142. Model Compounds for Rhodopsin and Bacteriorhodopsin: Synthesis, and ^1H- and ^{13}C-NMR Study

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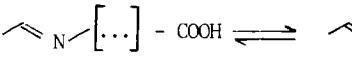
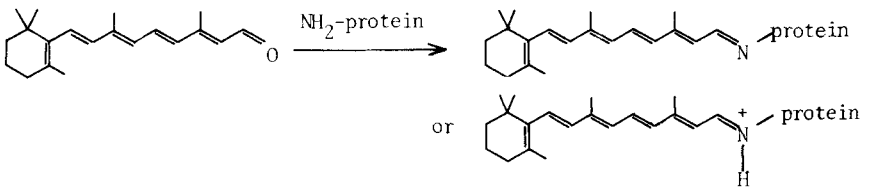
Summary

The reaction of all-*trans*-retinal and 5-(2,6,6-trimethylcyclohexenyl)-3-methyl-2,4-pentadienal with amino acids, amino esters and their salts was studied. The structure of the polyenic imines and iminium salts thus prepared was elucidated with the aid of ^1H- and ^{13}C-NMR spectroscopy. The condensation results in an equilibrium between the imine and its zwitterionic form  as shown by variable-temperature ^{13}C-NMR measurements. Addition of an inorganic salt ($LiClO_4$) favours the zwitterionic form. Comparison of the ^{13}C chemical shifts of these species with those obtained from the protonation of the corresponding imino-esters gave the percentage of the two forms. The species prepared from the amino acids constitute model compounds, and rhodopsin and bacteriorhodopsin are believed to exhibit similar behaviour.

Introduction. – Rhodopsin (the visual pigment) and bacteriorhodopsin (the major constituent of the purple membrane of *Halobacterium halobium*) have recently been the subject of much research. Rhodopsin results from the association of 11-*cis*-retinal with a protein, opsin, whereas all-*trans*- and 13-*cis*-retinal react with bacterioopsin to form bacteriorhodopsin. For these two pigments, the binding of retinal with the protein occurs between the carbonyl function of the former and the ε-amino group of a lysine fragment of the latter. The primary structure of these proteins has now been completely elucidated [1a, b] and furthermore the retinal has been located at the lysin 216 fragment in bacteriorhodopsin [2] [3]. The investigation of the nature of the binding of the retinal-protein association has been undertaken by several authors using *Raman* [4] [5], IR [6], UV [7] [8] and NMR [9] [10] spectrometry. These studies have shown that

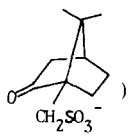
Scheme 1



retinal is attached to the ε-NH_2 *via* an aldimine bond or a protonated aldimine bond *(Scheme 1)*.

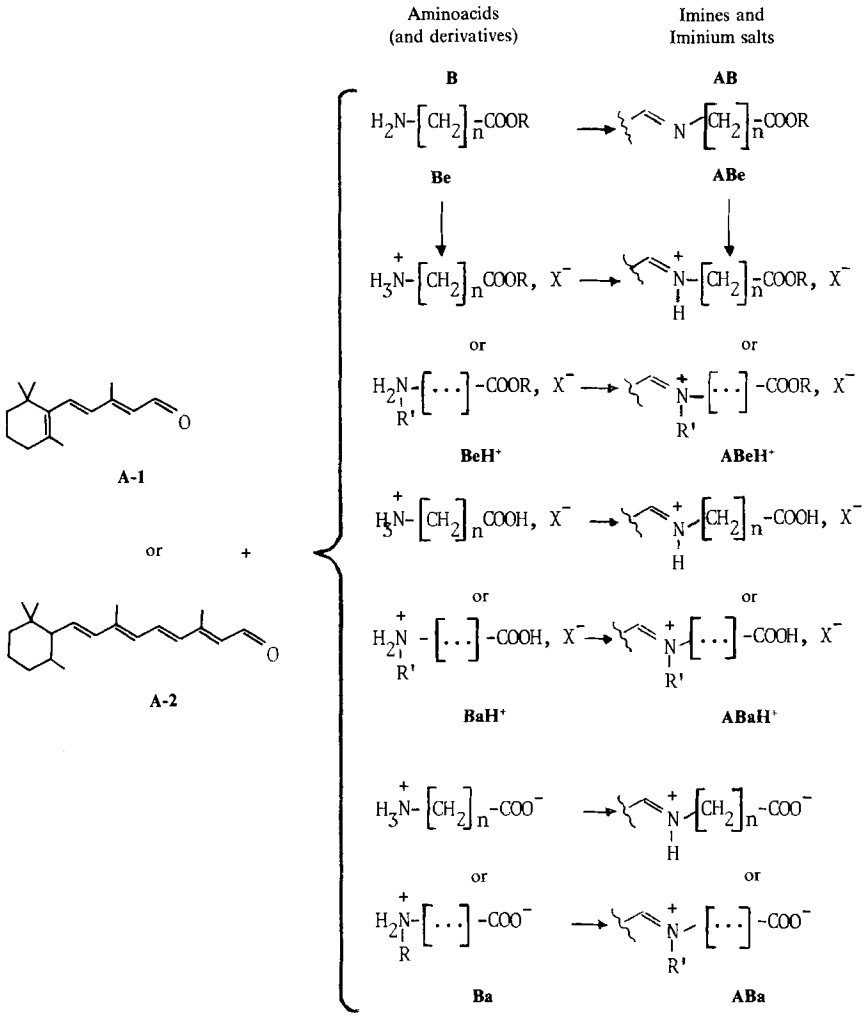
The study of these pigments by NMR spectroscopy is very difficult because of the problems of solubility and the great complexity of the spectra due to overlapping of the polyene chain and the aromatic protons of the protein. Therefore it is necessary to label selectively the different C- or H-atoms [9] [10]. To obtain NMR data on these species, several authors [11–18] have studied the *N*-butyl and *N*-propyl *Schiff* bases of retinal and their protonated derivatives (iminium ions). These models exhibit large red shifts in the UV spectra, indicating a great difference between them and the actual pigments: *e.g.* the *N*-butyl iminium salt of 11-*cis*-retinal absorbs at 440 nm in MeOH whereas the maximum absorption for rhodopsin occurs at 580 nm. This phenomenon, called 'opsin shift', has been studied by *Nakanishi et al.* They have proposed the external point charge model, which places a second negative charge near the *Schiff* base N-atom in addition to a counter-anion [19–26]. Their conclusions are based on UV studies of model iminium salts of retinal, including some amino-acid derivatives [27]. The latter seem to us appropriate models in the sense that they possess a potential negative charge in the neighbourhood of the polyene chain. Furthermore, the reaction between retinal and amino acids has not been extensively studied. This paper describes the best conditions for obtaining the imines and iminium salts by *Mannich*-type reaction and discusses their behaviour using NMR spectroscopy.

