Tetrahedron Vol. 38, No. 14, pp. 2089 to 2096, 1982
Printed in Great Britain.

0040-4020/82/142089-08$03.00/0
Pergamon Press Ltd.

DIRECT DETERMINATION OF ENANTIOMERIC PURITY USING CPL SPECTROSCOPY

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(*Received in U.K.* 15 *April* 1982)

Abstract The dependency of the Circular Polarization of the Luminescence (CPL) on the handedness of the excitation light potentially provides a means to determine enantiomeric purity. In literature two procedures have been outlined for such determinations, but as yet they have not been experimentally tested. These procedures, along with a third one we propose here, are evaluated from theory and confronted with experiment. It appears that the CPL technique provides a valuable tool for the determination of enantiomeric purity. The scope and limitations of the method are discussed.

INTRODUCTION

The determination of enantiomeric purity is an important issue in many fields of chemistry. During the years a number of methods have been elaborated to establish enantiomeric purities[1-5]. None of them appears to be universally applicable. Since the successful detection of the circular polarization of the luminescence (CPL)[6] of chiral molecules, two procedures have been suggested to use this technique for the determination of enantiomeric purity[7,8], but up till now neither of them has been tested in practice.

In the course of a study on the optical activity of β,γ-enones[9] in absorption and fluorescence, we needed to know the enantiomeric excess of a particular ketone. This prompted us to try out the CPL technique. The success of the method in this instance stimulated us to elaborate it further. The results are presented here.

The outline of the paper is as follows. First a general formalism is given, which enables a discussion of the procedures given before by Eaton[7] and by Kokke[8] and of a new version we propose here. Subsequently the experimental results from these methods are discussed and compared. Lastly we briefly discuss scope and limits of the CPL technique to determine enantiomeric purity.

FORMALISM

The enantiomeric purity p of a sample is defined by

$$p = \frac{c_l - c_r}{c_l + c_r} \quad \text{or}$$

$$\frac{c_l}{c_r} = \frac{1 + p}{1 - p} \qquad (0 \leq p \leq 1) \qquad (1)$$

where c_l denotes the molar concentration of the enantiomer in excess (chosen to be l), and c_r that of the other. To discuss the chiroptical properties of partially resolved samples it is convenient to consider them as a composition of a) an enantiomerically pure part (p=1) and b) a racemic part (p=0).

a) p=1.

For an enantiomerically pure sample (l-molecules) the different absorption probabilities for left (L) and right (R) circularly polarized light result in a non-vanishing dissymmetry factor g. Its magnitude is given by the ratio of observed circular dichroism and absorbance at a given wavelength:

$$g = \frac{(\varepsilon_L^l - \varepsilon_R^l)\, c_l\, d}{\tfrac{1}{2}(\varepsilon_L^l + \varepsilon_R^l)\, c_l\, d} = \frac{\Delta\varepsilon^l}{\varepsilon^l} \qquad (2)$$

In (2) c_l denotes the concentration and d the pathlength of the light in the sample solution.

Likewise in the spontaneous emission the intensities of both hands of circularly polarized light are different, $I_L \neq I_R$, giving rise to CPL. The associated dissymmetry factor, g_{em}, is given by (3).

$$g_{em} = \frac{(I_L^l - I_R^l)}{\tfrac{1}{2}(I_L^l + I_R^l)} = \frac{\Delta I^l}{I^l} \qquad (3)$$

Like g is a function of the excitation wavelength λ_{exc}, g_{em} depends on the emission wavelength λ_{em}. With the understanding that we always shall refer to a fixed pair of (λ_{exc}, λ_{em}), we can omit these wavelengths in the formulae. It is important to note that in obtaining g_{em} from experiment, the polarization state of the excitation light is of no consequence for the value of g_{em}; dealing with l-molecules only, photoselection effects cannot occur.

b) p=0.

