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Retinoids and Related Compounds. X.[1] Synthesis of Geometrical Isomers of (±)-2- and (±)-3-Hydroxyretinals and Identification of the Chromophore of the Fly Visual Pigment

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The geometrical isomers of (±)-2- and (±)-3-hydroxyretinals were synthesized and characterized. Subsequently, simultaneous separation of eight isomers of 2- and 3-hydroxyretinals by high-performance liquid chromatography (HPLC) was accomplished and, in addition, complete separation of eight *syn–anti* isomers of 3-hydroxyretinaloximes by HPLC was achieved. Under these analytical conditions, the chromophore of the fly (*Drosophila melanogaster*) visual pigment was identified as 11-*cis*-3-hydroxyretinal (**10**).

Keywords——2-hydroxyretinal; 3-hydroxyretinal; HPLC; 3-hydroxyretinaloxime; Emmons–Horner reaction; Wittig reaction; fly visual pigment; *Drosophila melanogaster*

It is generally accepted that 11-*cis* retinal (**1**) is a universal chromophore of the visual pigment in the animal kingdom with the exception of the vitamin A_2-based porphyropsins[2] in some animals. In 1984, Vogt and Kirschfeld proposed[3] that the chromophore of the fly visual pigment was not retinal but 3-hydroxyretinal (3-OH-RAL) on the basis of some chemical and ultraviolet (UV) spectral evidence and biosynthetic considerations. On the other hand, a carotenoid with an oxygen function at C-2 was firstly isolated and characterized in a single moth species, *Cerura*,[4] and these carotenoids were found to be typical of stick insects (*Plasmida*). However, the possibility of 2-hydroxyretinal (2-OH-RAL) as a chromophore remained unproven. In addition, there has been no confirmation of the geometry around the conjugated double bonds. In our previous paper,[5] we reported that the high-performance liquid chromatographic (HPLC) elution profile of the synthetic (±)-11-*cis* and all-*trans* 3-OH-RALoximes coincided with that of extracts from fly heads by the oxime method.[6] In this paper, we present full details of the synthesis of the geometrical isomers of (±)-2- and (±)-3-OH-RALs and the identification of the chromophore of the fly (*Drosophila melanogaster*) visual pigment.

The syntheses of (±)-all-*trans*[7] and (±)-9-*cis*[8] isomers, and (3*R*)-all-*trans* isomer[9] of 3-OH-RAL have already been reported independently. However, other isomers, particularly the 11-*cis* isomer, have not been synthesized yet. Further, no 2-OH-RAL isomer has yet been synthesized. Hence, the *trans* and *cis* isomers (all-*trans*, 13-*cis*, 11-*cis*, and 9-*cis*) of 2- and 3-OH-RAL were prepared by the $C_{13}+C_2+C_5 \rightarrow C_{20}$ route, in which 3-hydroxy-β-ionone (**2a**)[10] or 2-hydroxy-β-ionone (**3a**)[11] was the starting material.

First of all, 3-OH-RAL isomers were synthesized by usual methods with minor modifications (Chart 1). An Emmons–Horner reaction of *tert*-butyldimethylsilyl (TBDMS) oxy-β-ionone (**2b**) with the ester-phosphonate (a C_2-component) (**4**) in the presence of

