Tetrahedron. Vol. 31, pp. 201 to 206. Pergamon Press 1975. Printed in Great Britain

7-*CIS* ISOMERS OF RETINAL VIA 7-*CIS*- AND 7,9-DI*CIS*-β-C_{18}-TETRAENE KETONES

NEW GEOMETRIC ISOMERS OF VITAMIN A AND CAROTENOIDS—I

V. RAMAMURTHY and R. S. H. LIU*

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

(*Received in USA* 1 *July* 1974; *Received in the UK for publication* 12 *August* 1974)

Abstract—Based catalyzed condensation of 7-*cis*- or 7,9-di*cis*-β-ionylideneacetaldehyde with acetone gave either 7-*cis* or 7,9-di*cis*-β-C_{18}-tetraene ketone. Reaction of the tetraene ketone mixture with diethyl cyanomethylphosphonate gave a mixture of 4 isomers of retinonitrile, all believed to have the 7-*cis* geometry. Partial redution of the retinonitrile mixture with di-isobutylaluminum hydride gave a mixture of retinal isomers. Repeated column chromatography resulted in partial separation of the retinal isomers. Based on NMR data the geometry of the isomers prepared are believed to be 7-*cis*, 7,9-di*cis*, 7,13-di*cis*, and 7,9,13-tri*cis*.

In the preceding paper[1] we described the preparation of 7-*cis* isomers of dienes and trienes in the Vitamin A series by a photochemical method. In this paper we now describe our first effort to synthesize 4 of the 8 missing 7-*cis* isomers of retinal.† The results are preliminary in nature; but, we believe they firmly establish the stability of such highly hindered and long neglected isomers.

Because of the limitation of the photochemical method in preparing the 7-*cis* isomers of higher polyenes in the Vitamin A series[1] it is clear that in designing a synthetic route to 7-*cis* isomers of retinal, the introduction of the *cis* geometry will have to be done at the diene or triene stages. And, also because of the nagging doubt in the mind of some workers in this field of the possible existence of such isomers in vitamin A and carotenoids, to start out we chose a route involving chain lengthening by one double bond at a time so that the exact geometry and stability of compounds at all stages of synthesis will not be in doubt:

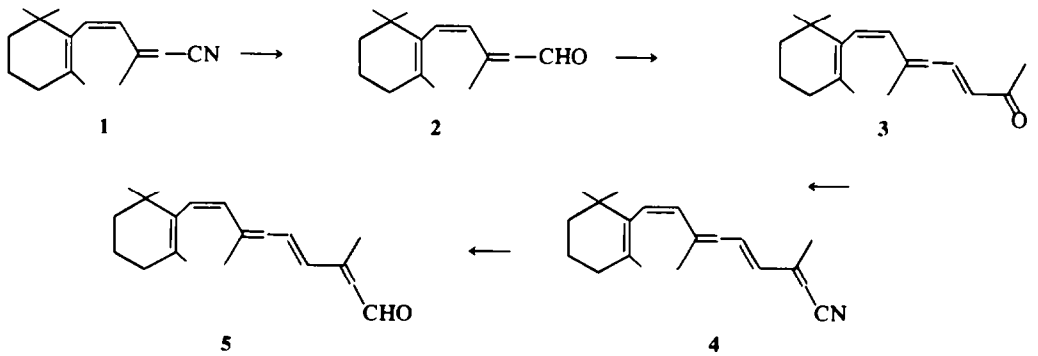
*Inquires to whom should be addressed. A preliminary account of the work was presented at the First Annual Meeting of the Americal Society for Photobiology, 11–14 June 1973, Sarasota, Florida.

†Only 6 of the possible 16 geometric isomers are known, all with the 7-*trans* geometry: All *trans*, 9-*cis*, 11-*cis*, 13-*cis*, 9,13-di*cis* and 11,13-di*cis*.