1. *Mannich* Reaction between all-*trans*-Retinal, 5-(2,6,6-Trimethylcyclohexenyl)-3-methyl-2,4-pentadienal and Amino Acids. – *Scheme 2* lists the synthetic routes used and *Table 1* compounds prepared.

Table 1. *Polyenic Imines and Iminium Model Compounds* (Y = )

	R^1	R^2	X^-	Compounds	
	CH_2–COOtBu	–	–	**A-1 Be-1**	**A-2 Be-1**
	$(CH_2)_3$–COOtBu	–	–	**A-1 Be-2**	**A-2 Be-2**
	$(CH_2)_5$–COOEt	–	–	**A-1 Be-3**	**A-2 Be-3**
	CH_2–COOtBu	H	Cl^-	**A-1 BeH^+-1 Cl^-**	
A-1B	$(CH_2)_3$–COOtBu	H	Cl^-	**A-1 BeH^+-2 Cl^-**	
	$(CH_2)_5$–COOEt	H	CF_3COO^-	**A-1 BeH^+-3 CF_3COO^-**	**A-2 BeH^+-3 CF_3COO^-**
	EtOCO–⟨⟩		ClO_4^-	**A-1 BeH^+-4 ClO_4^-**	**A-2 BeH^+-4 ClO_4^-**
	CH_2–COOEt	CH_3	CF_3COO^-		**A-2 BeH^+-5 CF_3COO^-**
	CH_2–COOH	H	CF_3COO^-	**A-1 BaH^+-1 CF_3COO^-**	**A-2 BaH^+-1 CF_3COO^-**
			Y^-	**A-1 BaH^+-1 Y^-**	**A-2 BaH^+-1 Y^-**
	$(CH_2)_3$–COOH	H	CF_3COO^-	**A-1 BaH^+-2 CF_3COO^-**	**A-2 BaH^+-2 CF_3COO^-**
			Y^-	**A-1 BaH^+-2 Y^-**	**A-2 BaH^+-2 Y^-**
A-2B	$(CH_2)_5$–COOH	H	CF_3COO^-	**A-1 BaH^+-3 CF_3COO^-**	**A-2 BaH^+-3 OF_3COO^-**
			Y^-	**A-1 BaH^+-3 Y^-**	**A-2 BaH^+-3 Y^-**
	HOOC–⟨⟩		ClO_4^-	**A-1 BaH^+-4 ClO_4^-**	**A-2 BaH^+-4 ClO_4^-**
			Y^-	**A-1 BaH^+-4 Y^-**	**A-2 BaH^+-4 Y^-**
	$(CH_2)_3$–COO^-	H	–	**A-1 Ba-2**	**A-2 Ba-2**
	$(CH_2)_5$–COO^-	H	–	**A-1 Ba-3**	**A-2 Ba-3**
	$^-$OOC–⟨⟩		–	**A-1 Ba-4**	**A-2 Ba-4**
	CH_2–COO^-	CH_3	–		**A-2 Ba-5**

Scheme 2

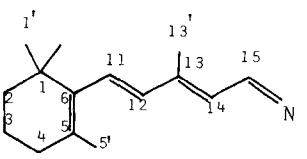


The main problem arises from the relative insolubility of the amino acids and their salts in organic solvents. Use of hydrophobic anions such as trifluoroacetate, or, better, the 10-camphorsulfonate anion makes the salts of all the amino acids soluble in $CHCl_3$ or MeOH. It has also been possible to solubilize some amino acids (*e.g.* proline, ε-aminohexanoic acid) in MeOH/H_2O solvent mixtures (see the *Exper. Part* and *Table 11*).

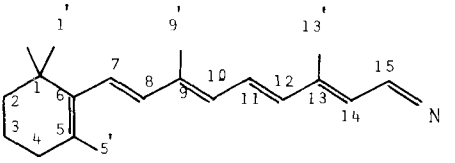
In the case of the imines **ABe**, the reaction is achieved by adding molecular sieves (an operation not necessary in the reactions with **BeH**$^+$, **BaH**$^+$, **Ba**). In spite of the formation of H_2O, the equilibrium is completely displaced towards the formation of **ABeH**$^+$, **ABaH**$^+$, **ABa** (if this is not the case a small amount of amino acid is added). These reactions complete in *ca.* 24 h at room temperature, but the presence of a big anion, such as 10-camphorsulfonate, slows the reaction down (see *Exper. Part, Table*

11). It is possible to isolate the imines **ABe** and keep them in the refrigerator for several days; this is not possible with **ABeH**$^+$, **ABaH**$^+$ and **ABa** which must be studied in the synthetic solution. Some of these solutions remain intact for a day at −10 °C at best. The second section shows that the spectroscopic behaviour of **A-1B** and **A-2B** compounds are similar. The former are generally more stable, especially for the **ABa** compounds; some experiments have only been possible with C_{15}-aldehyde derivatives. The colour of the solutions often indicates the nature of the product formed: the imine solutions are generally yellow, whereas the iminium solutions are characteristically red orange to deep red. The model compounds prepared are listed in *Table 1*.

2. NMR Study of Model Compounds ABe, ABeH$^+$, ABaH$^+$ and ABa *(Tables 2–6)*. – Because of the great similarity between the derivatives from **A-1** and **A-2**, we used the following numbering system:



A-1 B



A-2 B

The complete assignment of the proton spectra *(Table 2)* is easily carried out with the aid of J(H,H) and by comparison with previous models [18]. The differentiation between H–C(11) and H–C(12) in **A-1 B** compounds and between H–C(7) and H–C(8) in **A-2 B** compounds is based on the existence of the homoallylic coupling constant of H–C(7) or H–C(11) with 3H–C(5′). Similarly the ^{13}C-resonance signals

Table 2. *^{1}H-NMR Parameters of Imines and Iminium Salts Derived from all-*trans*-Retinal* **A-2** *and C_{15}-Aldehyde* **A-1** *at 250 MHz*. For numeration of the C-atoms, see *Formulae* **A-1B** and **A-2B**. **a** and **b** are *N*-methylimine and *N,N*-dimethyliminium iodide of **A-1**, respectively; **c** and **d** are *N,N*-dimethyliminium iodide and *N*-butyliminium trifluoroacetate of **A-2**, respectively. The concentration used was 0.5M; solvent: $CDCl_3$.

