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190. A Study of *N*-Butyl-(all-*trans*-retinylidene)amine and Its Protonated Species by ^{1}H- and ^{13}C-NMR. Spectroscopy[1])

by **Christian Pattaroni** and **Jürgen Lauterwein**

Institut de chimie organique de l'Université, 2, rue de la Barre, CH-1005 Lausanne

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Summary

The *Schiff* base of all-*trans*-retinal was investigated in organic solution by ^{1}H- and ^{13}C-NMR. at high field. Complete assignment of the ^{1}H-NMR. peaks of *N*-butyl-(all-*trans*-retinylidene)amine (**2**) and the *N*-butyl-(all-*trans*-retinylidene)-ammonium ion (**3**) was achieved by INDOR (internuclear double resonance). The vicinal proton coupling constants of the polyene chain show that the π-bond orders remain unchanged in *N*-butyl-(all-*trans*-retinylidene)amine relative to all-*trans*-retinal (**1**), but change towards larger π-delocalization in the *N*-butyl-(all-*trans*-retinylidene)ammonium ion. At $-61°$ only one isomer of *N*-butyl-(all-*trans*-retinylidene)ammonium was observed. This was shown to be *trans* at the imine linkage and independent of the solvent. The trifluoroacetic acid counter-ion can approach the positive charge of the N-atom in the weakly polar solvent dichloromethane but not in the leveling solvent methanol. In dichloromethane the nature of the 1:1 complex is a H-bonded ($O^-...H-N^+$) ion-pair whose rate of breaking and forming is rapid at RT. Strong stabilization of the ion-pair resulted from homo-conjugation with a second molecule of trifluoroacetic acid. Excess of acid efficiently diminished the isomerization rate at the C,N-bond.

Introduction. - The visual response in vertebrates is initiated by the *trans*-isomerization of the pigment 11-*cis*-retinal triggering a series of conformational changes in the apoprotein opsin [1] [2]. In conjunction with an investigation of the conformation of rhodopsin in detergent micelles we have recently performed a NMR. study of the conformation and dynamics of all-*trans*-retinal in organic solution and after incorporation into aqueous dodecyldimethylamine oxide micelles [3]. The ordered structure of the amphiphatic detergent or lipid molecules may better simulate the actual environment of the retinal chromophore [4]. Since the linkage to the retinal in rhodopsin is generally agreed to be *via* the primary amino group of a lysine [5] [6] we have also included in our NMR. studies the *Schiff*'s base of all-*trans*-retinal.

[1]) *Running title:* ^{1}H- and ^{13}C-NMR. of visual pigments. *Key words:* All-*trans*-retinal, *N*-butyl-(all-*trans*-retinylidene)amine, ^{1}H-NMR., ^{13}C-NMR.

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In the present work we give a complete assignment of the ^{1}H- and ^{13}C-NMR. spectra of the *Schiff* base of all-*trans*-retinal (ATR, **1**), *N*-butyl-(all-*trans*-retinylidene)amine (BATRA, **2**) and its protonated form, *N*-butyl-(all-*trans*-retinylidene)-ammonium ion (BATRAH$^+$, **3**). We refer to earlier literature [7–10] and discuss the chemical shifts and proton coupling constants in terms of alterations in the π-electronic charge distribution and bond orders in the polyene chain. Emphasis is given also to establish the energetically favourable configuration of **3** at the C,N-double bond and to discuss the influence of the solvent and of the counter negative charge on this positively charged cation.

Results. – *^{1}H-NMR. of all-*trans*-retinal* (**1**) *and* N*-butyl-(all-*trans*-retinylidene)-amine* (**2**). *Figure 1* shows the ^{1}H-NMR. spectra of the olefinic proton region of ATR (**1**) and BATRA (**2**) in $CDCl_3$. Using spin-decoupling and INDOR (internuclear double resonance) techniques all the protons of **1** and **2** have been assigned. The proton chemical shifts are listed in *Table 1* (see *Scheme 1* for the numbering). The proton-proton vicinal coupling constants are collected in *Table 2*.

^{1}H-NMR. spectra of **1** have been reported in $CDCl_3$ and $(CD_3)_2CO$ [13] [14]. Since no complete assignment of the ^{1}H-NMR. spectra of BATRA (**2**) has been previously made, we give a detailed description. The resonances of H–C(11) and H–C(15) are assigned unambiguously from their characteristic spin-spin splittings

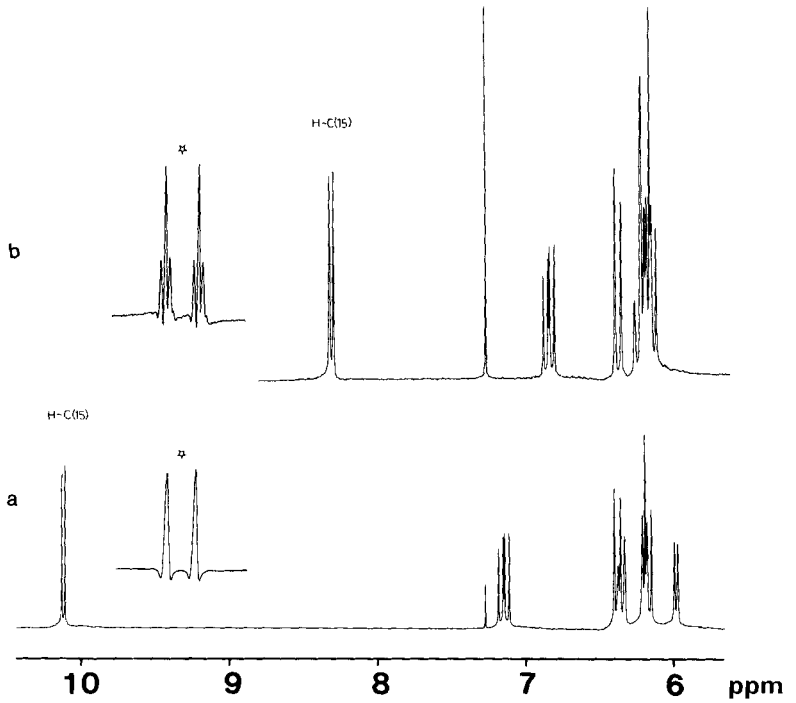


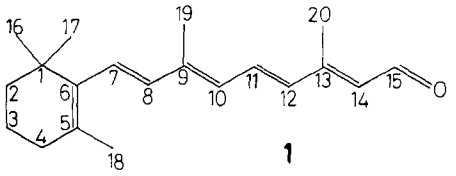
Fig. 1. *360-MHz-^{1}H-NMR. spectra of the resonances of the olefinic protons of (a) all-*trans*-retinal* (**1**) *and (b)* N*-butyl-(all-*trans*-retinylidene)amine* (**2**) *in $CDCl_3$ at 25°*. The H–C(15) resonance is also shown on an expanded scale after resolution enhancement (✲).

