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The synthesis of ^{13}C-labelled retinals

Johan Lugtenburg

Department of Organic Chemistry, University of Leiden,
P.O. Box 9502, 2300 RA Leiden, the Netherlands

Abstract - Sixteen different ^{13}C-labelled retinals in the all-*trans*, 13-*cis*, 11-*cis* and 9-*cis* configuration have been prepared.

INTRODUCTION

Many biological processes are triggered by the absorption of a quantum of light by target molecules, among these natural pigments.

In visual pigments and in the light-harvesting pigment of the halophilic Halobacterium halobium the light-absorbing moiety, *i.e.* the chromophore, is derived from retinal, as a protonated Schiff's base bound to be peptide chain of the protein (Ref. 1).

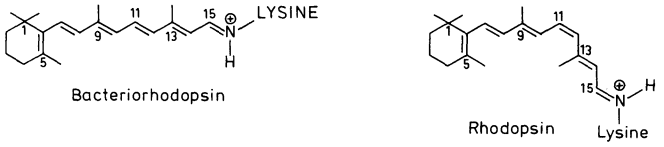


Fig. 1. Structure of the chromophoric part of bacteriorhodopsin and rhodopsin.

The chromophore of all visual pigments is an 11-*cis* retinylidene group. Bovine rhodopsin, a membrane protein of mol. weight = 38,000 D, comprises a peptide chain of 348 amino acid residues, its chromophore bound to the ε-amino group of lysine 296 (Ref. 2). Upon exposure to light this rhodopsin (λ_{max} = 498 nm, ε 40,000) is efficiently converted (ϕ = 0.67) into the colourless protein opsin and all-*trans* retinal, so-called bleaching.

While opsin interacts with 11-*cis* retinal with regeneration of rhodopsin, it interacts with 9-*cis* retinal to form isorhodopsin (Fig. 2a).
The primary photoproduct of both rhodopsin and isorhodopsin in bathorhodopsin (λ_{max} 543 nm) with a 35 Kcal/mol higher free enthalpy than rhodopsin. At physiological temperatures its half life is about 10^{-8} sec; in a thermal first-order reaction it converts into lumirhodopsin. Via the further intermediates metarhodopsin I and metarhodopsin II, opsin and free all-*trans* retinal are formed. Below -140°C bathorhodopsin is metastable and a photostationary state consisting of rhodopsin, isorhodopsin and bathorhodopsin is reached.

There is strong evidence that the presence of metarhodopsin II in the photoreceptor membrane induces electrical changes in this membrane, resulting in the generation of a nerve impulse. Light-information is converted into nerve information which precedes the sensation of vision.

Bacteriorhodopsin, the crystalline purple membrane of Halobacterium halobium (MW = 26,000 D) with a peptide chain of 248 amino residues may occur in a light- and a dark-adapted form. The chromophore of the light-adapted form is an all-*trans* retinal Schiff's base, linked to the ε-amino group of lysine 216 (Fig. 1). The photochemistry of light-adapted BR is sketched in Fig. 2b. The primary photoproduct K has taken up 15 Kcal/mol . It is thermally converted back into BR via a cycle of intermediates. The maximal cycling rate is 1000 times per second. During this photochemical cycle a proton is transported from inside the cell of the bacterium to the outside. In this way the light energy converted into the energy of a proton gradient across the cell membrane is utilized to synthesize ATP, *i.e.* to power life processes of the bacterium.

The features of rhodopsin, bacteriorhodopsin, their primary photoproducts and the other intermediates are the outcome of intimate interactions between the chromophore and the

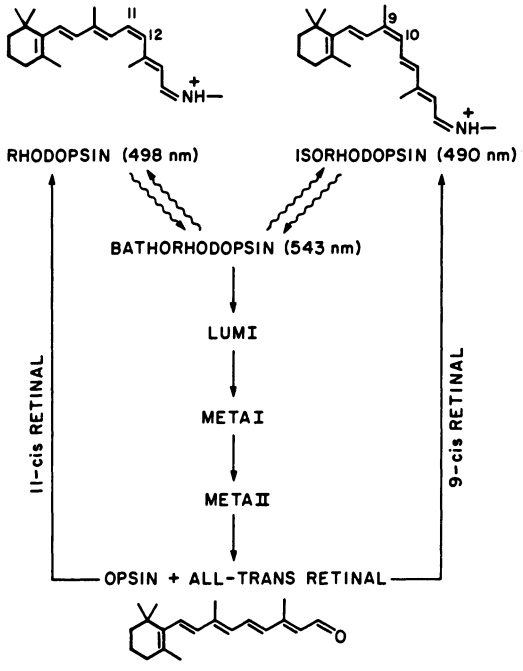


Fig. 2a. Scheme of the photochemistry of rhodopsin and isorhodopsin

Photoreactions (~~~~→)
Thermal reactions (——→)

