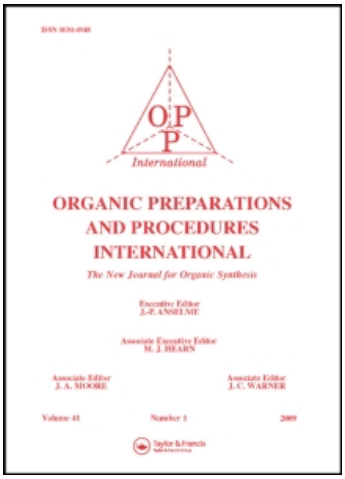

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PREPARATION OF THE 9-*cis*, 13-*cos*-. AND ALL *trans*-ISOMERS OF α- AND β_2-RETINAL

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PREPARATION OF THE 9-*cis*-, 13-*cis*-, AND ALL *trans*-ISOMERS OF α- AND β_2-RETINAL

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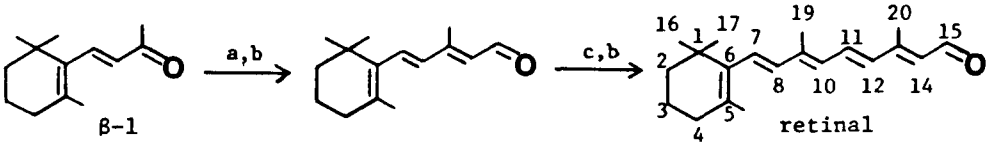
Vitamin A aldehyde (retinal, Scheme 1), the chromophore found in the light-sensitive pigments rhodopsin[1] and bacteriorhodopsin,[2] is bound to a protein in each case by a protonated Schiff base linkage involving one of the lysine residues. However, the specific noncovalent chromophore-protein interactions which give rise to shifted absorption maxima (relative to model protonated Schiff bases of this chromophore) in these systems are not fully understood. One common approach in studying these interactions has been to analyze analogues of the native chromophore which have been incorporated into the proteins. A wide variety of schemes have been modified to incorporate isotopic labels.[5] Here we describe the synthesis of two known retinoid analogues (Table 1), α-retinal[6] (α-7) and β_2-retinal[4] (β_2-7), by a synthetic procedure previously used only for the synthesis of ^{13}C-labeled β-retinals (Scheme 1).[7] While a number of HPLC systems have been

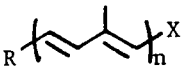


(a) NaH, $(C_2H_5O)_2POCH_2CO_2Et$. (b) $LiAlH_4$ followed by MnO_2.
(c) NaH, $(C_2H_5O)_2POCH_2C(CH_3)CHCO_2Et$.

Scheme 1

reported for the separation of β-retinal isomers,[4,8] only a few reports have described the separation of β_2-retinal[9] and no HPLC work has been reported for α-retinal or the synthetic intermediates **2-4** (Table 1). We report a systematic method employing isocratic solvent systems for the preparative HPLC separation (up to 400 mg of isomeric mixture per injection) of thirty major geometrical isomers for all intermediates and products in this synthesis (Table 1) as well as their characterization by ^{1}H NMR and UV/Vis spectroscopy.

TABLE 1. α - and 3,4-Dehydroretinoids Prepared via Scheme 1.



R	n = 1	n = 2	X
 ≡ α	2	5	CO_2Et
	3	6	CH_2OH
 ≡ β_2	4	7	CHO
		8	CO_2H

a = 9E, 13E; b = 9Z, 13E; c = 9E, 13Z

3,4-Dehydro-β-ionone (β_2-**1**) was synthesized from β-ionone (β-**1**, Scheme 1) according to the procedure of Surmatis and Thommen.[10] According to the procedure used by Iqbal *et al.*[7] for the conversion of β-**1** to an isomeric mixture of retinals, β-**7a-7c** (Scheme 1), compounds β_2-**1** and α-ionone (α-**1**) were then converted to β_2-retinal (β_2-**7**) and α-retinal (α-**7**), respectively. Briefly, for both the α- and β_2- system, **1** was reacted with triethylphosphonoacetate *via* a Horner-Emmons condensation to yield ester **2** as a mixture of all *trans*- and 9-*cis*-isomers. Reduction of **2** with $LiAlH_4$ provided the relatively unstable alcohol, **3**, which was oxidized with MnO_2 to