Table 1. *^{1}H-NMR. chemical shift data*[a]) *of all*-trans-*retinal* (**1**), N-*butyl-(all*-trans-*retinylidene)amine* (**2**) *and* N-*butyl-(all*-trans-*retinylidene)ammonium ion* (**3**)

Assignments	**1**	**2**		**3**				
	$CDCl_3$ 25°	$CDCl_3$ 25°	CD_2Cl_2 −61°	CD_2Cl_2, −61° b)	c)	d)	CD_3OD, −61° b)	c)
2 H–C(2)	1.476[e])	1.47	1.46	1.47	1.47	1.47	1.49	1.49
2 H–C(3)	1.622[e])	1.62	[f])	1.61	1.61	1.61	1.68	1.68
2 H–C(4)	2.032[e])	2.020	2.01	2.046	2.052	2.055	2.054	2.054
H–C(7)	6.342	6.224	6.238	6.456	6.506	6.522	6.484	6.490
H–C(8)	6.166	6.132	6.151	6.230	6.247	6.259	6.239	6.244
H–C(10)	6.186	6.156	6.196	6.294	6.307	6.324	6.352	6.357
H–C(11)	7.139	6.832	6.899	7.369	7.444	7.473	7.571	7.577
H–C(12)	6.371	6.364	6.425	6.589	6.566	6.579	6.643	6.644
H–C(14)	5.974	6.200	6.161	6.862	6.545	6.500	6.376	6.368
H–C(15)	10.112	8.301	8.336	8.269	8.338	8.288	8.966	8.964
3 H–C(16) 3 H–C(17)	1.036	1.027	1.022	1.034	1.040	1.041	1.039	1.038
3 H–C(18)	1.720	1.710	1.736	1.754	1.757	1.760	1.737	1.736
3 H–C(19)	2.029	1.983	2.014	2.096	2.112	2.123	2.110	2.109
3 H–C(20)	2.331	2.089	2.114	2.302	2.328	2.339	2.383	2.384
2 H–C(1′)		3.500	3.487	3.700	3.700	3.704	3.711	3.709
2 H–C(2′)		1.63	[f])	1.75	1.72	1.71	1.65	1.65
2 H–C(3′)		1.357	1.292	1.396	1.384	1.382	1.400	1.399
3 H–C(4′)		0.934	0.919	0.961	0.950	0.951	0.991	0.990
H–N=C(15)				14.841	12.137	11.500	[g])	[g])

[a]) In ppm; concentrations were 0.02M. [b]) 1:1 TFA addition salt. [c]) With 2 mol-equiv. of TFA. [d]) With 3 mol-equiv. of TFA. [e]) From spectral simulation (to be published). [f]) Overlapping *m*. [g]) Not observable.

Scheme 1



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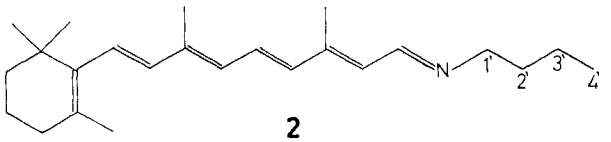

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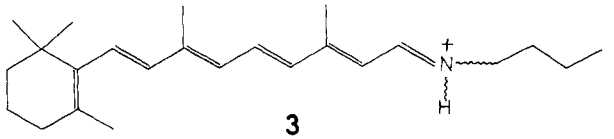


3

(Fig. 1b). H–C(11) occurs as a doublet of doublets; H–C(15) occurs as a doublet of triplets (long-range coupling of 1.2 Hz from the 2 H–C(1′) protons of butylamine). Then, applying the INDOR technique *(Fig. 2)* and saturating a transition of H–C(15) led to the progressive and regressive lines for H–C(14) *(Fig. 2a).* Saturation of the transitions of H–C(11) gave the positions of the H–C(10) and

Table 2. *H,H-coupling constants (Hz) of all-*trans*-retinal* (**1**), N-*butyl-(all-*trans*-retinylidene)amine* (**2**) *and* N-*butyl-(all-*trans*-retinylidene)ammonium ion* (**3**)

	1	**2**		**3**				
	$CDCl_3$ 25°	$CDCl_3$ 25°	CD_2Cl_2 −61°	CD_2Cl_2, −61° [a]	[b]	[c]	CD_3OD, −61° [a]	[b]
H–C(14), H–C(15)	8.0	9.7	9.7	11.1	11.4	11.3	11.1	11.0
H–C(11), H–C(12)	15.0	15.2	15.3	14.7	14.6	14.9	14.8	14.7
H–C(10), H–C(11)	11.5	11.3	11.3	11.8	12.0	11.8	11.8	11.8
H–C(7), H–C(8)	15.9	16.0	16.1	15.8	15.9	15.8	15.8	15.8
H–C(15), H–N=C(15)				14.3	14.9	15.3	[d]	[d]
H–N=C(15), 2 H–C(1′)				6.4	6.5	6.4	[d]	[d]
2 H–C(1′), 2 H–C(2′)		7.0	6.9	6.8	7.0	7.0	6.9	7.0

[a]) 1:1 TFA addition salt. [b]) With 2 mol-equiv. of TFA. [c]) With 3 mol-equiv. of TFA. [d]) Not observable.

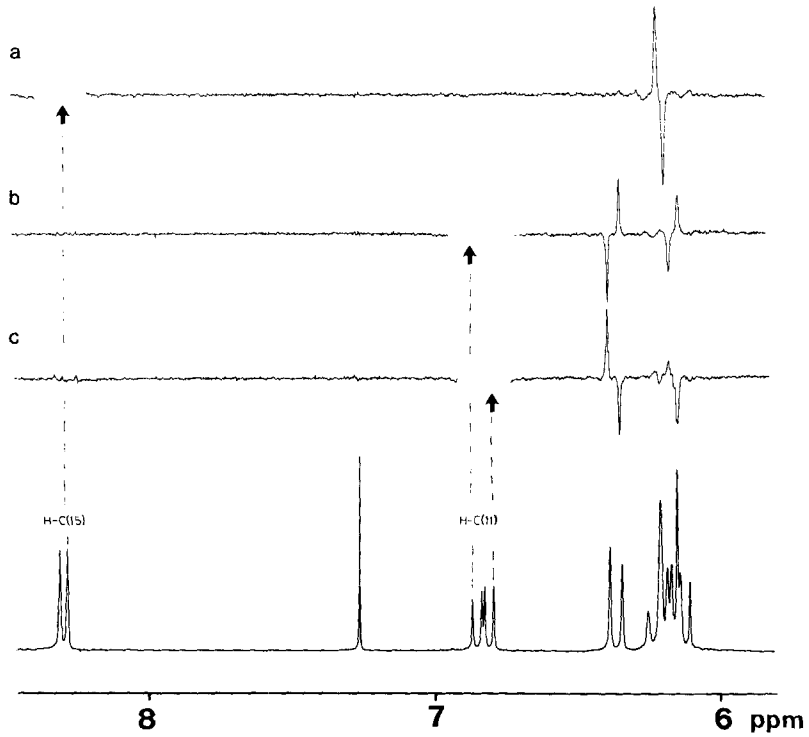


Fig. 2. *^{1}H-INDOR difference spectra a–c of the olefinic region of* N-*butyl-(all-*trans*-retinylidene)amine* (**2**) *in $CDCl_3$ at 25°.* Arrows indicate the individual transitions presaturated by $\gamma B_2 \sim 1$ Hz for 2 s. The bottom spectrum corresponds to that recorded in off-resonance.