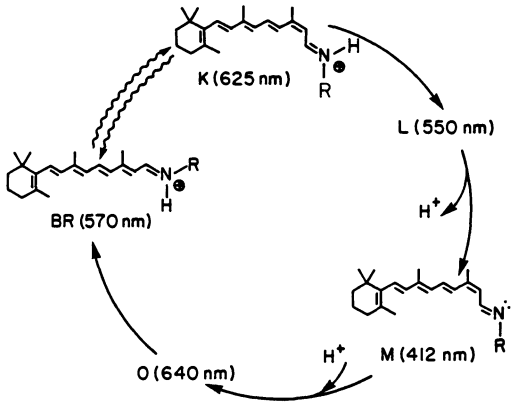


Fig. 2b. Photochemistry of light-adapted bacteriorhodopsin (BR 570).

protein moiety. To understand the photochemistry at molecular level it is necessary that the structure of the chromophore and its interaction with the protein moiety be elucidated. Resonance Raman (RR) spectroscopy has been used to obtain vibrational information about the chromophores in visual pigments and bacteriorhodopsin and in the labelled photointermediates (Ref. 3). Recently FT IR difference spectroscopy has also been applied to acquire vibrational difference spectra between the intermediates and the starting material (Ref. 4). In addition to information about the chromophore this technique gives information about the part of the protein that is subject to changes when going from starting material to the intermediates. The classical method of obtaining vibrational structural information is by use of isotopic substitution. The vibrational spectra of isotopic derivatives permit assignment and interpretation, whence the necessary structural information can be derived. The only way to obtain visual pigments and bacteriorhodopsin with chromophores carrying a specific label is by total synthesis of the labelled retinal and subsequent combination with the relevant apoprotein. Reaction of opsin with labelled 11-*cis* retinal yields rhodopsin with labelled chromophore; similarly labelled bacteriorhodopsins are obtained by reacting bacterio-opsin with labelled all-*trans* retinal.
As the latest development MASS ^{13}C and ^{15}N NMR solid-state spectroscopy has been applied (Ref. 5).

For the MASS ^{13}C NMR spectroscopy specific positions in the chromophore have to be enriched so as to be observable above the signals due to the 1.1% ^{13}C natural abundance level. Use of materials with 90% ^{13}C level will give 82 times more intense signals that are readily observed. The isotopic modification will not introduce changes in the electronic and steric interactions either in the chromophore or between the chromophore and the peptide part in any of these systems. Native pigments contain chromophores labelled at the natural abundance level, *e.g.* for ^{13}C 1.1%.

Free exchange of ideas and close cooperation with Prof. R. Mathies and his group of the University of California at Berkeley, who obtained and interpreted the RR spectra, and with Prof. R. Griffin and his group of M.I.T., Cambridge, Mass., who contributed the MASS ^{13}C NMR spectra, are gratefully acknowledged.

Synthesis of ^{13}C-labelled retinals

Our strategy of preparing all-*trans* retinal labelled with 90% ^{13}C incorporation on positions 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, 19 and 20 plus some dilabelled retinals will be discussed. We have based our synthesis on $K^{13}CN$, $^{13}CH_3I$, $^{13}CH_3CN$, $CH_3{-}^{13}CN$ and $^{13}CH_3{}^{13}CN$ with 90% as ^{13}C sources (Ref. 6). Different starting materials have been applied by other groups for the preparation of ^{13}C-labelled retinals (Ref. 7). The use of these one and two carbon containing starting materials keeps the expenses at affordable levels; the use of more

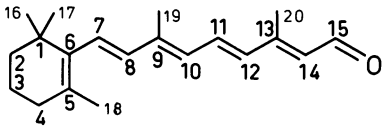


Fig. 3. All-*trans* retinal $C_{20}H_{28}O$.

complicated synthons will easily be prohibitively expensive. $K^{13}CN$ is used to introduce the label at C5; $^{13}CH_3I$ for the labelling of the methylcarbons 18, 19 and 20. The labelled acetonitriles are used for the introduction of ^{13}C at the positions 6, 7, 8, 9, etc. in the conjugated chain.

$14^{13}C$ retinal (1a), $15^{13}C$ retinal (1b) and $14.15^{13}C_2$ retinal (1c)

As starting material for the synthesis of retinal with ^{13}C label at positions 14 and 15 we needed C18 ketone(6). The most facile synthesis of C18 ketone(6) from β-ionone(2) is depicted in Fig. 4 (Ref. 8).

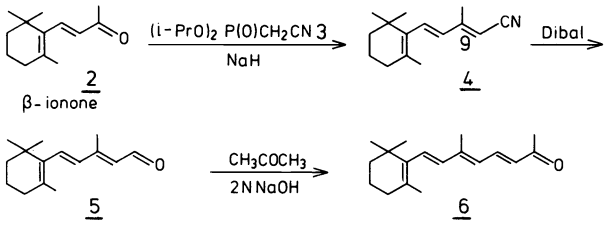


Fig. 4. Synthesis of C18 ketone(6) from β-ionone(2).

