Properties of 14-Methylretinal, 13-Desmethyl-14-methylretinal, and Visual Pigments Formed Therefrom

Sir:

11-*cis*-Retinal has been the subject of numerous studies because it is the chromophore of the visual pigments. Of particular importance is the distortion in the polyene side chain, which distinguishes 11-*cis*-retinal from the other planar retinal isomers.[1,2] X-Ray studies[3,4] have shown that in crystals, Φ_{12-13} is twisted by 39° from the planar 12-s-cis form **1**. Theoretical calculations[2,4] suggest that the 12-s-cis conformation **1** and the 12-s-trans conformation **2** may both be present in solution at room temperature. In this communication we report chemical and spectroscopic studies of two retinal analogs, 14-methylretinal (**3**) and 13-desmethyl-14-methylretinal (**4**), and pigments formed from their double bond isomers. The results suggest that the 12-s-cis conformation **1** of 11-*cis*-retinal makes an important contribution to its solution spectrum; however, in rhodopsin it may be possible that the 12-s-trans conformation **2** is present.

14-Methylretinal (**3**) was prepared by the Emmons reaction between the C_{18}-ketone (mixture of *all-trans*-**5** and 9-*cis*-**6**) and triethyl (2-carbethoxy)ethylphosphonate (**7**),[5] followed by LAH reduction and MnO_2 oxidation.

High pressure liquid chromatography[6] carried out in the dark (dark lc), coupled with recycling processes, was ideally suited for achieving the complete and efficient separation of the labile products in a pure state. Thus monochromatic irradiation at 400 nm[7] of the all-trans isomer afforded pure 13-*cis*-**8**, 9,13-di-*cis*-**10**, 11-*cis*-**11**/**12**, 9-*cis*-**9**, and *all-trans*-**3** (in sequence of elution).[8] Similar irradiation of all-trans retinal gave the corresponding 13-, 9,13-, 11-, 9-cis isomers and *all-trans*-retinal.

As shown in Table I and Figure 1, the absorption spectra of 14-methylretinals and corresponding retinals are very similar, and this indicates close resemblance in solution conformations. An exception is the 11-cis isomer whose absorption maximum at 338 nm (shortest wavelength observed for a retinal) is blue-shifted by 35 nm from that of the *all-trans*-**3**. In 11-*cis*-12-*s*-*trans*-retinal (**2**), the 12–13 bond is twisted in order to alleviate the 10-H/13-Me steric hindrance, and the calculated spectroscopic effect of this distortion is a hypsochromic shift of *ca.* 30 nm relative to the other isomers. However the observed shift is only *ca.* 5 nm, and this was attributed to the contribution of the 12-s-cis conformer **1**,[2] which would induce a red shift due to its cis-diene moiety.[9] In the 11-cis-14-methyl analog, the additional 14-Me group effectively blocks conformer **11** and hence the s-cis red shift should be absent. Thus this difference in uv data shows that in solution 11-*cis*-retinal exists largely in the 12-s-cis form **1**; on the other hand, its 14-methyl homolog probably has a 12-*s*-*trans*-**12** or a highly twisted ($\Phi_{12-13} \simeq 100°$) 12-s-cis conformation.

The absorption spectra of 11-*cis*-retinal is known to exhibit an anomalous temperature dependence.[10] This has been accounted for by a dynamic equilibrium

Table I. Uv ($^1B_u \leftarrow {}^1A_g$) of Retinals and Modified Retinals, *n*-Hexane, Room Temperature, nm ($\epsilon \times 10^{-4}$)

Isomers	Retinal[a]	14-Methylretinal	13-Desmethyl-14-methylretinal
All-trans	368 (4.75)	373 (4.6) **3**	368 (5.4) **4**
13-Cis	366 (3.86)	358 (3.5) **8**	366 (3.5) (*cf.* **8**)
11-Cis	363 (2.62) **1**	338 (1.8) **12**	362 (3.5) (*cf.* **12**)[b]
9-Cis	363 (3.96)	365 (3.5) **9**	
9,13-Di-cis	357 (3.58)	353 (2.8) **10**	

[a] Data from R. Hubbard, *J. Amer. Chem. Soc.*, **78**, 4662 (1956). [b] It is possible that this may be the 9-cis isomer.

