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Synthesis of New Heterocyclic and Polycyclic Aromatic Retinals and their Bacteriorhodopsin Analogues

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Abstract: New heterocyclic and polycyclic aromatic retinal analogues (Fig.1) were synthesised and their recombination with bacterioopsin was studied.

Retinoids are involved in many biological processes - photoreception; human and animal reproduction; development of tissues and organs during embryogenesis; regulation of cellular proliferation and differentiation. The key for this great diversity of biological functions is the existence of multiple specific binding proteins and nuclear receptors for Vitamin A metabolites.[1]

The processes of photoreception include participation of photoactive retinal proteins: bacterial rhodopsins (bacteriorhodopsin and newly discovered ion pumps or sensory rhodopsins)[2] and visual pigments (rod and cone visual pigments).[3] Studies of other members of photoactive proteins (circadian photopigments and retinochromes) are also in progress.[4] In particular, the study of artificial bacterial rhodopsins with a long-lived intermediate, generated by light illumination at room temperature, is directed also to the problem of the permanent optical storage of information, using molecular electronics devices.[5]

Structural investigations of the proteins - bacteriorhodopsin, halorhodopsin, bovine rhodopsin - including cryo-electron microscopy, revealed that the polypeptide chains of these proteins consist of seven transmembrane helices.[3] Chromophore analogues, including aromatic (phenyl, anthryl, 2-naphthyl etc.) analogues, have been used in structure-function studies of the retinal proteins.[6,7] In this work we report the synthesis of new analogues of retinal (Fig. 1), containing coumarin or polycyclic aromatic substituents, and their artificial bacteriorhodopsin analogues.

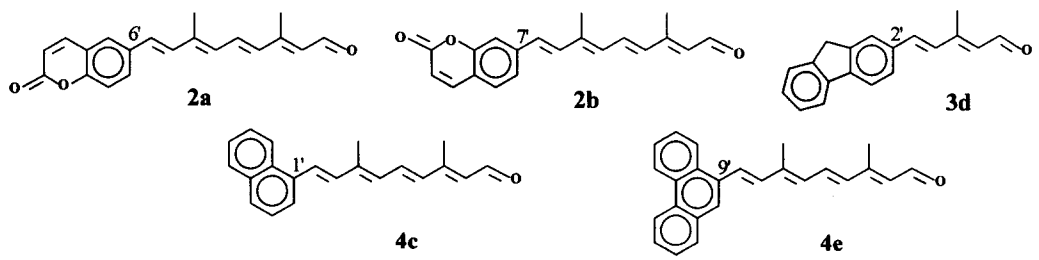

Fig. 1. Structures of the new coumarin (**2a, 2b**) and polycyclic aromatic (**3d, 4c, 4e**) retinal analogues.

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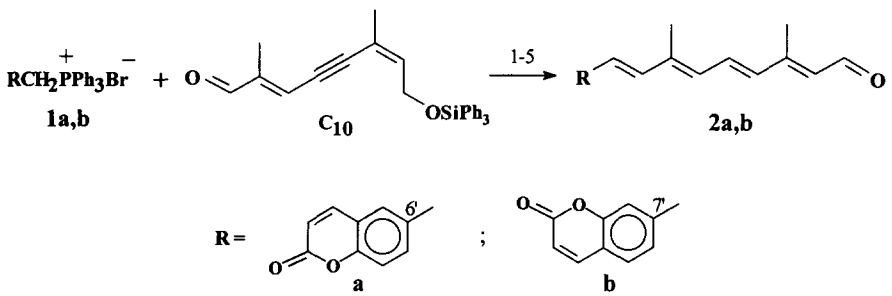
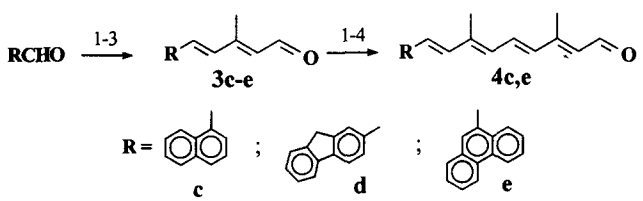
The new 6- and 7-coumarin analogues of retinal **2a** and **2b** were synthesised from the corresponding (2-oxo-*2H*-chromenyl)methyltriphenylphosphonium bromides **1a,b** and *(2E,6Z)*-2,6-dimethyl-8-triphenylsilyloxyocta-2,6-dien-4-yn-1-al (polyenal C_{10}) according to the olefination procedure (Scheme 1), described in our previous work for (3-coumarinyl)retinal.[8,9]



1. NaH/THF(-40°C). 2. NH_4F. 3. MnO_2. 4. H_2(Lindlar cat.). 5. Isomerization.

Scheme 1. Synthesis of the new coumarin analogues of retinal.