β-ionylidene-acetonitrile(4) is obtained in 95% yield by a Horner-Emmons reaction of β-ionone(2) with the C2-synthon diisopropylphosphonacetonitrile(3) (Ref. 9).
By using diisopropylphosphonate(3) synthesized by an Arbuzov reaction of chloroacetonitrile with triisopropylphosphite a high percentage 9E isomer of 4 is obtained (9E/Z = 84/16). Reduction of the nitrile with diisobutylaluminiumhydride (Ref. 10) and subsequent aldol-condensation of 5 with acetone and 2NNaOH gives the required C18 ketone(6) in 70% overall yield from β-ionone(2) (Ref. 11).

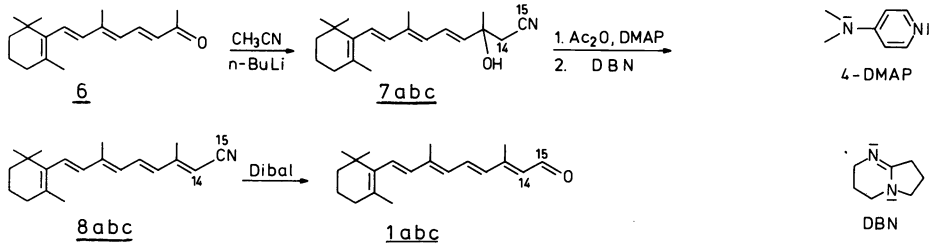


Fig. 5. Synthesis of $14^{13}C$(1a), $15^{13}C$(1b) and $14.15^{13}C_2$ retinal(1c): 4-DMAP = 4-dimethylaminopyridine; DBN = diazabicyclononene.

In Fig. 5 the scheme for the conversion of the C18 ketone into the retinals ^{13}C labelled at positions 14 and 15 is given. The addition of C18 ketone(6) at -60°C to ^{13}C lithium-acetonitrile (obtained by reacting 1 equiv. of n-butyllithium with ^{13}C acetonitrile in THF) gives the chain extension to the full skeleton of retinal in 80% yield.
The OH group in 7 was converted into the acetate with acetic anhydride and 4-dimethylamino-pyridine as base. This is followed by deacetylation with 1.5-diazabicyclo(4.3.0)-non-5-ene (DBN) in reflexing toluene to the labelled retinonitrile (8 a, b, c) as a 13E/Z mixture (2:1) in 80% yield. Dibal reduction of 8 a, b, c gives the required $14^{13}C$ (1a), $15^{13}C$ (1b) and $14,15^{13}C_2$ retinal(1c) in a facile way in 60% overal yield from the labelled acetonitriles (ref. 12).

$10^{13}C$ retinal(1d), $11^{13}C$ (1e), 10-11 $^{13}C_2$ (1f), $12^{13}C$ (1g) and $13^{13}C$ retinal(1h)

For the preparations of $10^{13}C$ (1d), $11^{13}C$ (1e) and 10-$11^{13}C_2$ retinal(1f) we used the reaction sequence of Fig. 6. Addition of ^{13}C lithiumnitrite to β-ionone(2) gives quantitatively the alcoholnitriles (9 d, e, f) as described for the unlabelled compound (Ref. 13). The dehydration is accomplished with a catalytic amount of N-bromosuccinimide, yielding after column-chromatography β-ionylideneacetonitriles (4 d, e, f) in 73% yield as a mixture of 9E/Z isomers (3:2) (Ref. 14). The β-ionylideneacetaldehydes (5 d, e, f) are obtained in 85% yield by

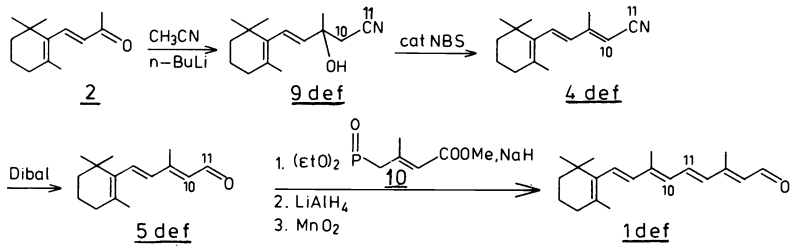


Fig. 6. Preparation of $10^{13}C$ retinal(1d), $11^{13}C$ retinal(1e) and $10\text{-}11^{13}C_2$ retinal(1f).

reduction of 4 d, e, f with diisobutylaluminiumhydride. The aldehydes 5 d, e, f were coupled with the C5 phosphonate 10 to the retinoic esters (Ref. 15). Subsequent reduction with $LiAlH_4$ and MnO_2 oxidation (Ref. 16) gives $10^{13}C$ (1d), $11^{13}C$ (1e) and $10,11^{13}C_2$ retinal(1f) in 55% yield starting from 5 d, e and f (Ref. 17).

