

responds to at least 20% of the material being a free radical, when checked against DPPH. Moreover, the optical and e.p.r. spectra are the same whether III is dissolved in ethylene chloride or iodine is added to I in ethylene chloride.

The marked contrast between the iodide ion, which is a good donor of electronic charge, and the nitrate ion which is not, and also the contrast between the non-polar ethylene chloride and the polar water, suggests the mechanism shown in the Chart. It is reasonable that an equilibrium favoring ionic species in polar solvents should shift in the direction favoring less charged forms in less polar solvents. This favorable effect, coupled with the favorable donor iodide ion and the favorable acceptor quinoindal dication, E_0 (polarographic pH 5 in water) + 0.075 v. vs. S.C.E., would facilitate the simple one electron transfer to give IIIb or the two electron transfer to give I. Our studies in this area are continuing and a report on the free radical behavior of the green and the black diiodide in the solid state, will be made later.

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ESTIMATION OF BASE PAIRING IN NUCLEIC ACIDS FROM HYPOCHROMISM

Sir:

Hypochromism in nucleic acids occurs as a low extinction coefficient of the native form relative to denatured forms in the absorption band whose maximum is at 260 $m\mu$.¹ The extinction coefficient

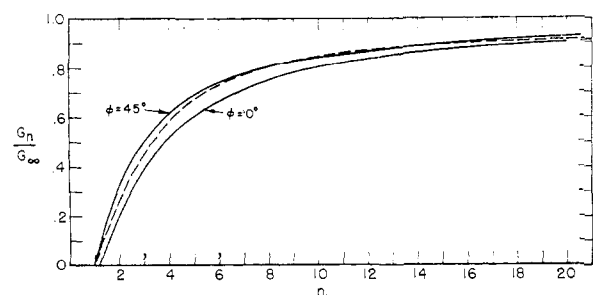


Fig. 1.—Relative hypochromism in DNA as a function sequence length. Solid curves are from Rich and Tinoco⁴ for two orientations (ϕ) of the transition dipole moment relative to the radial direction. Dashed curve represents equation (2).

has therefore been used to estimate the extent of base pairing,² which appears to be the main structural feature lost on denaturation. The calculation usually is based on the assumption that the

(1) R. Thomas, *Biochim. et Biophys. Acta*, **14**, 231 (1954).
(2) P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *Proc. Nat. Acad. Sci. U. S. A.*, **45**, 482 (1959).

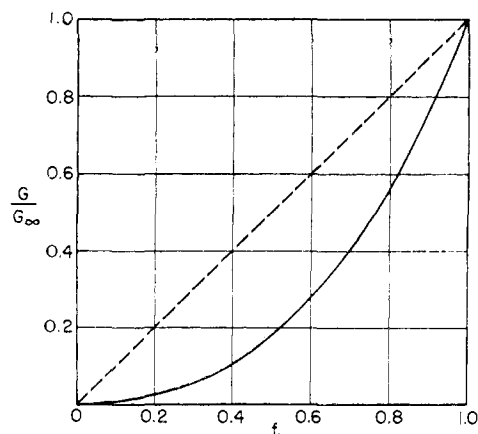


Fig. 2.—Relative hypochromism as a function of fraction of bases paired. Solid curve is from equation (3). Dashed line is for the case $G/G_\infty = f$.

hypochromism is a linear function of the number of base pairs. Since it has been shown by Tinoco³ that the observed hypochromism may be accounted for by interactions between neighboring base pairs along the nucleic acid chain, it appears that this assumption is not justified when the neighborhood of any base pair varies with the extent of pairing. Rich and Tinoco⁴ have shown on experimental and theoretical grounds that the hypochromism of a sequence of base pairs is a sensitive function of the length of the sequence, as shown in Fig. 1. From this it is concluded that the way in which the extinction coefficient (or hypochromism) varies with the degree of base pairing depends on the distribution of base pairs among sequences of various lengths. To illustrate this for a particular hypothetical case, a calculation is given for the hypochromism G as a function of the fraction f of bases paired, letting the pairing occur at random among complementary bases in two parallel strands.