		H–C(7)	H–C(8)	H–C(10)	H–C(11)	H–C(12)	H–C(14)	H–C(15)	H–C(N)
a	(*E*)				6.38	6.10	6.09	8.27	3.39
A-1 Be-1	(*E*)				6.39	6.03	6.13	8.21	4.11
A-2 Be-1	(*E*)	6.22	6.10	6.12	6.81	6.39	6.16	8.26	4.09
A-1 Be-2	(*E*)				6.40	6.07	6.10	8.25	3.50
b					7.13	6.48	6.35	9.18	3.94 3.60
A-1 BaH$^+$-4 ClO_4^-[a])	(*E*)				7.10	6.50	6.40	9.07	4.77 3.82
A-1 BeH$^+$-4 ClO_4^-[a])	(*E*)				7.16	6.50	6.35	8.75	5.23 3.95
A-1 Ba-4[a])	(*E*)				7.16	6.60	6.53	8.84	4.75 4.00
c		6.57	6.27	6.55	7.58	6.77	6.35	8.76	3.71 3.51
d[b])	(*E*)	6.46	6.23	6.29	7.37	6.59	6.86	8.27	3.68
A-2 Ba-4[a])	(*E*)	6.52	6.26	6.48	7.52	6.68	6.34	8.81	4.73 4.03
A-2 Ba-4[a])	(*Z*)	6.52	6.29	6.48	7.48	6.62	6.32	8.90	4.14 4.63

Table 2 (continued)

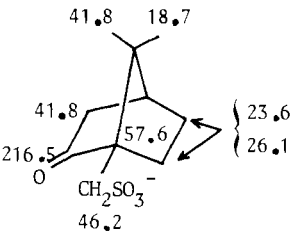
		^3J(7,8)	^3J(10,11)	^3J(11,12)	^3J(14,15)	^4J[H–C(15), C–N–C–H]	Solvent	T [°C]
a	(*E*)			16.2	9.3	1.6	$CDCl_3$	−10°
A-1 Be-1	(*E*)			16.0	9.2	1.7	$CDCl_3$	−10°
A-2 Be-1	(*E*)	16.0	11.4	15.2	9.6	1.7	$CDCl_3$	−10°
A-1 Be-2	(*E*)			16.2	9.3	1.7	$CDCl_3$	−10°
b				16.0	11.6		$CDCl_3$	−10°
A-1 BaH$^+$-4 ClO$_4^-$[a])	(*E*)			16.3	11.7		CD_3OD	−10°
A-1 BeH$^+$-4 ClO$_4^-$[a])	(*E*)			16.3	11.5		CD_3OD	−10°
A-1 Ba-4[a])	(*E*)			16.2	11.6		CD_3OD	−10°
c		16.0	11.75	14.75	11.5		CD_3OD	−10°
d[b])	(*E*)	15.8	11.8	14.7	11.1		CD_2Cl_2	−61°
A-2 Ba-4[a])	(*E*)	15.75	11.75	14.5	11.5		CD_3OD / $CDCl_3$ (v/v)	−10°
A-2 Ba-4[a])	(*Z*)	15.75	11.5	14.75	11.5			

[a]) Solvent: $CDCl_3/CH_3OH$.
[b]) Values for **d** are taken from [18].

(Tables 3–5) were assigned by comparison of our results with previous work [11–18], by the use of coupled, off-resonance spectra, and by running attached-proton-test (APT) spectra (*Fig. 1, 2* and *Exper. Part*). Some ambiguities remained concerning the differentiation between C(6) and C(9) for **A-2** derivatives and C(5), C(11) for **A-1** derivatives. These were resolved by considering the evolution of each on progressive addition of trifluoroacetic acid (TFA) (see *Fig. 3* and *4*).

The 10-camphorsulfonates (Y^- anion) were prepared to examine the influence of the nature of the anion on the iminium salts. The substitution of the trifluoroacetate anion by Y^- does not cause any significant change in the ^{13}C-shifts of the polyenic chain (*Tables 4* and *5*). Similarly, the nature of the cations has no appreciable influence on the two anions, which always possess the following ^{13}C-parameters:

CF_3COO^- { δCF_3 = 115.5 ppm; $^1J(^{13}C, F)$ = 293.5 Hz
{ δCOO^- = 161.2 ppm; $^2J(^{13}COO^-, F)$ = 34.7 Hz



Stereochemistry of the Imines **AB** *and of the Iminium Salts* **ABH$^+$**. The analysis of NMR parameters, (essentially J(H,H), see *Table 2*) proves that reactions involving the carbonyl group do not alter the structure of the polyene chain, which remains all-*trans* for both aldehydes.

The addition reaction of amino esters always produces the (*E*)-imine diastereoisomer **ABe**, whereas the same reaction with the salts of amino acids and esters gives a (*E*)/(*Z*)-iminium salt mixture **ABaH$^+$**, **ABeH$^+$** ((*E*) being the major product).