RESULTS AND DISCUSSION

The starting material (1) was obtained in good yield via either selective sensitized isomerization of the corresponding 7-*trans* isomers or reaction of diethyl cyanomethylphosphonate with *cis*-β-ionone.[1] Either route gave a mixture of 7-*cis* and 7,9-di*cis* isomers in an approximate ratio of 3:2. The two isomers could not be separated by column chromatography. In fact, because of the difficulty often encountered in separating such isomers in the main sequence that we have completed so far, we chose to work with isomeric mixtures of compounds obtained at all stages of reactions. Partial reduction of the nitrile with diisobutylaluminum hydride followed by hydrolysis with dilute HCl gave a similar isomeric mixture of β-ionylideneacetaldehyde, 2. Separation of the isomers (7-*cis* and 7,9-di*cis*) was accomplished by chromatographing on a silica gel column.[1]

Condensation of the two isomer mixture of 2 with acetone in the presence of sodium hydroxide gave the tetraene ketone 3, a yellow oil, in high yield (95%). Its IR spectrum showed the expected absorption for unsaturated ketones (1660 cm^{-1}) and the molecular ion in the mass spectrum agreed with that of the C_{18}-tetraene ketone. The geometry around the 7,8-bond of the isomers was revealed by its PMR spectrum. As noted in the previous



1 2 3 4 5

paper[1] the change of geometry from 7-*trans* to 7-*cis* in triene and higher polyenes is accompanied by an upfield shift of ~0·2 ppm of the Me-18 resonance as a result of increased shielding of the Me group by the side chain. This spectral characteristic is particularly useful in providing quick and reliable information regarding the stereochemistry at the 7,8-bond for higher polyenes. Therefore, the absence of singlets at δ 1·7 coupled with the retention of the singlet at 1·55 ppm strongly suggested that the 7-*cis* geometry remained intact during the reaction. It showed in addition to absorption at 740 (*cis* alkene) and 840 cm^{-1} (trisubstituted alkene) a band at 940 cm^{-1} indicative of the *trans* geometry at the newly formed 11,12-bond. This is in agreement with the known chemistry of *trans*-β-ionylideneacetaldehyde with acetone of which the new bond was formed stereospecifically to give the 11-*trans* isomer.[2] Therefore, these results suggested that the tetraene ketone mixture consisted of the 7-*cis* and the 7,9-di*cis* isomers.

Attempts to separate the two isomers by column chromatography were unsuccessful. The assignments were, however, unambiguously confirmed by comparison of the NMR spectrum with those of 7-*cis*- and 7,9-di*cis*-3 obtained from reactions of isomerically pure triene-aldehyde 2 with acetone. The spectra of the single isomers are shown in Figs 1 (7-*cis*) and 2 (7,9-di*cis*). The signals in the vinyl region, after the addition of Eu(fod)$_3$ shift reagent,[3] are readily analyzed based on first order coupling. The assignments are shown in the Figs and also listed in Table 1 for comparison with those of the corresponding 7-*trans* isomers. The vinyl coupling constants clearly show that the geometry around 7,8 and

Table 1. NMR spectral characteristics of β-C_{18}-tetraene ketone isomers[a]

Isomer	CH_3-19	CH_3-18	H_7 (d)	H_8 (d)	H_{10} (d)	H_{11} (dxd)	H_{12} (d)	$J_{7,8}$	$J_{11,12}$
all-*trans*[b]	2·04	1·68	6·20	6·20	6·09	7·46	6·06	16·0	16·0
9-*cis*[b]	2·04	1·75	6·25	6·70	6·02	7·50	5·99	16·0	16·0
7-*cis*[c]	1·93	1·50	5·99	6·05	6·18	7·46	6·06	12·5	15·0
7,9-di*cis*[c]	1·90	1·50	5·98	6·62	6·14	7·57	6·03	12·0	15·0

[a] In $CDCl_3$. Chemical shift in δ; coupling constant in Hz.
[b] M. Mousseron-Canet and J.-L. Olive, *Bull. Soc. Chim., Fr.* 3242 (1969).
[c] This work. HA-100.