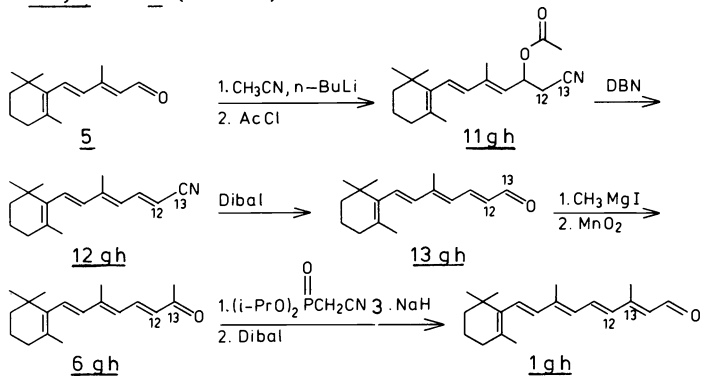


Fig. 7. Preparation of $12^{13}C$ retinal(1g) and $13^{13}C$ retinal(1h).

β-Ionylideneacetaldehyde(5) is added to lithiumacetonitrile in THF at -90°C. At this temperature base-catalyzed side reactions of 5 do not occur. The coupling product is then quenched with acetylchloride at -60°C to give the nitrile acetates (11 g and h) in 85% yield. Acetic acid elimination from 11 with DBN in refluxing toluene gives the tetraenenitriles (12 g, h) in 88% yield. Dibal reduction leads to the C17 aldehyde 13 gh in 95% yield. Reaction with excess methylmagnesiumiodide and subsequent MnO_2 oxidation give the desired C18 ketone (6g, 6h) in 60% yield. The one-step conversion of the nitrile 12 to the corresponding methyl ketone(6) with methyllithium does not give useful results. This conversion works well with aromatic and nonconjugated aliphatic nitriles (Ref. 18), but fails with conjugated aliphatic nitriles. Coupling with 3 and dibal reduction gives $12^{13}C$ (1g) and $13^{13}C$ retinal(1h) in 90% yield (85% 13E). These retinals are obtained in 38% yield based on the ^{13}C-labelled acetonitrile. From 0.25 g labelled acetonitrile 0.69 g ^{13}C-labelled retinal (1g, 1h) is obtained in eight steps.

$8^{13}C$ retinal(1i) and $9^{13}C$ retinal(1j)

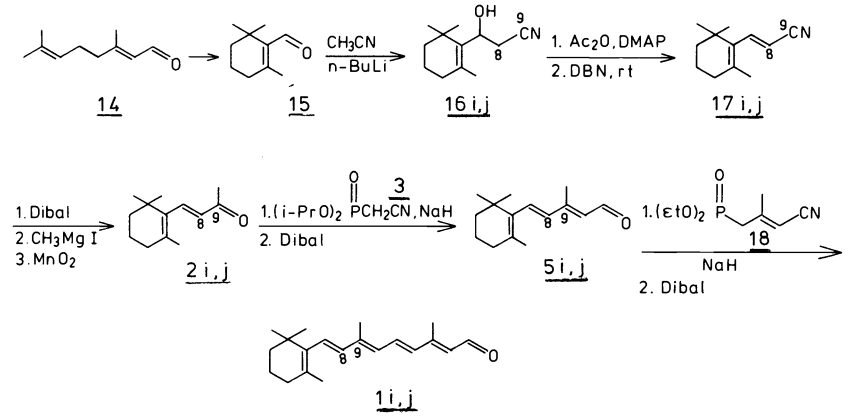


Fig. 8. Preparation of $8^{13}C$ retinal(1i) and $9^{13}C$ retinal(1j) from citral(14).

The starting aldehyde β-cyclocitral(15) is obtained by cyclization of the N-phenylimine of citral(14) with 95% sulfuric acid at -20°C (Ref. 19). Addition of β-cyclocitral(15) at -60°C to lithioacetonitrile gives the alcoholnitrile (16 i, j) in 95% yield, which was first converted into acetate. The deacetylation was carried out at room temperature with DBN in toluene. After three days the reaction was completed and the nitrile 17 i, j was obtained in 80% yield as a 7E/Z mixture (3:1) with no traces of *retro*-compounds. The nitrile 17 is converted in a three-step sequence into the labelled β-ionone (2 i, j) in 60% yield. First 17 is reduced with dibal to the 7E aldehyde (during this reaction the 7Z form isomerizes to 7E). The aldehyde reacts with excess methylmagnesiumiodide and the unsaturated alcohol is oxidized with MnO_2 to 2 i j. The β-ionone (2 i j) is converted into β-ionylideneacetaldehyde (5 i j) in 80% yield in two steps with the Wittig reagent 3 and subsequent dibal reduction similar to the reaction in Fig. 4. For the conversion of 5 i j into 1 i j we use a two-step procedure: the anion of the C5 synthon 18 (Ref. 20) is coupled with 5 i j to the retinonitrile and this is reduced with dibal to the retinals 1 i,j. 1i and 1j are formed in 80% yield from the aldehyde 5 i j which is a considerable improvement over the 55% yield for this conversion described in Fig. 6 with C5 ester synthon.