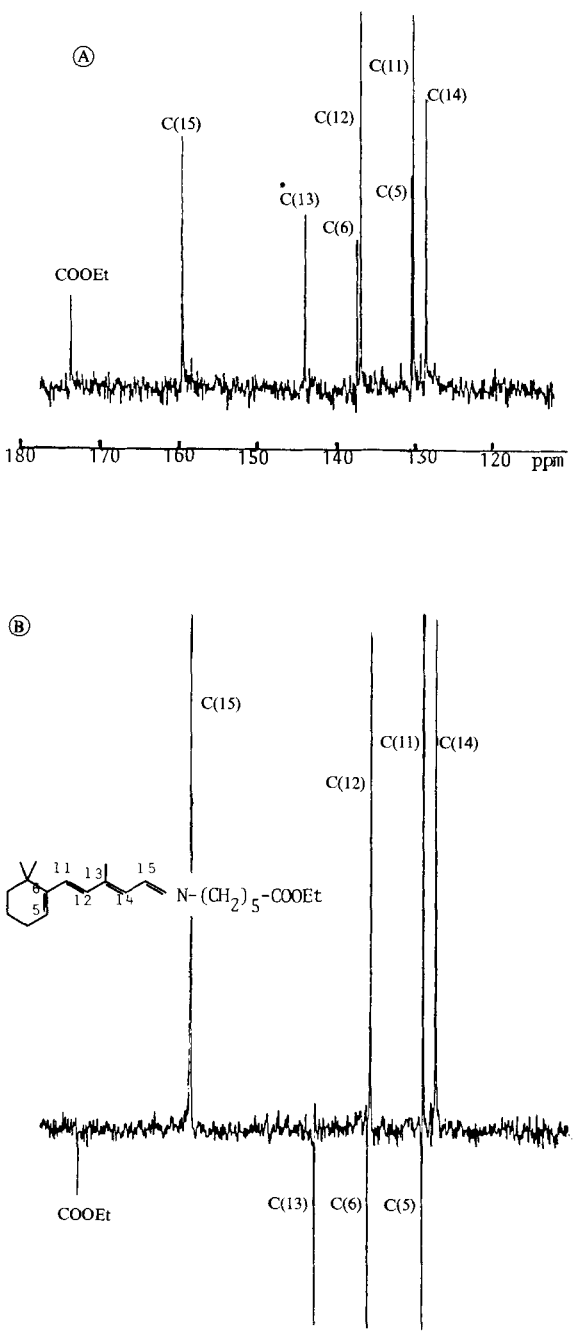
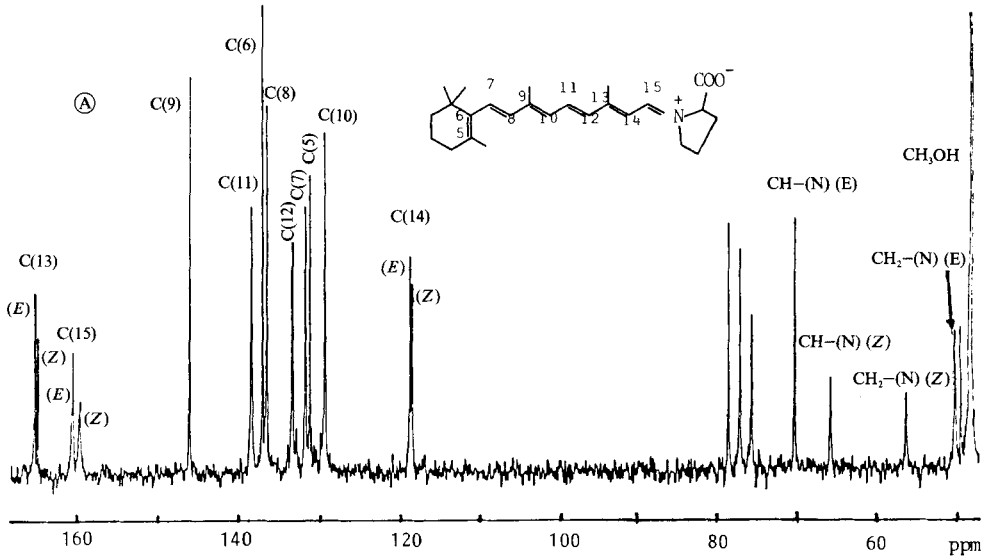


Fig. 1. *^{13}C-NMR spectra of imine* **A-1**–**Be-3** (olefinic C-atoms). Solvent: $CDCl_3$; T = −30°; (A) : normal decoupled spectra, (B) : APT spectra.

Table 3. *^{13}C-NMR Chemical Shifts of* **ABe** *Imines* (in $CDCl_3$ 0.5M, at 22.635 MHz, T = 10°). For numeration see *Formulae* **A-1B** and **A-2B**. **a** and **e** the *N*-methylimines of **A-1** and **A-2**, respectively.

	C(5)	C(6)	C(7)	C(8)	C(9)	C(10)	C(11)	C(12)	C(13)	C(14)	C(15)	C(5')	C(9')	C(13')	N–C	COO
A-1 Be-1	129.9	136.8					130.8	136.5	144.8	128.1	163.0	21.6		12.9	63.2	169.5
A-1 Be-2	130.1	137.0					130.3	136.2	145.5	128.0	160.0	21.7		12.9	60.9	172.7
A-1 Be-3	130.0	136.8					130.4	136.4	144.2	128.3	159.3	21.7		12.9	61.4	173.4
A-1 Be-3[a])	130.2	136.8					130.0	136.4	144.0	128.0	159.4	21.7		12.9	61.6	173.7
a	130.1	136.9					130.1	136.1	143.5	128.3	161.0	21.7		12.9	48.5	–
A-2 Be-1	129.5	138.0	128.5	137.5	138.5	129.8	128.1	135.7	145.5	129.0	163.2	21.7	12.8	13.1	63.2	169.5
A-2 Be-2	129.3	138.2	128.7	137.6	138.0	129.6	127.8	136.0	145.0	129.4	159.7	21.7	12.7	12.9	61.0	172.5
A-2 Be-3	129.3	138.2	128.2	137.6	138.3	129.8	127.8	135.8	144.9	129.9	159.6	21.7	12.8	12.9	61.6	173.2
A-2 Be-3[a])	129.4	138.1	128.0	137.6	138.1	130.0	128.0	135.9	144.5	129.4	159.8	21.7	12.8	13.0	61.7	173.4
e	129.3	138.2	127.9	137.6	137.8	129.5	127.7	136.1	143.6	130.1	159.6	21.8	12.8	12.9	48.6	–

[a]) T = −50°.



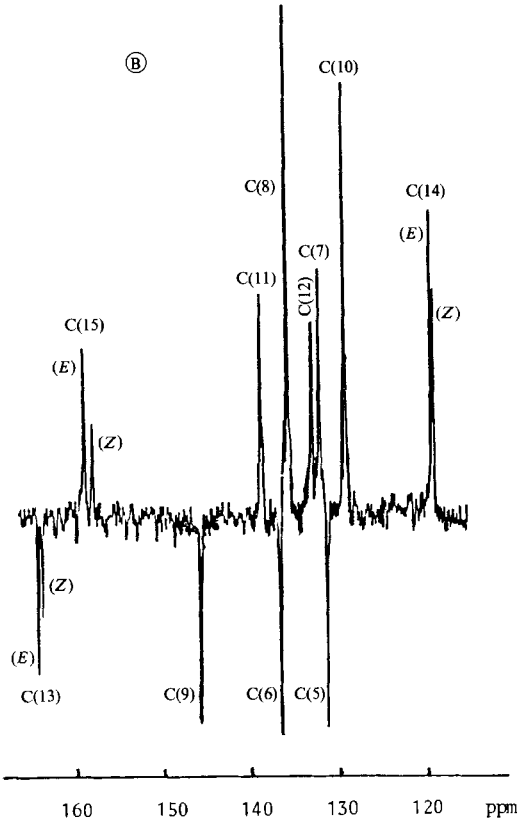


Fig. 2. *^{13}C-NMR spectra of* **A-2 Ba-4** *zwitterion at 22.635 MHz.* Solvent: $CDCl_3$ + CH_3OH; T = −50°. A: Normal decoupled spectra; B: APT spectra (olefinic C-atom); (*E*) = major isomer, (*Z*) = minor isomer.

