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Alkylated Azulenic Retinal and Bacteriorhodopsin Analogs

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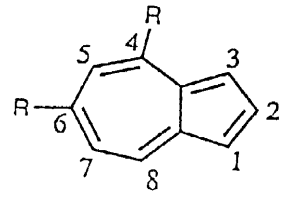
Abstract. The syntheses of 10 ring alkylated azulenedienal analogs of retinal are reported. They form bacteriorhodopsin pigment analogs at varying efficiency, in fact in a manner suggesting a possible t-butyl recognition site within the binding pocket.

Recently, the preparation of several azulenic and guaiazulenic retinal analogs was reported.[1] All of them yielded red-shifted bacteriorhodopsin (bR) analogs, some with the absorption maxima extended beyond 800 nm. Also, we noticed that the relative rates of pigment formation between the two series are different, suggesting a possible effect of alkyl substitution on the ring on pigment formation. In this paper we report a series of alkylated azulenic retinal analogs, other than those derived from the commercially available azulene and guaiazulene, and results of their interaction with bacterioopsin (bO).



First, the alkylated azulenes were prepared in the following manner. 6-Alkylazulenes were prepared from 4-alkylpyridines following the Hafner method modified by Koenig.[2] 4-Alkylazulenes were prepared by the addition of corresponding alkyl lithium where 4-alkylation was favored over 6-alkylation.[3a] The only exception being t-butyl lithium, where a low yield of 6-butyl azulene was obtained as the sole isolable product. This preference could be a reflection of a kinetic preference for the more open site by the bulky t-butyl group in agreement with an early study of nucleophilic substitution reactions involving azulenes.[3b] Thus, we prepared the following alkylated azulenes: 4-methyl, 4,6,8-trimethyl, 6-methyl, 6-isopropyl and 6-t-butyl. For the synthesis of analog **5** the 1-methyl group of guaiazulene was oxidized with DDQ to a formyl group using the procedure of Takase et al.[4]

Acylation at the 1-position of these alkylated azulenes, followed by C_5-chain extension reactions, typical of those employed in retinoid synthesis,[5] led to the azulenedienal analogs **3-8**.

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(We chose the dienal series because of their generally faster rate of formation of bR pigment analogs.) Ring fluorination of 6-isopropylazulene with Selectfluor™ gave 1-fluoro-6-isopropylazulene[6] which after the same sequence of chain extension reactions gave the retinal analog **9**. Chain fluorinated analog **10** was obtained by the use of C_2-fluorophosphonate reagent used earlier in fluororetinal synthesis.[7] Appended at the end of this paper (see ref. 13) are the characteristic H NMR data of analogs **3-10** (**1** and **2** are known compounds).[1]