Fig. 9 shows how the required phosphonate nitrile C5 synthon(18) is obtained firstly by a Horner-Emmons coupling of chloro-acetone with diethylphosphonoacetonitrile and secondly by an Arbusov reaction of the resulting chloride with triethylphosphite.

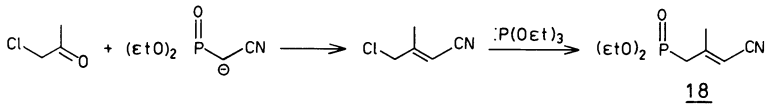


Fig. 9. Preparation of C5 synthon 18 in two steps from chloro-acetone.

$6^{13}C$ retinal(1k) and $7^{13}C$ retinal(1l)

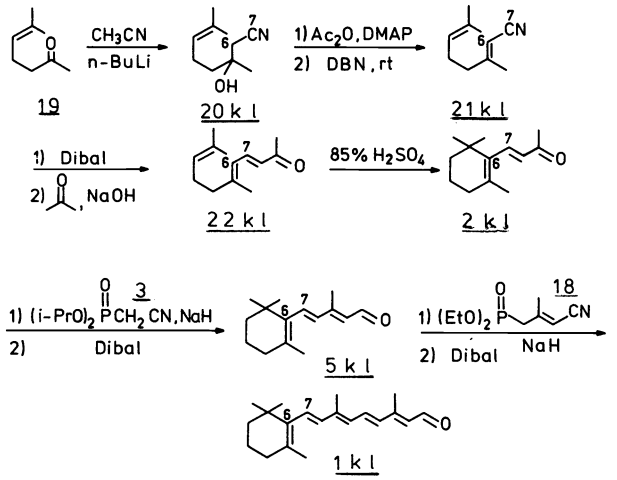


Fig. 10. Preparation of $6^{13}C$ retinal(1k) and $7^{13}C$ retinal(1l).

For the preparation of $6^{13}C$ (1k) and $7^{13}C$ retinal(1l), 6-methylhept-5-ene-2-one(19) is the starting material. Condensation with the anion of the labelled acetonitrile gives the alcoholnitrile (20 k, l). Removal of H_2O by the acetylation-deacetylation sequence affords the conjugated nitriles (21 k, l). Dibal reduction of 21 k, l gives the citral; the subsequent aldol condensation with acetone gives pseudo-ionone. Condensation of 22 to β-ionone is effected with 85% sulphuric acid at 0°C (Ref. 21). The yield of β-ionone is 67% based on the labelled acetonitrile. This β-ionone is then converted in a four-step sequence in 64% yield in $6^{13}C$ (1k) and $7^{13}C$ retinal(1l) as already discussed.

$5^{13}C$ retinal(1m) and $18^{13}C$ retinal(1n)

For the introduction of the $15^{13}C$ the SN_2 reaction of $K^{13}CN$ with the tosylate of 4-methylpent-4-ene-ol(25) was used. 25 was made in three steps starting from commercial 4-ketopentanol(23) via esterification to the acetate(24). Wittig coupling and saponification gives 25. This was converted into the corresponding tosylate and reaction with $K^{13}CN$ gives the nitriles (26m). This aliphatic nitrile reacts in high yield with methyllithium to form methylketone 27m (Ref. 18). Reaction with ^{13}C methyllithium (prepared from $^{13}CH_3I$ and 2Li) with (26) yields the methylketone (27n) with ^{13}C in the methyl group. Extension of the chain of 27 with the

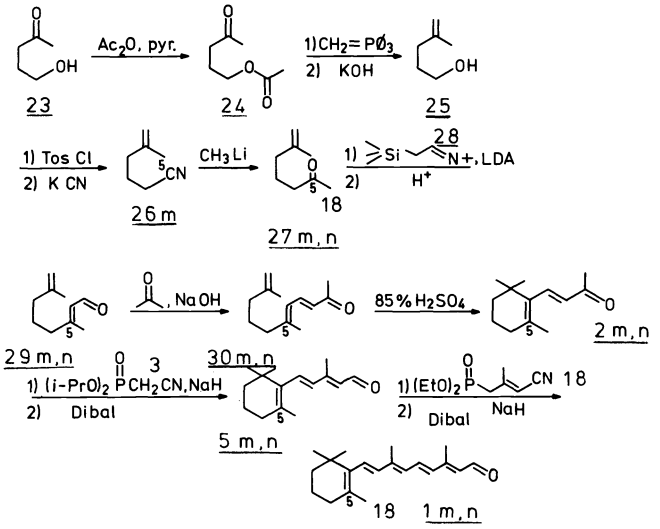


Fig. 11. Preparation of $5^{13}C$ retinal(1m) and $18^{13}C$ retinal(1n).

anion of synthon 28 and acid hydrolysis of the resulting tertbutylimine (Ref. 22) gives the isocitral 29 n, m. Aldol condensation of 29 n, m with acetone gives isopseudoionone 30 n, m. This was converted into the labelled β-ionone 2 m, n with sulphuric acid at 0°C. The yield of 2m based on $K^{13}CN$ is 39%. The β-ionone was converted in a four-step sequence into $5^{13}C$ (1m) and $18^{13}C$ retinal(1n).

