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Retro-γ-retinals and Visual Pigment Analogues

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As the part of the studies to clarify the interaction between 11-*cis* retinal and apoprotein opsin in the rhodopsin molecule, the retro-γ-retinal, possessing the dissected diene and trienal groups, has been investigated as a new chromophore in rhodopsin analogues. The syntheses, spectral properties of three retro-γ-retinal isomers [all-*trans* (VIIa), 11-*cis* (VIIa′) and 9-*cis* (VIIb)] and some chemical behaviours of VIIb have been described. Of these three isomers, VIIb has given a new artificial visual pigment.

Keywords——retro-γ-retinal; visual pigment analogue; rhodopsin; opsin; Wittig reaction

It is well known that the 11-*cis* isomer of retinal is the chromophore of the visual pigment rhodopsin (I). Interactions between this 11-*cis* retinal and the apoprotein opsin in the rhodopsin molecule can be investigated by examining the various properties of the artificial visual pigment analogues produced from the modified retinals and opsin. Therefore, the syntheses of particular retinal analogues are essential in order to elucidate the binding properties of opsin. The studies directed towards clarifying their mutual interactions are developing in several laboratories including our group[2] as one of the most attractive problems in the vision research.

We speculate from our experimental results so far that, at the first stage in the photolysis of rhodopsin, retro-γ-retinal structure (II) might be produced from the 11-*cis* retinal part of rhodopsin (I) by the 1,5-sigmatropic photorearrangement. This unique structure, possessing the dissected diene and trienal chromophores, is of particular interest in studying the steric requirements imposed on the retinal moiety by the apoprotein opsin. As the first part of our studies on retro-γ-retinal, we now wish to report the syntheses (Chart 1), spectral properties of three retro-γ-retinal isomers [all-*trans* (VIIa), 11-*cis* (VIIa′) and 9-*cis* (VIIb)] and some chemical behaviours of VIIb.

Irradiation of methyl β-ionylideneacetate (III) [*trans*: *cis*=*ca.* 3:2] with a 500W high pressure mercury lamp using Pyrex filter gave a mixture of 9-*trans* (IVa) and 9-*cis* (IVb) isomers of methyl retro-γ-ionylideneacetate.[3] Each isomer was clearly isolated by a careful chromatographic separation on a 5% deactivated alumina column and was identified on the basis of the nuclear magnetic resonance (NMR) data (Table I). Especially, chemical shift difference $\delta_{trans}-\delta_{cis}$ of C-9-methyl signals [IVa: δ 2.11 *cis* methyl with respect to CO_2Me; IVb: δ 1.79 *trans* methyl to CO_2Me] and that of C-8-methylene signals [IVa: δ 2.94 *trans* methylene to CO_2Me; IVb: δ 3.50 *cis* methylene to CO_2Me] in two isomers gave a distinct