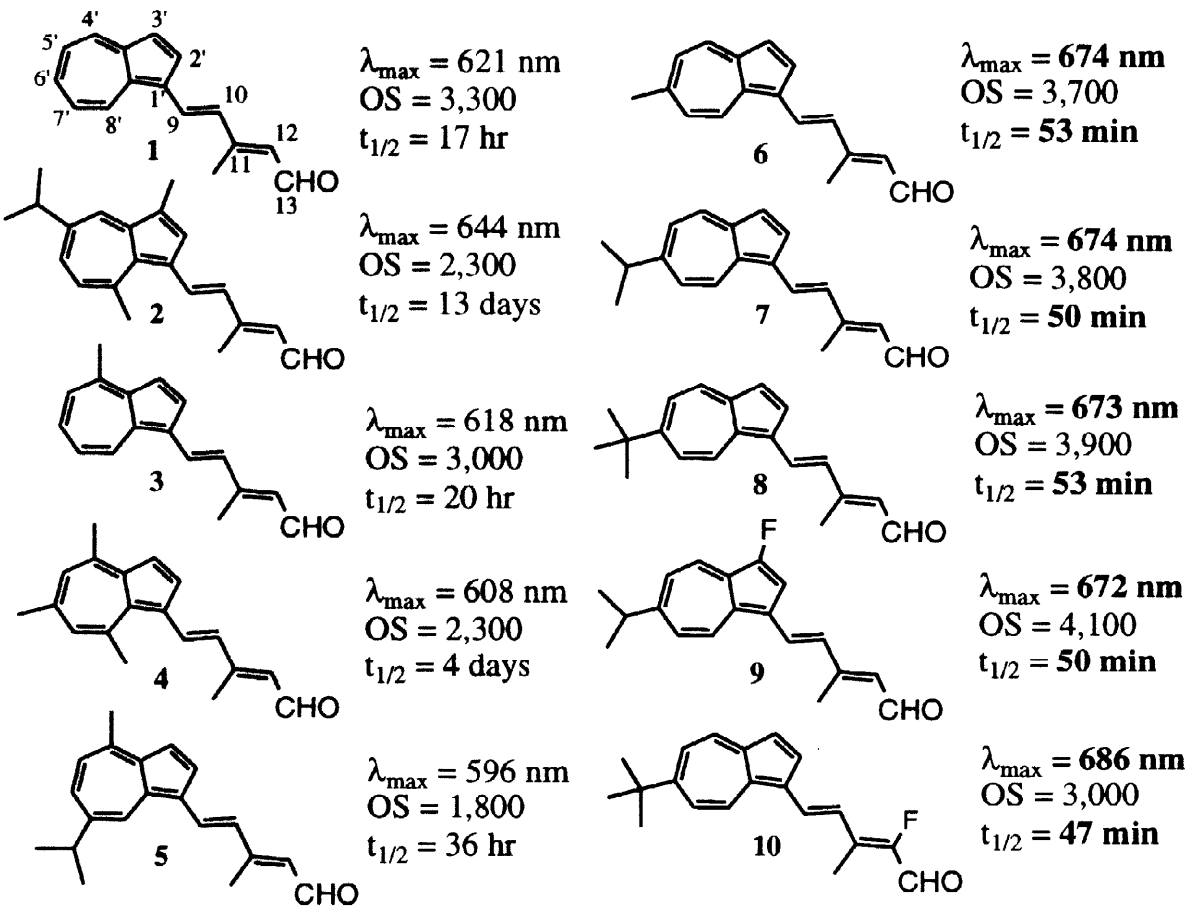

Figure 1. Azulenedienic retinal analogs **1-10**, their absorption maxima, opsin shift (OS in cm^{-1}) and time needed for 50% conversion to the bR pigment analogs.

Results of binding interaction of analogs **1-10** with bO are partially listed in Figure 1. It is interesting to note the significant variations in pigment absorption maxima and rates of pigment formation among these structurally similar analogs. In fact, there appears to be a correlation between the two quantities: those reacted faster yielded more red-shifted pigments. When these trends are compared to the substitution pattern of these alkylated analogs, it becomes clear that all 6-alkylated analogs yielded a more red-shifted pigment more rapidly (half life less than an hour while others require considerably longer time, some requiring days before half completion to the pigment analog). The 6-alklyl analogs also share similarly larger opsin shift values approaching that of native bR (5000 cm^{-1}),[9] suggesting similar protein substrate interactions. The implied

better fit is also reflected on their higher stability (by an average factor of 3) than other alkylated analogs toward external reagents such as hydroxyl amine. Due to their improved stability and optical properties the pigment from the 6-alkyl azulene analogs have the potential for protein based photonics applications.[10]

It is interesting to note that the bulky 6-t-butyl analog has the same half life in pigment formation as the corresponding methyl and isopropyl analogs (in fact, slightly shorter) even though all these are longer than that of all-trans retinal (23 min). In an attempt to identify structural similarity between 6-t-butyl azulenic analog and all trans retinal, we carried out computer matching by overlaying the two energy-minimized molecules in a way to allow close overlap of both the t-butyl centers (C-1 for all-trans retinal) and the aldehyde carbons. The computer simulated figures (Figure 2) indeed show a similar length between the two molecules. However, a more surprising feature is that a close match exists only for the 6-S-cis conformer of the retinal with analog **8** and not the commonly accepted 6-S-trans conformer in bR.[8] Perhaps this feature is partially reflected in the diminished rates of pigment formation for these azulenic analogs relative to native bR. We are currently pursuing molecular modelling studies of the analog pigments to understand the physical significance of the correlation between the rate of pigment formation and the absorption maximum.

