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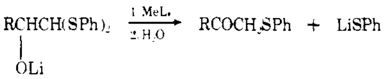
References and Notes

(1) T. Cohen, G. Herman, J. R. Falck, and A. J. Mura, Jr., *J. Org. Chem.*, **40**, 812 (1975).
(2) R. G. Salomon and J. K. Kochi, *J. Am. Chem. Soc.*, **95**, 3300 (1973).
(3) E. J. Corey and D. Seebach, *J. Org. Chem.*, **31**, 4097 (1966).
(4) A somewhat related transformation has been reported wherein the adduct of **1** with an aldehyde, upon treatment with MeLi, undergoes an apparent carbenoid rearrangement:



$$\underset{\textstyle |\atop \text{OLi}}{\text{RCH}}\text{CH(SPh)}_2 \xrightarrow[2.\ \text{H}_2\text{O}]{1.\ \text{MeLi}} \text{RCOCH}_2\text{SPh} + \text{LiSPh}$$

I. Kuwajima and Y. Kurata, *Chem. Lett.*, 291 (1972).
(5) (a) α-Thioalkyl or aryl ketones: D. Seebach and M. Teschner, *Tetrahedron Lett.*, 5113 (1973), and references cited therein; A. W. Johnson in "Organic Compounds of Sulphur, Selenium, and Tellurium", Vol 1, the Chemical Society, London, 1970, Chapter 6; B. M. Trost, K. Hiroi, and S. Kurozumi, *J. Am. Chem. Soc.*, **97**, 438 (1975); B. M. Trost and T. N. Salzmann, *ibid.*, **95**, 6840 (1973); R. L. Autrey and P. W. Scullard, *ibid.*, **87**, 3284 (1965); G. A. Russell and E. T. Sabourin, *J. Org. Chem.*, **34**, 2336 (1969); P. A. Grieco and C. S. Pogonowski, *J. Chem. Soc., Chem. Commun.*, 72 (1975); K. Rabindran and B. D. Tilak, *Curr. Sci.*, **20**, 207 (1951); F. Winternitz, N. J. Antia, M. Tumlirova, and R. Lachazette, *Bull. Chim. Soc. Fr.*, 1817 (1956); K. Hiroi, H. Matsuda, and Y. Kishida, *Chem. Pharm. Bull.*, **19**, 2207 (1971). (b) Corresponding ketosulfoxides: P. Grieco and C. Pogonowski, *J. Org. Chem.*, **39**, 732 (1974); G. A. Russell and G. J. Mikol, *J. Am. Chem. Soc.*, **88**, 5498 (1966); E. J. Corey and M. Chaykovsky, *ibid.*, **86**, 1639 (1964). (c) Alcohols from reduction of ketones: J. R. Shanklin, C. R. Johnson, J. Ollinger, and R. M. Coates, *ibid.*, **95**, 3429 (1973).
(6) C. D. Gutsche and D. Redmore, "Carbocyclic Ring Expansion Reactions", Academic Press, New York, N.Y., 1968.
(7) Two notable exceptions: W. L. Mock and M. E. Hartman, *J. Am. Chem. Soc.*, **92**, 5767 (1970); H. Taguchi, H. Yamamoto, and H. Nozaki, *ibid.*, **96**, 6510 (1974).
(8) Satisfactory elemental and spectroscopic analyses have been obtained for all new compounds.
(9) No intermediate was detectable from the adduct of cyclobutanone.
(10) Attempts to purify the other epoxides mentioned here by this method were not made.
(11) Y. Hayasi, M. Takaku, and H. Nozaki, *Tetrahedron Lett.*, 3179 (1969).
(12) D. F. Tavares and R. E. Estep, *Tetrahedron Lett.*, 1229 (1973).
(13) These authors reported that one of the epoxy thioethers from benzaldehyde rearranged in the presence of BF_3 etherate to α-*p*-tolylthiophenylacetaldehyde by apparent migration of the sulfur bearing group. This may be analogous to the rearrangement **6** (R,R′ = $-(CH_2)_6-$) → **5** observed when the former, produced in situ, is heated to 62° (Table I). It is of interest that the other epoxy thioethers under our conditions rearrange with carbon (or hydrogen) migration. We have also observed that **6** (R,R′ = $-(CH_2)_6-$) rearranges to **5** rapidly at room temperature in the presence of catalytic quantities of BF_3 etherate or of CF_3CO_2H or slowly (>30 hr) when heated at reflux in benzene containing cuprous triflate and amine. Surprisingly, however, the epoxide was recovered unchanged after treatment with LiBr-HMPA in refluxing benzene, conditions which isomerize other epoxides.[14]
(14) B. Rickborn and R. M. Gerkin, *J. Am. Chem. Soc.*, **93**, 1693 (1971).
(15) Mukaiyama has reported the activation of bivalent organosulfur compounds by cupric chloride in several reactions: T. Mukaiyama, *Int. J. Sulfur Chem., B*, **7**, 180 (1972); T. Mukaiyama, K. Narasaka, and H. Hokonoki, *J. Am. Chem. Soc.*, **91**, 4316 (1969); T. Mukaiyama, K. Narasaka, K. Maekawa, and H. Hokonoki, *Bull. Chem. Soc. Jpn.*, **43**, 2549 (1970); K. Maekawa, K. Narasaka, and T. Mukaiyama, *ibid.*, **46**, 3478 (1973); K. Narasaka, T. Sakashita, and T. Mukaiyama, *ibid.*, **45**, 3724 (1972).