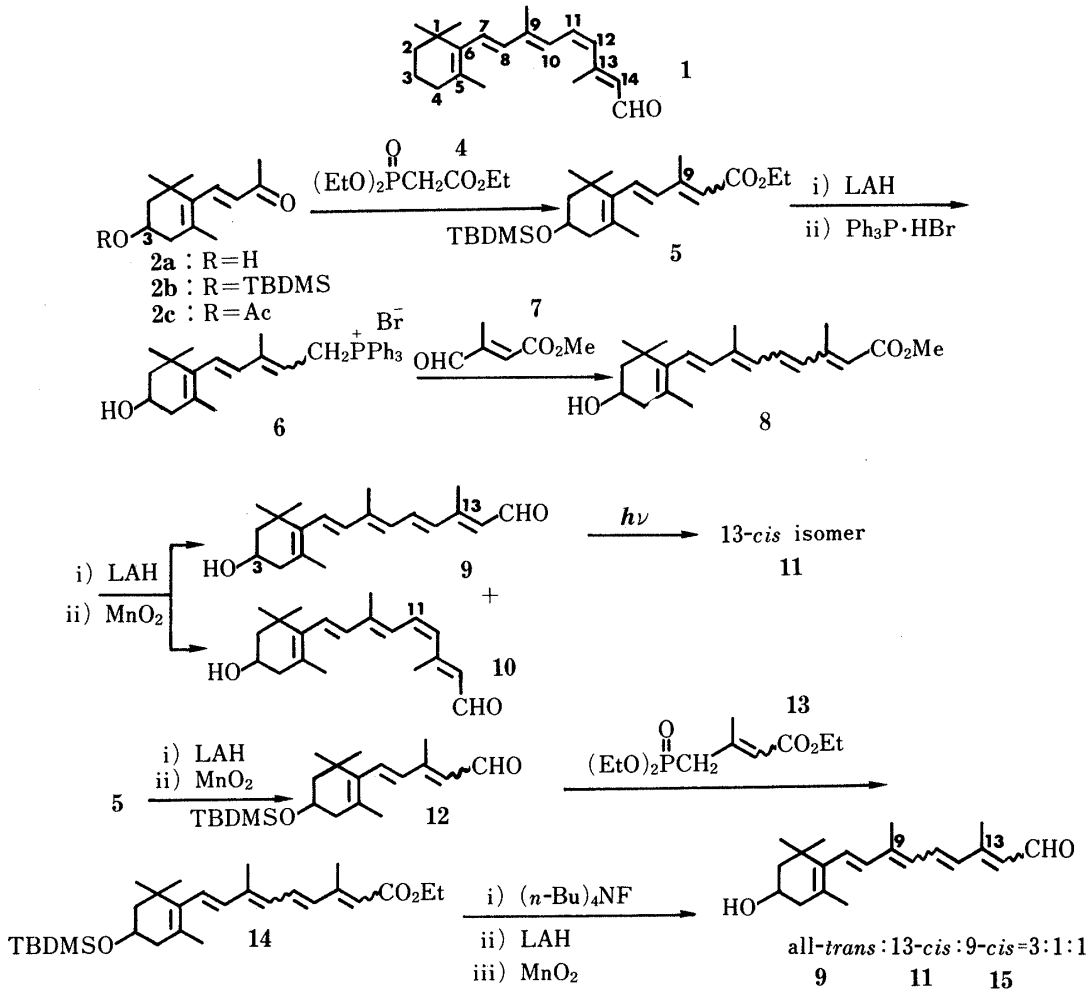

Chart 1

n-butyllithium (*n*-BuLi) gave a 3-hydroxy-*β*-ionylideneacetate derivative (**5**) as a mixture (*ca.* 3 : 1 from proton nuclear magnetic resonance (^{1}H-NMR)) of 9-*trans* and 9-*cis* isomers in high yield. The same condensation reaction between **2a** or 3-acetoxy-*β*-ionone (**2c**) and **4** resulted in low yields (37% or 27%). $LiAlH_4$(LAH) reduction of **5** and subsequent treatment of the resulting triene-alcohol with triphenylphosphonium bromide ($Ph_3P \cdot HBr$) gave the corresponding Wittig salt (**6**) which, without purification, was condensed with the formyl-ester (a C_5-component) (**7**) using sodium ethoxide as a base to afford methyl 3-hydroxyretinoate (**8**) as an isomeric mixture of the 11,12-double bond in 71% yield from **5**. Conversion of the ester group in **8** to the aldehyde group by LAH reduction and MnO_2 oxidation led to a mixture (*ca.* 1 : 1) of 3-OH-RAL isomers in 69% yield; these were separated and purified by a combination of column chromatography (CC) and preparative HPLC (pHPLC) in the dark to provide the all-*trans* aldehyde (**9**) and the 11-*cis* isomer (**10**), each in a pure state. The 13-*cis* isomer (**11**) was isolated in moderate yield (50%) from irradiation products of the all-*trans* isomer (**9**) in benzene. Alternatively, the 3-hydroxy-*β*-ionylideneacetaldehyde derivative (**12**) (9-*trans* : 9-*cis* = 3 : 1), derived from **5** by LAH reduction and subsequent MnO_2 oxidation, was condensed with the ester-phosphonate (a C_5-component, (*trans* : *cis* = 4 : 1)) (**13**)[12] in the presence of *n*-BuLi to give an isomeric mixture of the 3-hydroxyretinoate derivative (**14**) in 96% yield which, after deprotection using $(n\text{-Bu})_4NF$, was converted to a mixture of 3-OH-RAL isomers (all-*trans* (**9**) : 13-*cis* (**11**) : 9-*cis* (**15**) = 3 : 1 : 1) by the usual method (LAH and MnO_2) in 93% yield from **14**. The respec-

TABLE I. Characteristic Spectral Data for All-*trans* Retinal and 3-Hydroxyretinal Isomers

		All-*trans* retinal	All-*trans* 3-OH-RAL (**9**)	13-*cis* 3-OH-RAL (**11**)	11-*cis* 3-OH-RAL (**10**)	9-*cis* 3-OH-RAL (**15**)
UV λ_{max}^{EtOH} nm (ε)		383 (42884)[13]	379 (44000)	372 (31000) 257 (6500)	375 (21000) 282 (sh) 254 (14000)	372 (32000)
^{1}H-NMR δ (200 MHz, $CDCl_3$, J in Hz)	13-Me	2.33 (s)	2.34 (s)	2.15 (s)	2.36 (s)	2.32 (s)
	12-H	6.37 (d, J=15.4)	6.39 (d, J=16)	7.31 (d, J=15)	5.95 (d, J=12)	6.32 (d, J=15)
	11-H	7.15 (dd, J=15.4, 12)	7.14 (dd, J=16, 11)	7.04 (dd, J=15, 11.5)	6.69 (t-like, J=12)	7.21 (dd, J=15, 12)
	10-H	6.20 (d, J=12)	6.20 (d, J=11)	6.23 (d, J=11.5)	6.54 (d, J=12)	6.12 (d, J=12)
	8-H	6.18 (d, J=16.5)	6.15 (d, J=16)	6.16 (d, J=16.5)	6.13 (d, J=15)	6.67 (d, J=16)
	7-H	6.36 (d, J=16.5)	6.29 (d, J=16)	6.38 (d, J=16.5)	6.28 (d, J=15)	6.27 (d, J=16)
	3-H	—	*ca.* 4.02 (br m)	*ca.* 4.02 (br m)	*ca.* 4.03 (br m)	*ca.* 4.03 (br m)
	CHO	10.12 (d, J=8)	10.12 (d, J=8)	10.21 (d, J=8)	10.09 (d, J=8)	10.12 (d, J=8)
	OH[a]	—	1.60 (br s)	1.57 (br s)	1.62 (br s)	1.60 (br s)
	2-H_{ax}		1.48 (t-like, J=11.5)			
	4-H_{ax}		2.06 (dd, J=16.5, 10.5)			

a) D_2O treatment.

tive isomers were obtained in pure form by CC followed by pHPLC in the dark. Photoisomerization of the all-*trans* isomer (**9**) in several solvents gave a low yield of the 9-*cis* isomer (**15**). The structures of the four 3-OH-RAL isomers (all-*trans* (**9**), 13-*cis* (**11**), 11-*cis* (**10**), and 9-*cis* (**15**)) were confirmed on the basis of the UV absorption and ^{1}H-NMR spectral data (Table I) compared with those[13] of retinal isomers. The UV absorption maximum and the chemical shifts of olefinic and methyl protons in all-*trans* 3-OH-RAL (**9**) were consistent with those of all-*trans* retinal, and a 3-H signal and an OH signal were newly observed as shown in Table I. In addition, a decoupling experiment on **9** with addition of D_2O showed that 3-H was axial, based on the coupling constants, $J_{2ax,3} = J_{4ax,3} = ca.$ 11 Hz. The 13-*cis*-geometry is clearly evidenced by a strong downfield shift of the 12-H signal and the upfield shift of the 13-methyl signal. The 11-*cis*-geometry can be readily determined from the magnitude (12 Hz) of $J_{11,12}$. A 9-*cis*-configuration is recognizable from the downfield shift of the 8-H signal due to the anisotropic effect of the 11,12-double bond.

