Extrinsic Circular Dichroic Effects in Retinoates

Anil K. Singh
Department of Chemistry, Indian Institute of Technology, Powai, Bombay 400076, India

(−)-1-Phenylethyl retinoate shows a Cotton effect with c.d. bands at 360, 245, and 215 nm, whereas (+)-octan-2-yl retinoate has no such band in the 220—450 nm region. This can be explained either in terms of a dipole–dipole interaction between the phenyl and retinylidene moieties or in terms of the dissymmetric environment of the polyene. The results are pertinent to the origin of c.d. in rhodopsins.

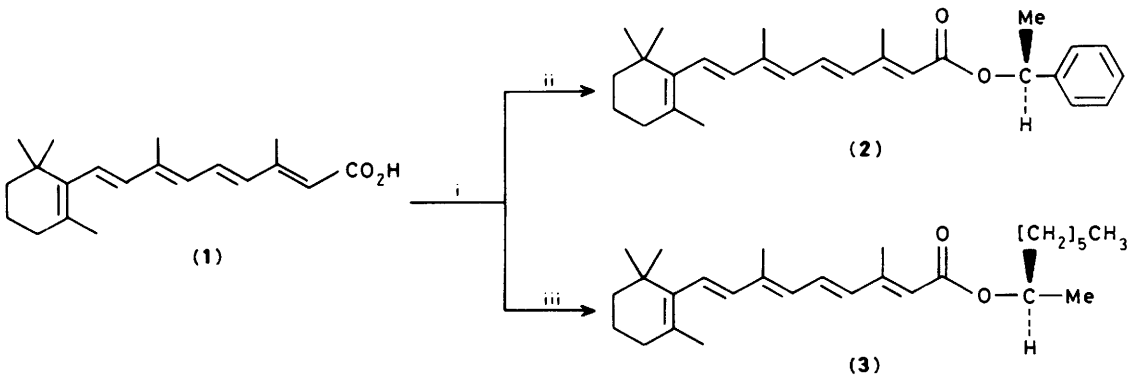
In both visual pigment protein-rhodopsin[1] and purple membrane protein-bacteriorhodopsin,[2] retinal binds to the apoprotein through a protonated Schiff's base linkage with the ε-amino group of a lysine residue. Monomeric bacteriorhodopsin has a broad absorption band in the visible region with a maximum near 550 nm and a positive c.d. band in the same region. Similarly, visual pigment rhodopsins exhibit absorption bands in the 500—580 nm region and c.d. bands in the 280—490 nm region. The 280 nm c.d. band of bovine rhodopsin has been attributed to the π–π* transitions of aromatic residues and the *n*–π* transitions of the cysteine moiety, whereas the 340 and 490 nm bands are believed to correspond to the *cis* and main peaks of the absorption spectrum. However, in solution, 11-*cis*-retinal (the chromophore of visual pigment rhodopsin), 13-*cis* and all-*trans*-retinal (the chromophores of bacteriorhodopsin), and the corresponding Schiff's bases of achiral amines do not show c.d. bands.

The mechanism through which these proteins acquire chirality and exhibit distinct Cotton effects in the u.v.–visible region has been discussed extensively; two mechanisms[3–9] have been put forward. First, optical activity is induced by the protein; upon binding of the retinal to the apoprotein, the retinylidene chromophore could become inherently chiral.[3–6] In the second, the electronic transitions of the bound chromophores are linked to the transitions of the protein residues (*e.g.* aromatic side chains and/or peptide bonds) by a coupled oscillator mechanism; as a result the retinylidene transitions may become optically active. However, it is not clear at present whether a twisted chromophore, a coupled oscillator mechanism, or a combination of both is responsible for the phenomenon. In this paper an attempt is made to determine the c.d. spectra of model retinoids and to correlate the results with the c.d. of rhodopsins.

Results and Discussion

C.d. spectra of (−)-1-phenylethyl retinoate (**2**) and (+)-octan-2-yl retinoate (**3**) have been determined in methanol. Both esters were prepared[10] by treating all-*trans*-retinoic acid (**1**) with the corresponding alcohols in the presence of dicyclohexylcarbodi-imide in dry dichloromethane at 25 °C under dim red light (Scheme). Compound (**2**) in methanol exhibits distinct c.d. bands at 360 (Δε 12.05), 245 (−11.48), and 215 nm (Figure); no c.d. bands were observed for the ester (**3**) in the 220—450 nm region. all-*trans*-Retinoic acid in methanol shows an absorption band at 348 nm (ε, 43 000), but no band in the 250 nm region. Similarly, the ester (**2**) shows an absorption band at 363 nm (ε, 65 585), with no appreciable absorption below 300 nm. (−)-1-Phenylethanol in methanol exhibits u.v. absorption bands with fine structure at 254 (ε 150) and 260 nm (ε 180), and c.d. bands at 254 (0.08), 260 (0.13), and 265 (0.14) nm (Table).[11,12]

The Cotton effects for compound (**2**) are noteworthy. all-*trans*-Retinoic acid in methanol is optically inactive and does not show a Cotton effect. (−)-1-Phenylethanol alcohol and (+)-octan-2-ol have no Cotton effects above 270 nm; hence the observed c.d. bands for compound (**2**) are a result of ester formation. The positive Cotton effect of 360 nm can be attributed to the transitions of the retinylidene moiety of the chiral ester (**2**). The negative c.d. band at 245 nm arises from coupling of the 254 nm band of the phenyl group with the 360 nm transition of the retinylidene group. The interaction is such that the 245 nm transition acquires a negative value. However, the possibility of an inherent c.d. band at 245 nm in the (−)-1-phenylethyl ester cannot be ruled out, as this compound would be expected to have some c.d. associated with the aromatic π–π* transition. Octan-2-yl retinoate (**3**) does not exhibit c.d. bands in the 220—450 region, since the (+)-octan-2-yl moiety has no strong transitions that could interact with the