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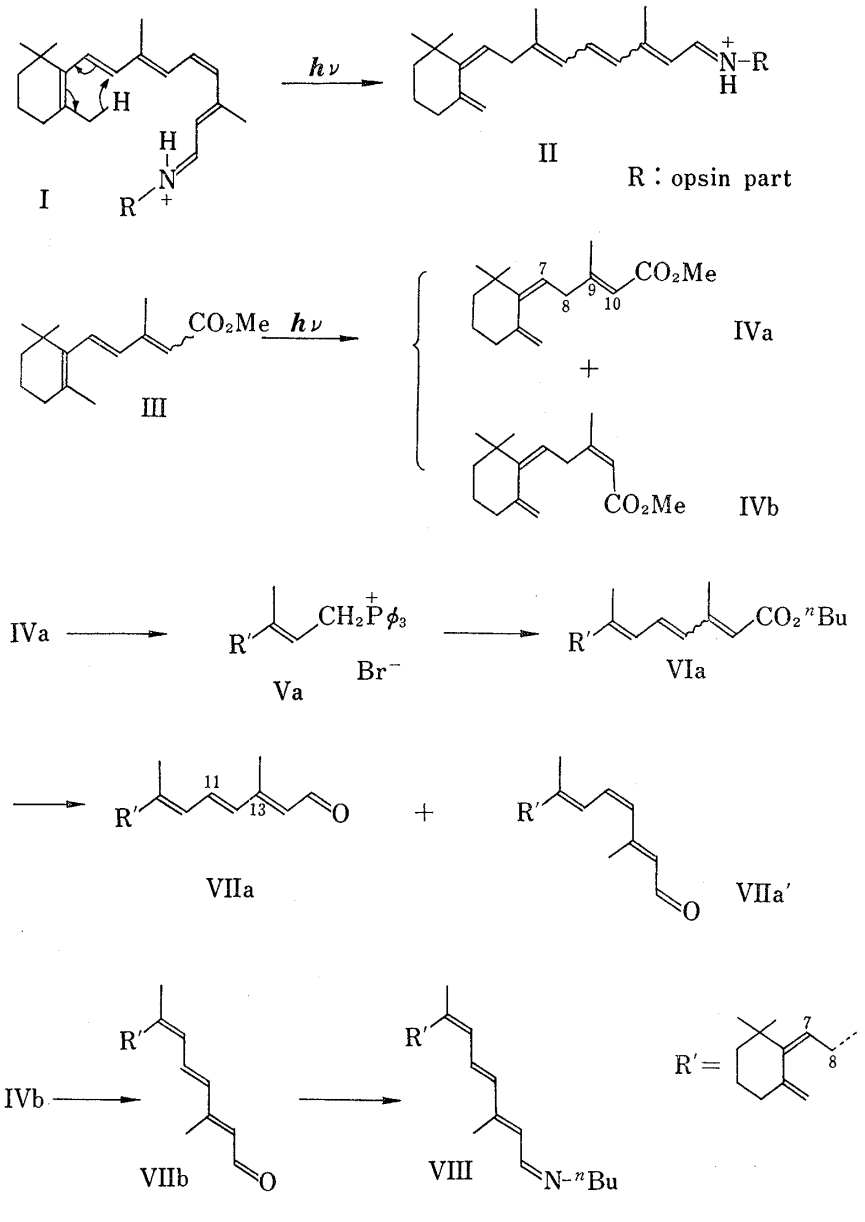

Chart 1

TABLE I. NMR Data of 9-*trans* (IVa) and 9-*cis* Retro-γ-ionylideneacetate (IVb)[a]

	IVa	IVb
gem.-CH_3	1.07 (s)	1.04 (s)
C_9–CH_3	2.11 (d, J=1)	1.79 (d, J=2)
C_8–H_2	2.94 (d, J=7)	3.50 (d, J=7.5)
CO_2CH_3	3.62 (s)	3.63 (s)
exo-CH_2	4.56 (d, J=3) 4.95 (m)	4.62 (d, J=3) 5.00 (m)
C_7–H	5.18 (t, J=7)	5.10 (t, J=7.5)
C_{10}–H	5.54 (q, J=1)	5.59 (br. s)

a) δ value in CCl_4, J in Hz, 60 MHz.

characterisation of each isomer. The 9-*trans* isomer (IVa) was converted by reduction with sodium bis-(2-methoxyethoxy)-aluminium hydride (SMEAH) in abs. ether, followed by treatment with triphenylphosphonium bromide into the Wittig salt (Va). This salt, without purification, was condensed in the presence of 5% KOH in *n*-propanol with *n*-butyl γ-oxosenecioate prepared from propionaldehyde and *n*-butyl glyoxalate to yield *n*-butyl retro-γ-retinoate (VIa). In this Wittig reaction, the use of NaOMe instead of KOH as a base gave no satisfactory result. The retro-γ-retinoate (VIa), on reduction with SMEAH and on subsequent oxidation with active manganese dioxide, furnished a mixture of two isomers of retro-γ-retinal. Preparative thin-layer chromatography (TLC) gave the all-*trans* retro-γ-retinal (VIIa) [lower band] and the 11-*cis* isomer (VIIa′) [upper band] in a pure form. Similarly, 9-*cis* retro-γ-retinal (VIIb) was prepared from the corresponding isomer (IVb), though 9,11-di-*cis* isomer was not isolated from IVb. All three isomers of the synthesised retro-γ-retinal showed ultraviolet (UV) absorption maxima at 343 nm and their characteristic features in NMR spectra were summarised in Table II. The chemical shifts of C-13-methyl protons in three retro-γ-retinals showed to be *trans* around the C-13,14 double bond. Compari-