Such photoselection effects do occur in racemic samples. When the excitation light is left circularly polarized, one of the enantiomers is preferentially excited resulting in a non-vanishing enantiomeric purity $p^* = (c_l^* - c_r^*)/(c_l^* + c_r^*)$ in the excited state. Provided no racemization occurs during the lifetime of this state, the emission will be circularly polarized to the extent

$$\left(\frac{(I_L - I_R)}{\tfrac{1}{2}(I_L + I_R)}\right)_L = G_L^{p=0} = p^* g_{em} \qquad (4)$$

where g_{em} is the dissymmetry factor of the l-enantiomer. The concentrations of the enantiomers in the excited state (c_l^* and c_r^*) directly relate to the absorbances of the l- and r-molecules for L-light:

$$\frac{c_l^*}{c_r^*} = \frac{\varepsilon_L^l\, c_l\, d}{\varepsilon_L^r\, c_r\, d} = \frac{\varepsilon_L^l}{\varepsilon_L^r} = \frac{\varepsilon + \tfrac{1}{2}\Delta\varepsilon^l}{\varepsilon + \tfrac{1}{2}\Delta\varepsilon^r} = \frac{1 + \tfrac{1}{2}g}{1 - \tfrac{1}{2}g} \qquad (5)$$

In deriving (5) it is used that $c_l = c_r$. When dealing with fluorescences and exciting with medium intensity light sources, this always applies since c_l^*, $c_r^* \ll c_l$, c_r. Further we have used relation (2) and the fact that $\Delta\varepsilon^r = -\Delta\varepsilon^l$.

Analogously to (1) one may define c_l^*/c_r^* in terms of p^*. Comparison with (5) then learns that $p^* = \tfrac{1}{2}g$. Thus the observed degree of circular polarization in the emission, $G_L^{p=0}$, may be expressed in terms of the dissymmetry factors of the pure enantiomer:

$$G_L^{p=0} = \tfrac{1}{2} g\, g_{em} \qquad (6a)$$

$$G_R^{p=0} = -\tfrac{1}{2} g\, g_{em} \qquad (6b)$$

Analogously one derives (6b), which describes the observed CPL when excitation is carried out with R-light.

From (6) one notes that $G^{p=0}$ changes sign when the sense of circular polarization of the excitation light is reversed. This is unlike the situation with enantiomerically pure samples and it constitutes the basic principle of the method to determine p from CPL measurements.

c) 0 < p < 1.

With partially resolved samples the ratio of CD and absorbance is given by equation (7).

$$G_{abs}^p = p\, g \qquad (7)$$

Likewise the CPL experiment, using unpolarized or linearly polarized excitation light, provides $p\, g_{em}$.

$$G^p = p\, g_{em} \qquad (8)$$

Both circular polarizations probe the enantiomeric purity of the sample. However, when in the emission experiment the excitation light is circularly polarized, the enantiomeric purity in the excited state is made unequal to that in the ground state, due to the photoselection effect. With L-excitation one has

$$\frac{c_l^*}{c_r^*} = \frac{c_l\, \varepsilon_L^l}{c_r\, \varepsilon_L^r} = \frac{1 + p}{1 - p} \cdot \frac{1 + \tfrac{1}{2}g}{1 - \tfrac{1}{2}g} = \frac{1 + \dfrac{p + \tfrac{1}{2}g}{1 + \tfrac{1}{2}pg}}{1 - \dfrac{p + \tfrac{1}{2}g}{1 + \tfrac{1}{2}pg}} \qquad (9)$$

where, again, we have used the fact that excited state concentration is low, i.e. c_l/c_r retains the value it has before irradiation.

Tetrahedron

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TETRAHEDRON PRIZE FOR CREATIVITY IN ORGANIC CHEMISTRY

RULES

The Tetrahedron Prize for Creativity in Organic Chemistry was founded in 1980 by the Executive Board of Editors and the Publisher of Tetrahedron Publications. It is intended to honour the memory of the founding co-Chairmen of these publications, Professor Sir Robert Robinson and Professor Robert Burns Woodward.

The Prize, which consists of an appropriate gold medal and citation as well as $10,000, is awarded every two years to an Organic Chemist who has contributed original ideas to Organic Chemistry.

The Jury is made up of the Executive Board of Editors of Tetrahedron Publications. Consulting Editors, who do *not* have executive function, do not vote for the Prize but are eligible as candidates.