(11-cis,12-s-cis) **1**, R = H; **11**, R = Me

(11-cis,12-s-trans) **2**, R = H; **12**, R = Me

5, all-trans; **6**, 9-cis

all-trans **3**, R = Me; **4**, R = H

(EtO)$_2$POCH(Me)CO$_2$Et **7**

13-*cis*-**8**

9-*cis*-**9**, R$_1$ = CHO; R$_2$ = Me

9,13-di-*cis*-**10**, R$_1$ = Me; R$_2$ = CHO

14-*s*-*cis*-**13**

14

(1) R. Hubbard and G. Wald, "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco and London, 1968, pp 545–554.

(2) B. Honig and M. Karplus, *Nature (London)*, **229**, 558 (1971).

(3) R. Gilardi, I. L. Karle, J. Karle, and W. Sperling, *Nature (London)*, **232**, 187 (1971); R. Gilardi, I. L. Karle, and J. Karle, *Acta Crystallogr., Sect. B*, **28**, 2605 (1972); T. Hamanaka, T. Mitsui, and M. Kakudo, *Acta Crystallogr., Sect. B*, **28**, 214 (1972).

(4) A. Warshel and M. Karplus, *J. Amer. Chem. Soc.*, in press.

(5) A. E. Arbuzov and A. F. Bazumov, *J. Russ. Phys. Chem. Soc.*, **61**, 623 (1929).

(6) Hplc was run under the following conditions: Corasil II, 6–9 ft preparative column, 3 ml/min, 1.5–3% Et$_2$O in hexane.

(7) A JASCO concave radiating monochromator was employed, 3-kW xenon source, 10–15 min irradiation. In view of the longer wavelength absorption of the all-trans isomer (Table I), usage of the 400-nm light resulted in a photoequilibrium mixture predominating in cis isomers.

(8) Characterization of double bond isomers is based on uv data, detailed nmr analysis, lc elution sequence, and binding properties to opsin.

(9) L. Dorfman, *Chem. Rev.*, **53**, 47 (1953); N. L. Allinger and M. A. Miller, *J. Amer. Chem. Soc.*, **86**, 2811 (1964); H. E. Simmons, *Progr. Phys. Org. Chem.*, **1**, 1 (1970).

(10) L. Jurkowitz, J. M. Loeb, P. K. Brown, and G. Wald, *Nature (London)*, **184**, 614 (1959); W. Sperling and C. N. Rafferty, *Nature (London)*, **224**, 591 (1969); W. Sperling, "Biochemistry and Physiology of Visual Pigments," W. Langer, Ed., Springer Verlag, Berlin, 1972, p 19; R. S. Becker, K. Inuzuka, and D. J. Balke, *J. Amer. Chem. Soc.*, **93**, 1 (1971).

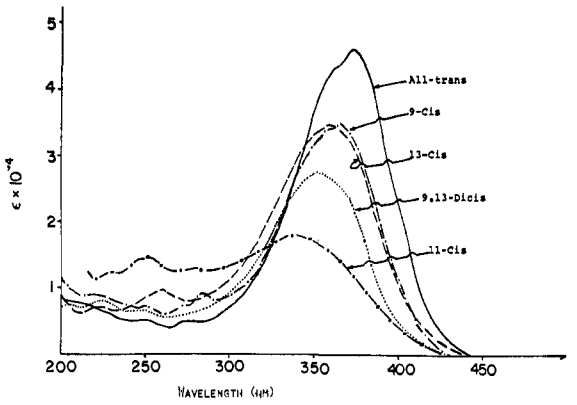

Figure 1. Uv of 14-methylretinal, *n*-hexane, room temperature: (—) *all-trans*-**3**, (-·-·-) 9-*cis*-**9**, (- - -) 13-*cis*-**8**, (. . . . . .) 9,13-di-*cis*-**10**, (-O-O-) 11-*cis*-**11**/**12**.

between 12-*s*-*cis*-**1** and 12-*s*-*trans*-**2** conformers[2,4] or by changes in the effective average value of Φ_{12-13} in going from the ground to excited state.[4] Even at the moderate temperature of −105°, the main band of 11-*cis*-retinal *in ethanol* is shifted from 376 to 387 nm with an ϵ increment of 1.31. In contrast, in 11-*cis*-14-methylretinal the shift is only from 350 to 353 nm and the increase in ϵ is only 1.13. This suggests that the increased long wavelength absorption of 11-*cis*-retinal at low temperatures is due to increased population of the 12-s-cis conformation; the small shift of the 14-methyl analog implies that its conformation remains relatively fixed as **12** between room temperature and −105°.