In analogy with 3-OH-RAL, the 2-OH-RAL isomers were synthesized (Chart 2) *via* 2-acetoxy-β-ionone (**3b**) prepared by us[14] and other groups.[11c] Condensation of **3b** with **4** in the presence of *n*-BuLi gave a mixture (9-*trans* : 9-*cis* = 3 : 1) of 2-acetoxy-β-ionylideneacetate (**16**) in 63% yield, which, on reduction with LAH and subsequent treatment of the resulting alcohol with $Ph_3P \cdot HBr$ in methanol, afforded the phosphonium salt (**17**). The Wittig condensation (NaOEt) of **17** with **7** (a C_5-component) resulted in the formation of a mixture of 2-hydroxyretinoate isomers (**18**). This ester mixture was converted into a mixture (*ca.* 1 : 1) of 2-OH-RALs by the usual method, and the all-*trans* isomer (**19**) and the 11-*cis* one (**20**) were

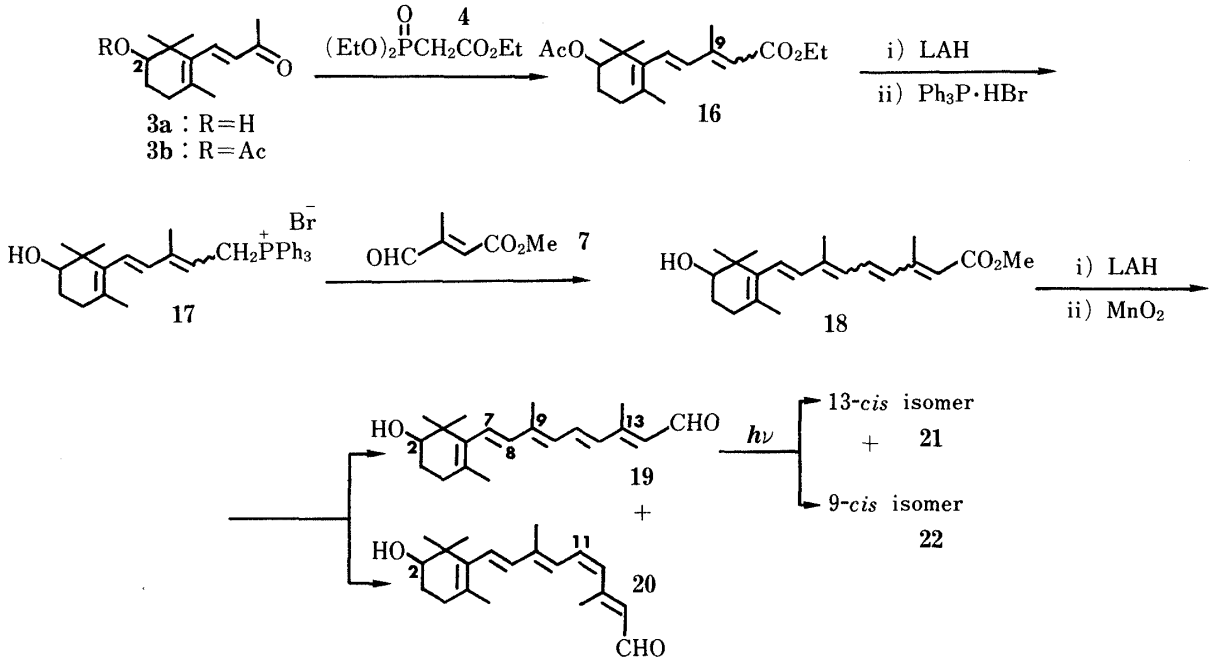

Chart 2

TABLE II. Characteristic Spectral Data for 2-Hydroxyretinal Isomers

		All-*trans* (**19**)	13-*cis* (**21**)	11-*cis* (**20**)	9-*cis* (**22**)
UV λ_{max}^{EtOH} nm (ε)		381 (42000)	375 (30000) 259 (8500)	376 (23000) 285 (sh) 254 (15000)	372 (32000)
^{1}H-NMR δ (200 MHz, $CDCl_3$, J in Hz)	13-Me	2.34 (s)	2.15 (s)	2.36 (s)	2.32 (s)
	12-H	6.39 (d, J=15)	7.31 (d, J=15)	5.95 (d, J=11.5)	6.31 (d, J=15)
	11-H	7.14 (dd, J=15, 11)	7.04 (dd, J=15, 11.5)	6.70 (t-like, J=12.5, 11.5)	7.22 (dd, J=15, 11.5)
	10-H	6.20 (d, J=11)	6.23 (d, J=11.5)	6.54 (d, J=12.5)	6.11 (d, J=11.5)
	8-H	6.18 (d, J=16.5)	6.16 (d, J=16.5)	6.13 (d, J=16)	6.67 (d, J=15.5)
	7-H	6.30 (d, J=16.5)	6.38 (d, J=16.5)	6.28 (d, J=16)	6.28 (d, J=15.5)
	2-H	3.57 (dd, J=9, 3.5)	3.57 (dd, J=9, 3.5)	3.55 (dd, J=9, 3.5)	3.58 (dd, J=9, 3.5)
	CHO	10.12 (d, J=8)	10.21 (d, J=8)	10.10 (d, J=8)	10.11 (d, J=8)
	OH[a]	1.54 (br s)	1.57 (br s)	1.52 (br s)	1.57 (br s)

a) D_2O treatment.

each isolated in pure form, in 73% total yield, by a combination of CC and pHPLC in the dark. Both the 13-*cis* (**21**, 15%) and 9-*cis* (**22**, 19%) isomers were isolated in pure form by using pHPLC from irradiation products of the all-*trans* isomer (**19**) in methanol. Spectral data (Table II) of the four 2-OH-RAL isomers gave satisfactory results. The coupling constants

(3.5, 9 Hz) for 2-H in the ^{1}H-NMR spectrum showed the presence of an equatorial hydroxyl group.

Simultaneous complete separation of a mixture of eight isomers of 2- and 3-OH-RALs was achieved by HPLC under the analytical conditions shown in Fig. 1 after examinations of various solvent combinations. The peaks of the four 2-OH-RAL isomers appear between 8 and 12 min, then the peaks of the four 3-OH-RAL isomers follow. Under these analytical

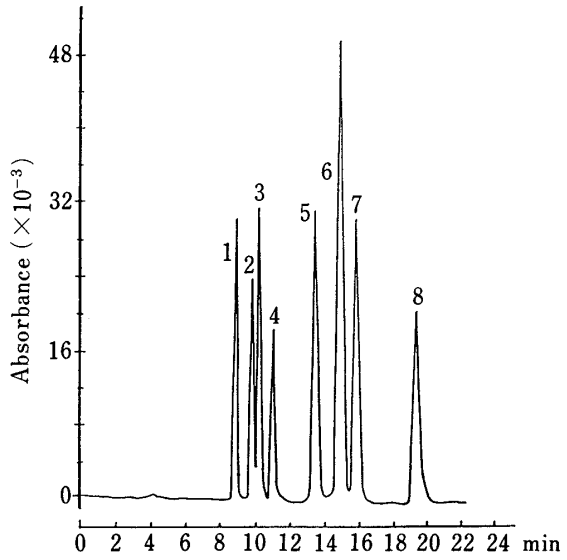

Fig. 1. HPLC Elution Pattern of a Mixture of 2- and 3-Hydroxyretinal Isomers

Column, LiChrosorb Si-60-5 (30 × 0.75 cm i.d.); eluent, MeOH : THF : hexane = 0.7 : 17 : 82.3; flow rate, 4 ml/min; UV detection, 350 nm. 1, 13-*cis* 2-OH-RAL; 2, 11-*cis* 2-OH-RAL; 3, all-*trans* 2-OH-RAL; 4, 9-*cis* 2-OH-RAL; 5, 13-*cis* 3-OH-RAL; 6, all-*trans* 3-OH-RAL; 7, 11-*cis* 3-OH-RAL; 8, 9-*cis* 3-OH-RAL.