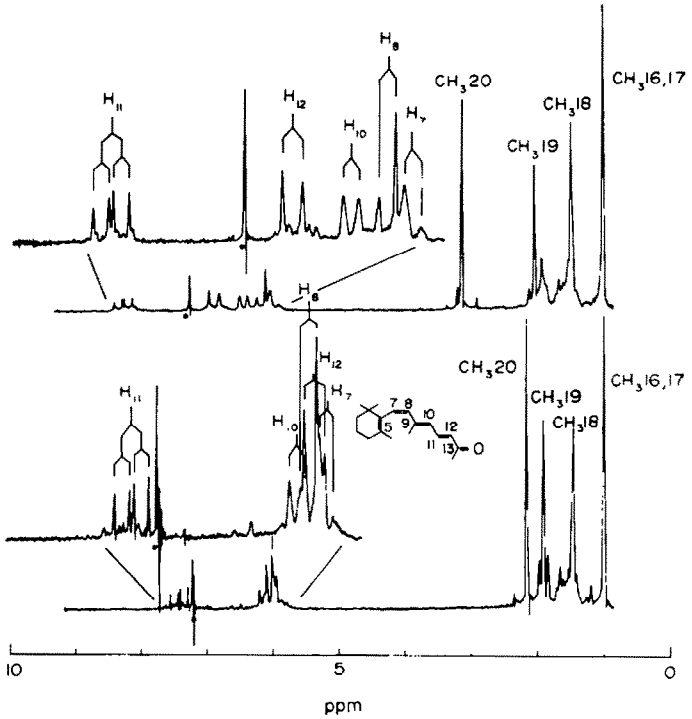


Fig. 1. 100 MHz PMR spectra of 7-*cis*-β-C_{18}-tetraene ketone, Ia: lower, in $CDCl_3$ without shift reagent; upper, with Eu(fod)$_3$. (* = benzene).

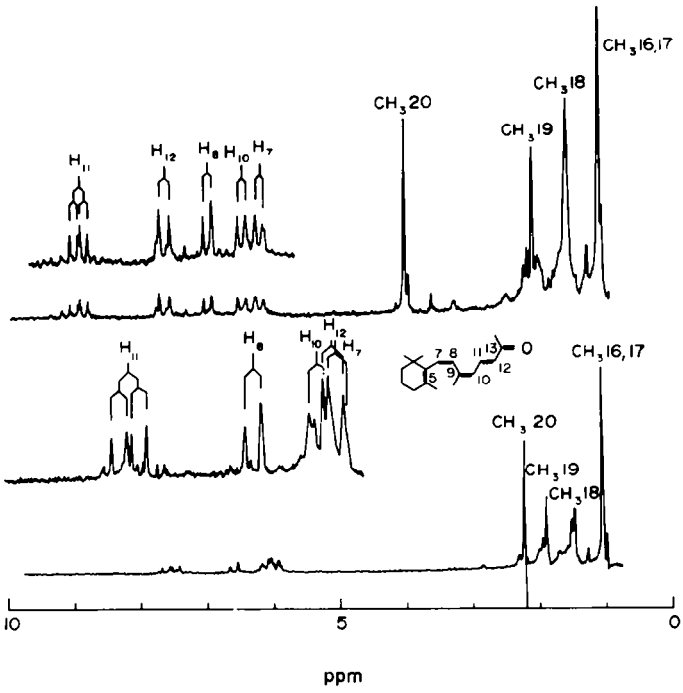


Fig. 2. 100 MHz PMR spectra of 7,9-di*cis*-β-C_{18}-tetraene ketone, Ib: lower, without shift reagent; upper, with $Eu(fod)_3$.

11.12 bonds are *cis* and *trans* respectively. The chemical shifts of H_8's are indicative of the geometry around the 9,10 bond: in the 9-*cis* isomers, such hydrogens being deshielded by the 11,12-double bond.

Reaction of the two isomer mixture of 3 with diethyl cyanomethylphosphonate gave a yellow oil in 85% yield. Its spectroscopic data agreed with the expected retinonitrile: MS: 281 for M^+, IR 2180 cm^{-1} for CN. The presence of the 7-*cis* geometry was again suggested by the Me-18 signals between 1·42 and 1·50 ppm. Since Horner reaction in forming trisubstituted double bonds was shown to proceed without stereoselectivity, a 4-isomer mixture was expected. Without further purification, the retinonitrile mixture was reacted with di-isobutylaluminum hydride followed by hydrolysis with dilute HCl. A yellow oil was obtained in 58% yield. The proton signals at δ 10·16 in PMR and CO absorption at 1650 cm^{-1} in IR clearly indicated the presence of an aldehyde group. The molecular weight by mass spec (284) is in agreement with that of retinal.