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Interpretation of Fluorescence Detected Circular Dichroism Data

Sir:

Recently Turner et al.[1] reported on the use of fluorescence for detecting circular dichroism (CD) in molecular systems. We have also been investigating this technique and wish to report on some similarities and differences between their results and ours. In particular the approach taken by us on the analysis of results provides some guidance on how the contributions of various competing mechanisms might be disentangled.

It is known that circular dichroism is a powerful technique for investigating molecular structure. Until recently all such investigations were carried out in transmission. More recently Schlessinger and Steinberg[2,3] have shown that measurement of the circular polarization of the light emitted in fluorescence can often provide the same type of information. In the event that there is molecular rearrangement or energy transfer while the molecule is in the excited state, the Schlessinger and Steinberg technique can provide information otherwise not obtainable through other means. In another development Pao[4] and Einhorn et al.[5] have shown that CD may also be measured in reflection. However the reflection method suffers from some practical limitations, the need for extreme accuracy in optical alignment being one of these.

The transmission experiment is perfectly satisfactory except when the samples of interest become nearly opaque and/or when nonhomogeneous samples (such as cellular structure of nonuniform composition) are involved. Under such circumstances the technique reported by Turner et al. and studied by us constitutes a powerful alternate approach. Another circumstance of interest is the one emphasized by Turner et al., namely, the possibility of disentangling two overlapping CD species, only one of which is fluorescent.

However, use of the fluorescence technique brings with it certain complexities in the interpretation of results. Some of these are mentioned in the Turner et al. communication and we wish to expand on these in this note.

Laser excitation by an Argon ion laser at three discrete wavelengths, namely, 458, 476, and 488 nm, were used in our experiment. An Isomet Model 401-A Pockel cell is used to modulate the polarization of the incident beam. The fluorescence output is detected either in the forward direction and/or at 90° to the excitation beam. The light output is detected with a Hamatsu Type R375 photomultiplier, and the resulting electrical signal is processed through a P.A.R. Model HR-8 lock-in amplifier. The DC output voltage of the PMT is held constant by automatic control of the photomultiplier high voltage supply. The sensitivity and other CD measurement characteristics of this spectropolarimeter are similar to those of commercially available instruments, *provided laser excitation is used.*

The system studied by us is the polysaccharide chondroitin sulfate Type A (CSA) complexed with acridine orange dye. The system turned out to be a very difficult system. As we proceeded, we found that results varied appreciably depending on the ratio of the concentrations of CSA and dye and on whether the dye was added to the polymer solution or whether polymer was added to a dye solution. Furthermore CD varied drastically with temperature. After we had unraveled or at least had characterized these trends, we find that the CD detected in fluorescence agreed with that obtained in transmission in general trends but could be off by a scaling factor (0.5-6) which varied from case to case.

In interpretation of data we represented possible systems somewhat differently compared to the scheme used in the Turner publication. We feel that two basic types of situation may be present in the FDCD experiment.