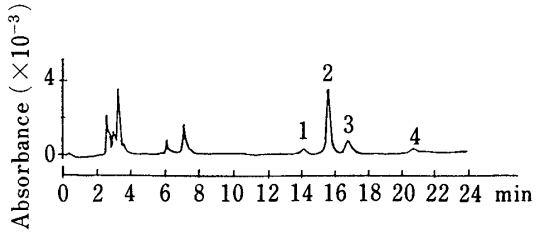

Fig. 2. HPLC Elution Profile of Head Extracts (Formaldehyde Method) of *D. melanogaster*

HPLC conditions: see Fig. 1. 1, 13-*cis* 3-OH-RAL; 2, all-*trans* 3-OH-RAL; 3, 11-*cis* 3-OH-RAL; 4, 9-*cis* 3-OH-RAL.

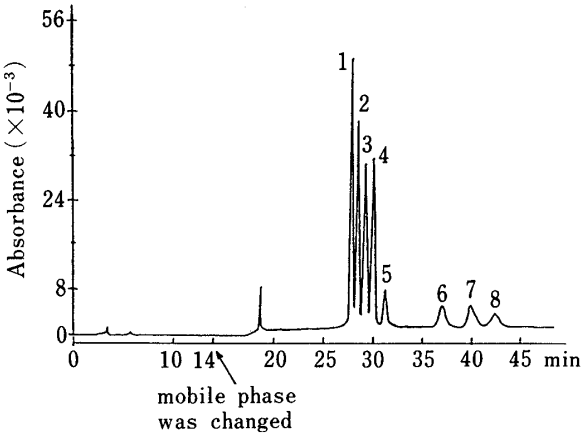

Fig. 3. HPLC Elution Pattern of a Mixture of Eight Geometrical Isomers of 3-Hydroxyretinaloxime

Column, YMC-Pack A-012-3 (S-3 SIL) (15 × 0.6 cm i.d.); eluent, MeOH : methyl *tert*-butyl ether : benzene = 0.1 : 1.2 : 98.7 then MeOH : methyl *tert*-butyl ether : benzene = 1 : 6 : 93; flow rate, 1.7 ml/min; UV detection 350 nm. 1, 9-*cis-syn*; 2, 13-*cis-syn*; 3, 11-*cis-syn*; 4, all-*trans-syn*; 5, all-*trans-anti*; 6, 13-*cis-anti*; 7, 9-*cis-anti*; 8, 11-*cis-anti*.

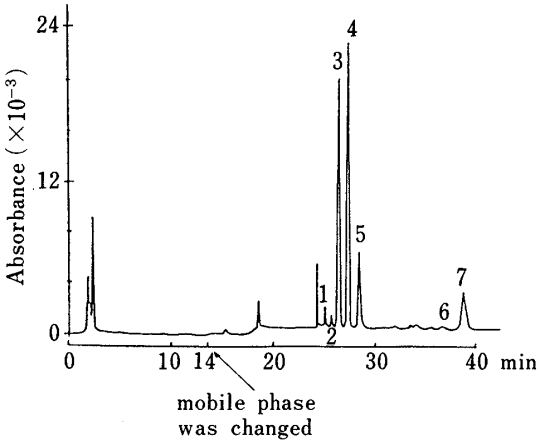

Fig. 4. HPLC Elution Profile of Head Extracts (Oxime Method) of *D. melanogaster*

HPLC conditions: see Fig. 3. 1, 9-*cis-syn* (ε 39000); 2, 13-*cis-syn* (ε 41000); 3, 11-*cis-syn* (ε 28000); 4, all-*trans-syn* (ε 44000); 5, all-*trans-anti*; 6, 9-*cis-anti*; 7, 11-*cis-anti*.

conditions, an extract from heads of *Drosophila melanogaster* was examined (Fig. 2). The extraction was done by the formaldehyde method[15] which was recently recommended instead of the oxime method[6] for retinal analysis of biologically native samples. As shown in Fig. 2, no peak was observed in the area where the peaks of 2-OH-RAL isomers should appear and then the all-*trans* form was observed as a main peak with other *cis* isomers in the area of 3-OH-RALs. Namely, the visual pigment chromophore of *D. melanogaster* was found to be 3-OH-RAL, but not 2-OH-RAL. The possibility of isomerization during the extraction had to be considered. Therefore, in order to determine the geometry around the conjugated double bond, we adopted the oxime method[5,6] in the extraction of fly heads, since the method is generally accepted to achieve almost complete recovery of retinal without nonspecific isomerization. Before the extract was subjected to HPLC analysis, conditions for simultaneous separation of the eight isomers of 3-OH-RALoxime by HPLC were established (Fig. 3) after repeated examinations of various kinds of solvent systems. Authentic specimens of 3-OH-RALoximes were prepared by the known procedure.[6,16] An HPLC chromatogram (Fig. 4) of a native sample from *D. melanogaster* showed the existence of the two main *syn*-peaks of the 11-*cis* and all-*trans* isomers. Four peaks (peak Nos. 3, 4, 5, and 7) in Fig. 4 were identified as corresponding to authentic *syn* and *anti* isomers of 11-*cis* and all-*trans* 3-OH-RALoximes, respectively, by coinjection with the extract from the fly heads. Based on the ε values of both *syn* isomers at the wavelength of detection, the 11-*cis* isomer was found to be a main component of the extract. Since the all-*trans* isomer was found to give no pigment formation in the fly body,[17] the isomer might be assumed not to be the chromophore of the fly visual pigment. Therefore, this is the first synthetic confirmation that the chromophore of the fly visual pigment is 11-*cis*-3-OH-RAL. The significance for the presence of a relatively large amount of all-*trans* form will be discussed elsewhere.