The PMR spectrum of the oil showed a strong singlet at δ ~ 1·55 corresponding to Me-18 in 7-*cis* isomers. However at δ 1·7, there was another singlet of about one tenth in intensity. Its chemical shift is characteristic of Me-18 in 7-*trans* isomers. These observations suggested that the 7-*cis* geometry was mostly retained during the reduction reaction, and now we have reasons to believe that the partial loss of stereochemistry was due to absorption of room light during the work-up process (see below).

The mixture of retinal isomer was chromatographed twice on a silica gel column (Bio Sil A) using a hexane-benzene (3:1) solvent mixture. partial separation of isomers was thus achieved. So far under no circumstances have we succeeded in isolating all isomers. Among all aldehyde containing fractions collected, the one eluted first contained essentially two isomers believed to be the two with the 13-*cis* geometry. The PMR spectrum of this fraction shown in Fig 3, is sufficiently well resolved for assingment of all the vinyl and methyl hydrogens. Examination shows that all major peaks are attributable to two isomers. In making assignments, we found the 220 MHz spectra for the known 7-*trans* isomers of retinal reported by Patel[4] very helpful. Therefore these data are reproduced in Table 2. The singlet at 1·54 is due to Me-18. The value is again indicative of the 7-cis geometry. The singlet (with fine structures) at 2·10 is due to Me-20. And, the value is only consistent with the 18-*cis* geometry because the aldehyde carbonyls in the 13-*trans* isomers are known to deshield the Me-20 causing it to resonate at ~0·2 ppm lower field than that in 13-*cis* (see Patel's data in Table 2). The two split singlets (δ 1·90 and 1·94) must be due to Me-19 of two isomers. The geometry around the 9,10 bond in the two isomers becomes known after examination of the vinyl signals: the H_8's in the 9-*cis* isomers are expected to be deshielded by the 11,12 bond. For example in the known 7-*trans* series (Table 2), the signals are deshielded by about 0·5 ppm. Therefore the two pairs of doublets (J = 12·0 Hz) in the spectrum at δ 6·12 and 6·68, are attributable to H_8's in 9-*trans* and 9-*cis*

Table 2. NMR spectra of retinal isomers in $CDCl_3$[a]

Isomer	Me-20	Me-19	Me-18	Me-16,17	14 H	12 H	11 H	10 H	8 H	7 H	$J_{11,12}$	$J_{10,11}$	$J_{7,8}$	$J_{14,15}$
all-t[b]	2·33	2·03	1·72	1·04	5·98	6·37	7·15	6·20	6·18	6·36	15·4	12·0	16·5	8·0
9-*cis*[b]	2·30	2·00	1·72	1·05	5·94	6·27	7·20	6·06	6·64	6·31	15·4	11·8	15·9	8·2
11-*cis*[b]	2·36	1·99	1·71	1·02	6·07	5·92	6·69	6·54	6·14	6·32	11·5	13·0	16·0	8·0
13-*cis*[b]	2·14	2·02	1·72	1·04	5·85	7·28	7·05	6·23	6·18	6·35	15·0	11·1	15·9	8·0
9,13-di*cis*[b]	2·15	2·05	1·77	1·05	5·87	7·25	7·16	6·16	6·68	6·36	15·0	10·5	16·0	8·0
11,13-di*cis*[b]	2·07	1·96	1·68	1·01	5·98	6·11	6·77	6·20	6·08	6·28	11·8	12·5	16·0	8·1
7-*cis*[c]	2·32	1·97	1·55	1·08	5·86	?[d]	?	?	?	?	?	?	?	8·0
7,9-di*cis*[c]	2·32	1·91	1·55	1·08	5·81	?[d]	?	?	?	?	?	?	?	8·0
7,13-di*cis*[c]	2·10	1·94	1·54	1·07	5·80	7·22	6·92	6·36	6·11	5·98	15·5	11·0	11·5	8·0
7,9,13-tri*cis*[c]	2·10	1·90	1·54	1·04	5·80	7·18	7·00	6·23	6·56	5·97	15·5	11·5	12·0	8·0

[a] Chemical shift in δ, coupling constant in Hz. Solvent: $CDCl_3$.
[b] Data of D. J. Patel (220 MHz), *Nature*, **221**, 825 (1969).
[c] This work, 100 MHz.
[d] Not sufficiently well revolved for assignment of vinyl hydrogens.