Scheme. Reagents: i, dicyclohexylcarbodi-imide, dry CH_2Cl_2, 0—25 °C; ii, (−)-(*S*)-1-phenylethanol; iii, (+)-(*R*)-octan-2-ol

Table. U.v. and c.d. absorption data

Compound	U.v.(MeOH) $\lambda_{max.}$/nm (ε)	C.d.(MeOH) $\lambda_{max.}$/nm (Δε)
all-*trans*-Retinoic acid (**1**)	348 (43 000)	
(−)-1-Phenylethanol	254 (150), 260 (180)	254 (0.08), 260 (0.13), 265 (0.14)
(+)-Octan-2-ol	None in 220—450 nm region	
(−)-1-Phenylethyl retinoate (**2**)	363 (65 585)	360 (12.05), 245 (−11.48), 215
(+)-Octan-2-yl retinoate (**3**)	360 (46 000)	None in 220—450 nm region

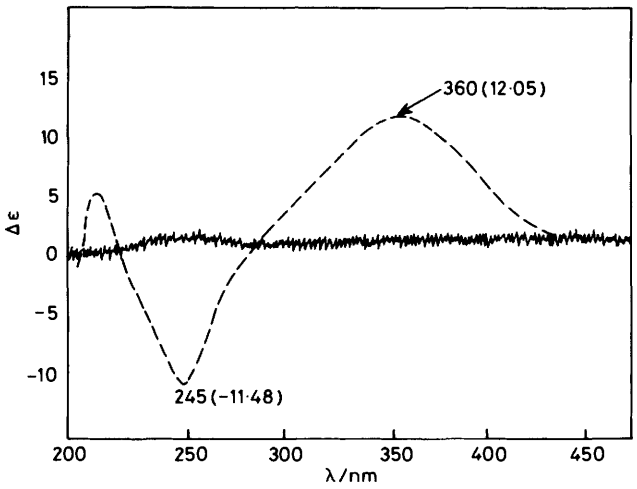


Figure. C.d. spectrum of 1-phenylethyl retinoate (**2**) in MeOH

retinylidene group to induce optical activity. The optical activity induced in the ester (**2**) can be interpreted in terms of dipole–dipole interaction between the phenyl group and the polyene, which, owing to the pressure of the chiral centre, occurs more with one side of the polyene than the other. This will result in a chiral environment giving rise to a c.d. maximum.

The present results indicate that inherent chirality of the retinyl component of rhodopsins is not essential for a c.d. band to appear. The observed c.d. spectra of rhodopsins can arise from dipole–dipole coupling of the polyene chain with an aromatic amino acid residue. However, these results do not rule out the possibility of some c.d. contribution from twisting of the retinyl chromophore.

Experimental

(−)-(*S*)-1-Phenylethanol and (+)-(*R*)-octan-2-ol were obtained from Norse Laboratories Inc. all-*trans*-Retinoic acid was obtained from Sigma Chemical Company. Compounds (**2**) and (**3**) were prepared, purified, and characterised by the literature procedure.[10] U.v. spectra were measured with a Beckman DU-6 spectrophotometer. C.d. measurements were carried out with a JASCO J-40 spectropolarimeter. All experiments were performed under dim red light in order to avoid unwanted photoisomerization of the polyenes.

Acknowledgements

I thank the Department of Science and Technology, Government of India for financial support and Professor K. Nakanishi for providing the c.d. analyses. Critical suggestions from the referee are gratefully acknowledged.

References

1 V. B. Nair and K. Nakanishi, 'Comprehensive Biochemistry,' vol. 3, ed. Ch. Tamm, Elsevier, Amsterdam, 1982, p. 283.
2 (*a*) W. Stoeckenius, R. H. Lozier, and R. A. Bogomolni, *Biochim. Biophys. Acta*, 1979, **505**, 215; (*b*) A. K. Singh, *J. Sci. Ind. Res.*, 1982, **41**, 665.
3 W. Sperling and C. N. Rafferty, *Nature (London)*, 1969, **224**, 519.
4 M. J. Burke, D. C. Pratt, R. R. Faulkner, and A. Moscowitz, *Exp. Eye Res.*, 1973, **17**, 557.
5 T. G. Ebrey and T. Yoshizawa, *Exp. Eye Res.*, 1973, **17**, 545.
6 B. Honig, P. Kahn, and T. G. Ebrey, *Biochemistry*, 1973, **12**, 1637.
7 A. S. Waggoner and L. Stryer, *Biochemistry*, 1971, **10**, 3250.
8 E. M. Johnston and R. Zand, *Biochem. Biophys. Res. Commun.*, 1972, **47**, 712.
9 A. Kropf, P. Whittenberger, S. Goff, and A. S. Waggoner, *Exp. Eye Res.*, 1973, **17**, 519.
10 A. K. Singh, *Synth. Commun.*, 1982, **12**, 447.
11 O. Cervinka, M. Kral, and P. Malon, *Collect Czech. Chem. Commun.*, 1976, **41**, 2406.
12 L. Verbit, *J. Am. Chem. Soc.*, 1965, **87**, 1617.

Received 10*th February* 1984; *Paper* 4/236