13-Desmethyl-14-methylretinal (**4**) was prepared by a similar procedure starting from C_{17}-aldehydes corresponding to the C_{18}-ketones **5** and **6**, and the isomers were prepared by dark lc separation of the irradiation mixture. Although five isomers were separated, the amounts of 9-cis and 9,13-di-cis compounds were insufficient to be fully characterized; uv data are given in Table I. Significantly a 0.7 ppm high field shift in the nmr aldehyde protons (9.43–9.53 ppm, in $CDCl_3$) was observed as compared to all other retinals (10.22–10.34 ppm).[11,12] We suggest that this is due to a 14-s-cis conformation **13**[13] in which 15-H is not in the anisotropic deshielding zone of the polyene system. In turn, this suggests that in retinal[11,12] the aldehyde carbonyl in 14-methylretinal adopts the 14-s-trans conformation **14**.

The binding of retinal to opsin is highly restrictive, and only several slightly modified retinals are known to form visual pigment analogs.[14] The reactivity of compounds **3** and **4** with opsin was therefore tested in order to gain further information regarding the binding site. A pigment regenerating system modified after Zorn and Futterman[15] was used. After the incubation period the cattle rods containing the regenerated pigments were freeze dried and then extracted two or three times with cold petroleum ether to remove excess chromophore; otherwise its strong 380-nm absorption can cause serious errors in CD measurements.

(11) R. Rowan, A. Warshel, B. Sykes, and M. Karplus, *J. Amer. Chem. Soc.*, in press.
(12) D. Patel, *Nature (London)*, **221**, 825 (1969).
(13) Presence of the 13-methyl group in retinal and 14-methylretinal would favor conformer **14** over **13**.
(14) *Cf.* B. H. Honig, T. G. Ebrey, and P. Kahn, *Biochemistry*, **12**, 1637 (1973); B. H. Honig and T. G. Ebrey, *Annu. Rev. Biophys. Bioeng.*, in press.
(15) M. Zorn and S. Futterman, *J. Biol. Chem.*, **224**, 881 (1971).

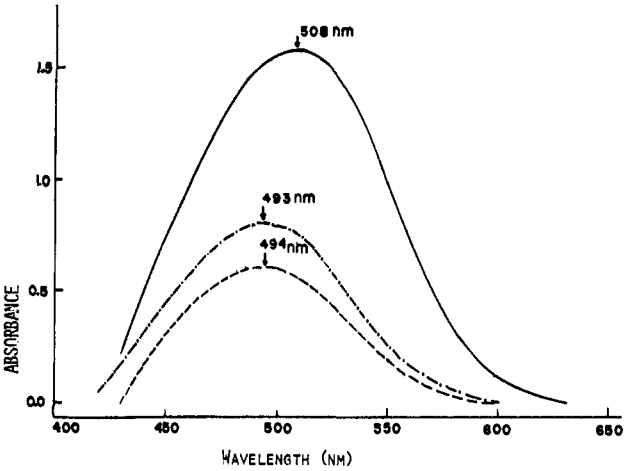

Figure 2. Visible absorption spectra of visual pigments formed from 14-methylretinal, 2% Triton X-100, room temperature; (—) 11-cis, (-·-·-) 9,13-di-cis, (- - -) 9-cis. Approximately threefold excess of the chromophores were added to equal quantities of opsin.

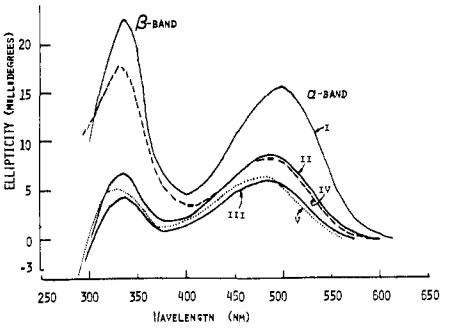

Figure 3. Cd of some visual pigments, 2% Triton X-100, room temperature. "Ellipticity" stands for the ratio of ellipticity at λ_{max} to absorbance at λ_{max}, θ_{max}/A_{max}. Curve I (—) 14-*m*-rhodopsin, 497, 335 nm; curve II (—) 14-*m*-isorhodopsin II, 484, 334 nm; curve III (—) 14-*m*-isorhodopsin I, 485, 334 nm; curve IV (- - - -) regenerated cattle rhodopsin, 487, 331 nm; curve V (. . . . . .) regenerated cattle isorhodopsin, 480, 328 nm.