Table II. Characteristic NMR Data[a] of Compounds VIIa, VIIa′ and VIIb

	VIIa	VIIa′	VIIb
gem.-CH_3	1.03 (s, 6H)	1.04 (s, 6H)	1.02 (s, 6H)
C_{13}-CH_3	2.31 (s, 3H)	2.38 (s, 3H)	2.30 (s, 3H)
C_8-H_2	2.97 (d, 2H, J=8)	2.99 (d, 2H, J=8)	3.14 (d, 2H, J=7)
exo-CH_2	4.58 (d, 1H, J=3)	4.62 (d, 1H, J=3)	4.64 (d, 1H, J=3)
	4.99 (m, 1H)	5.01 (m, 1H)	5.09 (m, 1H)
C_{11}-H	7.04 (dd, 1H, J=11, 15)	6.63 (dd, 1H, J=10, 12)	6.94 (dd, 1H, J=11, 15)
CHO	10.14 (d, 1H, J=8)	10.12 (d, 1H, J=8)	10.13 (d, 1H, J=8)

a) δ value in $CDCl_3$, J in Hz, 90 MHz.

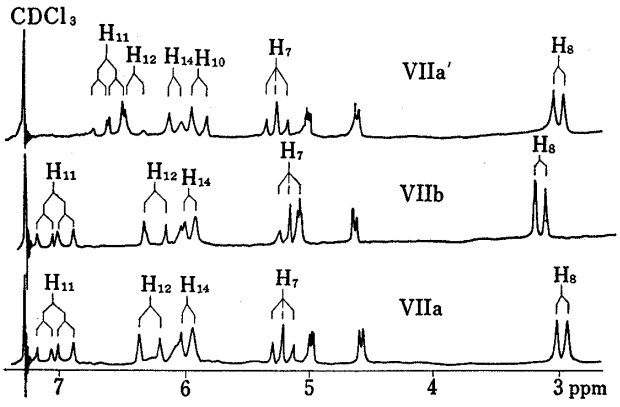

Fig. 1. Characteristic NMR Peaks of Compounds VIIa, VIIa′ and VIIb

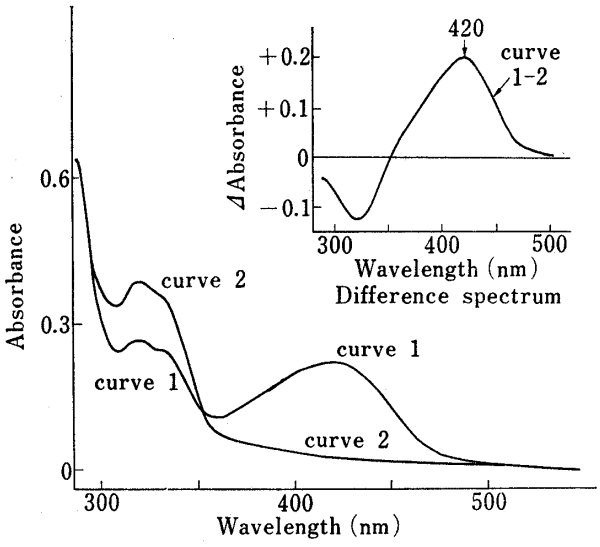

Fig. 2. Spectrum of 9-*cis* Retro-γ-retinal Pigment

Curve 1: before bleaching.
Curve 2: after bleaching.

son of the chemical shifts of the protons at C-11 in the three isomers exhibited that the protons in the all-*trans* and the 9-*cis* isomers appeared further downfield and that in the 11-*cis* isomer relatively upfield, suggesting that both all-*trans* and 9-*cis* isomers have a high coplanarity in the trienal part and the 11-*cis* isomer possesses a twisting chain.