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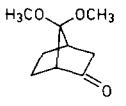
ISSN 0040-4020
TETRAB 38(14) 1971–2220 (1982)

Printed in Great Britain by A. Wheaton & Co. Ltd., Exeter

concentration dependency of the results.

RESULTS AND DISCUSSION

The course of the determination will be illustrated in some detail using (1S,4R)-(+)-7,7-dimethoxybicyclo[2.2.1]heptan-2-one (1) as an example. Absorption and CD spectra of a spectroscopic solution of partially enriched 1 in n-heptane are recorded (figure 1; at this stage



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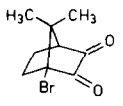

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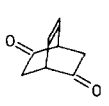

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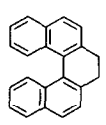

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the scale of Δε is not yet known). Next the fluorescence and CPL spectra are measured using small bandwidths (fig. 1). With these data the optimum excitation and emission wavelength and bandwidth for the measurement of the G values are chosen. With a racemic sample solution of 1 we determine the weighting function in order to obtain the effective value of G^{p}_{abs}, and the quantities $G_L^{p=0}$ and/or $G_R^{p=0}$. With the sample solution of partially resolved 1 a more accurate value of G^{p} is obtained as well as the quantities G_L^{p} and G_R^{p}. An idea of the magnitudes of the various quantities may be gathered from inspection of the data obtained with 1. With excitation at 300 nm (bandwidth 20 nm) and wavelength of observation at 420 nm (bandwidth 40 nm) we have $G^{p}=+(314\pm5)\times10^{-5}$, $G_L^{p=0}-G_R^{p=0}=+(67\pm5)\times10^{-5}$, $G_L^{p}=+(345\pm4)\times10^{-5}$, $G_R^{p}=+(287\pm4)\times10^{-5}$. The uncertainties in these values are the standard errors originating from the photon noise. The effective value of G^{p}_{abs} is determined to be 3.12×10^{-2}, the percentage circular polarization of the excitation light is 97.5. The value of p obtained in the procedures A, B, and C is 36.7±13.5, 38.4±1.5, and 38.2±1.4 percent, respectively (table 2). The knowledge of p allows the scaling of the previously obtained CD[13] and CPL spectra of 1 (fig. 1). Table 2 also gives the values of the dissymmetry factors in absorption and fluorescence at the wavelengths used in the experiments (but obtained with small bandwidths), as well as the value of the fluorescence quantum yield ϕ_F.

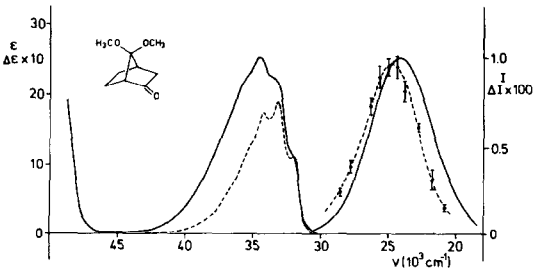

Figure 1. Spectra of 1 in n-heptane. (——): absorption (ε) and fluorescence (I); (- - -): circular dichroism (Δε) and circular polarization of the fluorescence (ΔI). The vertical bars represent the standard error in the ΔI values. CD and CPL have been corrected towards 100 % enantiomeric purity.

In order to probe the linearity of the methods we repeated the analysis on a second sample of 1 that was available from another synthesis (table 2). As expected, it appears that within experimental error the ratio of the p values of the samples equals that of the $[\alpha]_D$ and CD values. From table 2 it is clear that in procedure A the standard error exceeds that in procedures B and C by an order of magnitude. As explained above, this is due to neglect of the possibility to obtain the quantity pg directly. In line with this we shall omit in the remainder of the table the results from method A.

To explore the technique in the region of high enantiomeric purity we have studied (1S)--(-)-1-bromo-α-fenchocamphoronequinone (2), synthesized earlier in this laboratory by Kokke[15]. Being chemically correlated to camphor, 2 is expected to be virtually enantiomerically pure as is borne out by the measurements (procedure B, table 2). Since racemic 2 was not available to us, we could not apply procedure C.