H–C(12) resonances (*Fig. 2b* and *2c*). Individual assignment of H–C(10) and H–C(12) was made from the coupling of 1.3 Hz between H–C(10) and 3 H–C(19) observed as a splitting of the methyl resonance. The residual *AB*-system corresponds to the protons H–C(7) and H–C(8), with H–C(7) at higher frequency as shown by its homoallylic coupling with 3 H–C(18) [15]. The butylamine resonances satisfied the condition of 1:1 stoichiometry with the retinal. Their chemical shifts increase with the proximity of the protons to the imine group. Saturating the H–C(15) proton gave rise to a nuclear *Overhauser* enhancement of the 2 H–C(1′) protons comparable to that of the 3 H–C(20) protons. Applying the centroid model [14] for a freely rotating methylene group we estimated the *distance* between the H–C(15) and 2 H–C(1′) protons: about 3 Å for the *trans*-isomer with respect to the C,N-bond and 4 Å for the *cis*. Comparison with the distance of about 3 Å between H–C(15) and 3 H–C(20) confirmed therefore the existence of the *trans*-isomer. The assignment of the methyl groups of **2** were achieved from nuclear

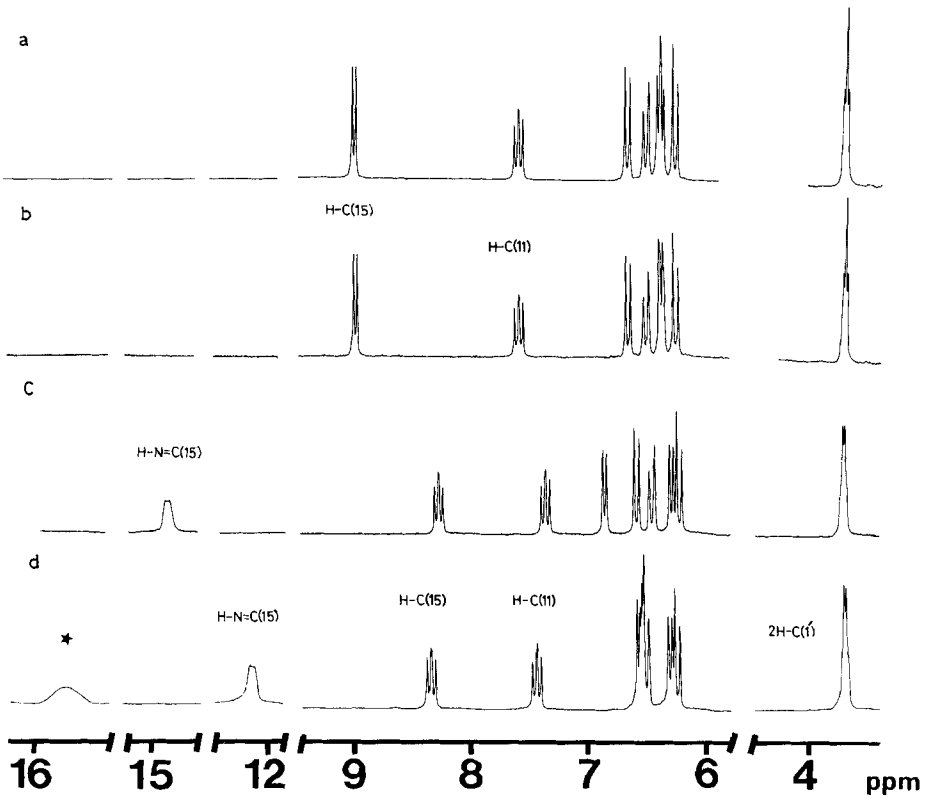


Fig. 3. *The ^{1}H-NMR. spectra at 360 MHz of* N-*butyl-(all-*trans-*retinylidene)ammonium ion* (**3**) *at* $-61°$; (a) **2**/TFA 1:1 and (b) **3**/TFA 1:2 in CD_3OD; (c) **2**/TFA 1:1 and (d) **2**/TFA 1:2 in CD_2Cl_2. The resonances of the olefinic proton region and of the 2 H–C(1′) protons are shown. In spectra (c) and (d) the resonance of the iminium proton H–N=C(15) is also seen. The resonance in (d) marked by ✱ arises from free TFA proton.

Overhauser effects observed at the olefinic protons with close proximity in space. They were in agreement with those observed in all-*trans*-retinal [14].

^{1}H-NMR. of N-*butyl-(all-*trans-*retinylidene)ammonium ion* (**3**). The ^{1}H-NMR. spectrum of the 1:1 trifluoroacetic acid (TFA) addition salt (**3**) of BATRA (**2**) was recorded in CD_2Cl_2 and CD_3OD at $-61°$, $-53°$, $-40°$, $-17°$, $-3°$ and 24°. All resonances were assigned from spin-coupling patterns and by double resonance. No appreciable changes of the chemical shifts were observed in this temperature range. BATRA-solutions in CD_2Cl_2 and CD_3OD were also titrated with TFA up to a five-fold molar excess of the counter-ion and the ^{1}H-NMR. spectra of these solutions determined at $-61°$. All spectra at $-61°$ *(Fig. 3)* consisted of one set of peaks indicating that only one isomer with respect to the imine linkage of the protonated species **3** was present in appreciable concentration. The chemical shifts and coupling constants are listed in *Table 1* and *2*, respectively.

Figures 3a-b show the high frequency proton region of the *Schiff* base in CD_3OD at $-61°$ acidified with one and two mol-equivalents of TFA. BATRA (**2**) can be considered to be completely protonated to **3** by equimolar amounts of TFA since no further chemical shift changes occurred in this solvent when excess of TFA was added (see also *Table 1*). The resonances of H–C(15) and 2 H–C(1′) appear as a doublet and a triplet. No coupling was observed with the iminium proton. The latter must undergo a rapid exchange with the solvent despite the presence of a molar excess of the strongly acidic TFA.