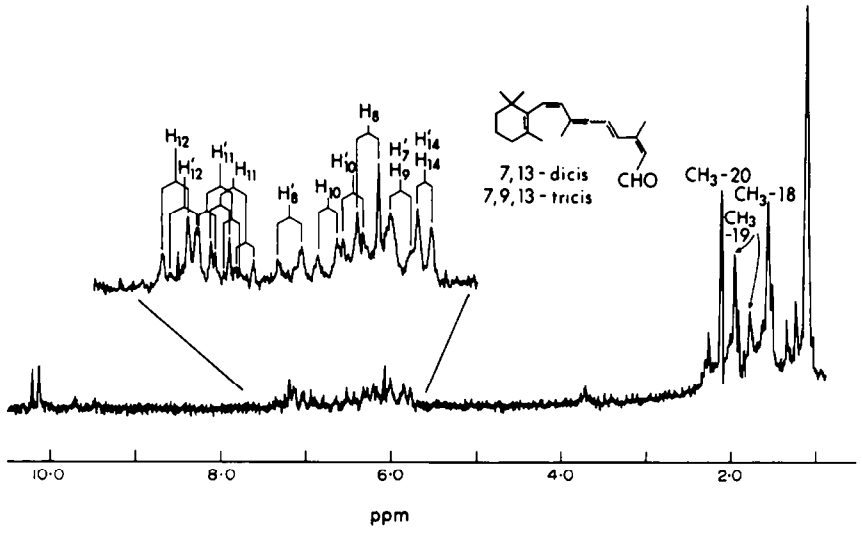


Fig. 3. 100 MHz PMR spectra of a mixture of 7,13-di*cis*- and 7,9,13-tri*cis*-retinal; upper, vinyl region expanded.

isomers. Clearly, they are both coupled with a broad doublet at 5·96 (J = 12·0). The latter is therefore assigned to the H_7's. That these peaks are broad is characteristic of H_7 in compounds in this series—due to long range coupling with the CH_2-4 and Me-18.[5] Also, its chemical shift is expectedly not sensitive to geometric variation at 9,10. The signals for other vinyl hydrogens are fortunately sufficiently separated for complete assignments. This is partly due to H_{12} in the 13-*cis* isomers being characteristically deshielded by the C=O group, thus not overlapping with other signals. The assignments are listed in Table 2. The values of coupling constants ($J_{11,12}$ = 15·5 Hz) show that the *trans* geometry at the 11-12 bond is retained. Therefore, the two retinal isomers present in this fraction must be 7,13-di*cis* and 7,9,13-tri*cis*. It is worth noting that the chromatographic behavior of these isomers parallel those of 7-*trans*. On silica gel column, the 13-*cis* isomers among the latter group also eluted first.[6]

Another later fraction was found to contain mostly two but different isomers. But it also contained about 15% of a 7-*trans* isomer as indicated by the singlet at δ 1·70 in the PMR spectrum. The spectrum is unfortunately rather complex, particularly in the vinyl region. We only succeeded with some degree of certainty in assigning peaks due to Me hydrogens. They are consistent with those of 7-*cis*- and 7,9-di*cis*-retinal. The assignments are listed in Table 2. Again, the δ for Me-18 agrees with the 7-*cis* geometry and those for Me-20 (2·32 due to deshielding by C=O) agree with the 13-*trans* geometry. The absence of vinyl signals below δ ~ 7·0 for H_{12} negates the possible presence of the 13-*cis* geometry. They superimpose with signals of remaining vinyl hydrogens to make this region too complex for analysis. The UV spectra of these two fractions (Fig 4) show features similar to those of the known *cis*-isomers—in addition to the major band at ~370 mμ the presence of *cis*-bands at shorter wavelengths.[7]

The fractions in between these two appeared to contain varying amounts of these 4 isomers (by NMR).