Of the five isomers of 14-methylretinal tested, the 11-*cis*-**11**/**12**, 9-*cis*-**9**, and 9,13-di-*cis*-**10** isomers formed pigments, which we shall call 14-*m*-rhodopsin and 14-*m*-isorhodopsin I and II, respectively, whereas the *all-trans*-**3** and 13-*cis*-**8** isomers did not. Excepting the 9,13-di-cis isomer, this pattern of isomeric specificity of pigment formation is similar to that of retinal. As it has been reported that 9,13-di-*cis*-retinal (**10**) does not couple with opsin to form a pigment,[16] compound **10** is under reinvestigation.

The uv and CD spectra of the three 14-methylretinal based pigments are shown in Figures 2 and 3. Two CD bands can be seen for the 14-*m*-rhodopsin, one at 497 nm (α band) and the other at 335 nm (β band). The ratio of the rotational strengths of the α band to β band are 1.4, as compared to 1.6 for rhodopsin. If the photosensitivity (to bleaching) of natural rhodopsin is taken as 1, then those of the three pigment analogs were found to be: 14-*m*-rhodopsin, 1.5; 14-*m*-isorhodopsin I and II, *ca.* 0.3. The higher sensitivity of the 14-m homolog as compared to rhodopsin should be noted.

The close resemblance in the shape of CD spectra of rhodopsin and 14-*m*-rhodopsin, and their similar photo-

(16) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952).

sensitivities suggest that the chromophores of these two pigments have similar conformations when bound to opsin. Since steric hindrance prohibits the 11-cis isomer of 14-methylretinal from assuming the crystal conformation of 11-*cis*-retinal, it is clear that this conformation of 11-*cis*-retinal is not the only one that can fit into the binding site. The 12-s-trans conformation **2** is a possibility but determination of the exact conformation requires further studies.

In line with a general phenomenon seen for other natural and artificial pigments,[14] 14-*m*-isorhodopsin I absorbs at shorter wavelength (494 nm) than the 11-cis pigment (508 nm). Of the three geometric isomers of 13-desmethyl-14-methylretinal tested for their reactivity with opsin, only the 11-cis isomer (*cf.* **12**)[17] formed a pigment, but in low yield and unstable: uv 492 nm, CD 487 and 300 nm (positive signs) (both measured in 2% Triton X-100).

Acknowledgments. We thank Sally Chambers for technical assistance.[18]

(17) *Cf.* footnote *b* in Table I.

(18) This work was supported by NIH Grants EY-00433 and EY-60000 and by the Hoffmann-La Roche Foundation.

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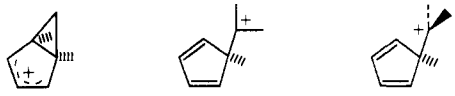
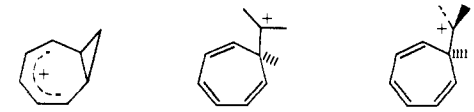
Degenerate Rearrangement in Homocyclopropenyl Cation. Violation of Orbital Symmetry Control for a Sigmatropic Migration[1]

Sir:

The stereospecific circumambulation of cyclopropane about the cyclopentadienyl ring in bicyclo[3.1.0]hexenyl cation (I) provided one of the earliest examples of orbital symmetry control over a sigmatropic migration process.[2,3] The maintenance of positive orbital overlap during these suprafacial [1,4] shifts necessitates passing through a bisected as opposed to an eclipsed cyclopentadienylcarbinyl transition state (II and III, respectively) at each step of the way. Although experimental data are presently unavailable on the stereochemistry of the [1,6] rearrangement in the next higher cationic analog, homotropylium[4] (IV), theoretical molecular orbital calculations indicate that it too falls



