain bis[*p*-phenylenetetramethylene(2-

(1) This work was supported by a grant from the National Institutes of Health (AM 11404).

(2) J. H. Wang, *Accounts Chem. Res.*, **3**, 90 (1970), and references cited therein.

(3) (a) M. Weizmann, E. Bergmann, and E. Bogradov, *Chem. Ind. (London)*, **59**, 402 (1940); (b) D. H. Hey and R. Wilkinson, *J. Chem. Soc.*, 1030 (1940).

(4) We list analyses and spectra for key intermediates. However, nmr spectra of all intermediates are consistent with structures written.

(5) J. Cason, "Organic Syntheses," Collect. Vol. 3, Wiley, New York, N. Y., 1955, p 169.

(6) (a) H. Fischer, "Organic Syntheses," Collect. Vol. 2, Wiley, New York, N. Y., 1943, p 202; (b) A. H. Corwin and W. M. Quattlebaum, Jr., *J. Amer. Chem. Soc.*, **58**, 1081 (1936).

(7) H. Fisher and H. Orth, "Die Chemie des Pyrrols," Vol. I, Akademie Verlag, Leipzig, 1934, p 239.

(8) H. Fischer and F. Schubert, *Z. Physiol. Chem.*, **155**, 110, 1 (1926).

(9) *Anal.* Calcd, for $C_{38}H_{48}N_2O_4$: C, 76.47; H, 8.11; N, 4.70. Found: C, 76.33; H, 8.28; N, 4.80. For the nmr spectrum ($CDCl_3$), see Table I.

(10) (a) K. M. Biswas, L. E. Houghton, and A. H. Jackson, *Tetrahedron, Suppl.*, 7, 261 (1966); (b) T. A. Ballantine, A. H. Jackson, G. W. Kenner, and G. McGilivray, *ibid.*, 7, 241 (1966).