The absolute configuration at the C-3-position could be 3*R*, considering that xanthophylls with 3-hydroxy groups in flies can be used as precursors for the chromophore. Work is in progress on the determination of the absolute structure.

Experimental

UV spectra were recorded on Shimadzu UV 200S and UV-160 instruments and infrared (IR) spectra on a Shimadzu IR-27G spectrometer. ^{1}H-NMR spectra at 60 or 200 MHz were determined on a JEOL JNM-PMX 60 or a Varian XL-200 superconducting FT-NMR spectrometer using tetramethylsilane as an internal reference. Mass spectra (MS) were determined on a Hitachi M-80 double-focusing GC mass spectrometer. CC was performed on silica gel (Merck Art 7739) using a short column under reduced pressure. Analytical HPLC was carried out in the dark on a Shimadzu LC-3A (Figs. 1, 2, and 3) or a Hitachi 655 (Fig. 4) instrument with a UV detector using columns of LiChrosorb Si-60-5 (30 × 0.4 or 0.75 cm i.d.) and YMC-Pack A-012-3(S-3 SIL) (15 × 0.6 cm i.d.). pHPLC was carried out in the dark on a Shimadzu LC-3A with a UV detector (Yamazen UV detector UVILOG-10V) using a column of LiChrosorb Si-60-5 (1.0 × 30 cm i.d.). Unless otherwise stated, solvent extracts were dried over anhydrous Na_2SO_4 and all operations were carried out under nitrogen or argon. Ether refers to diethyl ether and hexane to *n*-hexane.

3-*tert*-Butyldimethylsilyloxy-β-ionone (2b)——TBDMSCl (1.65 g) was added at 0 °C to a stirred solution of 3-hydroxy-β-ionone (**2a**,[10] 1.9 g), Et_3N (1.5 ml), and 4-dimethylaminopyridine (DMAP) (2.23 g) in dry CH_2Cl_2 (16 ml). The mixture was stirred at room temperature for 1.5 h. The reaction was quenched with water and extracted with CH_2Cl_2. The extracts were washed with brine, dried, and evaporated *in vacuo* to give an oil which was purified by CC (eluent, ether : hexane = 1 : 1) to afford the TBDMS ether (**2b**, 2.53 g (86%)) as a yellow oil. UV λ_{max}^{EtOH} nm: 287, 235. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1665 (conj. C=O), 1595 (conj. C=C). ^{1}H-NMR δ (60 MHz, $CDCl_3$): 0.07 (6H, s, $(CH_3)_3CSi(CH_3)_2$), 0.90 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 1.09 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 2.26 (3H, s, 9-CH_3), 3.93 (1H, m, 3-H), 6.03 (1H, d, J = 16 Hz, 8-H), 7.17 (1H, d, J = 16 Hz, 7-H). MS *m/z*: 322.2336 (M^+, $C_{19}H_{34}O_2Si$ requires 322.2327).

Ethyl 3-*tert*-Butyldimethylsilyloxy-β-ionylideneacetate (5)——A solution (0.86 ml) of *n*-BuLi (15% (w/v) in hexane solution) was added to a stirred solution of the ester-phosphonate (**4**, 444 mg) in dry tetrahydrofuran (THF) (2 ml) at 0 °C. The mixture was stirred at room temperature for 0.5 h and a solution of **2b** (300 mg) in dry THF (1 ml) was added dropwise at 0 °C. The mixture was refluxed for 3 h. After cooling, the reaction was quenched by the addition of saturated aqueous NH_4Cl solution. The mixture was extracted with ether, and the extract was washed

with brine, then dried. Removal of the solvent *in vacuo* gave an oil, which was purified by CC (eluent, ether : hexane = 5 : 95) to afford a mixture (9-*trans* : 9-*cis* = 3 : 1 from HPLC (LiChrosorb Si-60-5 (0.4 × 30 cm)/ether : hexane = 5 : 95)) of isomers of the ester (**5**, 340 mg (93%)) as a yellow oil. UV λ_{max}^{EtOH} nm: 298, 257 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (conj. CO_2Et), 1595 (conj. C=C). ^{1}H-NMR δ (60 MHz, $CDCl_3$): 0.08 (6H, s, $(CH_3)_3CSi(CH_3)_2$), 0.90 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 1.05 (6H, s, *gem*-CH_3), 1.27 (3H, t, J = 7 Hz, $CO_2CH_2CH_3$), 1.68 (3H, s, 5-CH_3), 2.02 (3/4H, s, 9-CH_3 (9-*cis*)), 2.26 (9/4H, s, 9-CH_3 (9-*trans*)), 4.13 (2H, q, J = 7 Hz, $CO_2CH_2CH_3$), 5.60 (1/4H, s, 10-H (9-*cis*)), 5.70 (3/4H, s, 10-H (9-*trans*)), 5.97 (3/4H, d, J = 16 Hz, 8-H (9-*trans*)), 6.47 (1H, d, J = 16 Hz, 7-H), 7.62 (1/4H, d, J = 16 Hz, 8-H (9-*cis*)). MS *m/z*: 392.2737 (M^+, $C_{23}H_{40}O_3Si$ requires 392.2744).

Methyl 3-Hydroxyretinoate (8)——A solution of the ester (**5**, 0.5 g) in dry ether (3 ml) was added dropwise to a stirred suspension of LAH (97 mg) in dry ether (2 ml) at 0 °C and the mixture was stirred at room temperature for 0.5 h. The excess of LAH was destroyed by the addition of moist ether and water. The mixture was extracted with ether, and the extract was washed with brine and dried. Evaporation of the solvent gave a light yellow oil, which was dissolved in MeOH (5 ml), and this solution was added to a solution of $Ph_3P \cdot HBr$ (0.51 g) in MeOH (5 ml). The mixture was stirred at room temperature for 36 h. Evaporation of MeOH gave a residue, which was washed with ether to afford the Wittig salt (**6**). This salt and the formyl-ester (**7**, 0.2 g) were dissolved in dry $CHCl_3$ (10 ml), and a solution of NaOEt (0.1 g) in EtOH (0.3 ml) was added at 0 °C. After the addition was complete, the mixture was stirred at room temperature for 0.5 h, poured into chilled water, and extracted with CH_2Cl_2. The organic layer was washed with brine and dried. Evaporation of the solvent gave an oil, which was purified by CC (eluent, ether : hexane = 1 : 3) to afford the 3-hydroxyretinoate (**8**, 0.3 g (71%)) as a yellow oil. UV λ_{max}^{EtOH} nm: 348, 244. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3450 (OH), 1705 (conj. CO_2Me). MS *m/z*: 330.2195 (M^+, $C_{21}H_{30}O_3$ requires 330.2193).