$19^{13}C$ retinal(1o) and $20^{13}C$ retinal(1p)

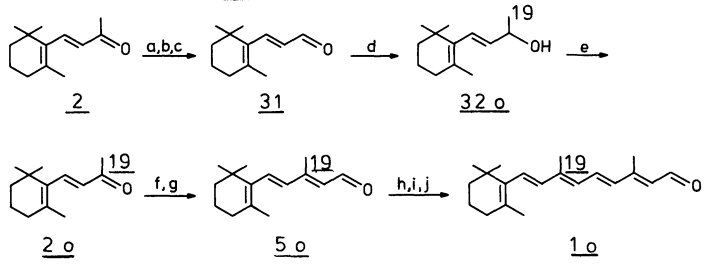


Fig. 12. Scheme for the preparation of $19^{13}C$ retinal(1o): a = NaOCl; b = $LiAlH_4$; c = MnO_2; d = $^{13}CH_3MgI$; e = MnO_2; f = $(iPrO)_2P(=O)CH_2$-CN, NaH; g = dibal; h = $(EtO)_2P(=O)CH_2(CH_3)C=CHCO_2CH_3$; i = $LiAlH_4$; j = MnO_2.

For the introduction of ^{13}C at 19 and 20 $^{13}CH_3MgI$ was used that was prepared from $^{13}CH_3I$ and Mg. Fig. 12 shows that β-ionone(2) is the starting material for $19^{13}C$ retinal(1o). β-Ionone(2) is oxidized by NaOCl to the corresponding acid (Ref. 23); this is reduced by $LiAlH_4$ and the resulting alcohol oxidized to the aldehyde 31. Grignard reaction of 31 with $^{13}CH_3MgI$ gives the labelled β-ionol 32o; this is oxidized with MnO_2 to labelled β-ionone(2o) which is converted with the known sequence into the retinal (1o) (Ref. 17).

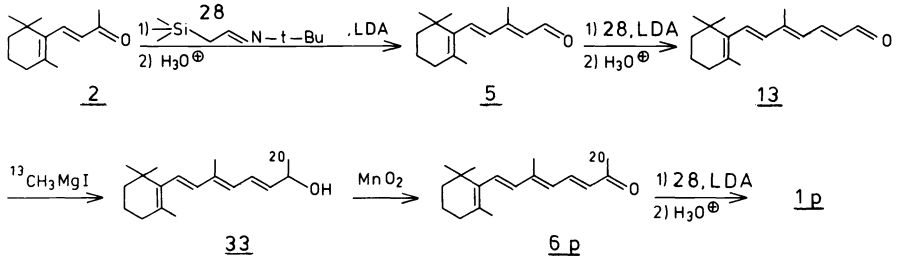


Fig. 13. Scheme for the preparation of $20^{13}C$ retinal(1p).

Fig. 13 shows how $20^{13}C$ retinal is made. β-Ionone(2) is converted into β-ionylidine acetaldehyde(5) by chain extension with the anion of synthon 28. This extension is repeated to give the aldehyde(13). Grignard reaction of (13) with $^{13}CH_3MgI$ gives the labelled alcohol (33). Oxidation with MnO_2 gives the labelled ^{18}C ketone(6p). One final chain extension with

the tert-butylimine of trimethylsilylacetaldehyde(28) gives 20^{13}C retinal(1p) (Ref. 17). The advantage of the use of the Peterson olefination with 28 is that it is a one-pot reaction to be performed at low temperature leading to a high yield (95%) of the required aldehyde. A disadvantage is that the newly formed double bond occurs in a 1:1 ratio of Z and E isomers.

*300 MHz ^1H NMR and 75.5 MHz ^{13}C NMR spectroscopy of the ^{13}C-labelled all-*trans *retinals*

HPLC purification yielded 99% pure ^{13}C-labelled all-*trans* retinal. From the 300 MHz ^{1}H NMR spectra the position and amount of incorporation is evident. (The percentage of incorporation was also checked by double-focus mass spectrometry.) This is illustrated in Fig. 14 for the ^{1}H NMR spectrum of 14,15 $^{13}C_2$ retinal.