The polycyclic aromatic retinal analogues were synthesised by Horner-Wadsworth-Emmons (HWE) olefination of the corresponding polycyclic aldehyde with the C_5-phosphonate, subsequent reduction of the ester with diisobutylaluminium hydride and oxidation of the alcohol to the aldehyde with manganese dioxide. The same procedure (C_5-elongation) was applied to synthesise the desired 1-naphthyl and 9-phenanthryl retinal analogues **4c** and **4e** (Scheme 2), from the pentadienals **3c,e** of the first stage (C_{15}^{R}-retinal analogues). Finally, thermal isomerization in the presence of traces of iodine was used to obtain the most stable all-*E* isomer of the corresponding retinal analogue. The 2-fluorenylretinal **3d** was used as a C_{15}^{R}-retinal analogue.



1. $(EtO)_2P(O)CH_2$-C(CH_3) = CH-$COOCH_3$, NaH / THF. 2. DIBAL-H /-60°C, 30 min/.
3. MnO_2 /2 hrs, r.t./. 4. Isomerization: reflux(1 h) in hexane/EtOAc=1:1, traces I_2, Ar-atm.;

Scheme 2. Synthesis of the new polycyclic aromatic retinal analogues.

All new retinal analogues were characterized by their HRMS, ^{1}H-NMR-, UV- and IR-spectral data.[12]

The newly synthesised retinal analogues were studied in binding experiments with bacterioopsin (Table 1).

The retinal analogues **2a,b** and **4c,e** with polyenic side chains, identical to that of all-*E* retinal, bind to bacteroopsin to form artificial bacteriorhodopsin pigments. These chromoprotein analogues possess a hypsochromic shift of their absorption maxima in comparison with that of the natural bacteriorhodopsin, which is due to the steric and electronic effects of the cyclic component of the chromophore molecule.

Table 1. Spectral Data of the New Retinals and of their Bacteriorhodopsin Analogues.

Compd. No.	λ_{max} *abs*[a]	λ_{max} *SB*[b]	λ_{max} *PSB*[c]	λ_{max} BR_{ag}[d]	*opsin shift*[e]
all-*E* retinal[13]	381 nm	365 nm	440 nm	568 nm	5120 cm^{-1}
13-*cis* retinal[13]	375 nm		440 nm	558 nm	4810 cm^{-1}
2a	391 nm	388 nm	464 nm	478 nm	631 cm^{-1}
2b	401 nm	395 nm	460 nm	497,438,413nm	1619 cm^{-1}
3d	365 nm	353 nm	420 nm	432* nm	*
4c	398 nm	382 nm	460 nm	490 nm	1331 cm^{-1}
4e	399 nm	385 nm	461 nm	499 nm	1651 cm^{-1}

a) registered in methanol. b) SB - Schiff base of the retinal analogues with *n*-butylamine in methanol. c) PSB - Schiff bases, protonated by HCl (g)-saturated methanol. d) bacteriorhodopsin analogues from bleached purple membranes of *Halobacterium salinarium (strain S-9)*. e) OS = [1/ λ_{max} PSB -1/ λ_{max} BR_{ag}] x 10^7 cm^{-1}. * poor pigment formation, only small shoulder at this wavelength.

In summary, new retinal analogues, containing coumarin or polycyclic aromatic fluorophores, were synthesised and their recombination with bacterioopsin was tested. These new retinoids could be successfully used as artificial cofactors in structure-function studies of rhodopsins, the newly discovered members of the bacterial rhodopsins' family[2], and may be applied in the search for molecular electronic devices.[5]