Three isomeric retro-γ-retinals were tested with cattle opsin as to their possible formation of an artificial visual pigment. Of these, the 9-*cis* isomer (VIIb) slowly gave a new pigment showing a clear absorption maximum at 420 nm (Fig. 2). The same possibilities for two other isomers are under careful examinations. The significances and characterisations of the reconstituted pigment from VIIb should be published elsewhere in the very near future. In addition, the reaction of VIIb with *n*-butylamine using tri-*n*-butyl borate as a catalyst afforded the corresponding imine (VIII) whose UV absorption maximum was 323 nm. Upon addition of acid, a bathochromic shift of *ca.* 70 nm was observed. Similar shift had been reported[4] between all-*trans*-N-retinylidene-*n*-butylamine and its iminium salt. The difference between the maxima of the reconstituted pigment from VIIb and the iminium salt of VIII suggests that there would be steric interactions between retro-γ-retinal chromophore and opsin in the molecule. In the UV spectrum, the maximum value (420 nm) of the reconstituted pigment is very close to the value (430 nm) reported for hypsorhodopsin which is believed to be the preceding intermediate of bathorhodopsin at the first step in the photolysis of rhodopsin.[5] The new visual pigment analogue prepared by us will be expected to contribute to clarification of the early stages in the photobleaching of rhodopsin.

Experimental

Unless otherwise stated, spectroscopic measurements were performed as follows. UV spectra were recorded on a Shimadzu UV 200S instrument. NMR spectra at 60 or 90 MHz were determined on Varian A-60 D or NEVA-NV 21 spectrometers, tetramethylsilane being used as internal standard. Mass spectra (MS) were determined on a JEOL JMS-01SG mass spectrometer; high resolution measurements were made relative to perfluorokerosene as reference. Preparative TLC was carried out on a silica gel plate (Merck silica gel $60F_{254}$ precoated plate, 0.25 or 0.5 mm thickness). Alumina for column chromatography was Merck aluminium oxide 90 standardised (Aktivitätsstufe II—III). Irradiation was conducted with Eikosha Halōs 500 watt lamp (type PIH-500).

Methyl Retro-γ-ionylideneacetate (IVa and IVb)——A solution of methyl β-ionylideneacetate (III[6]; 1.55 g) in ethanol (350 ml) was irradiated with a 500 W high pressure mercury lamp attached with Pyrex filter under bubbling of N_2 at the temperature cooled with ice-water. The reaction was followed by UV and the irradiation was stopped when the peak corresponding to the starting material disappeared and the formation of a new peak having λ_{max} at 220 nm was observed. Evaporation of the solvent below 30° gave a pale yellow oil (1.5 g) which was submitted to column chromatography (5% deactivated alumina; *n*-hexane) to afford the 9-*cis* isomer (IVb; 468 mg), the mixture of IVb and IVa (*ca.* 200 mg) and the 9-*trans* isomer (IVa; 776 mg). IVa: UV λ_{max}^{EtOH} 218 nm; NMR (Table I). IVb: UV λ_{max}^{EtOH} 218 nm; NMR (Table I).

***n*-Butyl Retro-γ-retinoate (VIa)**——A 70% solution (1.3 ml) of SMEAH in benzene was diluted with abs. ether and then added dropwise under cooling to a stirred solution of IVa (776 mg) in abs. ether (20 ml) in a stream of N_2. After complete addition, the reaction mixture was stirred at room temperature for 30 min. The excess of hydride was destroyed with moist ether, followed by water and the product was extracted with ether. After drying the extracts, evaporation of the solvent gave the hydroxy compound (663 mg) which was dissolved in MeOH and $CHCl_3$ (1: 1) (*ca.* 20 ml). To this solution was added triphenylphosphonium bromide (1.1 g) and the mixture was stirred at room temperature for 48 hr. After shaking once with water, evaporation of the dried organic layer gave a viscous oil (Va; 2.175 g). To a solution of the crude Wittig salt (Va) in *n*-propanol (*ca.* 30 ml), was added a solution of *n*-butyl γ-oxosenecioate[7] (676 mg) in *n*-propanol (*ca.* 20 ml) under stirring in a stream of N_2 at 0°. After complete addition, 5% KOH was added to the reaction mixture at −30°. After making sure that the mixture became to weak alkali, it was stirred at room