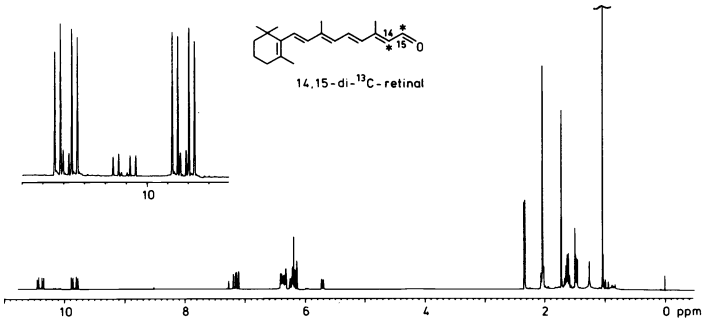
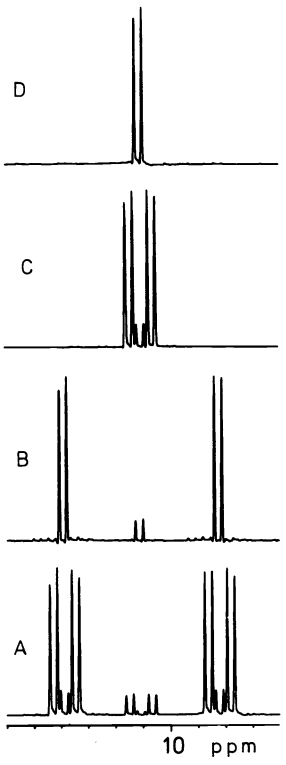


Fig. 14. 300 MHz ^{1}H NMR spectrum of 14,15 di ^{13}C retinal.

From the signals of the aldehyde proton (H15) at 10,11 ppm the isotope composition can be measured. The eight intense signals are due to the 83% molecule with ^{13}C on both 14 and 15, while the smaller signals are due to the 8% ^{13}C on 14 and on 15. In the centre at 10.11 ppm the doublet of the 1% 14,15 $^{12}C_2$ retinal is observable (Ref. 24).
From the intense peaks the following coupling constant values can be determined: $J_{H_{14}-H_{15}} = 8.0$ Hz, $J_{C_{14}-H_{15}} = 24.5$ Hz and $J_{C_{15}-H_{15}} = 169.7$ Hz.



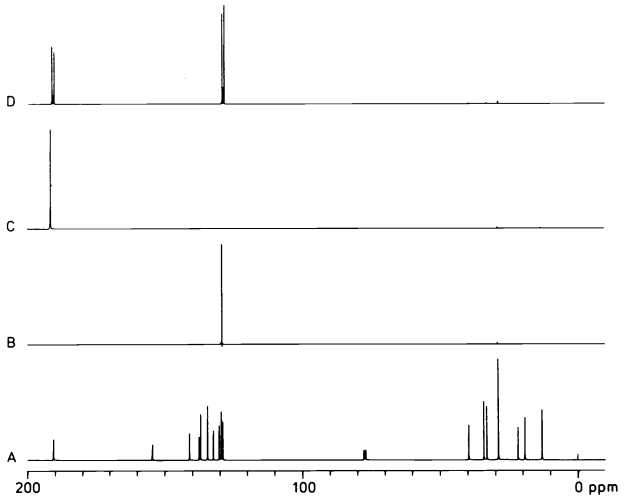


Fig. 16. ^{1}H noise-decoupled 75.5 MHz ^{13}C NMR spectra of all-*trans* retinal (A), 14^{13}C all-*trans* retinal (B), 15^{13}C all-*trans* retinal (C) and 14,15$^{13}C_2$ all-*trans* retinal (D).

Fig. 15. The aldehyde region in the 300 MHz ^{1}H NMR spectrum of all-*trans* (D), 14^{13}C all-*trans* (C), 15^{13}C all-*trans* (B) and 14,15 $^{13}C_2$ all-*trans* retinal (A).

Fig. 15 presents the H15 signals of all-*trans* (D), of $14^{13}C$ all-*trans* (C: 92% incorporation), of $15^{13}C$ all-*trans* (B: 92% incorporation) and again $14,15\ ^{13}C_2$ all-*trans* retinal (A).
In Fig. 16 the ^1H noise-decoupled 75.5 MHz spectra of normal all-*trans* (A) (Ref. 25) and $14^{13}C$ retinal (B), $15^{13}C$ all-*trans* (C) and $14,15^{13}C_2$ all-*trans* retinal (D) are drawn.
From the spectra the position of the label is immediately evident. In D the AB spectrum with $J_{C_{14}-C_{15}} = 56.9$ Hz is clearly visible. In the centre of the AB pair the signals due to the two times 8% mono-labelled molecules are visible. The labelled retinals contain 1.1% (natural abundance) of ^{13}C at the other positions. The labelled retinals have $0.9 \times 1.1\% = 1.0\%$ doubly labelled positions. The carbon-carbon coupling constants can be obtained from the labelled retinals at the natural abundance level.
In Table 1 the ^{13}C-^{13}C coupling constant values are tabulated. The $^1J_{^{13}C-^{13}C}$ values show a good relation to the bond character of the bond between the two carbons (Ref. 26).
They are around 70 Hz for carbons linked via a double bond, around 55 Hz for a single bond in the polyene chain, and 40 Hz for sp2-sp3 single bonds. These values are in good agreement with the values for small systems as butadiene and 1-methylcyclohexene and are a measure of the hybridization and bond lengths of the C-C bond in question (Ref. 27).