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9. The heterocyclic triphenylphosphonium bromides **1a,b** - precursors for ylide formation in the Wittig condensation - were obtained in a reaction sequence including bromination of the corresponding 6- or 7-methylcoumarins with N-bromosuccinimide (NBS)[10] followed by interaction of the 6- or 7-(bromomethyl)coumarins with triphenylphosphine (yields 90-91%). The method used for bromination of methylcoumarins with NBS, led to reaction products which contain *ca.*10% (dibromomethyl)coumarins in addition to the main products: 6- or 7-(bromomethyl)coumarins. In this case, the synthesis must be modified in order to avoid the presence in the main product of (dibromomethyl)coumarins, which lead to a second phosphonium salt. Thus, the reaction product containing the corresponding bromomethylcoumarin as the main product and (dibromomethyl)coumarin as the by-product was hydrolysed (boiling in water, 4 hrs). Using this procedure, the corresponding bromomethylcoumarin was completely converted to the hydroxymethylcoumarin, but the corresponding (dibromomethyl)coumarin was converted to the formylcoumarin. Then, the corresponding hydroxymethyl- and formylcoumarins were separated by column chromatography (CC). The hydrolysed products have larger differences in their R_f-values in comparison to the original bromomethyl- and (dibromomethyl)coumarins. After separation by CC the corresponding hydroxymethylcoumarin was rebrominated (boiling in conc. HBr, 30 min.) to give 6- or 7-bromomethylcoumarin (96-100%) and was converted by interaction with triphenylphosphine to the desired heterocyclic precursor **1a or 1b**[11] for the Wittig reaction.

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11. ***Data for the heterocyclic precursors in the Wittig condensation:*** **(2-oxo-*2H*-chromen-6-yl)methyltriphenylphosphonium bromide 1a:** ^{1}H-NMR δ ($CDCl_3$)ppm: 5.76 (d, $J_{P,H}$=14.6 Hz, 2H, $C\underline{H}_2P^+$), 6.31 (d, J=9.5 Hz, 1H, H-3), 6.90-8.00 (m, 18H, H-arom), 7.50 (d, J=9.5 Hz, 1H, H-4). ^{31}P-NMR (24.5 ppm). IR (KBr): 689, 695, 725, 747, 837, 850, 909, 998, 1108, 1439 (P-Phenyl), 1570, 1725 (CO, lactone), 2785, 2853, 2880, 2923 (C-H) cm^{-1}. Anal. calc. for $C_{28}H_{22}BrO_2P$ (501.362): Br 15.94, P 6.18; found: Br 15.65, P 6.02. Yield: 91 %. M. p.: 335-336°C.

(2-oxo-*2H*-chromen-7-yl)methyltriphenylphosphonium bromide 1b: ^{1}H-NMR δ ($CDCl_3$) ppm: 5.74 (d, $J_{P,H}$=15.3 Hz, 2H, $C\underline{H}_2P^+$), 6.34 (dd, J=9.5 Hz, J=0.9 Hz, 1H, H-3), 6.75 (d, J=1.8 Hz, 1H, H-arom), 7.30 (d, J=7.8 Hz, 1H, H-arom), 7.54 (d, J=7.8 Hz, 1H, H-arom), 7.60-7.90 (m, 16H, H-arom + H-4). ^{31}P-NMR (24.4 ppm). IR (KBr): 690, 720, 729, 744, 1110, 1438 (P-Phenyl), 1613, 1723sh, 1735, 2784, 2858, 2880, 2924 (C-H) cm^{-1}. Anal. calc. for $C_{28}H_{22}BrO_2P$ (501.362): Br 15.94, P 6.18; found: Br 16.02, P 6.08. Yield: 90 %. M. p.: 310°C.

12. ***Data for the retinal analogues:*** **all-*E* 3,7-Dimethyl-9-(2'-oxo-*2'H*-chromen-6-yl)-2,4,6,8-nonatetraenal 2a.** ^{1}H-NMR δ ($CDCl_3$) ppm: 2.11 (s, 3H, CH_3), 2.35 (d, J=1.0 Hz, 3H, CH_3), 6.01 (d, J=8.1 Hz, 1H, H-2), 6.39 (d, J=11.5 Hz, 1H, H-6), 6.44 (d, J=9.5Hz, 1H, H-3' coum), 6.45 (d, J=15.0 Hz, 1H, H-4), 6.71 (d, J=16.0 Hz, 1H, H-9 or H-8), 6.89 (d, J=16 Hz, 1H, H-8 or H-9), 7.14 (dd, J=15.0 Hz, J=11.5 Hz, 1H, H-5), 7.25-7.66 (m,3H, H-arom), 7.70 (d, J=9.5 Hz, 1H, H-4' coum), 10.13 (d, J=8.1 Hz, 1H, CHO). HRMS: exact mass calcd for $C_{20}H_{18}O_3$ [M+] 306. 1256, found 306. 1255. UV (CH_3OH) λ_{max}: 391 nm. M. p.: 181-183°C. IR (KBr): 1568, 1639, 1720 (CO-lactone), 2859, 2917 (C-H) cm^{-1}.