Figure 3c shows the ^{1}H-NMR. spectrum of the synthetically prepared 1:1 complex of TFA with the *Schiff* base in CD_2Cl_2 at $-61°$. It is obvious that the exchange of the iminium proton is slow under these conditions. The H–C(15) resonance occurs as a doublet of doublets and the 2 H–C(1′) resonance in the form of a quartet. The H–N=C(15) proton itself is observed at 14.8 ppm as a broad but structured resonance of relative intensity approaching one. The ^{1}H-NMR. spectrum

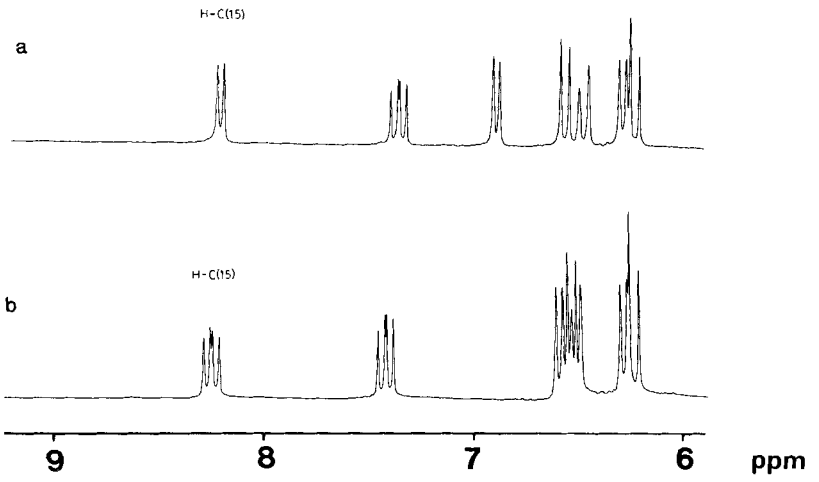


Fig. 4. *The ^{1}H-NMR. spectra at 360 MHz of (a)* **2***/TFA 1:1 and (b)* **2***/TFA 1:2 in CD_2Cl_2 at $-3°$.* Olefinic proton region only.

of the *Schiff* base acidified by two mol-equivalents of TFA *(Fig. 3d)* shows similar, if not identical features.

The coupling constant between the iminium proton and H–C(15) measured in CD_2Cl_2 at $-61°$ was between 14.3 Hz at equimolar TFA concentration and 15.3 Hz with three mol-equivalents of TFA *(Table 2)*. This enabled an attribution of *trans*-configuration at the imine linkage for the only isomer of $BATRAH^+$ (**3**) [16]. For the 1:1 complex in CD_2Cl_2 the splitting of H–C(15) due to the iminium proton disappeared above $-61°$. At $-53°$ the H–C(15) resonance occurred as a broad triplet. At this coalescence temperature the N,H-bond must break and reform at least 14.3 times per second. After a further increase in temperature H–C(15) appeared as a doublet (coupling with H–C(14): 11.1 Hz, *Fig. 4a*).

When BATRA (**2**) was dissolved in CD_2Cl_2 containing two equivalents of TFA, the exchange rate of the iminium proton was considerably reduced and the coupling between H–C(15) and the iminium proton could be observed over the whole temperature range studied *(Fig. 4b)*. Coalescence of the coupling was finally observed at 20°.

Since the coalescence temperatures for the iminium proton splitting of H–C(15) in CD_2Cl_2 varied so remarkably with the molar ratio of acid to base, two different chemical exchange processes must be present. Indeed, *N*-protonation of 0.02 M BATRA (**2**) in CD_2Cl_2 by one equivalent of TFA is incomplete (see below) and the exchange is governed by the protonation equilibrium

$$BATRAH^+ \rightleftharpoons BATRA + H^+ \tag{1}$$

The coalescence temperature observed at 20° in CD_2Cl_2 when BATRA was completely protonated by excess of TFA requires another mechanism by which free TFA molecules can exchange with the 1:1 complex. As a possible explanation we discuss below the formation of a homoconjugate ion [17] of TFA with the BATRA/TFA ion-pair.

Depending on their proximity to the charged N-atom the chemical shifts of the protons and C-atoms exhibited two different phenomena on being titrated with TFA in CD_2Cl_2 at $-61°$. The chemical shifts for most of the protons and C-atoms increased uniformly to give a plateau at 3 mol-equivalents of TFA. However, the high frequency shifts of H–C(12), H–C(14) and the butyl protons no longer increased when a two or three-fold excess of TFA was added but instead reversed towards lower frequencies. At the same time the shielding observed for H–C(15) in the 1:1 TFA addition salt was reduced by excess of TFA. In the rapid exchange limit between the non-protonated and *N*-protonated forms of the *Schiff* base the chemical shift δ at a given proton concentration can be written as

$$\delta = X(\delta_p - \delta_n) + \delta_n \tag{2}$$

where X is the molar fraction of the *N*-protonated form with the chemical shift δ_p, and δ_n is the chemical shift of the non-protonated base. From the observed shifts of H–C(7), H–C(8), H–C(10) and H–C(11) we calculated X = 0.78 for the 1:1 TFA addition salt and X = 0.92 at a two-fold excess of TFA. *Table 3* gives the

Table 3. *^{1}H-NMR. chemical shifts of the* N-*butyl-(all-*trans-*retinylidene)ammonium ion forming a 1:1 complex with TFA.* Influence of the solvent and excess of TFA.

Assignments	δ(ppm)	$\Delta\delta$(ppm)[b]		
	[a]	[c]	[d]	[e]
2 H–C(2)	1.47			−0.02
2 H–C(3)	1.61	[f]		−0.07
2 H–C(4)	2.056	0.046		
H–C(7)	6.518	0.280		0.034
H–C(8)	6.252	0.101		0.013
H–C(10)	6.322	0.127		−0.030
H–C(11)	7.502	0.680	0.029	−0.069
H–C(12)	6.635	0.210	0.056	
H–C(14)	7.060	0.899	0.560	0.684
H–C(15)	8.250	−0.086	−0.038	−0.716
3 H–C(16)	1.037			
3 H–C(17)				
3 H–C(18)	1.759	0.023		0.027
3 H–C(19)	2.119	0.105		
3 H–C(20)	2.355	0.241	0.016	−0.029
2 H–C(1′)	3.760	0.273	0.056	0.051
2 H–C(2′)	[f]	[f]	[f]	[f]
2 H–C(3′)	1.425	0.133	0.043	0.026
3 H–C(4′)	0.973	0.054	0.022	−0.017

[a]) Chemical shift values of the 1:1 TFA addition salt in CD_2Cl_2 at −61° corresponding to a molar fraction of *N*-protonation, X = 0.78, were extrapolated to complete protonation, X = 1. [b]) Changes in chemical shifts < 0.01 ppm are suppressed. [c]) Changes in chemical shifts relative to **2**. [d]) Changes in chemical shifts relative to excess of TFA. [e]) Changes in chemical shifts relative to CD_3OD. [f]) Could not be determined.

chemical shifts of the 1:1 complex after extrapolation for X = 1. Comparison with the chemical shifts in the presence of an excess of TFA revealed a particularly large shielding of H–C(14) by 0.56 ppm, with only minor changes (< 0.06 ppm) for H–C(12), H–C(15) and the butyl protons *(Table 3)*.