give the aldehyde **4**. This was condensed with ethyl-γ-diethylphophonosenecioate, which provided ester **5** as a mixture consisting mainly of all trans-, 13-cis-, and 9-cis-isomers. Finally, the ester was converted to the corresponding retinol **6** and retinal **7** via the procedures previously employed for the conversion of **2** to **4**. The yields at each step were comparable to those reported by Iqbal et al.[7] for the β-system.

As summarized in Table 2, preparative HPLC on a Whatman M9 or M20 silica gel column resulted in the separation of the individual isomers of the compounds **2**-**7** (Table 1). The Whatman M20 column was used at 9mL/min flow rates which gave similar retention times to those of the Whatman M9 column at 2mL/min. The isomeric assignments were initially inferred from the HPLC results. The all trans-isomers were expected to predominate for the Horner-Emmons condensation[11] which is also consistent with HPLC results on the β-system.[4,8] The remaining two isomers were tentatively assigned by their relative retention times, since the 9-cis-isomer has a longer retention time than the 13-cis-isomer in the β-system.[9] These tentative assignment were then confirmed by proton NMR data. Based upon previous studies with the β-system,[12] a downfield shift for proton 8 (H-8) and an upfield shift (with respect to the all trans-isomer) for the 9-methyl group (H-19) is characteristic of the 9-cis-isomers, while a downfield shift for proton 12 (H-12) and an upfield shift for the 13-methyl group (H-20) is characteristic of the 13-cis-isomers. These shifts have been attributed to steric interactions in the β-system,[12] which should be nearly identical in the α- and β_2-systems. Tables 3 and 4 contain the proton chemical shift assignments for thirty-six isomers of the α- and β_2-retinoids. Complete ^{13}C-NMR assignments for the compounds in Table 1 will be reported separately.

TABLE 2. Preparative HPLC Separation on Whatman M9 Column and UV/Vis Data for Compounds **1-7**.[a]

Compound	Solvent[b]	Retention Time (min.)		Relative Peak Area (%)[c]		λ_{max} (nm)[d]		
		α	β_2	α	β_2	α	β	β_2
1	A	20.5	23.4	100 (222)	100 (333)	227	290	337
2a	C	29.4	39.9	85 (265)	81 (340)	264	302[e]	343
2b	C	25.6	33.8	15	19	270	302[e]	354
3a	A	37.7	46.6	79 (237)	79 (310)	242	259[f]	313
3b	A	31.8	37.3	21	21	242	266[f]	317
4a	B	22.4	25.6	80 (277)	82 (357)	281	326[f]	366
4b	B	18.3	19.5	20	18	280	321[f]	362
5a	C	31.5	41.4	73 (360)	67 (360)	344	354[g]	383
5b	C	27.5	35.5	15	21	340	348[g]	373
5c	C	24.5	30.9	12	12	342	359[g]	375
6a	A	47.0	62.9	58 (312)	64 (350)	316	325[g]	352
6b	A	45.8	61.1	24	17	315	323[g]	353
6c	A	38.4	49.5	18	19	319	328[g]	354
7a	B	29.4	34.6	60 (350)	58 (380)	358	381[g]	392
7b	B	24.6	27.4	22	18	354	373[g]	383
7c	B	22.0	24.0	18	24	354	375[g]	379

a) See Experimental Section for details. b) A = 10% THF, 10% Et_2O in hexane; B = 3% THF, 10% Et_2O in hexane; C = 1.5% Et_2O in hexane. c) Numbers in parantheses are the wavelengths (nm) at which HPLC separations were monitored. d) In cyclohexane. e) Mixture of isomers (see reference 14). f) Values obtained from reference 15. g) Values in EtOH (see reference 16).