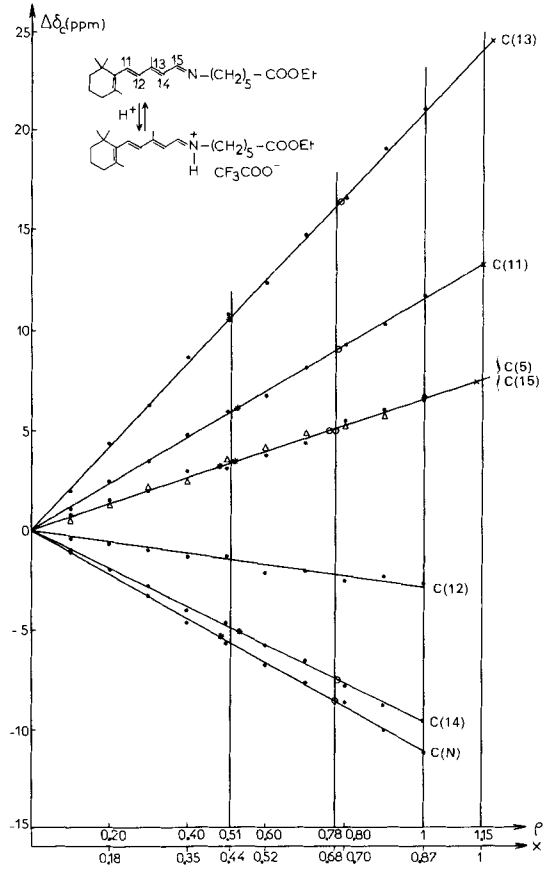


Fig. 3. *^{13}C chemical shift variations* vs. *TFA concentration for the* **A-1 Be-3** *imine.* T = −50°, solvent: $CDCl_3$, concentration 0.5M; for details, see *Exper. Part.* $\Delta\delta_C$ [ppm] = δ_C (imine + TFA) − δ_C (pure imine); ρ = molar fraction of TFA; X = molar fraction of the iminium salt. For each C-atom the points obtained are represented by a black circle (●). To distinguish C(15) and C(5) variations the C(5) points are shown as triangles (△). The circle (○) and the asterisks (*) represent the carbon shift variations for **A-1 Ba-3** after correction (see *Tables 7* and *10*) at −50° and +15°, respectively. Crosses (X) represent ^{13}C shift values for **A-1 BeH$^+$-4** (C(13), C(11) and C(5) only).

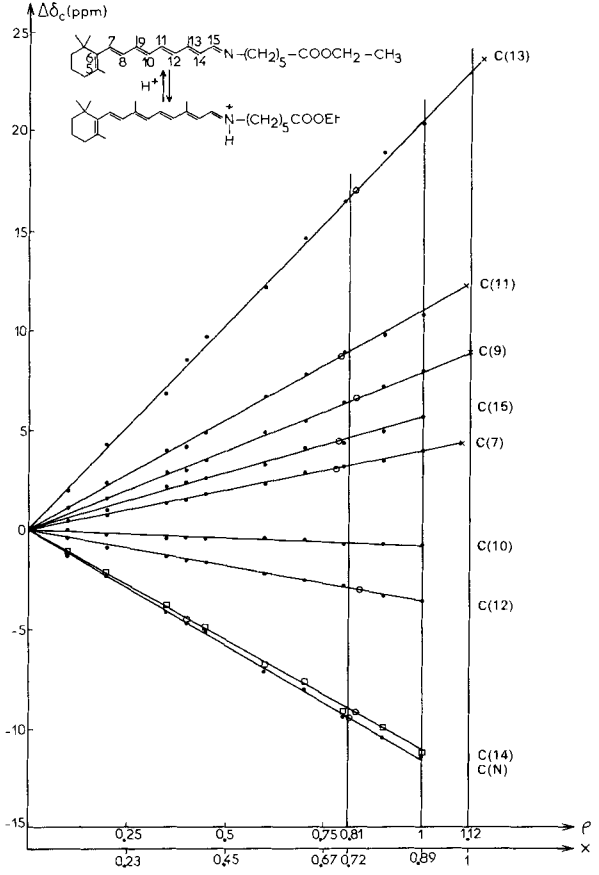


Fig. 4. *^{13}C chemical shift variations* vs. *TFA concentration for the* **A-2 Be-3** *imine.* T = −50°, 0.5M in $CDCl_3$, for details see *Exper. Part.* $\Delta\delta_C$ [ppm] = δ_C (imine + TFA) − δ_C (pure imine); ρ = molar fraction of TFA; X = molar fraction of iminium salt. For each C-atom the points obtained are represented by a black circle (●). To distinguish the C(14) and C(N), the C(14) points are shown as squares (□), the circles (○) represent the carbon shift variations for **A-2 Ba-3** at T = −50° after correction (see *Tables 5* and *10*). Crosses (X) represent the ^{13}C shift values of **A-2 BeH$^+$-4** (for C(7), C(9), C(11), C(13) only).

Table 4. *^{13}C-NMR Chemical Shifts* (in ppm from TMS at 22.635 MHz) *of Iminium Salts Derived from* **A-1**. For numeration of the C-atoms, see *Formulae* **A-1B** and **A-2B**. **b** and **b′** are *N,N*-dimethyliminium iodide and bromide, respectively, derived from **A-1**. Y^- = 10-Camphorsulfonate anion. Solvent: $CH_3OH/CDCl_3$ (see *Table 11*); T = −50°; concentration: 0.5M.