The hypochromism is defined as $G = (\epsilon_0 - \epsilon)/\epsilon_0$, where ϵ_0 is the average extinction coefficient of the isolated bases and ϵ is that of the nucleic acid. For base pairs in sequences of n pairs we define $G_n = (\epsilon_0 - \epsilon_n)/\epsilon_0$. Then

$$G = \sum_{n=1}^{\infty} G_n W_n \quad (1)$$

where W_n is the weight fraction of base pairs in sequences of length n . Equation (1) implies no particular distribution of sequence lengths, but does contain the assumption that there is no interaction between neighboring sequences. For a random distribution of sequence lengths, $W_n = nf^n(1-f)^2$. An approximation to the theoretical curves of Rich and Tinoco⁴ for G_n/G_∞ is obtained in the exponential form (2)

$$G_n/G_\infty = 1 - 0.18e^{-0.0345(n-1)} - 0.82e^{-0.332(n-1)} \quad (2)$$

The dashed curve in Fig. 1 is a plot of equation (2), showing this to be an acceptable approximation. From equations (1) and (2) one then obtains expression (3) useful for numerical calculations

(3) I. Tinoco, Jr., *J. Am. Chem. Soc.*, **82**, 4785 (1960).
(4) A. Rich and I. Tinoco, Jr., *ibid.*, **82**, 6409 (1960).

$$G/G_{\infty} = f \left[1 - 0.18 \left(\frac{1-f}{1-0.9661f} \right)^2 - 0.82 \left(\frac{1-f}{1-0.6761f} \right)^2 \right] \quad (3)$$

Equation (3) is plotted in Fig. 2. It is seen that, for this case, there is a significant correction to the usual assumption that $G/G_{\infty} = f$.

The degree to which the example above approximates any real system is uncertain. It is likely that the probability that a given base be paired is influenced significantly by the presence or absence of pairs among neighboring bases. The result would be that the distribution of sequence lengths would not be random as assumed above. Any attempt to account for this in the type of calculation performed here must introduce structural parameters which are not known at present. An indication of the nature of this effect has been given by theoretical treatments of the thermal denaturation process for two-stranded nucleic acids,⁶ where it has been shown that the process occurs via states involving a significant proportion of base pairs in relatively short sequences. For this case it appears likely that the sequence length distribution is weighted more heavily in the long sequences than is the random distribution, as a result of the cooperative nature of the denaturation. This effect would decrease the correction necessary in the assumption of $G/G_{\infty} = f$. In the case of ribonucleic acid, thermal denaturation occurs over a much broader range than in deoxyribonucleic acid,² suggesting that the destruction of base pairs is more nearly random in the former, in which case the above example would apply to a better degree of approximation. The example serves at least to place an upper limit on the correction necessary to the calculation of f from the hypochromism in cooperative melting. In denatured DNA, on the other hand, where a partial restoration of hypochromism occurs on reversal of the denaturing conditions, it appears that the cooperative nature of the base pairing has been destroyed, as evidenced by the broadening of the transition.² It is expected that the example used here would be a reasonable approximation to this case.

One significant result of these observations is the possible necessity to reinterpret the melting temperature, T_m , for nucleic acids. This has been defined² as the temperature at which $G/G_{\infty} = 0.5$. This definition remains adequate for most purposes, where T_m is required only as an operational quantity. However, a melting temperature of simpler thermodynamic significance would be defined as the temperature at which $f = 0.5$. At this temperature T_m' , ΔF^0 for the transition is zero, and $T_m' = \Delta H^0/\Delta S^0$. From Table I it is seen that $G/G_{\infty} = 0.18$ at this temperature for the present example. For the broad thermal transitions in ribonucleic acids, it appears² that T_m' might be around 10° higher than T_m , although this is assumed to be an approximate upper limit.

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(5) B. H. Zimm, *J. Chem. Phys.*, **33**, 1349 (1960), and references cited therein.

TRANSANNULAR AND HYDROGEN-REARRANGEMENT REACTIONS IN CARBENOID DECOMPOSITION OF DIAZOCYCLOALKANES

Sir:

Diazo compounds decompose to carbenes which give olefins by hydrogen-migration and cyclopropanes by 1,3-intramolecular insertion¹; carbon-skeleton rearrangement does not occur extensively in carbenoid systems of the neopentyl type.^{1a} Rearrangements of carbon-skeleton² are major reactions, however, in thermal decomposition of diazocyclopropane,³ cyclopropyldiazomethane,⁴ and diazocyclobutane⁴ in that allene (ring opening), cyclobutene (ring expansion), and methylenecyclopropane (ring contraction), respectively, are the principal products.