all-*E* 3,7-Dimethyl-9-(2'-oxo-*2'H*-chromen-7-yl)-2,4,6,8-nonatetraenal 2b. ^{1}H-NMR δ ($CDCl_3$) ppm: 2.12 (d, J=1.0 Hz, 3H, CH_3), 2.35 (d, J=1.0 Hz, 3H, CH_3), 6.03 (d, J=8.1 Hz, 1H, H-2), 6.39 (d, J=9.5 Hz, 1H, H-3' coum), 6.48 (d, J=11.7 Hz, 1H, H-6), 6.49 (d, J=15.0 Hz, 1H, H-4), 6.71 (d, J=15.9 Hz, 1H, H-9 or H-8), 7.00 (d, J=15.9 Hz, 1H, H-8 or H-9), 7.15 (dd, J=15.0 Hz, J=11.7 Hz, 1H, H-5), 7.33-7.70 (m, 4H, H-arom + H-4' coum), 10.14 (d, J=8.1 Hz, 1H, CHO). HRMS: exact mass calcd for $C_{20}H_{18}O_3$ [M+] 306. 1256, found 306. 1260. UV (CH_3OH) λ_{max}: 401, 420(sh) nm. M. p.: 189°C (dec.). IR (film): 1610, 1656, 1730 (CO-lactone), 2855, 2928 (C-H) cm^{-1}.

all-*E* 3-Methyl-5-(2-fluorenyl)-2,4-pentadienal 3d. ^{1}H-NMR δ ($CDCl_3$) ppm: 2.41 (d, J=1.0 Hz, 3H, CH_3), 3.93 (s, 2H, CH_2), 6.11 (d, J=8.1 Hz, 1H, H-2), 6.96 (d, J=16.1 Hz, 1H, H-4 or H-5), 7.17 (d, J=16.1 Hz, 1H, H-5 or H-4), 7.28-7.85 (m, 7H, H-arom), 10.17 (d, J=8.1 Hz, CHO). Anal. calc. for $C_{19}H_{16}O$ (260.12): C 87.66, H 6.20; found: C 87.38, H 6.22. UV (CH_3OH) λ_{max}: 365, 208 nm. M. p.: 159-161°C. IR (KBr): 730, 763, 837, 956, 1594, 1613, 1653, 2847 (C-H) cm^{-1}.

all-*E* 3,7-Dimethyl-9-(1-naphthyl)-2,4,6,8-nonatetraenal 4c. ^{1}H-NMR δ ($CDCl_3$) ppm: 2.23 (d, J=0.7 Hz, 3H, CH_3), 2.36 (d, J=1 Hz, 3H, CH_3), 6.02 (d, J=8 Hz, 1H, H-2), 6.42 (d, J=11 Hz, 1H, H-6), 6.46 (d, J=15 Hz, 1H, H-4), 6.97 (d, J=16 Hz, 1H, H-9 or H-8), 7.20 (d, J=15 Hz, J=11 Hz, 1H, H-5), 7.40-8.20 (m, 8H, H-arom + H-8 or H-9), 10.13 (d, J=8 Hz, 1H, CHO) HRMS: exact mass calcd for $C_{21}H_{20}O$ [M+] 288. 1514, found 288.1519. UV (CH_3OH) λ_{max}: 398, 219 nm. M. p.: 140-142°C. IR (film): 1575, 1607, 1654, 2851, 2922, 3045 (C-H) cm^{-1}.

all-*E* 3,7-Dimethyl-9-(9-phenanthryl)-2,4,6,8-nonatetraenal 4e. ^{1}H-NMR δ ($CDCl_3$) ppm: 2.25 (s, 3H, CH_3), 2.37 (d, J=1 Hz, 3H, CH_3), 6.02 (d, J=8 Hz, 1H, H-2), 6.45 (d, J=11 Hz, 1H, H-6), 6.46 (d, J=15 Hz, 1H, H-4), 7.05 (d, J=16 Hz, 1H, H-9 or H-8), 7.20 (dd, J=15 Hz, J=11 Hz, 1H, H-5), 7.51 (d, J=16 Hz, 1H, H-8 or H-9), 7.55-8.80 (m, 9H, H-arom), 10.14 (d, J=8 Hz, 1H, CHO). HRMS: exact mass calcd for $C_{25}H_{22}O$ [M+] 338. 1671, found 338. 1674. UV (CH_3OH) λ_{max}: 399, 249, 212 nm. M. p.: 150°C (dec.). IR (KBr): 960, 1115, 1156, 1552, 1586, 1646, 2850, 2919 (C-H) cm^{-1}.

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