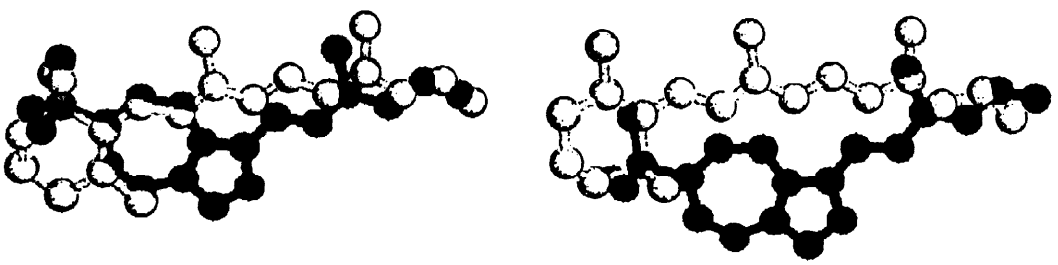

Figure 2. Overlay of energy minimized structures (Hyperchem 3, AM1) of 6-t-butylazulene dienal, **8** (solid circles), on 6-s-cis retinal (left) and 6-s-trans retinal (right).

For the two fluoroanalogs, they again reflect the earlier findings with fluoro-rhodopsin[11] or bR[12] in that the 14F derivatives, where the electron withdrawing F-atom is located more closely to the charged iminium nitrogen in the pigment, are more red-shifted than those with the F-atom relocated to a more remote site on the conjugated system.[14]