All-*trans* and 11-*cis* 3-Hydroxyretinals (9 and 10)——A solution of the retinoate (**8**, 0.3 g) in dry ether (2 ml) was treated with a suspension of LAH (52 mg) in dry ether (3 ml) by the same procedure as described for reduction of **5** to yield a yellow amorphous product (0.25 g) (UV λ_{max}^{EtOH} nm: 323, 316 (sh), 241. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (OH)). A mixture of the resulting hydroxy compound in dry CH_2Cl_2 (10 ml) and active MnO_2 (2.66 g) was shaken at room temperature for 2 h, and then filtered through Celite. Evaporation of the filtrate gave an oil, which was purified by CC (eluent, THF : benzene = 15 : 85) to afford an isomeric mixture of 3-OH-RAL (188 mg, (69%)) as an orange-yellow oil. Separation of the mixture by pHPLC (eluent, MeOH : THF : Hexane = 0.7 : 17 : 82.3) gave an all-*trans* isomer (**9**) and an 11-*cis* isomer (**10**), each in a pure state, in a ratio of *ca.* 1 : 1. All-*trans* 3-OH-RAL (**9**): UV, see Table I. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table I, 1.08 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 1.79 (1H, ddd, J = 2, 4, 11.5 Hz, 2-H_{eq}), 2.03 (3H, s, 9-CH_3), 2.41 (1H, dd, J = 6, 16.5 Hz, 4-H_{eq}), 5.99 (1H, d, J = 8 Hz, 14-H). MS *m/z*: 300.2075 (M^+, $C_{20}H_{28}O_2$ requires 300.2087). 11-*cis* 3-OH-RAL (**10**): UV, see Table I. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table I, 1.07 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 1.99 (3H, s, 9-CH_3), 6.09 (1H, d, J = 8 Hz, 14-H). MS *m/z*: 300.2078 (M^+, $C_{20}H_{28}O_2$ requires 300.2087).

13-*cis* 3-Hydroxyretinal (11)——A solution of all-*trans* 3-OH-RAL (**9**, 15 mg) in benzene (15 ml) was exposed under stirring to light from a 43 cm log fluorescent lamp (30 W) at a distance of 15 cm for 2 h. Evaporation of benzene followed by pHPLC (*cf.* conditions described for separation of **9** and **10**) gave a 13-*cis* isomer (**11**) in 50% yield. UV, see Table I. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table I, 1.08 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 2.03 (3H, s, 9-CH_3), 5.86 (1H, d, J = 8 Hz, 14-H). MS *m/z*: 300.2108 (M^+, $C_{20}H_{28}O_2$ requires 300.2088).

3-*tert*-Butyldimethylsilyloxy-β-ionylideneacetaldehyde (12)——The ionylideneacetate derivative (**5**, 1.63 g) was treated with LAH (refer to the procedure described for reduction of **5**) to give the hydroxy compound (1.46 g), which was dissolved in dry CH_2Cl_2 (25 ml) and shaken with active MnO_2 (73.4 g). The mixture was worked up by the same method as described for the preparation of **9** and **10**. Evaporation of the solvent gave a residue, which was purified by CC (eluent, ether : hexane = 1 : 9) to yield the aldehyde (**12**, 1.18 g (81%)). UV λ_{max}^{EtOH} nm: 318, 275 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1665 (conj. CHO), 1605 (conj. C=C). ^{1}H-NMR δ (60 MHz, $CDCl_3$): 0.08 (6H, s, $(CH_3)_3CSi(CH_3)_2$), 0.90 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 1.07 (6H, s, *gem*-CH_3), 1.71 (3H, s, 5-CH_3), 2.10 (3/4H, s, 9-CH_3 (9-*cis*)), 2.28 (9/4H, s, 9-CH_3 (9-*trans*)), *ca.* 3.98 (1H, m, 3-H), 5.83 (1/4H, d, J = 8 Hz, 10-H (9-*cis*)), 5.87 (3/4H, d, J = 8 Hz, 10-H (9-*trans*)), 6.10 (3/4H, d, J = 16 Hz, 8-H (9-*trans*)), 6.63 (1H, d, J = 16 Hz, 7-H), 7.03 (1/4H, d, J = 16 Hz, 8-H (9-*cis*)), 10.00 (1H, d, J = 8 Hz, CHO). MS *m/z*: 348.2487 (M^+, $C_{21}H_{36}O_2Si$ requires 348.2482).

Ethyl 3-*tert*-Butyldimethylsilyloxyretinoate (14)——A solution (3.6 ml) of *n*-BuLi (15% (w/v) in hexane solution) was added dropwise to a stirred solution of the C_5-phosphonate (**13**,[12] 2.24 g) in dry THF (20 ml) at 0 °C and the mixture was stirred at room temperature for 0.5 h. Then a solution of **12** (1.18 g) in dry THF (20 ml) was added dropwise at 0 °C. After the addition was complete, the mixture was stirred at room temperature for a further 1.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution. The mixture was extracted with ether, and the organic extract was washed with brine and dried. Evaporation of the solvent gave an oil which was purified by CC (eluent, ether : hexane = 8 : 92) to yield the retinoate mixture (**14**, 1.49 g (96%)) as a yellow oil. UV λ_{max}^{EtOH} nm: 353. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1695 (conj. CO_2Et), 1605, 1585 (conj. C=C). MS *m/z*: 458.3215 (M^+, $C_{28}H_{46}O_3Si$ requires 458.3213).

All-*trans*, 13-*cis*, and 9-*cis* 3-Hydroxyretinals (9, 11, and 15)——A solution (2.6 ml) of $(n\text{-Bu})_4NF$ (1(mol/l) in THF solution) was added dropwise to a solution of **14** (150 mg) in dry THF (3 ml) at 0 °C. The mixture was stirred at room temperature for 2.5 h and poured into ice-water. The mixture was washed with 5% HCl and brine, and dried.