Iminium salts			C(5)	C(6)	C(11)	C(12)	C(13)	C(14)	C(15)	C(5′)	C(13)	N–C	COO
b[a])			137.2	139.3	142.8	134.2	168.1	116.9	164.9	22.4	15.9	50.3; 42.2	–
b′[a])			137.3	139.0	142.8	134.3	168.9	116.9	165.4	22.2	15.4	49.7; 41.5	–
A-1 BeH$^+$-1	Cl^-[a])	(*E*)	137.6	137.6	141.7	134.5	166.0	120.1	168.9	22.0	14.5	51.1	165.6
A-1 BaH$^+$-1	CF_3COO^-	(*E*)	136.0	136.4	141.7	133.6	166.5	118.7	168.1	21.0	12.7	50.9	168.2
		(*Z*)					169.8	115.8	163.7			46.1	
	Y^-	(*E*)	136.0	137.4	141.2	133.6	166.0	119.1	168.0	21.0	13.0	50.6	168.4
		(*Z*)					169.4	116.0	164.1			46.3	
A-1 BeH$^+$-2	Cl^-[a])	(*E*)	136.7	137.1	140.9	134.5	164.1	119.9	166.3	22.0	14.4	51.0	171.7
A-1 BaH$^+$-2	CF_3COO^-	(*E*)	136.6	137.0	141.3	133.8	164.9	118.7	166.2	21.1	13.0	50.7	174.9
		(*Z*)					168.9	115.5	163.0			44.4	
	Y^-	(*E*)	136.5	138.0	141.2	133.7	165.3	119.0	166.4	21.0	12.9	50.8	175.0
		(*Z*)					168.9	115.8	163.1			44.3	
A-1 Ba-2[b])			134.7	136.8	138.9	135.8	160.7	120.3	164.6	21.0	12.5	54.4	179.0
A-1 BeH$^+$-3	CF_3COO^-[a])	(*E*)	136.8	137.0	141.8	133.8	165.1	118.5	166.0	21.0	12.7	50.5	173.7
A-1 BaH$^+$-3	CF_3COO^-	(*E*)	136.5	136.5	141.0	133.8	164.4	118.7	165.8	21.0	12.8	51.3	175.9
		(*Z*)					168.0	115.3	162.6			45.1	
	Y^-	(*E*)	136.6	137.6	140.7	133.7	164.0	119.0	165.9	21.1	12.9	51.1	175.7
		(*Z*)					168.7	115.7	162.8			45.3	
A-1 Ba-3[b])		(*E*)	134.6	137.4	137.7	136.0	157.5	120.5	162.6	21.0	12.4	55.8	180.0
A-1 BeH$^+$-4	ClO_4^-	(*E*)	137.4	137.5	143.4	133.8	168.7	118.0	163.0	21.5	13.4	67.2; 50.9	168.9
		(*Z*)						117.6	162.5			62.4; 56.2	169.8
A-1 BaH$^+$-4	ClO_4^-	(*E*)	136.6	138.9	142.2	133.9	167.4	118.1	162.2	21.4	13.3	68.6; 51.0	171.3
		(*Z*)					166.9	117.5	161.2			64.3; 56.9	170.7
	Y^-	(*E*)	136.6	138.4	142.2	133.8	167.7	118.0	162.9	21.0	13.2	67.5; 50.7	169.8
		(*Z*)					167.3	117.5	162.3			62.2; 56.4	168.8
A-1 Ba-4		(*E*)	137.0	137.4	142.0	133.6	165.6	118.0	161.2	21.0	12.6	70.3; 50.5	172.2
		(*Z*)					165.3	118.0	160.2			65.8; 56.6	171.6

[a]) Solvent: $CDCl_3$.

[b]) Solvent: $CH_3OH/H_2O/CDCl_3$.

Table 5. *^{13}C-NMR Chemical Shifts* (in ppm from TMS at 22.635 MHz) *of Iminium Salts Derived from* **A-2**. For numeration of the C-atoms, see *Formulae* **A-1B** and **A-2B**. **c** and **d** are *N,N*-dimethyliminium iodide and *N*-butyliminium trifluoroacetate (from [18]) derived from **A-2**. Y^- = 10-Camphorsulfonate anion. T = −50°; solvent: $CH_3OH/CDCl_3$ (see *Table 11*); concentration: 0.5M.

Iminium salts			C(5)	C(6)	C(7)	C(8)	C(9)	C(10)	C(11)	C(12)	C(13)	C(14)	C(15)	C(5′)	C(9′)	C(13′)	N–C	COO
c[a])			132.4	137.7	133.0	136.7	147.2	129.8	139.6	133.9	167.7	117.2	164.5	21.9	13.4	16.2	50.0; 41.9	–
d[b])			132.1	137.8	132.1	137.3	145.6	129.0	137.6	133.9	162.2	120.4	163.5	22.1	13.3	14.2	53.1	–
A-2 BaH$^+$-1	CF_3COO^-	(*E*)	131.2	137.2	131.8	136.4	145.9	129.1	138.1	132.9	166.0	118.9	167.2	21.0	12.3	13.0	50.9	168.3
		(*Z*)									169.2	117.3	163.2				46.2	
	Y^-	(*E*)	131.0	137.0	131.4	136.2	145.4	129.0	138.0	133.0	165.9	119.2	167.4	20.9	12.3	13.0	50.6	168.2
		(*Z*)									168.9	117.7	163.5				46.0	
A-2 BaH$^+$-2	CF_3COO^-	(*E*)	131.1	137.1	131.6	136.4	145.5	129.0	138.0	132.8	164.6	118.7	165.3	20.8	12.2	13.0	50.6	174.5
		(*Z*)									167.9	117.2	162.3				44.2	
	Y^-	(*E*)	130.6	136.8	131.0	136.2	144.7	129.0	137.4	132.9	164.0	119.0	165.3	20.6	12.0	13.0	50.4	174.1
		(*Z*)									167.6		162.6					
A-2 Ba-2[c])			130.4	136.8	130.4	136.5	144.1	129.1	136.4	135.2	160.7	120.4	164.2	20.8	12.1	12.6	53.6	178.8
A-2 BeH$^+$-3	CF_3COO^-[a])		131.6	137.2	131.9	136.7	146.1	129.3	138.8	132.5	164.8	118.9	165.5	20.7	12.5	13.1	50.2	173.8
A-2 BaH$^+$-3	CF_3COO^-	(*E*)	131.0	137.0	131.5	136.4	145.3	129.0	137.8	132.8	164.2	118.7	165.0	20.8	12.1	13.0	51.2	175.7
		(*Z*)									167.7	116.3	162.0				44.9	
	Y^-	(*E*)	130.8	136.6	130.3	136.6	144.2	129.0	137.1	132.6	163.4	118.9	165.2	20.6	11.9	12.6	50.6	175.4
A-2 Ba-3[c])		(*E*)	129.7	136.8	130.4	136.1	143.4	129.1	136.7	135.0	158.1	120.7	162.9	21.0	12.2	12.3	55.2	180.3
A-2 BeH$^+$-4	ClO_4^-	(*E*)	131.4	136.7	132.3	136.8	147.0	129.5	140.2	133.5	168.3	118.0	161.9	20.9	12.2	13.3	67.1; 50.7	169.0
		(*Z*)									168.0	117.3	161.2				62.3; 53.2	
A-2 BaH$^+$-4	ClO_4^-	(*E*)	131.1	136.7	131.7	136.3	146.0	129.3	138.8	133.4	166.6	118.1	161.1	20.9	12.1	13.1	68.4; 50.5	171.2
		(*Z*)									165.9	117.6	160.2				63.2; 56.0	170.9
	Y^-	(*E*)	131.3	136.9	132.1	136.2	146.6	129.2	139.2	133.4	167.5	118.0	161.9	20.8	12.2	13.2	67.4; 50.7	169.8
		(*Z*)									167.1	117.3	161.0				62.7; 56.4	168.9
A-2 Ba-4		(*E*)	130.9	136.7	131.7	136.0	145.7	129.1	138.6	133.1	164.9	118.2	160.5	20.8	12.1	12.9	70.2; 50.4	171.4
		(*Z*)									164.6	117.9	159.3				65.7; 56.4	
A-2 BeH$^+$-5	CF_3COO^-	(*E*)	131.6	136.8	132.2	136.9	147.6	129.5	140.4	133.5	170.9	117.5	165.1	21.1	12.2	12.0	60.8; 41.6	172.1
		(*Z*)									170.5	117.0	164.5				54.0; 46.8	
A-2 Ba-5[c])		(*E*)	131.0	136.9	131.7	136.5	146.4	129.0	138.7	133.3	168.0	117.5	163.8	21.1	12.2	12.3	64.0; 41.4	169.8
		(*Z*)									166.4	117.0	163.1				57.1; 50.0	

[a]) Solvent: $CDCl_3$.
[b]) Solvent: CD_2Cl_2 (T = −10°).
[c]) Solvent: $CH_3OH/H_2O/CDCl_3$.