The next entry in the table relates to (1R,4R)-bicyclo[2.2.2]oct-2-ene-5,7-dione (3), which was partially resolved by photolysis of racemic 3 with circularly polarized light[16]. As shown by Kuhn[17], the enantiomeric purity resulting from an asymmetric photodestruction experiment is governed by ½g and therefore is relatively low. The observed p data (procedures B and C), which agree with the enantiomeric purity expected from Kuhn's theory[17] on partial photodestruction, illustrate the usefulness of

Table 2. Obtained enantiomeric purities, and values of the fluorescence quantum yields and dissymmetry factors.

	ϕ_F	g (λ) [a]	g_{em} (λ) [a]	Enantiomeric purity (%) A	B	C
1	3×10^{-3} [b]	0.083 (300 nm)	0.088 (420 nm)	37 ± 14	38.4 ± 1.5	38.2 ± 1.4
				21 ± 23	25.7 ± 0.7	25.7 ± 1.0
2	$\sim 10^{-3}$ [c]	-0.061 (450 nm)	-0.028 (520 nm)		100 ± 2	
3	4×10^{-4} [b]	0.080 (300 nm)	0.044 (440 nm)		3.3 ± 0.1	3.4 ± 0.1
4	~ 1 [c]	0.025 (255 nm)	-7.5×10^{-4} (380 nm)		110 ± 30	110 ± 30

a Values of g and g_{em} observed with small monochromator slits and corrected towards 100 % enantiomeric purity (1 and 3).

b Fluorescence quantum yields determined using adamantanone ($\phi_F = 5.2\times10^{-3}$)[24] as a reference.

c Estimates.

the CPL method to determine low enantiomeric purities. It also demonstrates that the method can succussfully be applied to compounds which are only weakly fluorescent and prone to photochemical rearrangements.

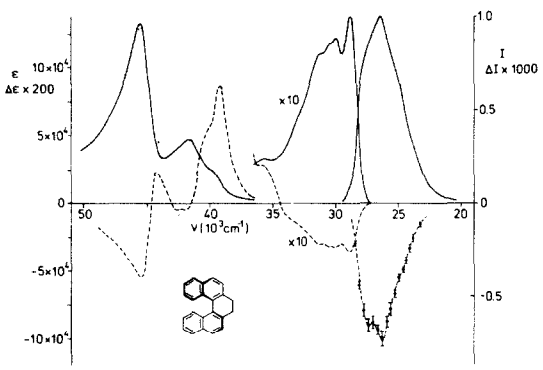

Figure 2. Spectra of 4 in n-heptane. (——): absorption (ε) and fluorescence (I); (- - -): circular dichroism (Δε) and circular polarization of the fluorescence (ΔI). The vertical bars represent the standard error in the ΔI values.

A main restriction of the method is illustrated by (S)-(+)-9,10-dihydrodibenzo[c,g]phenanthrene (4, fig. 2). A straightforward evaluation of the observed G data for this compound yields p=(110±30)%, both from procedure B and C. The large error which results for this highly fluorescent compound is due to the small g_{em} value (table 2). Whereas the excitation wavelength has been chosen so as to yield a maximum value of g (255 nm, ~ 39,000 cm^{-1}, fig. 2), the low dissymmetry factor in emission definitely poses a limit to the applicability of the method. That the large ϕ_F cannot effectively compete with a small dissymmetry factor is easily understandable: the relative accuracy in the value of e.g. $G_L^{p=0}$ depends linearly on the magnitude of g and g_{em}, but only on the square root of the fluorescence intensity.

CONCLUSION

With regard to the various procedures to determine enantiomeric purity using the CPL technique, it appears that the data in table 2 support the predictions from theory. The procedures B and C, which are very similar, are by far superior to A. An advantage of B is that in principle all measurements can be performed on a single sample: no additional racemic sample solution is needed. Occasionally this advantage can turn into a disadvantage, e.g. with photolabile compounds if only a limited amount of partially enriched, but a sufficient amount of racemic material is available. When both options are open, we have a slight preference for procedure C: it is more transparent (the quantities gg_{em}

and pg_{em} are directly observed) and it is somewhat faster (pg_{em} can be obtained using unpolarized, and therefore more intense, excitation light).