Evaporation of the solvent quantitatively gave a yellow oil (UV λ_{max}^{EtOH} nm: 352. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3625, 3450 (OH), 1695 (conj. CO_2Et), 1610, 1585 (conj. C=C)) which was converted to a mixture (91.5 mg (93%)) of 3-OH-RAL isomers by the same method as described for the preparation of **9** and **10**. pHPLC separation yielded the all-*trans* isomer (**9**), 13-*cis* form (**11**), and 9-*cis* form (**15**) in a ratio of 3 : 1 : 1, each in a pure state. 9-*cis* 3-OH-RAL (**15**): UV, see Table I. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table I, 1.09 (6H, s, *gem*-CH_3), 1.78 (3H, s, 5-CH_3), 2.02 (3H, s, 9-CH_3), 5.98 (1H, d, J=8 Hz, 14-H). MS *m/z*: 300.2100 (M^+, $C_{20}H_{28}O_2$ requires 300.2088).

2-Acetoxy-β-ionone (3b)——A solution of 2-hydroxy-β-ionone (**3a**,[11] 650 mg) in Et_3N (10 ml) and Ac_2O (638 mg) was added to a solution of DMAP (858 mg) in Et_3N (15 ml). The mixture was stirred at room temperature for 2 h, poured into ice-water, and extracted with ether. The organic layer was washed with 5% HCl, neutralized with saturated aqueous $NaHCO_3$, washed with brine, and dried. Evaporation of the solvent gave an oil, which was purified by CC (eluent, ether : hexane = 1 : 1) to afford **3b** (709 mg, (91%)) as a yellow oil. UV λ_{max}^{EtOH} nm: 290, 217. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (OAc), 1660 (conj. C=O). ^{1}H-NMR δ (60 MHz, $CDCl_3$): 1.08 (6H, s, *gem*-CH_3), 1.77 (3H, s, 5-CH_3), 2.05 (3H, s, $OCOCH_3$), 2.30 (3H, s, 9-CH_3), 4.75 (1H, dd, J=4, 6 Hz, 2-H), 6.05 (1H, d, J=16 Hz, 8-H), 7.13 (1H, d, J=16 Hz, 7-H). MS *m/z*: 250.1576 (M^+, $C_{15}H_{22}O_3$ requires 250.1567).

Ethyl 2-Acetoxy-β-ionylideneacetate (16)——A solution of **4** (0.9 g) in dry THF (3 ml), a solution (1.71 ml) of *n*-BuLi (15% (w/v) in hexane solution), and a solution of **3b** (0.5 g) in dry THF (2 ml) were treated as described for the preparation of **5**. After evaporation of the solvent, the residue was purified by CC (eluent, ether : hexane = 3 : 7) to give **16** (0.4 g, (63%)) as a yellow oil and the starting material (**3b**, 0.15 g (30%)). **16** was determined to be a mixture (3 : 1) of 9-*trans* and 9-*cis* from its ^{1}H-NMR spectrum. UV λ_{max}^{EtOH} nm: 293, 255 (sh). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (OAc), 1705 (conj. CO_2Et). ^{1}H-NMR δ (60 MHz, $CDCl_3$): 1.03 (6H, s, *gem*-CH_3), 1.28 (3H, t, J=7 Hz, $CO_2CH_2CH_3$), 1.70 (3H, s, 5-CH_3), 2.05 (3H, s, $OCOCH_3$), 2.15 (3/4H, s, 9-CH_3 (9-*cis*)), 2.33 (9/4H, s, 9-CH_3 (9-*trans*)), 4.13 (2H, q, J=7 Hz, $CO_2CH_2CH_3$), 4.75 (1H, dd, J=4, 6 Hz, 2-H), 5.62 (1/4H, s, 10-H (9-*cis*)), 5.70 (3/4H, s, 10-H (9-*trans*)), 6.00 (3/4H, d, J=16 Hz, 8-H (9-*trans*)), 6.47 (1H, d, J=16 Hz, 7-H), 7.59 (1/4H, d, J=16 Hz, 8-H (9-*cis*)). MS *m/z*: 320.1965 (M^+, $C_{19}H_{28}O_4$ requires 320.1985).

Methyl 2-Hydroxyretinoate (18)——In a manner similar to that used for the preparation of **8** from **5**, a yellow oil **18** (72 mg, (71%)) was obtained from **16** (98 mg). UV λ_{max}^{EtOH} nm: 352, 245. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3625, 3400 (OH), 1705 (conj. CO_2Me), 1610, 1585 (conj. C=C). MS *m/z*: 330.2180 (M^+, $C_{21}H_{30}O_3$ requires 330.2193).

All-*trans* and 11-*cis* 2-Hydroxyretinals (19 and 20)——The retinoate (**18**, 46 mg) was treated with LAH (6.5 mg) in dry ether (3 ml) in the same manner as used for the reduction of **5** to give an oil (38.5 mg), which was oxidized with active MnO_2 (410 mg) in dry CH_2Cl_2 (3 ml) as described for the preparation of **9** and **10**. Purification of the crude products by CC (eluent, THF : hexane = 3 : 7) afforded an isomeric mixture (all-*trans* (**19**) : 11-*cis* (**20**) = ca. 1 : 1 from HPLC) of 2-OH-RAL which was separated by pHPLC (*cf.* the case of the purification of **9** and **10**). All-*trans* 2-OH-RAL (**19**): UV, see Table II. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table II, 1.05 and 1.09 (each 3H, each s, *gem*-CH_3), 1.72 (3H, s, 5-CH_3), 2.04 (3H, s, 9-CH_3), 5.98 (1H, d, J=8 Hz, 14-H). MS *m/z*: 300.2083 (M^+, $C_{20}H_{28}O_2$ requires 300.2087). 11-*cis* 2-OH-RAL (**20**): UV, see Table II. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table II, 1.04 and 1.08 (each 3H, each s, *gem*-CH_3), 1.72 (3H, s, 5-CH_3), 1.99 (3H, s, 9-CH_3), 6.09 (1H, d, J=8 Hz, 14-H). MS *m/z*: 300.2089 (M^+, $C_{20}H_{28}O_2$ requires 300.2088).

13-*cis* and 9-*cis* 2-Hydroxyretinals (21 and 22)——A stirred solution of **19** (20 mg) in MeOH (20 ml) was irradiated in the same manner as described for the preparation of **11** from **9**. Evaporation of MeOH gave a yellow oil, which was separated by pHPLC to afford a 13-*cis* isomer (**21**, 15%) and a 9-*cis* isomer (**22**, 19%), each in a pure state. 13-*cis* 2-OH-RAL (**21**): UV, see Table II. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table II, 1.05 and 1.09 (each 3H, each s, *gem*-CH_3), 1.72 (3H, s, 5-CH_3), 2.03 (3H, s, 9-CH_3), 5.86 (1H, d, J=8 Hz, 14-H). MS *m/z*: 300.2098 (M^+, $C_{20}H_{28}O_2$ requires 300.2087). 9-*cis* 2-OH-RAL (**22**): UV, see Table II. ^{1}H-NMR δ (200 MHz, $CDCl_3$): see Table II, 1.06 and 1.10 (each 3H, each s, *gem*-CH_3), 1.76 (3H, s, 5-CH_3), 2.03 (3H, s, 9-CH_3), 5.98 (1H, d, J=8 Hz, 14-H). MS *m/z*: 300.2081 (M^+, $C_{20}H_{28}O_2$ requires 300.2087).