The first and simplest situation arises when an optically active *nonfluorescent* material is present with another fluorescent nonactive material. Near the front surface of the sample where the intensity of the alternately left then right circularly polarized incident beam is constant, the fluorescence output is also constant. However, for an interior point of the sample there are variations in the incident light inten-

Table I. Summary of CD Properties[a]

	Transmission Case 1	Fluorescent nonoptically active material and nonfluorescent optically active material Case 2 $\eta_2 = 0, \eta_1 \neq 0, A_1 \neq 0$	Nonfluorescent nonoptically active absorber and fluorescent optically active material Case 3 $\eta_1 = 0$	Nonfluorescent optically active material and fluorescent optically active material Case 4 $\eta_1 = 0$
I_{DC}	$10^{-A}I_0$	$\frac{I_0\eta_1\epsilon_1'(1 - 10^{-A})}{\epsilon'}$	$\frac{\eta_2 I_0\epsilon_2'(1 - 10^{-A})}{\epsilon'}$	$\frac{\eta_2 I_0\epsilon_2'(1 - 10^{-A})}{\epsilon'}$
I_{AC}	$\frac{-I_0\Delta A \ln 10 \times 10^{-A}}{2}$	$\frac{-I_0\eta_1\epsilon_1'\Delta\epsilon'}{2\epsilon'^2}[1 - 10^{-A} - 10^{-A}A \ln 10]$	$\frac{\eta_2 I_0\Delta\epsilon'\epsilon_1'}{2\epsilon'^2}\left(1 - 10^{-A} + \frac{10^{-A}AA_2 \ln 10}{A_1}\right)$	$\frac{\eta_2 I_0}{2\epsilon'^2}[(\epsilon_1'\Delta\epsilon_2' - \epsilon_2\Delta\epsilon_1')(1 - 10^{-A}) + 10^{-A}\Delta A\epsilon_2'\epsilon' \ln 10]$
$\frac{I_{AC}}{I_{DC}}$	$\frac{-\Delta A \ln 10}{2}$	$\frac{\Delta A 10^{-A} \ln 10}{2(1 - 10^{-A})} - \frac{\Delta\epsilon'}{2\epsilon'}$	$\frac{\Delta A 10^{-A} \ln 10}{2(1 - 10^{-A})} + \frac{\Delta\epsilon'\epsilon_1'}{2\epsilon'\epsilon_2'}$	$\frac{\Delta A 10^{-A} \ln 10}{2(1 - 10^{-A})} + \frac{\Delta\epsilon_2'\epsilon_1'}{2\epsilon'\epsilon_2'} - \frac{\Delta\epsilon_1'}{2\epsilon'}$
S/N	$\frac{\sqrt{I_0 10^{-A}\Delta A \ln 10}}{2K_1}$	$\frac{\Delta\epsilon'}{2K_2\epsilon'}\sqrt{\frac{I_0\eta_1\epsilon_1'}{\epsilon'(1 - 10^{-A}}}[1 - 10^{-A}(1 + A \ln 10)]$	$\frac{\epsilon_1'\Delta\epsilon'}{2\epsilon' K_3}\sqrt{\frac{\eta_2 I_0}{\epsilon'\epsilon_2'(1 - 10^{-A})}}\Big(1 - 10^{-A} + \Big[10^{-A}\frac{AA_2 \ln 10}{A_1}\Big)\Big]$	$\frac{1}{2K_4\epsilon'}\sqrt{\frac{\eta_2 I_0}{\epsilon'\epsilon_2'(1 - 10^{-A})}}[(\epsilon_1'\Delta\epsilon_2' - \epsilon_2'\Delta\epsilon_1')(1 - 10^{-A}) + 10^{-A}\Delta A\epsilon_2'\epsilon' \ln 10]$

[a] Note: $A = A_1 + A_2$, $\epsilon' = \epsilon_1' + \epsilon_2'$, $\epsilon_1' = \epsilon_1 C_1$, $A_1 = \epsilon_1 C_1 l$, η = efficiency of material = (incident beam power)/(fluorescent output power).

sity as the circular polarization state of the incident beam changes due to the presence of the optically active material between the front surface and the interior point. This variation in intensity of the incident beam on the fluorescent molecules at interior points causes a variation in the intensity of emitted fluorescence light. The fluorescent material acts as an indicator or tracer for the active material. The output signal from the total sample is an average of the contributions from each point in the cell.