TABLE 3. ^{1}H-NMR ASSIGNMENTS FOR THE ISOMERS OF α-RETINOIDS

^{1}H CHEMICAL SHIFT IN $CDCl_3$(ppm,TMS)[a]

α-Isomer	2a	2b	3	4	6	7	8	10	11	12	14	15	16	17	18	19	20
1	1.22	1.45	2.06	5.50	2.29	6.62	6.05						0.86	0.09	1.57	2.25	
2a	1.18	1.45	2.03	5.45	2.22	5.93	6.08	5.73					0.82	0.91	1.56	2.27	
2b	1.19	1.46	2.03	5.44	2.30	5.91	7.54	5.61					0.83	0.91	1.59	1.98	
3a	1.18	1.44	2.01	5.41	2.16	5.50	6.04	5.59	4.27				0.81	0.90	1.57	1.78	
3b	1.19	1.44	2.02	5.42	2.18	5.57	6.37	5.48	4.30				0.82	0.90	1.58	1.85	
4a	1.22	1.45	2.04	5.48	2.27	6.11	6.19	5.92	10.11				0.84	0.92	1.58	2.26	
4b	1.23	1.44	2.05	5.49	2.30	6.01	7.04	5.83	10.19				0.86	0.94	1.59	2.07	
5a	1.17	1.45	2.02	5.42	2.21	5.63	6.12	6.13	6.96	6.27	5.77		0.82	0.91	1.58	1.94	2.34
5b	1.21	1.47	2.03	5.44	2.25	5.64	6.62	6.00	7.09	6.21	5.77		0.84	0.93	1.60	1.93	2.36
5c	1.19	1.45	2.02	5.42	2.20	5.62	6.12	6.22	6.94	7.76	5.63		0.82	0.91	1.58	1.93	2.06
6a	1.20	1.45	2.00	5.40	2.17	5.52	6.09	6.08	6.58	6.25	5.65	4.24	0.82	0.90	1.58	1.81	1.88
6b	1.23	1.45	2.02	5.43	2.24	5.55	6.60	5.95	6.73	6.21	5.69	4.31	0.83	0.92	1.61	1.88	1.90
6c	1.23	1.43	2.01	5.41	2.18	5.53	6.10	6.11	6.63	6.63	5.56	4.32	0.82	0.90	1.58	1.90	1.93
7a	1.20	1.45	2.03	5.44	2.26	5.70	6.14	6.16	7.11	6.36	5.97	10.10	0.83	0.91	1.58	1.97	2.32
7b	1.23	1.48	2.04	5.46	2.27	5.70	6.63	6.04	7.26	6.23	5.98	10.11	0.84	0.94	1.61	1.96	2.30
7c	1.21	1.47	2.03	5.44	2.21	5.71	6.15	6.20	7.00	7.27	5.85	10.20	0.83	0.91	1.58	1.97	2.14
8a	1.19	1.45	2.01	5.43	2.20	5.66	6.13	6.13	7.01	6.30	5.79		0.82	0.90	1.58	1.95	2.36
8b	1.22	1.47	2.03	5.44	2.26	5.66	6.63	6.01	7.15	6.24	5.80		0.84	0.93	1.61	1.94	2.38
8c	1.20	1.45	2.02	5.42	2.20	5.65	6.15	6.25	7.00	7.74	5.66		0.83	0.91	1.58	1.94	2.10

a) The chemical shifts of the $-OCH_2CH_3$ and $-OCH_2CH_3$ protons were 4.16 and 1.28 ppm, respectively for **2a** and **2b** and 4.17 and 1.29 ppm, respectively, for **5a - 5c**.