Table 6. *Coupling Constants* [$^1J(^{13}C,H)$ in Hz] *of Some Imines and Iminium Salts Derived from the Aldehyde* **A-1** *and* **A-2** (for experimental specifications see *Tables 3, 4* and *5*; **a** and **b** are *N*-methylimine and *N,N*-dimethyliminium iodide, both derived from the aldehyde **A-1**)

Compounds	^1J(C(14),H)	^1J(C(15),H)	^1J(C(N),H)
a	154.0	155.0	137.0
A-1 Be-1	156.0	154.0	136.0 (CH_3–N)
b	162.0	174.0	145.0 $(CH_3)_2N$
A-1 BeH^+-1 Cl^-	162.0	176.0	145.0 (CH–N)
A-1 BeH^+-4 ClO_4^-	162.0	176.0	153.0 (CH–N)
			144.0 (CH_2–N)
A-1 BaH^+-2 CF_3COO^-	163.0	173.0	144.0 (CH_2N)
A-1 Ba-4	163.0	171.5	149.0 (CH–N)
			144.0 (CH_2–N)
A-2 BaH^+-4 ClO_4^-	160.0	179.0	150.0 (CH–N)
			144.0 (CH_2N)

The *Mannich* reaction in the presence of primary and secondary amino acids **Ba** results in the (*E*)-isomer and an (*E*)/(*Z*)-mixture of the zwitterionic isomers **ABa**, respectively. If the reaction is run under mild conditions, the protonation of (*E*)-imines **ABe**, only results in (*E*)-iminium salts. The (*E*)/(*Z*)-structure of the C=N bond was elucidated by using the nuclear *Overhauser* effect as shown in *Table 7*. In the *N,N*-dimethyliminium salts **b′** and **c**, the lower-field CH_3-group remains in the (*E*)-position. This is corroborated by the lanthanide induced shift. Lanthanide salts can complex the anions [28]. Such associations are able to transmit induced shifts through space if the anion and cation are very close to one another. Increasing amounts of (+)-tris[3-(heptafluorobutyryl)-D-camphorato]europium III, [(+)-Eu(hfbc)$_3$] added to $CHCl_3$-solutions of **b′** result in the shifts represented in *Fig. 5*. The shift variations of N–CH_3(E) (lower field) are similar to those of H–C(15) while N–CH_3-(*Z*) and H–C(14) shift variations lie on the same straight line. This graph also indicates that the complexed bromide anion is probably located between N–CH_3-(*E*) and H–C(15). The bromide rather than the iodide was preferred for this experiment because of its size; when complexed with (+)-Eu(hfbc)$_3$, the larger iodide ion produces lower induced shifts than the bromide ion.

Table 7. *NOE Experiments on Imines and Iminium Salts* (for solvents and concentrations, see *Exper. Part*). **a**, **b** and **b′** are *N*-methylimine, *N,N*-dimethyliminium iodide and bromide, respectively, all derived from **A-1**; **c** is the *N,N*-dimethyliminium bromide derived from **A-2**.

Compounds	Irradiated protons	Intensity enhancement on H–C(15) [%]
a	N–CH_3	19
A-1 Be-1	N–CH_2	10
A-1 BaH^+-3 CF_3COO^-	N–CH_2	11
b′	N–CH_3 (3.94 ppm)	13
	N–CH_3 (3.60 ppm)	2
A-2 Be-3	N–CH_2	10
A-2 BeH^+-3 CF_3COO^-	N–CH_2	12
c	N–CH_3 (3.71 ppm)	13
	N–CH_3 (3.51 ppm)	≃ 0

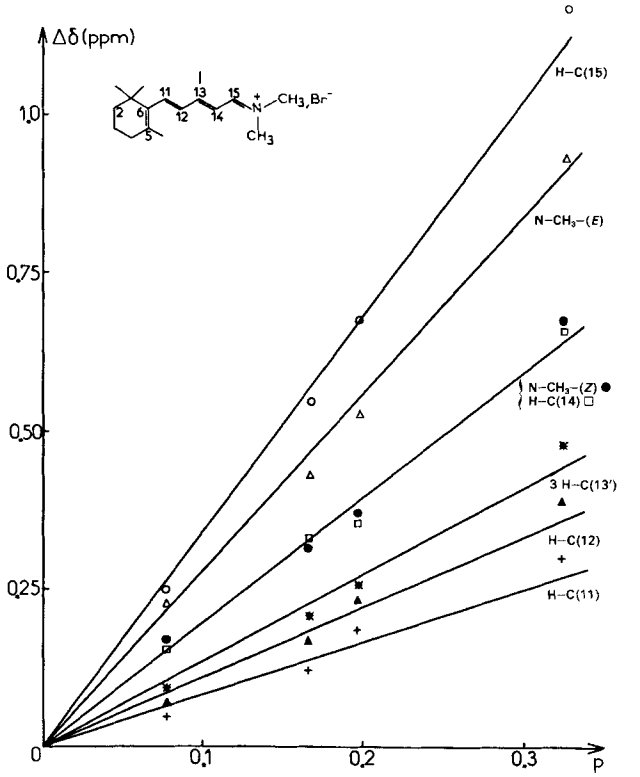
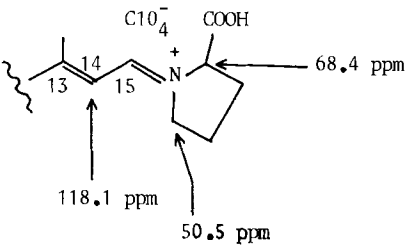
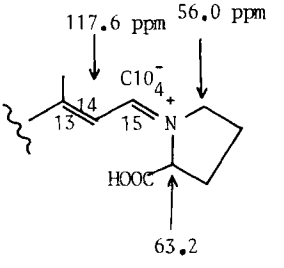


Fig. 5. *^1H chemical shift variations for iminium bromide* (**b'**) vs. *molar fraction of* (+)-Eu(hfbc)$_3$. ρ = Number of moles of (+)-Eu(hfbc)$_3$/Number of moles of salt.