The CPL technique provides an absolute method for the determination of enantiomeric purity: the resolving agent is not a chiral molecule or chiral chemical environment, but the circularly polarized light. Since the chiral interaction relates to spectroscopic properties, it is of a quite different type. Therefore the technique may provide an answer, where other methods to determine p fail. For instance with complex molecules having badly resolved NMR spectra, or with molecules lacking functional groups to interact with resolving agents or media. There is still another distinction with other techniques. Whereas the highest accuracy with most other methods is in the range of medium and high enantiomeric purity, the CPL method gives best results in the low p region.

Of course the method has intrinsic limitations. An obvious prerequisite is that the compound under investigation should fluoresce, although the quantum yield of fluorescence need not necessarily be high. A second criterion relates to the magnitude of the dissymmetry factors in absorption and fluorescence. For weak fluorescers, measurements become intractable if the product gg_{em} is much smaller than 10^{-4}, say; for strongly fluorescent compounds if it is smaller than $\sim 10^{-5}$. One may predict that the technique provides a viable means to study many saturated and unsaturated ketones, diones, and other classes of compounds whose longest wavelength absorption has $n \rightarrow \pi^*$ character. It should work also with metal-complexes having fluorescence of d - d or f - f type. Possibilities are not restricted to magnetic dipole allowed fluorescences, however, since we found that the dissymmetry factors of aromatic molecules like hexahelicene and [6.6]vespirene also satisfy the criteria set above.

EXPERIMENTAL

(1S,4R)-(+)-dimethoxybicyclo[2.2.1]heptan-2-one (1). 7,7-dimethoxynorbornene[18] was hydroborated with (-)-diisopinocampheylborane[19]. Hydrolysis and oxidation yielded the ketone 1. $[\alpha]_D^{25}$ +72±3° (CH_2Cl_2, c=2.22), corrected towards 100% enantiomeric purity. The detailed synthesis will be published elsewhere[20]. Racemic 1 was synthesized according to literature[21].

(1S)-(-)-1-bromo-α-fenchocamphoronequinone (2) was synthesized by Kokke[15]. $[\alpha]_D$ -366° ($CHCl_3$, c=0.38).

Racemic bicyclo[2.2.2]oct-2-ene-5,7-dione (3) was prepared according to literature[22]. The asymmetric photodestruction experiment and the chiroptical spectra,as well as the determination of the absolute configuration are described elsewhere[16].

(S)-(+)-9,10-dihydro-dibenzo[c,g]phenanthrene (4) and racemic 4 were available from stock. The CD spectrum of (+)-4 agrees with that in literature[23].

The solvents used in the experiments were n-heptane (1, 3, 4) and $CHCl_3$ (2) of spectroscopic quality. Concentrations were $\sim 3 \times 10^{-2}$ M (1); $\sim 1 \times 10^{-2}$ M (2); $\sim 2 \times 10^{-3}$ M (3); $\sim 4 \times 10^{-5}$ M (4).

The CD measurements were performed on a Jobin-Yvon MIII spectrometer, the absorption spectra on a Cary 219 spectrophotometer. The fluorescence and CPL measurements were carried out on a home-built instrument[12]. Circular polarization of the excitation light was achieved by inserting a circular polarizer (Glan polarizer and quarter wave plate, Halle) in the excitation channel of the CPL spectrometer. The excitation and emission wavelengths (bandwidths) used in the determination of the enantiomeric purities were: 1: 300 (20), 420 (40); 2: 450 (13), 520 (20); 3: 300 (20), 420 (40); 4: 255 (10), 380 (20), where the values are in nm.

Acknowledgement: We like to thank Dr. H.J.C. Jacobs for critically reading and Mrs. E.J. Bos for typing the manuscript.