These 7-*cis* isomers appear to be reasonably stable. An ethanol solution of the compound when kept under nitrogen at 0° for 6 months showed no change in its absorption spectrum. On the other hand, they are quite light sensitive. Upon UV irradiation of solutions of either mixture of isomers, isomerization took place immediately leading to the formation of a mixture of 7-*trans* isomers. This was indicated by rapid disappearance of the singlet at δ ~ 1·55 replaced by a singlet at δ ~ 1·7. This preliminary observation of the photochemistry of 7-*cis* isomers of retinal (in agreement with other polyenes in this series)[1,8] seems to support the assumptions and findings of previous workers in that 7-*cis* isomers are not present in the photostationary mixture of retinal.[7] This conclusion, however, will have to be verified in a more thorough photochemical study and to await more definitive structural assignments of all isomers. Also, conditions for a more sensitive analytical method to assay isomer mixtures will have to be found. A likely candidate will be high pressure lc.[9]

EXPERIMENTAL

7-cis and 7,9-Dicis-β-ionylideneacetonitrile, 1. The compounds could be obtained either by way of photosensitized irradiation of the corresponding 7-*trans* isomers or by Horner modified Wittig reaction by reacting *cis*-β-ionone with diethyl cyanomethylphosphonate. Both procedures were described.[1]

7-cis and 7,9-Dicis-β-ionylideneacetaldehyde, 2. The compounds were prepared by reduction of 1 with di-isobutylaluminum hydride followed by acid hydrolysis.[1] Conditions for separating the isomers were also reported.

7-cis and 7,9-Dicis-β-C_{18}-tetraene ketone, 3. A procedure similar to that used for preparation of all-*trans*-C_{18}-tetraene ketone was followed.[2]

A mixture of 7-*cis* isomers of 2 (5 g) in 100 ml acetone was stirred at room temp with 40 ml of 10% NaOH for 12 hr. The

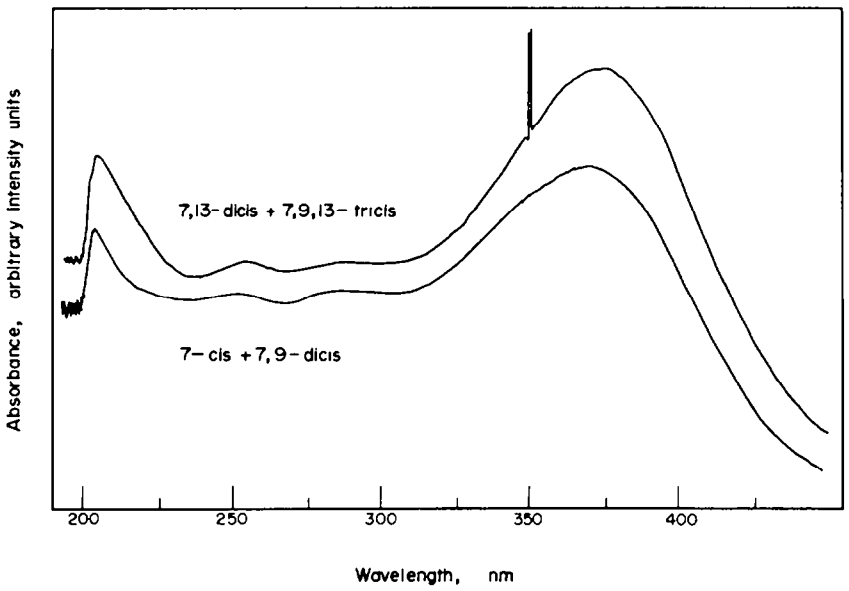


Fig. 4. UV spectra of mixtures of retinal isomers: upper, fraction I containing 7,13-di*cis*- and 7,9,13-tri*cis*-retinal; lower, fraction II containing mostly 7-*cis*- and 7,9-di*cis*-retinal. Solvent: ethanol.

mixture was extracted with ether. After usual work-up, a yellow oil of the tetraene ketone was obtained (yield: 95%). The mixture was purified by passing through a silica gel column. No separation of isomers was achieved. [MS: 258 for M^+; IR (neat) 1660 (C=O), 1590 (conj. C=C), 960 (*trans* alkene), 890 (trisubstituted alkene) and 740 (*cis* alkene), NMR (Table 1) agreed with a mixture of 7-*cis* and 7,9-di*cis* isomers]. Single isomers of the tetraene ketone were prepared in a similar manner by starting with isomerically pure 2: [7-*cis*: UV (EtOH) 347 nm (20,600); 7,9-di*cis*: UV (EtOH) 330 (16,600); NMR Figs 1, 2].