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temperature overnight. Water was then added and the product was extracted with ether. Evaporation of the dried ether extracts gave an oil which was purified by column chromatography (5% deactivated alumina; ether: *n*-hexane=1: 30) and subsequent preparative TLC (silica gel plate (0.5 mm); 3% ether/*n*-hexane) to afford a yellow oil (VIa; 300 mg (21%)). VIa: MS *m/e* 356 (M^+); UV λ_{max}^{EtOH} 318 nm.

Retro-γ-retinals(All-*trans* VIIa, 11-*cis* VIIa′ and 9-*cis* VIIb)——A 70% solution (0.36 ml) of SMEAH in benzene was diluted with abs. ether and then added dropwise under cooling to a stirred solution of the retinoate (VIa; 300 mg) in abs. ether (*ca.* 20 ml) in a stream of N_2. After complete addition, the mixture was stirred at room temperature for 1 hr and then the excess of hydride was destroyed by the addition of moist ether, followed by water. The product was extracted with ether, and the ethereal extracts were combined, washed with saturated aqueous sodium chloride, dried (Na_2SO_4) and evaporated. The resulting crude alcohol (225 mg) was dissolved in *n*-hexane (*ca.* 20 ml) and was shaken with active manganese dioxide (1.15 g) at room temperature for 1 hr. Filtration of the precipitate and evaporation of the solvent gave a yellow oil which was purified by preparative TLC (silica gel (0.5 mm); 10% ether/*n*-hexane) to yield a pale yellow oil (VIIa; 30 mg) and (VIIa′; 10 mg). VIIa: MS *m/e* 284.214 (M^+, $C_{20}H_{28}O$ requires 284.214); UV λ_{max}^{EtOH} 343 nm; NMR δ ($CDCl_3$, 90 MHz), 1.03 (6H, s, *gem*.CH_3), 1.86 (3H, s, C-9-CH_3), 2.19 (2H, t, J=6 Hz, C-4-H_2), 2.31 (3H, s, C-13-CH_3), 2.97 (2H, d, J=8 Hz, C-8-H_2), 4.58 (1H, d, J=3 Hz, *exo*CH_2), 4.99 (1H, m, *exo*CH_2), 5.22 (1H, t, J=8 Hz, C-7-H), 5.99 (1H, d, J=8 Hz, C-14-H), 6.02 (1H, d, J=11 Hz, C-10-H), 6.28 (1H, d, J=15 Hz, C-12-H), 7.04 (1H, dd, J=11, 15 Hz, C-11-H), 10.14 (1H, d, J=8 Hz, C-15-H). VIIa′: MS *m/e* 284.214 (M^+, $C_{20}H_{28}O$ requires 284.214); UV λ_{max}^{EtOH} 343 nm; NMR δ ($CDCl_3$, 90 MHz), 1.04 (6H, s, *gem*. CH_3), 1.84 (3H, s, C-9-CH_3), 2.38 (3H, s, C-13-CH_3), 2.99 (2H, d, J=8 Hz, C-8-H_2), 4.62 (1H, d, J=3 Hz, *exo*CH_2), 5.01 (1H, m, *exo*CH_2), 5.26 (1H, t, J=8 Hz, C-7-H), 5.89 (1H, d, J=10 Hz, C-10-H), 6.09 (1H, d, J=8 Hz, C-14-H), 6.41 (1H, d, J=12 Hz, C-12-H), 6.63 (1H, dd, J=10, 12 Hz, C-11-H), 10.12 (1H, d, J=8 Hz, C-15-H). Similarly, the 9-*cis* isomer (VIIb; 50 mg) was obtained from the corresponding 9-*cis* retro-γ-ionylideneacetate (IVb; 468 mg). VIIb: MS *m/e* 284.215 (M^+, $C_{20}H_{28}O$ requires 284.214); UV λ_{max}^{EtOH} 343 nm; NMR δ ($CDCl_3$, 90 MHz), 1.02 (6H, s, *gem*.CH_3), 1.84 (3H, s, C-9-CH_3), 2.20 (2H, t, J=6 Hz, C-4-H_2), 2.30 (3H, s, C-13-CH_3), 3.14 (2H, d, J=7 Hz, C-8-H_2), 4.64 (1H, d, J=3 Hz, *exo*CH_2), 5.09 (1H, m, *exo*CH_2), 5.17 (1H, t, J=7 Hz, C-7-H), 5.97 (1H, d, J=8 Hz, C-14-H), 5.99 (1H, d, J=11 Hz, C-10-H), 6.25 (1H, d, J=15 Hz, C-12-H), 6.94 (1H, dd, J=11, 15 Hz, C-11-H), 10.13 (1H, d, J=8 Hz, C-15-H).