Previous studies with selected β_2-compounds[13] suggested that purified isomers may be carried through subsequent reactions (reduction and oxidation) without significant double bond isomerization. This we confirmed with both the β_2- and α-systems by converting the individual pure isomers of

TABLE 4. ^{1}H-NMR ASSIGNMENTS FOR THE ISOMERS OF β_2-RETINOIDS

^{1}H CHEMICAL SHIFTS IN $CDCl_3$(ppm,TMS)[a]

β_2-Isomer	2	3	4	7	8	10	11	12	14	15	16	17	18	19	20
1	2.11	5.88	5.88	7.28	6.20						1.08	1.08	1.90	2.30	
2a	2.08	5.75	5.83	6.56	6.23	5.78					1.03	1.03	1.85	2.35	
2b	2.01	5.70	5.79	6.52	7.71	5.59					1.00	1.00	1.85	1.98	
3a	2.07	5.71	5.83	6.13	6.17	5.65	4.31				1.01	1.01	1.84	1.86	
3b	2.08	5.75	5.84	6.20	6.53	5.57	4.31				1.02	1.02	1.86	1.93	
4a	2.10	5.82	5.87	6.76	6.32	5.96	10.13				1.06	1.06	1.88	2.33	
4b	2.12	5.84	5.88	6.65	7.23	5.90	10.17								
5a	2.08	5.74	5.85	6.28	6.28	6.19	7.00	6.30	5.78		1.04	1.04	1.87	2.01	2.36
5b	2.10	5.77	5.88	6.27	6.81	6.08	7.08	6.23	5.77		1.05	1.05	1.91	2.01	2.33
5c	2.09	5.75	5.85	6.28	6.28	6.29	6.98	7.79	5.64		1.04	1.04	1.87	2.00	2.07
6a	2.07	5.72	5.85	6.16	6.27	6.14	6.61	6.30	5.70	4.31	1.03	1.03	1.86	1.97	1.86
6b	2.10	5.76	5.87	6.18	6.80	6.04	6.72	6.24	5.69	4.31	1.04	1.04	1.84	1.98	1.91
6c	2.09	5.71	5.84	6.14	6.22	6.23	6.77	6.66	5.56	4.31	1.03	1.03	1.89	1.96	2.00
7a	2.09	5.76	5.86	6.31	6.37	6.23	7.15	6.39	5.98	10.11	1.05	1.03	1.89	2.04	2.34
7b	2.11	5.80	5.89	6.32	6.82	6.12	7.23	6.32	5.97	10.10	1.06	1.06	1.92	2.04	2.30
7c	2.11	5.77	5.86	6.33	6.33	6.26	7.04	7.30	5.86	10.20	1.05	1.05	1.88	2.04	2.15
8a	2.08	5.76	5.86	6.30	6.30	6.20	7.05	6.33	5.81		1.04	1.04	1.88	2.02	2.37
8b	2.11	5.77	5.88	6.30	6.81	6.09	7.14	6.25	5.80		1.05	1.05	1.92	2.02	2.34
8c	2.11	5.77	5.86	6.32	6.32	6.32	7.03	7.78	5.67		1.05	1.05	1.88	2.02	2.11

a) The chemical shifts of the $-OCH_2CH_3$ and $-OCH_2CH_3$ protons were 4.17 and 1.28 ppm, respectively, for **2a**, 4.08 and 1.20 ppm for **2b**, and 4.17 and 1.28 ppm, respectively, for **5a-5c**.

esters **5** stereoselectively to the corresponding alcohol **6** and aldehyde **7**. In all cases, HPLC confirmed that isomeric purity was largely maintained. This is a useful procedure which allows for separation and storage of the isomers at the more stable ester stage (**5a-5c**) with conversion to the less stable alcohol **6** and aldehyde **7** when needed. Finally, the isomers of esters

5 were also converted to the corresponding acids 8 by base hydrolysis[6], again with retention of the isomeric state of the compounds as indicated by NMR spectroscopy (Tables 2 and 3).