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13. H-NMR data (in $CDCl_3$ at 300 MHz) for compounds **3-10** is given below. **3**. δ 10.15 (C13-H, d, 8.4 Hz, 1H), 8.5 (C8'-H, d, 9.9 Hz, 1H), 8.18 (C3'-H, d, 4.5 Hz, 1H), 7.72 (C9-H, d, 15.6 Hz, 1H), 7.6 (C7'-H, t, 9.8 Hz), 7.53 (C6'-H, t, 10.5 Hz, 1H), 7.48 (C2'-H, d, 4.5 Hz, 1H), 7.22 (C5'-H, d, 9.6 Hz), 7.00 (C10-H, d, 15.6 Hz, 1H), 6.11 (C12-H, d, 8.4 Hz, 1H), 2.9 (C4'-CH_3, s, 3H), 2.49 (C11-CH_3, s, 3H) ppm. **4**. δ 10.13 (C13-H, d, 8.1 Hz, 1H), 8.05 (C9-H, d, 15.6 Hz, 1H), 7.93 (C3'-H, d, 3.9 Hz, 1H), 7.34 (C2'-H, d, 4 Hz, 1H), 7.05 (C5'-H & C7'-H, s, 2H), 6.8 (C10-H, d, 15.6 Hz, 1H), 6.08 (C12-H, d, 8.1 Hz, 1H), 3.07 (C8'-CH_3, s, 3H), 2.84 (C4'-CH_3, s, 3H), 2.58 (C6'-CH_3, s, 3H), 2.24 (C11-CH_3, s,3H) ppm. **5**. δ 10.26 (C13-H, d, 8.1 Hz, 1H), 8.35 (C8'-H, d, 1.8 Hz, 1H), 8.08 (C3'-H, d, 4.2 Hz, 1H), 7.84 (C9-H, d, 15 Hz, 1H), 7.45 (C6'-H, dd, 10 Hz & 1.8 Hz, 1H), 7. 32 (C2'-H, d, 4.2 Hz, 1H) 7.13 (C5'-H, d, 10.8 Hz, 1H), 7.09 (C10-H, d, 15 Hz, 1H), 6.1 (C12-H, d, 8.1 Hz, 1H), 3.26 (CH_3-CH-CH_3, m, 1H), 2.62 (C11-CH_3, s, 3H), 1.3 (CH_3-CH-CH_3, d, 7 Hz, 6H) ppm. **6**. δ 10.14 (C13-H, d, 8.4 Hz, 1H), 8.32 (C8'-H, d, 10.5 Hz, 1H), 8.12 (C4'-H, d, 10.1 Hz, 1H), 8.1 (C3'-H, d, 4.0 Hz, 1H), 7.66 (C9-H, d, 15.6 Hz, 1H), 7.33 (C2'-H, d, 4.0 Hz, 1H), 7.14 (C7'-H, d, 10 Hz, 1H), 7.11 (C5'-H, d, 10.3 Hz 1H), 6.97 (C10-H, d, 15.6 Hz, 1H), 6.1 (C12-H, d, 8.4 Hz, 1H), 2.64 (C6'-CH_3, s, 3H), 2.47 (C11-CH_3, s, 3H) ppm. **7**. δ 10.07 (C13-H, d, 8.1 Hz, 1H), 8.34 (C8'-H, d, 10.2 Hz 1H), 8.11 (C4'-H, d, 10Hz, 1H), 8.03 (C3'-H, d, 4.1 Hz 1H), 7.6 (C9-H, d, 15.6 Hz, 1H), 7.25 (C2'-H, d, 4.1 Hz, 1H), 7.11 (C7'-H, d, 10.2 Hz, 1H), 7.07 (C5'-H, d, 10 Hz, 1H), 6.88 (C10-H, d, 15.6 Hz, 1H), 6.02 (C12-H, d, 8.1 Hz, 1H), 3.01 (CH_3-CH-CH_3, m, 1H), 2.38 (C11-CH_3, s, 3H), 1.28 (CH_3-CH-CH_3, d, 6.8 Hz, 6H) ppm. **8**. δ 10.15 (C13-H, d, 8.4 Hz, 1H), 8.44 (C8'-H, d, 10.8 Hz, 1H), 8.21 (C4'-H, d, 10.2 Hz, 1H), 8.14 (C3'-H, d, 4.2 Hz, 1H), 7.7 (C9-H, d, 15.6 Hz, 1H), 7.44 (C7'-H, d, 10.8 Hz, 1H), 7.38 (C5'-H, d, 10.5 Hz, 1H), 6.97 (C10-H, d, 15.6 Hz, 1H), 6.10 (C12-H, d, 8.4 Hz, 1H), 2.48 (C11-CH_3, s, 3H), 1.5 (C6-t-butyl, s, 9H) ppm. **9**. δ 10.14 (C13-H, d, 8.4 Hz, 1H), 8.34 (C8'-H, d, 3 Hz & 10.5 Hz, 1H), 8.13 (C7'-H, d, 10 Hz, 1H), 7.65 (C2'-H, s, 1H), 7.63 (C9-H, d, 15.6 Hz, 1H), 7.03 (C7'-H, d, 10Hz, 1H), 7.01 (C5'-H, d, 10Hz, 1H), 6.86 (C10-H, d, 15.6 Hz, 1H), 6.1 (C12-H, d, 8.4 Hz, 1H), 3.01 (CH_3-CH-CH_3, m, 1H), 2.46 (C11-CH_3, s, 3H), 1.3 (CH_3-CH-CH_3, d, 6.9 Hz, 6H) ppm. **10**. δ. 9.86 (C13-H, d, 17.5 Hz, 1H), 8.45 (C8'-H, d, 10.34Hz 1H), 8.22 (C4'-H, d, 10.5 Hz, 1H) 8.2 (C3'-H, d, 4.5 Hz, 1H), 7.73 (C9-H, d, 14.8 Hz, 1H), 7.53 (C2'-H, d, 4.5 Hz, 1H), 7.41 (C7'-H, d, 10.2 Hz, 1H), 7.4 (C10-H, d, 14.8 Hz, 1H), 2.41 (C11-CH_3, d, 3.0 Hz, 3H), 1.48 (C6-t-butyl, s, 9H) ppm.

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