The second and somewhat more complex situation arises when an optically active fluorescent material is present in a solution either by itself or together with a nonactive nonfluorescent absorbing material. The absorbed energy and the emitted fluorescence light are dependent upon the intensity and circular polarization state of the light incident upon the active fluorescent material. For example, if $A_L > A_R$ the energy absorbed and the fluorescence light emitted by a molecule at the front surface of the sample, where the intensity of the incident beam is constant, will be a maximum when the incident beam is LCP (left circularly polarized). The transmitted beam which is incident on molecules not at the front surface will be of minimum intensity when the incident beam is LCP. For interior points the molecule's ability to more readily absorb energy from LCP light is negated by the reduction in the intensity of LCP light relative to the RCP light. For molecules less than $1/(\epsilon c \ln 10)$ distant from the front of the sample where ϵ and c represent the extinction coefficient and concentration, respectively, of the optically active fluorescent material, the absorption preference of the molecule for LCP prevails and fluorescence output from these molecules is a minimum when the incident beam is LCP. For molecules further than $1/(\epsilon c \ln 10)$ from the front surface, the intensity difference between the LCP and RCP incident light is more important and the fluorescence output from these molecules is a minimum when the incident beam is LCP. For the entire sample cell the fluorescence output will be a maximum when the incident beam is LCP but the magnitude of the variation in fluorescence output with incident beam polarization changes will be strongly dependent on cell length. The maximum variation in the fluorescence output as the incident beam polarization changes will occur when the cell length is $1/(\epsilon c \ln 10)$. For long cells the variation in the fluorescence signal may be increased by adding a nonactive nonfluorescent absorber to the solution. The absorber attenuates the incident beam as it traverses the sample cell and thereby decreases disproportionately the fluorescence contribution from the rear of the cell. The net result is that the variation in the fluorescence output is significantly larger than it would have been had the absorber been absent.

In Table I the signals of experimental interest are tabulated for the transmission case, the two fundamental cases just described, and the case of an optically active fluorescent material in solution with a nonfluorescent active material as discussed in the Turner paper. The quantities of experimental interest are: (1) I_{DC}, the DC or average value of the output light; (2) I_{AC}, the AC or peak value of the variations in output light which result from the polarization variations in the incident beam; (3) I_{AC}/I_{DC}, the directly observed quantity which in the transmission case is directly proportional to ΔA; (4) S/N, the signal to noise ratio based on the assumption that the noise is due entirely to shot noise; the noise is equal to $K\sqrt{I_{DC}}$ where K is a proportionality constant which is a function of system bandwidth and wavelength of light being observed.

Several important conclusions can be drawn from the equations in Table I. (1) The signal to noise ratio for fluorescence experiments can be greater than the S/N ratio for transmission experiments for optically dense materials. Case 3 with $\epsilon_1' = 0$ is the only exception to this. (2) For case 2 (the tracer or indicator effect) the AC/DC ratio will be of the same polarity as the transmission ratio and will approach a constant value of $-\Delta\epsilon'/2\epsilon'$ for optically dense samples. (3) For case 3 (the absorber effect) the AC/DC ratio will have the polarity opposite to that of the transmission ratio and will approach a constant value of $\Delta\epsilon'\epsilon_1'/2\epsilon'\epsilon_2'$ for optically dense samples. If $\epsilon_1' = 0$ (no absorber) the ratio will go to zero for optically dense samples. (4) Case 4 may be viewed as a combination of case 2 and case 3 with the AC/DC ratios for optical dense samples approaching $(\Delta\epsilon_2'\epsilon_1'/2\epsilon'\epsilon_2') - (\Delta\epsilon_1'/2\epsilon')$. (5) For a sample of unknown classification the polarity of the fluorescence CD compared to that of the transmission CD along with the manner in which the CD varies as cell length is changed may provide the information required to correctly classify the sample.

The results of Turner, Tinoco, and Maestre (eq 1 and 2 of ref 1) are identical with those exhibited for cases 3 and 4 of Table I except for a factor of ln 10/2 which seems to be missing in eq 2 of ref 1. We note that case 2 and 3 of Table I could be obtained from case 4 if the appropriate combination of limiting procedures is used.

The thrust of our program is the detection of backward scattered fluorescence light, an experimental configuration suitable for the scanning of opaque samples, possibly solid and possibly of nonuniform composition. Focused laser excitation is used for this purpose, and further results will be submitted for publication in the near future.

Acknowledgment. Work was supported by NIH Grant GM 16268.

References and Notes

(1) D. H. Turner, I. Tinoco, and M. Maestre, *J. Am. Chem. Soc.*, **96**, 4340 (1974).
(2) J. Schlessinger and I. Z. Steinberg, *Proc. Nat. Acad. Sci. U.S.A.*, **69**, 769 (1972).
(3) I. Z. Steinberg and A. Gafni, *Rev. Sci. Instrum.*, **43**, 409 (1972).
(4) Y. H. Pao and J. R. Onstott, *Int. J. Quantum Chem., Symp.*, **3**, 119 (1969).
(5) A. J. Einhorn, Y. H. Pao, and F. W. Phelps, *Chem. Phys.*, **1**, 277 (1973).