Intramolecular 1,2-, 1,3-, 1,5- and 1,6 hydride shifts occur extensively in carbonium ion reactions of medium-sized saturated rings; transannular alkylations to give bicyclic products occur only to a limited extent.⁵ A study has now been made of decomposition of homologous C₅ through C₁₀ diazocycloalkanes in order to determine the kind and extent of transannular and rearrangement reactions which simple cyclic carbenes give. Diazocyclopentane⁶ and diazocyclohexane⁶ decompose thermally to cyclopentene (100%) and cyclohexene (100%), respectively; there were no detectable quantities of hydrocarbons derived from transannular insertion or ring contraction in these systems. The decomposition of diazocyclohexane to cyclohexene is contrasted with the quantitative

(1) (a) L. Friedman and H. Shechter, *J. Am. Chem. Soc.*, **81**, 5512 (1959); (b) J. W. Powell and M. C. Whiting, *Tetrahedron*, **7**, 305 (1959); (c) 1,5- and 1,6-intramolecular insertion occurs in decomposition of 2-(β-phenylethyl)-phenyldiazomethane to 2-phenylindan and of 2-(γ-phenylpropyl)-phenyldiazomethane to 2-benzylindan and 2-phenyltetralin: C. D. Gutsche and H. E. Johnson, *J. Am. Chem. Soc.*, **77**, 5933 (1955); C. D. Gutsche, E. F. Jason, R. S. Coffey and H. E. Johnson, *ibid.*, **80**, 5756 (1958).

(2) Carbon-skeleton rearrangement of a carbene is equivalent to intramolecular insertion of the carbenic center across a carbon-carbon bond.

(3) (a) Private communication by S. S. Rawalay of this laboratory. Diazocyclopropane was generated as a presumed intermediate in reaction of N-cyclopropyl-N-nitroso-p-toluenesulfonamide and lithium ethoxide in diethyl Carbitol. (b) See also W. M. Jones, *ibid.*, **82**, 6200 (1960).

(4) L. Friedman and H. Shechter, *ibid.*, **82**, 1002 (1960).

(5) (a) Solvolysis of cyclodecyl arylsulfonates,^{5a} diazotization of cyclodecylamine,^{5d} and formolysis of cycloheptene oxide,^{5e} *cis*- and *trans*-cyclooctene oxides,^{5f,6} *cis*- and *trans*-cyclononene oxides,^{5h} and *cis*-cyclodecene oxide⁵ⁱ involve marked transannular hydride shifts; no bicyclic products were reported. (b) *cis*- and *trans*-decalins^{5j} are minor products in acid-catalyzed isomerization of *trans*- to *cis*-cyclodecene. Formolysis of *trans*-cyclodecene oxide yields *trans*-1-decalol^{5j} (see footnote 13b), a transannular alkylation product (21%), along with cyclodecanone, 1,6-cyclodecanediol and other products. (c) H. J. Urich and V. Prelog, *Helv. Chim. Acta*, **40**, 477 (1957). (d) V. Prelog, H. J. Urich, A. A. Bothner-By and J. Wursch, *ibid.*, **38**, 1095 (1955). (e) A. C. Cope, T. A. Liss and G. W. Wood, *J. Am. Chem. Soc.*, **79**, 6289 (1957). (f) A. C. Cope, J. M. Grisar and P. E. Peterson, *ibid.*, **81**, 1640 (1959). (g) A. C. Cope, A. Fournier and H. E. Simmons, *ibid.*, **79**, 3905 (1957). (h) V. Prelog, K. Schenker and W. King, *Helv. Chim. Acta*, **36**, 471 (1953). (i) V. Prelog and K. Schenker, *ibid.*, **35**, 2044 (1952). (j) A. C. Cope, D. C. McLean and N. A. Nelson, *J. Am. Chem. Soc.*, **77**, 1628 (1955).

(6) (a) The diazocycloalkanes were generated by decomposing tosylhydrazones of the cycloalkanonones with sodium methoxide (1.2 equivalents) in diethyl Carbitol. The yields of hydrocarbons are 60–70%. The percentages reported are product compositions of the mixture of hydrocarbons formed as determined by gas chromatography. (b) The C_{8–10} cycloalkanonone tosylhydrazones (analytical samples) melted at 180–181, 155–156, 144–146, 140–141, 135–136, and 137–138°, respectively.