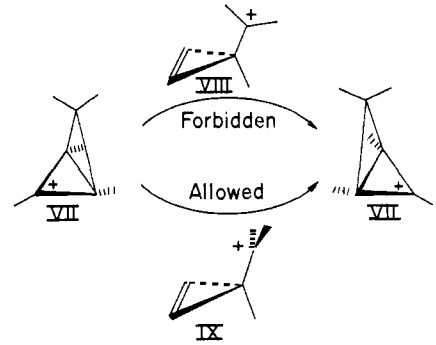
I II III



IV V VI

under orbital control, this time the eclipsed cycloheptatrienylcarbinyl cation (VI) being the preferred intermediate structure.[5] In each of these examples the best available level of theory indicates the symmetry forbidden transition state (III and V respectively for rearrangement in the bicyclo[3.1.0]hexenyl and homotropylium cations) to lie approximately 10 kcal/mol higher in energy.[5a,c,6]

Our attention in this communication focuses on the circumambulation process in the lower homolog of bicyclo[3.1.0]hexenyl cation, the homocyclopropenyl system (VII). Here again two transition state pathways



are conceivable, and, as before, orbital symmetry arguments (this time for a [1,2] migration) should enable us to choose between them. Thus on this basis alone, we would anticipate an eclipsed cyclopropenylcarbinyl type structure (IX) to be preferred over a bisected one (VIII). Realize, however, that the manifold of high-lying occupied molecular orbitals on cyclopropene contains not only a π component but also a pair of in-plane orbitals analogous to the Walsh functions of cyclopropane[7] (Figure 1). It has already been noted that interaction between the asymmetric Walsh cyclopropane orbital and the carbonium center in cyclopropylcarbinyl results in far greater net stabilization than the corresponding interaction involving the symmetric component.[8-10] As the magnitude of this differential stabilization is fairly large (theoretical molecular orbital calculations

(1) Presented as part of the Symposium on Theoretical Organic Chemistry, American Chemical Society 29th Southwest Regional Meeting, El Paso, Texas, Dec 6–7, 1973.

(2) P. Vogel, M. Saunders, N. M. Hasty, Jr., and J. A. Berson, *J. Amer. Chem. Soc.*, 93, 1551 (1971), and references therein.

(3) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, 8, 781 (1969).

(4) For a discussion of the difficulties involved see J. A. Berson and J. A. Jenkins, *J. Amer. Chem. Soc.*, 94, 8907 (1972).

(5) (a) W. J. Hehre, *J. Amer. Chem. Soc.*, 94, 8908 (1972); (b) *ibid.*, 95, 5807 (1973); (c) *ibid.*, submitted for publication.

(6) Experimentally, the symmetry forbidden transition state for circumambulatory rearrangement in heptamethyl bicyclo[3.1.0]hexenyl cation lies at least 5.7 kcal/mol above the allowed structure: R. F. Childs and S. Winstein, *J. Amer. Chem. Soc.*, 90, 7146 (1968).

(7) A. D. Walsh, *Trans. Faraday Soc.*, 45, 179 (1949); for a discussion see R. Hoffmann and R. B. Davidson, *J. Amer. Chem. Soc.*, 93, 5699 (1971).

(8) For reviews of the experimental literature see H. G. Richey, Jr., in "Carbonium Ions," G. A. Olah and P. v. R. Schleyer, Ed., Wiley, New York, N. Y., 1972, p 1201; K. B. Wiberg, B. A. Hess, Jr., and A. J. Ashe, III, *ibid.*, p 1295.

(9) Theoretical molecular orbital calculations: (a) L. Radom, J. A. Pople, V. Buss, and P. v. R. Schleyer, *J. Amer. Chem. Soc.*, 92, 6380 (1970), and references therein to earlier work; (b) W. J. Hehre and P. C. Hiberty, *ibid.*, 94, 5917 (1972); (c) L. Radom, J. A. Pople, and P. v. R. Schleyer, *ibid.*, 94, 5935 (1972); (d) W. J. Hehre and P. C. Hiberty, *ibid.*, 96, 302 (1974).

(10) The conjugative interaction present in bisected cyclopropylcarbinyl displays itself in terms of an extremely short ring-carbonium center bond length (1.384 *vs.* ~1.52 Å in methylcyclopropane). The corresponding linkage in eclipsed cyclopropylcarbinyl (1.480 Å) is indicative of a much diminished interaction. Theoretical ring-carbonium center bond lengths for bisected and eclipsed cyclopropenylcarbinyl (1.384 and 1.524 Å, respectively) show similar effects.