EXPERIMENTAL SECTION

The chromatographic separations were performed on a Rainin Rabbit HPLC system which included an Apple IIe-controlled Gilson Data Master and a Hitachi Model 100-40 variable wavelength UV/Vis detector. The columns employed were a Whatman Partisil 10 M9/50 (500 x 9.4 mm ID) with a flow rate of 2mL/min with separability of about 20 mg per injection and a Partisil 10 M20/50 (500 x 22.00 mm ID) with a flow rate of 9mL/min with separability of between 100-400 mg per injection. All solvents were HPLC grade (Fisher Omni-Solve), saturated with dry nitrogen, and degassed by filtering through a Millipore filter (0.45 μm) prior to use. The UV/Vis data were obtained on a Beckman Model 26 spectrophotometer. The ^{1}H NMR spectra were obtained on a GE wide bore spectrometer (NT series) equipped with an 1180e processor and 293c pulse programmer. The resonance frequency for ^{1}H was 300.1 MHz. The proton resonances were referenced internally to tetramethylsilane (TMS) and spectra were obtained in $CDCl_3$ at ambient temperatures. Assignments were made by selective homonuclear decoupling and by COSY 90 two dimensional NMR experiments. The COSY 2D experiment utilized the following pulse sequence[17]: (π/2)-(t1)-(π/2)-(FID,t2). Quadrature phase detection was employed and the transmitter was placed at 7.9 ppm. The spectral width in the F1 and F2 domains was 1529 Hz and contained 1K data points. Two hundred and fifty-six spectra were acquired in about 5 hrs. Processing involved sine multiplication in each dimension and zero filling to yield a 512x512 data set. The π/2 pulse was 7.5 μs.

3,4-Dehydro-β-ionone (β_2-1) was synthesized from β-ionone (β-1) (Aldrich) according to an established procedure[10]. α-Ionone (α-1) was purchased from Aldrich. The α-and β_2-retinoids were prepared from the corresponding ionones exactly as described by Iqbal *et al.*[7] for the conversion of β-ionone to ^{13}C-labelled-retinals. Pertinent IR and R_f data are given in Table 5 on the isomeric mixtures of compounds **1-7**. Crude synthetic samples were placed on a short silica column and eluted with 10% acetone/hexane to remove the more polar components prior to HPLC separation. The separated isomers were collected in flasks which were cooled in an ice bath and shielded from light. UV/Vis maxima of the long-wavelength band and ^{1}H-NMR assignments for the separated isomers of 1-7 are given in Table 2-4. The data presented here are consistent with previous characterizations of these compounds.[6,13,15,16] The acids 8 were prepared from the corresponding esters 5 by hydrolysis with potassium hydroxide according to the procedure of Hale *et al.*[6] By using isomerically pure esters, the acids were prepared without E-Z isomerization. ^{1}H-NMR assignments (Table 3 and 4) are also consistent with previously published 60 MHz assignments[13] on β_2-retinoic acid.

TABLE 5. R_f and Infrared Data on Isomeric Mixtures of α- and β_2-Retinoids

Compound Number	R_f [a]		Infrared (cm^{-1})[b]					
			R = α			R = β_2		
	R=α	R= β_2	OH	C=C	C=O	OH	C=C	C=O
1	0.65	0.50	-	1675 1620	1690	-	1585	1655
2	0.86	0.76	-	1600 1620	1705	-	1595	1695
3	0.17	0.11	3320	1630	-	3325	1625	-
4	0.49	0.40	-	1580 1610	1660	-	1595	1655
5	0.70	0.75	-	1650	1705	-	1660	1710
6	0.18	0.15	3320	-	-	3410	1630	-
7	0.43	0.50	-	1645	1655	-	1560	1650

a) In 10% acetone/hexane on silica gel. b) Liquid films.

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