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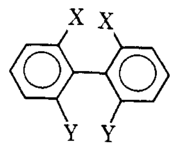
An Optical Rotatory Dispersion Study of the Conformation of Optically Active Biphenyls in Solution

Sir:

The preferred geometry of biphenyl varies considerably with experimental conditions. The torsional angle between the rings has been variously reported to be 42-45° (gas phase),[1] 0° (crystalline),[2] and 20° (solution).[3] When the torsional angle, ϕ, is neither 0 or 90°, biphenyl exists in two enantiomeric conformations, IIa and IIIa (with torsional angles ϕ and $180° - \phi$), that can interconvert by traversing a transition state at either 0 or 90° (see Figure 1).

Although substituted biphenyls of the type I have long been known to have torsional angles different from 0, 90, and 180° (0° is defined as X eclipsing X) in both the gas[4] and solution[5] phases, no evidence for the exisence of more than one conformation between $\phi = 0$ and 180° was presented. We present evidence in support of the existence of two equilibrating conformations ($0° < \phi < 180°$) for several optically active biphenyls. If two conformations ($0° < \phi < 180°$) exist for optically active biphenyls such as Ib-e, the relationship between energy and torsional angle becomes more complicated than that for biphenyl, itself (see Figure 2).



Ia, $X = Y = H$
b, $X = CH_3$; $Y = NO_2$
c, $X = COOH$; $Y = NO_2$
d, $X = CH_2OH$; $Y = NO_2$
e, $X = CH_2Br$; $Y = NO_2$

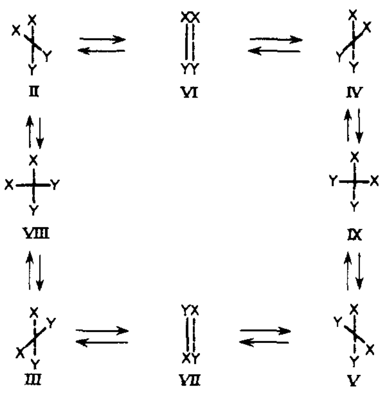


Figure 1. Possible conformations of substituted biphenyls.

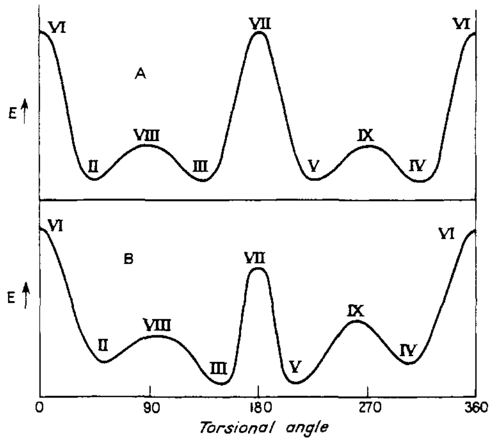


Figure 2. Energy vs. torsional angle for (A) biphenyl; (B) optically active biphenyls such as Ib or Ic. The values of the extrema are chosen for illustrative purposes only.

The potential barriers at 0 and 180° no longer are of equal magnitude. The potential barriers that were at 90 and 270° are generally shifted somewhat from these angles. Notably, IIb-e and IIIb-e are no longer enantiomers but diastereomers. Thus, IIb-e and IIIb-e differ in energy. Assuming the barrier at 90° to be small, IIb-e and IIIb-e will be in equilibrium, with their relative populations determinable from a Boltzmann distribution.

If we consider the groups X and Y to be perturbations on Ia, we expect IIb-e and IIIb-e to have opposite chirality (and molecular rotation) since IIa and IIIa are enantiomers (this has been predicted[5a] from the theory of Kirkwood[6] for the transition near 260 nm). Thus, a small change in the relative populations of IIb-e should result in a significant change in the observed molecular rotations of Ib-e.

The ORD spectra of Ib-e have been measured as a function of temperature. The results for the λ_{max} nearest 260 nm (the transition due to the chirality of the phenyl-phenyl twist[5]) are collected in Table I. The spectra were measured in a thermostated cell using a Durrum-Jasco J-20 spectrometer. After the temperature was varied, the initial spectrum was remeasured. No racemization was detected for any of the compounds studied. The behavior of Ib in *n*-octane was essentially identical with that in methanol (the 254 nm rotation at +45.0° decreased to 0.76 of its −15.0° value). Unfortunately the low solubility of the other biphenyls in *n*-octane precluded further studies in this solvent.

The data indicate that large reversible changes in rotation occur. In the case of Id, for example, the rotation decreases by more than one-third upon going from −15 to +45°. Such a phenomenon strongly suggests that there be a