7-cis *Isomers of retinonitrile*, 4. To a suspension of NaH (2·4 g as ~57% oil dispersion) in 50 ml DMF was added diethyl cyanomethylphosphonate (9 g). After 30 min, a mixture of 7-*cis* and 7,9-di*cis*-β-3 (13 g) was added slowly while maintaining the temp at 30°. After complete addition the mixture was stirred at room temp for about 10 hr. The mixture was worked up by slow addition of 200 ml water and extracted with ether. The ether layer, dried over $MgSO_4$, upon evaporation gave a yellow oil. Spectral data (see discussion) agreed with a mixture of retinonitrile of the 7-*cis* geometry. The isomers could not be separated by column chromatography (Bio-Sil A; hexane/benzene solvent mixture): [IR: 2,180 (CN), 730, 785, 960 cm^{-1}; MS: m/e = 281 for M^+, calc. for $C_{20}H_{27}N$ = 281].

7-cis *Isomers of retinal*, 5. A mixture of retinonitrile isomers, 4, (6 g) was taken in a 250 ml round bottomed flask with 50 ml hexane and cooled to 0°. To this 3 g (as 20% soln in hexane) of diisobutylaluminum hydride cooled to 0° was added slowly. After complete addition the mixture was stirred at room temp for 3 hr. Then the mixture was poured over ice and stirred. Dil HCl was added to hydrolyze the complex, the mixture extracted with ether. The ether extract was dried over $MgSO_4$. Evaporation of ether gave a mixture of retinal isomers: [MS: m/e = 284 for M^+, calc. for $C_{20}H_{28}O$ = 284; IR (film) ~1650 (CO) 720, 950, 1102, 1435, 1527 cm^{-1}].

The isomeric mixture of retinal was purified by chromatography over silica gel (Bio-Sil A) using hexane-benzene (3:1) as solvent. The first passage resulted in partial separation. The partially separated mixture upon re-chromatographing over Bio-Sil A using hexane-benzene (3:1) as solvent gave 7,13-di*cis* and 7,9,13-tri*cis* retinal in the first fraction [UV: Fig 4 and NMR: Fig 3 and Table 2] and predominantly 7-*cis* and 7,9-di*cis* retinal in a later fraction [UV: Fig 4; NMR: Table 3].

Acknowledgement—The work was supported by grants from the Public Health Service (RO1 EY AM 00918) and the Alfred P. Sloan Foundation. V. R. acknowledges the support of the NSF-DSD grant to the chemistry department (No. NSF GU 3855) in the form of a pre-doctoral research fellowship.

REFERENCES

[1]V. Ramamurthy, G. Tustin, C. C. Yau and R. S. H. Liu, *Tetrahedron* **31**, 193 (1975)

[2a]H. O. Huisman, R. Smit, P. H. Van Leeuwen and J. H. Van Rig, *Recl. Trav. Chim.* **75**, 977 (1956); [b]N. L. Wendler, H. L. Slates, N. R. Trenner and M. J. Tishler, *J. Am. Chem. Soc.* **73**, 719 (1951); [c]M. Mousseron-Canet and J.-L. Olive, *Bull. Soc. Chim., Fr.* 3242 (1969)

[3]R. E. Sievers and R. E. Rondeau, *J. Am. Chem. Soc.* **93**, 1522 (1971)

[4]D. Patel, *Nature* **221**, 825 (1969)

[5a]V. Ramamurthy, T. T. Bopp and R. S. H. Liu, *Tetrahedron Letters* 3915 (1972); [b]B. Honig, B. Hudson, B. D. Sykes and M. Karplus, *Proc. Nat. Acad. Sci.* **68**, 1289 (1971); the latter for 7-*trans* isomers

[6]C. Planta, U. Schwieter, L. Chopard-dit-Jean, R. Rüegg, M. Kofler and O. Isler, *Helv. Chem. Acta* **45**, 548 (1962)

[7]A. Kropf and R. Hubbard, *Photochem. Photobiol.* **12**, 249 (1970)

[8]V. Ramamurthy and R. S. H. Liu, *J. Am. Chem. Soc.* **96**, 5625 (1974)

[9a]M. Vecchi, J. Vesely and G. Oesterhelt, *J. Chromatog.* **83**, 447 (1973); [b]W. K. Chan, K. Nakanishi, T. G. Ebrey and B. Honig, *J. Am. Chem. Soc.* **96**, 3642 (1974)