Isomerization of the N-*butyl-(all-*trans-*retinylidene)ammonium ion solutions.* When protected from light and stored below 0° the BATRA-solutions containing one or two equivalents of TFA were stable in CD_2Cl_2 and showed no evidence for the formation of new isomers. After warming the solutions to RT., the ^{1}H-NMR. spectra *(Fig. 5)* revealed a rapid isomerization of the 1:1 complex (12% after 5 min). The same isomer was also formed when BATRA-solutions were acidified by two mol-equivalents of TFA, but this took considerably longer (12% after 8 h). Isomerization of **3** in CD_3OD occurred rapidly at RT. (14% after 5 min) and was independent of the TFA equivalents added.

Allowing isomerization of the BATRA/TFA 1:2 sample in CD_2Cl_2 at RT. and cooling to −10° permitted measurement of the coupling of H(15) with HN=C(15) in the newly formed isomer *(Fig. 5c)*. The value of 9.9 Hz implies that the imine linkage of this protonated *Schiff* base has the *cis*-configuration at the C,N-double bond. *Table 4* compares some characteristic chemical shifts and coupling constants obtained for the *trans* and *cis* isomers of **3** in CD_2Cl_2 and CD_3OD at 25°.

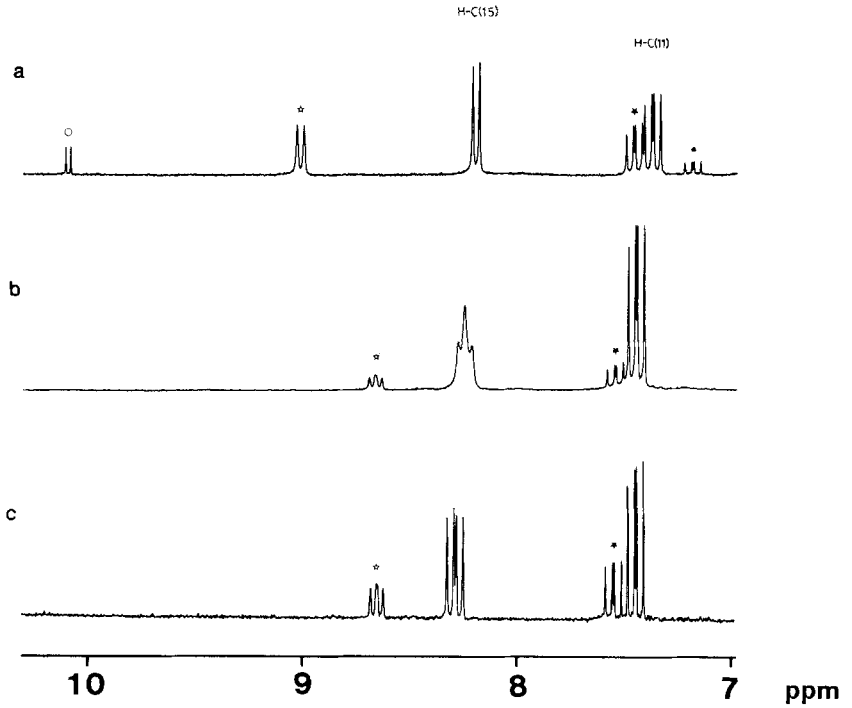


Fig. 5. *Partial isomerization of the* N-*butyl-(all-*trans-*retinylidene)ammonium ion in* CD_2Cl_2. ^{1}H-NMR. spectra of the H–C(11) and H–C(15) resonances after 8 h at 25°, (a) **2**/TFA 1:1; (b) **2**/TFA 1:2. ✶ and ✡ indicate the newly formed resonances of the isomer with the *cis*-configuration at the aldimine linkage. Slow hydrolysis of the protonated *Schiff* base due to traces of residual water also produced some all-*trans*-retinal, the resonances of which are marked by ● and ○. (c) Spectrum of the **2**/TFA 1:2 solution after cooling to −10°. Spectral resolution in (c) was improved by multiplication of the free induction decay by a gaussian function [29].

When BATRAH$^+$ (**3**) was dissolved in CD_3OD, neither the iminium proton nor its coupling with H–C(15) could be observed *(Fig. 3a–b)*. Nevertheless we can assign the isomers in CD_3OD by analysis of the long-range coupling between H–C(15) and the 2 butyl protons at C(1′). This coupling was not observable (<0.8 Hz) in the spectra of the originally prepared **3** in CD_2Cl_2 or in CD_3OD. It could however be measured for the isomer at RT. (1.4 Hz in both solvents). Assuming that there is no large solvent effect on this coupling constant, the isomer obtained from protonation at low temperature must be *trans* independent of the solvent.

*^{13}C-NMR. of all-*trans-*retinal* (**1**), N-*butyl-(all-*trans-*retinylidene)amine* (**2**) *and* N-*butyl-(all-*trans-*retinylidene)ammonium ion* (**3**). The ^{13}C-NMR. spectra of ATR (**1**) were recorded in $(CD_3)_2CO$ and CD_2Cl_2 at 31° and −10° and those of BATRAH$^+$ (**3**) with one or more mol-equivalents of TFA in CD_2Cl_2 at −10°. *Figure 6* illustrates the chemical shift changes of the olefinic C-atoms upon protonation of the

Table 4. *^{1}H-NMR. parameters*[a]) *of the protonated* Schiff *base* **3** *exhibiting either* cis *or* trans *configuration at the C, N-double bond*

	trans				*cis*			
	CD_2Cl_2		CD_3OD		CD_2Cl_2		CD_3OD	
	[b])	[c])	[b])	[c])	[b])	[c])	[b])	[c])
Chemical shifts (ppm)								
H–C(15)	8.189	8.242	8.842	8.838	9.012	8.657	8.649	8.647
H–C(11)	7.362	7.439	7.562	7.568	7.447	7.536	7.658	7.661
2 H–C(1′)	3.665	3.668	3.723	3.727	3.624	3.620	3.714	3.720
Coupling constants (Hz)								
H–C(14), H–C(15)	11.1	11.4[e])	11.2	11.2	11.7	11.7[e])	11.6	11.6
H–C(11), H–C(12)	14.9	14.6	14.8	14.8	14.6	14.6	14.8	14.8
H–C(10), H–C(11)	11.7	11.7	11.6	11.6	11.6	11.7	11.6	11.6
H–C(15), H–N=C(15)	[d])	15.3[e])	[f])	[f])	[d])	9.9[e])	[f])	[f])
2 H–C(1′), 2 H–C(2′)	7.3	7.0	7.2	7.2	7.3	7.0	7.2	7.2

[a]) At 25°; total concentrations of the isomer mixtures were 0.02 M. [b]) 1:1 TFA addition salt. [c]) With 2 mol-equiv. of TFA. [d]) Not observable at +20°. [e]) Measured at −10°. [f]) Not observable at −61°.