All-*trans*-N-retinylidene-*n*-butylamine (VIII)——A solution of *n*-butylamine (0.1 ml) in ethyl acetate (*ca.* 10 ml) was added over a period of 5 min to a stirred solution of 9-*cis* retro-γ-retinal (18 mg) and tri-*n*-butyl borate (15 mg) in ethyl acetate (*ca.* 20 ml) in a stream of N_2. The mixture was stirred at room temperature for 2 hr and the course of reaction followed by UV. On completion of the reaction, water (15 ml) and ether (15 ml) were added. The ethereal layer was separated off, washed with water, dried and evaporated to give a yellow oil (VIII; 20 mg). VIII: MS *m/e* 339 (M^+); UV λ_{max}^{EtOH} 323 nm, $\lambda_{max}^{EtOH+HCl}$ 390 nm; NMR δ ($CDCl_3$, 90 MHz), 1.00 (6H, s, *gem*.CH_3), 1.81 (3H, s, C-9-CH_3), 2.06 (3H, s, C-13-CH_3), 3.11 (2H, d, J=7 Hz, C-8-H_2), 3.51 (2H, t, J=6 Hz, C=N-CH_2-), 4.64 (1H, d, J=3 Hz, *exo*CH_2), 5.07 (1H, m, *exo*CH_2), 5.17 (1H, t, J=7 Hz, C-7-H), 5.89—6.33 (3H, m, olefinic protons), 6.76 (1H, dd, J=12, 16 Hz, C-11-H), 8.34 (1H, d, J=10 Hz, CH=N).

Formation of 9-*cis* Retro-γ-retinal Pigment——Opsin was extracted with 0.7% digitonin dissolved in 10 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (pH 7.0) from light-adapted cattle retinas using an usual method.[8] The 9-*cis* isomer of retro-γ-retinal (VIIb) in ethanol was incubated with the opsin extract in the dark at 25° for 6 hr under nitrogen atmosphere. The final concentration of ethanol was 1%. Such low concentration of ethanol did not affect the pigment formation. After completion of the reaction, NH_2OH (50 mM in the final concentration) was added to an aliquot of the reaction mixture. Then the absorption spectrum was recorded using Hitachi 323 recording spectrophotometer (curve 1 in Fig. 2). After bleaching the pigment by white light (50 W tungsten lamp), the spectrum was recorded again (curve 2). The difference spectrum between before and after bleaching (curve 1- curve 2) was calculated and shown in Fig. 2. The longer wavelengths light than 440 nm bleached also the pigment formed from VIIb and opsin (data not shown). $NaBH_4$ did not bleach the pigment. Extraction of the chromophore from the pigment was done using the method of Pilkiewicz *et al.*[9] The HPLC analysis of the extract showed only the 9-*cis* retro-γ-retinal peak. Thus no isomerisation or modification of VIIb occurred during the pigment formation.

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