Table 1. In this Table the ^{13}C-^{13}C coupling constant values of the ^{13}C-labelled retinals are tabulated. The signs of the values have not been determined. The values are in Hz.

$^1J_{^{13}C-^{13}C}$		$^2J_{^{13}C-^{13}C}$		$^3J_{^{13}C-^{13}C}$	
1-6	40.3	9-18	2.9	1-8	0
4-5	40.6	7-9	0	3-6	3.1
5-6	76.4	8-10	2.4	4-7	4.0
5-18	43.9	8-19	1.9	5-8	0
6-7	56.4	9-11	0	6-9	5.4
7-8	71.1	10-12	0	7-10	7.0
8-9	56.0	10-19	0	7-18	2.7
9-10	70.4	11-13	0	7-19	3.1
9-19	43.3	12-14	0	8-11	7.1
10-11	58.7	12-20	1.9	9-12	9.4
11-12	69.8	13-15	3.0	10-13	7.5
13-14	66.7	14-20	0	11-14	8.2
13-20	40.4			11-19	4.3
14-15	56.9			11-20	3.3
				12-15	7.2
				15-20	4.7

*Preparation of 9*cis-, *11*cis- *and 13*cis-*retinal*

The ^{13}C-labelled all-*trans* retinals upon irradiation with light in acetonitrile undergo *cis-trans* isomerization. The photostationary state has 13*cis*, 11*cis*, 9*cis* and all-*trans* as the main components (Ref. 28). These can be isolated in pure form by HPLC separation. Their UV-vis spectra are identical with the corresponding unlabelled isomers.

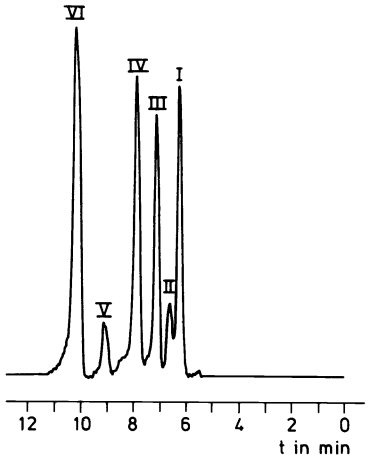


Fig. 17. HPLC analysis of the isomeric mixture obtained by irradiation of all-*trans* retinal(VI) in CH_3CN: I = 13*cis*; II = 9.13di*cis*; III = 11*cis*; IV = 9*cis*; V = 7*cis*.

MASS ^{13}C solid-state NMR and RR studies

In this section we will mention some of the results obtained from the study of bacteriorhodopsin with ^{13}C-labelled chromophores. MASS ^{13}C solid-state NMR spectroscopy of dark-adapted bacteriorhodopsin shows that the chromophore in this system occurs in a 4:6 ratio in the all-*trans* and 13*cis* protonated Schiff's base structure. The signals of the carbon atoms 10, 11, 12, 15, 19 and 20 are in agreement with an unperturbed Schiff's base structure. The signals of the 14 carbon indicate that the 13*cis* occurs in the 13,15 di*cis* structure (Ref. 29). The resonance of the 14C is 8 ppm shifted to higher field due to the γ effect by the ε-CH_2 group of the lysine 216. In the light-adapted bacteriorhodopsin the all-*trans* 15(C=N)

trans structure is present. This means that light-dark adaptation involves isomerization around two bonds (13 and 15). These results could be confirmed by Resonance Raman studies (Ref. 30). With this technique the structures of the chromophores in the intermediates could also be established. In the intermediates K and L (Fig. 2b) the C=N bond has a *trans* structure. This means that the primary photochemical event from light-adapted bacteriorhodopsin involves 13*trans-cis* isomerization only. The use of ^{13}C-labelled retinals has been essential to effect the complete vibrational analysis of all-*trans* - 13*cis*, 11*cis*, 9*cis* and 9,13di*cis* retinal (Ref. 31). The complete vibrational analysis of the chromophores in bacteriorhodopsin, rhodopsin and their photoproducts are in progress.

CONCLUSIVE REMARKS

Up to now 16 different ^{13}C-labelled retinals in all-*trans*, 13*cis*, 11*cis* and 9*cis* configurations have been prepared by our group. The synthetic strategy allows us to prepare thus far unknown ^{13}C-labelled retinals.
The RR and solid-state ^{13}C NMR spectroscopy of bacteriorhodopsin and its photoproducts with ^{13}C label have given thus far unattainable information about the action of bacteriorhodopsin. Similar information is to be expected from the study of ^{13}C-labelled rhodopsins.
MASS ^{13}C NMR spectroscopy, especially, promises to become an outstanding technique for the study of the interaction of small molecules with the active site of (receptor) proteins.

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