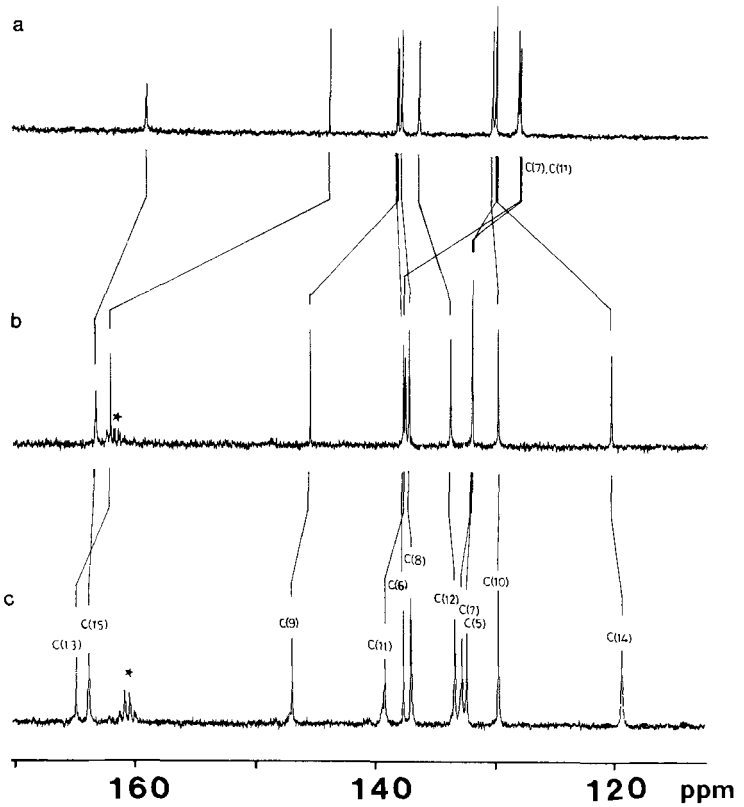


Fig. 6. *The ^{13}C-NMR. spectra at 90.5 MHz of the olefinic region of (a)* N*-butyl-(all-*trans*-retinylidene)-amine, (b)* N*-butyl-(all-*trans*-retinylidene)ammonium ion,* **2***/TFA 1:1 and (c)* **2***/TFA 1:2 in CD_2Cl_2 at −10°.* The quadruplet marked by ✱ in spectra (b) and (c) is from the acidic carbon of TFA.

Schiff base. Only one isomer with respect to the protonation site was observed which, according to ^{1}H-NMR., has *trans*-configuration at the C, N-double bond.

Proton off-resonance decoupling was used to assign the ^{13}C-resonances. Furthermore, proton-coupled ^{13}C-NMR. spectra enabled classification of the olefinic proton-bearing C-atoms. Assignment depended on the number of protons in the *meta*-position and their long-range couplings (results to be published). Our results are in agreement with those made earlier [8] [9] except that assignments of C(7) and C(12) of **3** have to be inversed in [9]. Assignments of the quaternary ^{13}C-resonances in **1** and **2** are based on previous data [7] [8] and were extended to **3** by chemical shift correlation on titration with TFA. The methyl and methylene ^{13}C-resonances were readily assigned by proton decoupling. Differentiation between the butyl C-atoms C(2′) and C(3′) and the ring C-atoms C(1), C(2), C(3) and C(4) was easy since the latter were practically insensitive to the formation of the *Schiff* base linkage or its protonation.

The chemical shifts and assignments of individual C-atoms are given in *Table 5*. In CD_2Cl_2 at $-10°$, at the concentration used for ^{13}C-NMR., the *Schiff* base was completely protonated at a twofold excess of TFA. Then, applying equation 2 a

Table 5. *^{13}C-NMR. chemical shift data*[a]) *of all*-trans-*retinal* (**1**), N-*butyl-(all*-trans-*retinylidene)amine* (**2**), *and* N-*butyl-(all*-trans-*retinylidene)ammonium ion* (**3**)

Assignments	**1**		**2**		**3**	
	$(CD_3)_2CO$ 31°	CD_2Cl_2	CD_2Cl_2 31°	$-10°$	CD_2Cl_2, $-10°$ [b])	[c])
C(1)	34.92	34.64	34.62	34.56	34.54	34.54
C(2)	40.42	40.11	40.13	40.00	39.83	39.85
C(3)	19.91	19.65	19.71	19.63	19.47	19.43
C(4)	33.66	33.55	33.51	33.45	33.58	33.64
C(5)	130.62	130.82	130.09	130.08	132.06	132.52
C(6)	138.58	138.10	138.23[d])	138.13[d])	137.80	137.80
C(7)	129.83	129.34	128.10[e])	128.05[e])	132.06	132.92
C(8)	138.34	137.56	137.94	137.91	137.31	137.16
C(9)	141.45	141.64	138.31[d])	138.31[d])	145.61	147.09
C(10)	130.75	130.08	130.39	130.37	129.01	129.88
C(11)	133.29	132.85	128.23[e])	128.15[e])	137.64	139.31
C(12)	135.90	134.94	136.50	136.44	133.89	133.53
C(13)	155.15	155.03	144.00	144.03	162.23	165.08
C(14)	129.83	129.77	130.12	130.08	120.37	119.50
C(15)	191.20	191.15	159.30	159.32	163.47	164.01
C(16) C(17)	29.30	29.13	29.14	29.09	29.08	29.06
C(18)	21.92	21.86	21.86	21.89	22.05	22.00
C(19)	12.98	13.13	12.94	12.94	13.34	13.39
C(20)	13.05	13.30	13.14	13.13	14.21	14.35
C(1′)			62.24	62.25	53.10	52.85
C(2′)			33.74	33.68	31.59	31.41
C(3′)			20.92	20.90	20.14	19.94
C(4′)			14.07	14.10	13.77	13.59

[a]) In ppm; concentrations were 0.05 M. [b]) 1:1 TFA addition salt. [c]) With 3 mol-equiv. of TFA. [d])[e]) Assignments may be interchanged.

molar fraction of X = 0.85 of **3** was determined for the 1:1 TFA addition salt. No additional chemical shifts due to the varying number of TFA equivalents were observed for the carbon resonances.