REFERENCES

1. M.Raban and K.Mislow in *Topics in stereochemistry, Vol.2*, N.L.Allinger and E.L.Eliel eds., Wiley-Interscience,New York (1967), pp 199-230.
2. Y.Izumi and A.Tai, *Stereo Differentiating Reactions*, Kodansha/Academic Press,Tokyo/New York (1977), chapter 8.
3. C.Kutal in *NMR Shift Reagents*, R.E.Sievers ed.,Academic Press,New York (1973), pp 87-98.
4. A.F.Cockerill,G.L.O.Davies,R.C.Harden and D.M.Rackham, *Chem. Rev.*, 73, 553 (1973).
5. G.R.Sullivan in *Topics in Stereochemistry Vol.10*, E.L.Eliel and N.L.Allinger eds., Wiley-Interscience,New York (1978), pp 287-

329.

6. a C.A.Emeis and L.J.Oosterhoff, *Chem. Phys. Letters*, 1, 129; 268 (1967).
   b C.A.Emeis and L.J.Oosterhoff, *J. Chem. Phys.*, 54, 4809 (1971).
   c C.A.Emeis, *Ph.D. Thesis*, University of Leiden, The Netherlands (1968).
7. S.S.Eaton, *Chem. Phys. Letters*, 8, 251 (1971).
8. W.C.M.C.Kokke, *J. Am. Chem. Soc.*, 96, 2627 (1974).
9. P.H.Schippers,J.P.M.van der Ploeg and H.P.J.M.Dekkers, *J. Am. Chem. Soc.*, submitted for publication.
10. P.H.Schippers and H.P.J.M.Dekkers, *Anal. Chem.*, 53, 778 (1981).
11. a I.Z.Steinberg and A.Gafni, *Rev. Sci. Intrum.*, 43, 409 (1972).
    b R.A.Shatwell and A.J.McCaffery, *J. Phys. E: Sci. Instrum.*, 7, 297 (1974).
    c B.C.Cavenett and G.Sowersby, *J. Phys. E: Sci. Instrum.*, 8, 365 (1975).
    d F.S.Richardson and J.P.Riehl, *Chem. Rev.*, 77, 773 (1977).
    e H.G.Brittain, *J. Am. Chem. Soc.*, 102, 3693 (1980).
12. P.H.Schippers,A.van den Beukel and H.P.J.M. Dekkers, *J. Phys. E: Sci. Instrum.*, submitted for publication.
13. Recently, Lightner et al.[14] reported $[\alpha]_D$ $+59^o$, whereas we find $[\alpha]_D^{25}+72\pm3^o$ (c=2.22; CH_2Cl_2) for enantiomerically pure 1.
14. D.A.Lightner,J.D.Gawroński and T.D.Bouman, *J. Am. Chem. Soc.*, 102, 1983 (1980).
15. W.C.M.C.Kokke and F.A.Varkevisser, *J. Org. Chem.*, 39, 1653 (1974).
16. P.H.Schippers and H.P.J.M.Dekkers, *J. Chem. Soc. Perkin Trans. II*, submitted for publication.
17. W.Kuhn and E.Knopf, *Z. Phys. Chem.*, 7B, 292 (1930).
18. P.G.Gassmann and J.L.Marshall, *Org. Synth.*, 48, 68 (1968).
19. a H.C.Brown and G.Zweifel, *J. Am. Chem. Soc.*, 83, 486 (1961).
    b H.C.Brown, N.R.Ayyangar and G.Zweifel, *J. Am. Chem. Soc.*, 86, 1071 (1964).
    c H.C.Brown, *Organic Synthesis via Boranes*, Wiley, New York (1975).
20. J.P.M.van der Ploeg, P.H.Schippers and H.P.J.M.Dekkers, to be published.
21. D.A.Lightner and D.E.Jackman, *J. Am. Chem. Soc.*, 96, 1938 (1974).
22. C.A.Grob and A.Weiss, *Helv. Chim. Acta* 43, 1390 (1960).
23. S.F.Mason,R.H.Seal and D.R.Roberts, *Tetrahedron*, 30, 1671 (1974).
24. A.M.Halpern and R.B.Walter, *Chem. Phys Letters*, 25, 393 (1974).