Extraction of the Native Sample from Fly Heads by the Formaldehyde Method——About 400 heads of *D. melanogaster* were homogenized in a glass homogenizer containing 6 M formaldehyde in 50% MeOH (2 ml) with a motor-driven Teflon pestle. After standing at room temperature for 5 min, retinoids were extracted by addition of CH_2Cl_2 (2 ml) and water (2 ml) and vigorous shaking, and the CH_2Cl_2 layer was diluted with hexane (5 ml). The mixture was centrifuged at 3000 rpm for 10 min and the CH_2Cl_2–hexane layer was collected with a pipette. The extraction with CH_2Cl_2–hexane was repeated twice more, and the extracts were combined, washed with water by centrifugation to remove formaldehyde and dehydrated by centrifugation with anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a residue, which was taken up in CH_2Cl_2 (1 ml), dried under N_2 gas and dissolved in the eluent for HPLC.

General Procedure for the Oxime Formation——A methanol solution of 3-OH-RAL was mixed with a 50- to 100-fold molar excess of aqueous hydroxylamine hydrogen carbonate (prepared by neutralization of the aqueous hydrochloride with saturated aqueous $NaHCO_3$) and the final concentration of methanol was maintained at 70% by volume. The solution was stirred in the dark for 5 min at room temperature and then extracted twice with CH_2Cl_2–MeOH–H_2O (1 : 1 : 1). The lower layer was washed twice with brine and dried. Evaporation of the solvent *in vacuo* left a mixture of *syn*- and *anti*-isomers in a ratio of about 3 : 1. These isomers were purified by the following pHPLC

technique. Column, LiChrosorb Si-60-5 (30 × 1.0 cm i.d.); eluent, MeOH : methyl *tert*-butyl ether : benzene = 1 : 3 : 96. Spectral data for *syn*-isomers of 3-OH-RALoxime are shown below. UV spectra were measured in a mixed solvent (MeOH : methyl *tert*-butyl ether : benzene = 1 : 6 : 93).

All-*trans* 3-OH-RALoxime——UV λ_{max} nm (ε): 362 (48000), 350 (sh). ^{1}H-NMR δ (200 MHz, $CDCl_3$): 1.08 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 1.98 (3H, s, 9-CH_3), 2.01 (3H, s, 13-CH_3), *ca.* 4.01 (1H, br m, 3-H), 6.15 (1H, d, J = 11 Hz, 10-H), 6.15 (1H, d, J = 10.5 Hz, 14-H), 6.16 (1H, d, J = 17 Hz, 8-H), 6.20 (1H, d, J = 17 Hz, 7-H), 6.37 (1H, d, J = 15 Hz, 12-H), 6.79 (1H, dd, J = 11, 15 Hz, 11-H), 8.19 (1H, d, J = 10.5 Hz, 15-H). MS *m/z*: 315.2213 (M^+, $C_{20}H_{29}NO_2$ requires 315.2197).

13-*cis* 3-OH-RALoxime——UV λ_{max} nm (ε): 359 (41000), 349 (sh). ^{1}H-NMR δ (200 MHz, $CDCl_3$): 1.08 (6H, s, *gem*-CH_3), 1.74 (3H, s, 5-CH_3), 1.98 (3H, s, 9-CH_3), 2.04 (3H, s, 13-CH_3), *ca.* 3.93 (1H, br m, 3-H), 6.02 (1H, d, J = 11 Hz, 14-H), 6.12 (1H, d, J = *ca.* 16 Hz, 8-H), 6.17 (1H, d, J = *ca.* 10 Hz, 10-H), 6.20 (1H, d, J = *ca.* 16 Hz, 7-H), *ca.* 6.76 (2H, m, 11-H, 12-H), 8.32 (1H, d, J = 11 Hz, 15-H). MS *m/z*: 315.2197 (M^+, $C_{20}H_{29}NO_2$ requires 315.2197).

11-*cis* 3-OH-RALoxime——UV λ_{max} nm (ε): 351 (28000), 360 (sh). ^{1}H-NMR δ (200 MHz, $CDCl_3$): 1.07 (6H, s, *gem*-CH_3), 1.73 (3H, s, 5-CH_3), 1.95 (3H, s, 9-CH_3), 2.07 (3H, s, 13-CH_3), *ca.* 4.02 (1H, br m, 3-H), 5.95 (1H, d, J = 10.5 Hz, 12-H), 6.11 (1H, d, J = 16 Hz, 8-H), 6.19 (1H, d, J = 16 Hz, 7-H), 6.20 (1H, d, J = 10.5 Hz, 14-H), 6.46 (1H, t-like, J = 10.5, 11.5 Hz, 11-H), 6.58 (1H, d, J = 11.5 Hz, 10-H), 8.15 (1H, d, J = 10.5 Hz, 15-H). MS *m/z*: 315.2215 (M^+, $C_{20}H_{29}NO_2$ requires 315.2197).

9-*cis* 3-OH-RALoxime——UV λ_{max} nm (ε): 358 (42000), 350 (sh). ^{1}H-NMR δ (200 MHz, $CDCl_3$): 1.09 (6H, s, *gem*-CH_3), 1.77 (3H, s, 5-CH_3), 1.99 (6H, s, 9-CH_3 and 13-CH_3), *ca.* 4.08 (1H, br m, 3-H), 6.06 (1H, d, J = 11.5 Hz, 10-H), 6.14 (1H, d, J = 11 Hz, 14-H), 6.18 (1H, d, J = 16 Hz, 7-H), 6.30 (1H, d, J = 15.5 Hz, 12-H), 6.65 (1H, d, J = 16 Hz, 8-H), 6.87 (1H, dd, J = 11.5, 15.5 Hz, 11-H), 8.18 (1H, d, J = 11 Hz, 15-H). MS *m/z*: 315.2214 (M^+, $C_{20}H_{29}NO_2$ requires 315.2197).

Details of the extraction of the native sample from fly heads by the oxime method were described in the previous paper.[5]

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