UV. spectra. Visible and UV. spectra were recorded in $CHCl_3$ at 25° (**1**, **2**) and −5° (**2**, **3**) for identification purposes only [18] [19]. The transition from ATR (**1**) to BATRA (**2**) was reflected by a hypsochromic shift of λ_{max} from 390 to 367 nm and an increase of the molar extinction coefficient of *ca.* 11000 cm^{-1} mol^{-1}. Protonation of the N-atom of the *Schiff* base induced the characteristically large bathochromic shift of λ_{max} [20]. The spectra of a solution of BATRA containing either one or two mol-equivalents or a 600-fold excess of TFA, showed only little variation in their extinction coefficients, however, they varied in their λ_{max} from 456 to 459 to 469 nm.

Discussion. – *^1H and ^{13}C chemical shifts.* Conversion of the carbonyl group of all-*trans*-retinal to an imine group, and subsequent protonation of the imine results in little change in the chemical shifts of the ring protons and C-atoms. However, the replacement of the O-atom of all-*trans*-retinal by a N-atom of its *Schiff* base introduces lower electronegativity and leads to a release of electronic charge density into the polyene chain [8]. The chemical shifts of the olefinic C-atoms, odd-numbered C-atoms being shifted to low frequency and even-numbered C-atoms being shifted to high frequency (*Table 5, cf.* those reported [8] [9]) and their linear correlation with the local excess π-electron density is confirmed. The alternating charge distribution at the C-atoms is reflected also by the proton chemical shifts. On going from ATR (**1**) to BATRA (**2**) we see a shielding of H–C(7), H–C(11) and H–C(15) and a deshielding of H–C(14) *(Table 1).* As observed in aromatic systems [21], the proton resonance shifts and the local electron densities on the bonded C-atoms appear to be correlated in these conjugated polyene chains by an electrostatic polarization of the C,H-σ-bond.

The changes in the olefinic C-atoms chemical shifts which take place upon protonation of **2** are opposite *(Fig. 6).* The resonances of even-numbered C-atoms shift to low frequency and those of odd-numbered C-atoms to high frequency. This alternation has been attributed [9] to the delocalization of the positive charge on the N-atom over the polyene chain and is explained by several limiting resonance structures [22]. In contrast to the C-atoms no alternation in the sign of the chemical shifts was found for the olefinic protons *(Table 3).* Most resonances are deshielded on going from BATRA (**2**) to $BATRAH^+$ (**3**). However, since the proton shifts are much larger for H–C(7) and H–C(11) than for H–C(8), H–C(10) and H–C(12), the delocalization of the positive charge of $BATRAH^+$ is also reflected in the ^1H-NMR. spectrum.

The proton chemical shifts of H–C(14) and H–C(15) do not fit into the given picture *(Table 3).* Both are strongly dependent on the solvent and on the concentration of the TFA counter-ions. The position of H–C(15) being only slightly affected upon protonation of the imine N-atom (displacement to even lower frequency in CD_2Cl_2!), we adopt the explanation of *Sharma et al.* [15]. They proposed that the deshielding induced by the residual positive charge on the N-atom is balanced by the decrease of the double-bond character and hence by

the decrease of the anisotropy of the C,N-bond. The large deshielding of H–C(14) which is observed in CD_2Cl_2 but not in CD_3OD, cannot be explained on the grounds of charge delocalization. We discuss below the possibility of formation of a specific complex with the TFA counter-ion.

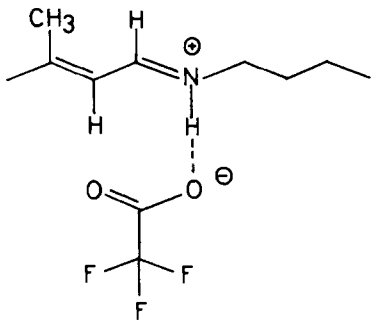
Proton coupling constants. On going from ATR to BATRA the π-electron charges remain well localized on the C-atoms. This can be seen from the vicinal proton coupling constants (H–C(7),H–C(8); H–C(10),H–C(11); H–C(11), H–C(12)) which were unaltered within 2% *(Table 2)*. The increase of the coupling constant H–C(14),H–C(15) parallels the decrease in the electronegativity of the functional group [23]. In conclusion, the alternating character of single and double bonds in ATR [13][14] is fully maintained in BATRA.

On the contrary, small differences between the coupling constants of the *Schiff* base and its protonated form could be observed over the whole polyene chain *(Table 2)*. These differences fall outside the experimental error of ± 0.3 Hz. We have compared the changes in the coupling constants on going from BATRA to $BATRAH^+$ with those of the π-bond orders calculated by *Inoue et al.* [9] using the CNDO/2-MO method. The coupling constants H–C(7),H–C(8) and H–C(11), H–C(12) decreased by 1.9 and 3.3% (π-bond orders by -1.4 and -3.7%); on the contrary, the coupling constants H–C(10),H–C(11) and H–C(14),H–C(15) increased by 4.4 and 16.5% (π-bond orders by $+10$ and $+34.6$%). This demonstrates a surprisingly good correlation. ^{1}H-NMR. proves therefore that there is a remarkable decrease of the degree of bond alternation, if no collapse, in the protonated *Schiff* base. The finding is supported by X-ray [24] and resonance-enhanced *Raman* [25] spectral data and can be supposed to induce the bathochromic shift of about 90 nm observed for λ_{max} in the electronic absorption spectrum [9].

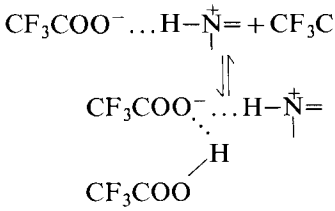
Effects of the solvent and counter-ions. An association of the *N*-butyl-(all-*trans*-retinylidene)ammonium ion with counter-ions may be anticipated in a weakly polar solvent like CD_2Cl_2, but would be considered negligeable in leveling solvents [26] such as CD_3OD. Since the positive charge in **3** is largely concentrated on the N-atom, association of TFA in CD_2Cl_2 should occur selectively at this point and involve a close approach to the charge at the N-atom. The chemical shifts of $BATRAH^+$ in a solution containing a one-to-one proportion of TFA indicate (in CD_2Cl_2 with respect to CD_3OD) a deshielding by 0.7 ppm of H–C(14) and a similar shielding of H–C(15) *(Table 3)*. Since the iminium proton is observed in CD_2Cl_2 at very high frequency (14.8 ppm), we conclude that the TFA counter-ion forms a hydrogen-bonded ($O^-\ldots H-N^+$) ion-pair with $BATRAH^+$. Related complexes have been reported between phenolic acids and triethylamine [27]. An ion-pair as shown in *Scheme 2* can explain the deshielding of H–C(14) since the anisotropic carbonyl group of TFA must be temporarily directed towards this proton. Proximity of the carbonyl group of TFA to the butyl protons appears also responsible for their deshielding, although a little smaller because of free rotation. The shielding of H–C(15), on the other hand, can be interpreted by the lack of a uniform polarization by the electronegative hydroxyl groups of CD_3OD.

Several facts show the influence of a second TFA equivalent in CD_2Cl_2. On going from one to two equivalents of TFA the iminium proton and the H–C(14)

Scheme 2



proton are strongly shielded *(Fig. 3d)* whilst other chemical shifts change very little *(Table 3)*. When the conjugate acid of the anionic partner of the ion-pair is in excess it can readily form a homoconjugate ion [17]. Thus, we may represent



(3)

a possible 2:1 complex as shown in equation 3 and attribute the shielding of the imine resonance relative to the 1:1 complex to a weakening of the H-bond in the ion-pair. The shielding of H–C(14) can be explained by a decrease in the polarizability power of the TFA carbonyl group and/or a steric rearrangement which removes the carbonyl group from the H–C(14) bond. Homoconjugation and consequent exchange between the two TFA molecules involving the breaking and reforming of the N,H-bond could explain the coalescence temperature for the H–C(15), H–N=C(15) spin-spin coupling observed at 20°.

Isomerization of N-*butyl-(all-*trans-*retinylidene)ammonium ion* (**3**). Our results show clearly that no free rotation about the C,N-bond of the protonated *Schiff* base can exist at temperatures below 0° in CD_2Cl_2 and CD_3OD *(Fig. 3* and *4)*. The only isomer with respect to the imine linkage was found to be *trans*, independent of the solvent and the concentration of TFA counter-ions. This is also valid for the 1:1 TFA addition salt when prepared and handled as described above, and is in contradiction to the earlier ^{1}H-NMR. study of *Sharma et al.* [15] who found for this species a mixture of *trans* and *cis* isomers at −50° in $CDCl_3$. Hence, the *trans*-configuration is favoured in organic solution and partial *cis*-isomerization can be observed only at elevated temperatures *(Fig. 5)*. The rate of isomerization depends on the solvent and the TFA equivalents added. Most important is the strong stabilization of the *trans*-form in CD_2Cl_2 by a second equivalent of TFA. Since this parallels an increase of the H–C(15), H–N=C(15) coupling *(Table 4)* and a deshielding of H–C(15) the second counter-ion should increase the double-bond

character of the polyenic C,N-bond and prevent it from rotating. Homoconjugation by the second molecule of TFA appears to favour the residence time of the H-atom in a tautomeric molecular (O–H...N) complex. No influence of the number of TFA equivalents on the isomerization rate was exerted in the leveling solvent CD_3OD.

Comparison between the trans- *and* cis-*isomer of the protonated* Schiff *base.* The ^{1}H-coupling constants for the two isomers of the protonated *Schiff* base being *trans* or *cis* at the C(15)=N linkage *(Table 4)* show the same alternation of single and double bonds from C(7) to C(15). Furthermore, their values are in agreement with an essentially planar all-*trans* conformation for this part of the molecule [14]. The slightly larger coupling H–C(14),H–C(15) in the *cis*-isomer can be attributed, as in the analysis of retinal isomers [14] to a smaller H–C(15)–C(14) angle in the *cis*-isomer as a result of the steric interaction of 2 H–C(1′) with H–C(14) which, although admittedly small, may lead to some opening of the C(14)–C(15)–N angle. The difference in the coupling H–C(15),H–N=C(15) for the two isomers is that expected for a *cis/trans* isomerization about an ethylene-type linkage [28]. The deshielding of H–C(11) is larger in the *cis*-isomer and independent of the solvent *(Table 4)*, indicating that the positive charge delocalization into the polyene chain is slightly increased. The chemical shift of H–C(15) is more difficult to interpret because of its solvent dependence also in the *cis*-isomer. When the *cis*-isomer is obtained in CD_2Cl_2 the approach of the TFA counter-ion towards the iminium proton apparently can no longer polarize the H–C(14) but rather the H–C(15) proton. This would explain the large deshielding of H–C(15) in case of the 1:1 TFA addition salt. More data are needed to discuss the interaction of the TFA molecules with the *cis*-isomer.

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Experimental Part

Materials. All-*trans*-retinal (**1**) was purchased from *Fluka* or *Sigma*. Other ATR was a gift of *Hoffmann-La Roche*. The products were free of other isomers (NMR.). Ether was dried over sodium wire, distilled and conserved over 4 Å mol sieves. TFA was distilled from P_2O_5.

Preparation of N-*butyl-(all-*trans-*retinylidene)amine* (**2**). The procedure was slightly modified from that reported [11]. ATR (440 mg, 1.55 mmol) was dissolved in cold anhydrous ether (3 ml) and kept at –20° in the dark. To this stirred solution cold butylamine (680 mg, 9.32 mmol) was added dropwise. The mixture was stirred at –20°, in the presence of 3 Å mol sieves for 24 h. The reaction was monitored by UV. BATRA (510 mg, 97%) was isolated by evaporation of the solvent and excess of butylamine *in vacuo*. The yellowish crystals were conserved at 0° under N_2.

Preparation of N-*butyl-(all-*trans-*retinylidene)ammonium ion* (**3**). a) *1:1 TFA addition salt.* BATRA (510 mg, 1.50 mmol) was dissolved in the dark at –20° in 3 ml of ether and one mol-equivalent of TFA (170 mg, 1.50 mmol) was added. The red precipitate (660 mg, 98%) was filtered and dried *in vacuo*.

b) *BATRA*H^+ *with excess of TFA.* Two or three mol-equivalents of TFA were added at –20° directly to a solution of BATRA in CD_2Cl_2 or CD_3OD.

NMR.-spectroscopy. The ^{1}H- and ^{13}C-NMR. spectra were recorded at 360 and 90.5 MHz, respectively, using a *Bruker* WH-360 spectrometer. Variable temperature was maintained at a

precision of $\pm 1°$. Chemical shifts were measured in ppm relative to internal tetramethylsilane. INDOR spectra were recorded in the *Fourier* transform mode by pre-saturating individual transitions with weak power. Free induction decays of the pre-saturated and the unperturbed (off-resonance) system were subtracted before transformation [12].

UV. spectroscopy. UV. absorption spectra were taken on a *PYE Unicam* SP8-100 spectrometer. The temperature was controlled by the passage of cooled methanol through a jacketed cell.

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