The assignment of ^{13}C N-methyl group resonances (*Z* and *E*) in *N,N*-dimethyliminium salts **b** and **c** is carried out by considering the γ-effects. There is a strong γ-interaction between C(14) and N–CH$_3$-(*Z*). Therefore N–CH$_3$-(*E*) must be situated at a lower-field position than N–CH$_3$-(*Z*). The (*E,Z*) structures of proline (**Ba-4**) derivatives are determined using the nature of this effect. Considering for example **A-2 BaH$^+$-4** ClO_4^- the N–*C*H–COOH resonance is located at a lower frequency for the



A-2 BaH$^+$-4 ClO_4^-
(*E*)-isomer (major product)

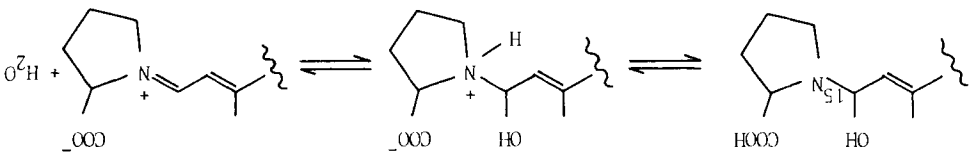


A-2 BaH$^+$-4 ClO_4^-
(*Z*)-isomer (minor product)

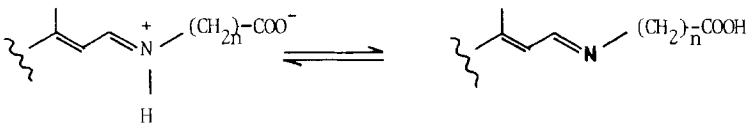
(*Z*)-isomer than for the (*E*)-isomer. The reverse situation is encountered for the N–CH_2 resonances.

Structural Proofs of the Zwitterionic Models **ABa**. The most stable iminium models are those formed from proline (**Ba-4**). This relative stability is probably due to the absence of a labile hydrogen on the N-atom. The ^{13}C chemical shifts of **A-1 BaH$^+$-4 ClO_4^-** or **A-2 BaH$^+$-4 ClO_4^-** and those of **A-1 Ba-4** or **A-2 Ba-4**, the ^{13}C parameters are very similar. In this case, formation of imines is impossible, the first identifiable species being the addition product, ($\delta_{C(15)} = 99.0$ ppm) which immediately loses one molecule of H_2O *(Scheme 3)*.

Scheme 3



The addition of perchloric acid to a solution of **A-1 Ba-4** (*E,Z*) produces another compound which possesses the same ^{13}C-NMR parameters as **A-1 BaH$^+$-4 ClO_4** (*E, Z*). The reaction of **A-1** or **A-2** with primary amino acids, 4-aminobutyric acid (**Ba-2**) or 6-aminohexanoic acid (**Ba-3**) follows a similar pathway, but the variable temperature



^{13}C chemical shifts of the zwitterions obtained reflect the equilibrium between the imine and the iminium form. The ^{13}C chemical shift variations of **A-1 Ba-3** *vs.* temperature (between 15°C and −50°C, *Fig. 6*) give a straight line, the lower the temperature, the higher the chemical shifts of the odd-numbered C-atoms and the lower those of the even numbered C-atoms of the polyenic chain *(Table 8)*. At lower-temperatures the zwitterion is the favoured form (^{13}C-NMR chemical shifts are then near those of the iminium salts). The same experiment conducted on the **A-2 Ba-4** zwitterion shows no

Table 8. *^{13}C Chemical Shift Variations* (in ppm) *with Temperature for the* **A-1 Ba-3** *Zwitterion* (for experimental conditions see *Table 4*)

T(K)	C(5)	C(6)	C(11)	C(12)	C(13)	C(14)	C(15)	C–N
288	133.0	137.5	134.7	136.3	151.5	122.9	160.8	58.9
273	133.2	137.5	135.4	136.3	152.9	122.5	161.3	58.3
263	133.5	137.5	135.9	136.3	153.9	122.1	161.7	57.7
253	133.8	137.5	136.5	136.2	154.8	121.7	161.9	57.2
243	134.1	137.4	136.9	136.2	155.7	121.2	162.1	56.7
233	134.2	137.4	137.3	136.1	156.6	120.8	162.4	56.2
223	134.6	137.4	137.7	136.0	157.5	120.5	162.6	55.8

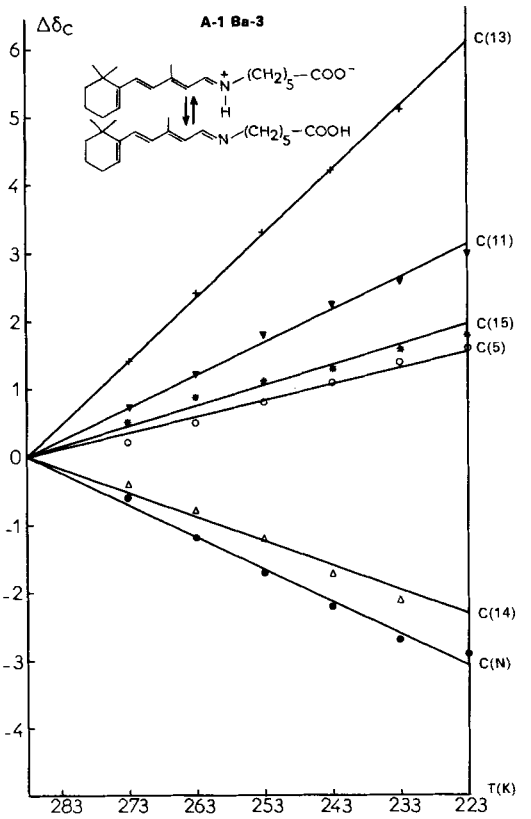
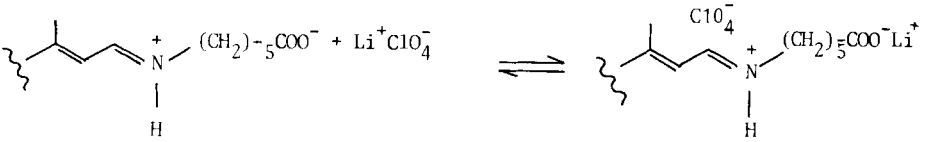


Fig. 6. *^{13}C chemical shift variations* [ppm] vs. *temperature for* **A-1 Ba-3** *zwitterion*. Solutions: 0.5M in $CDCl_3$/CH_3OH/H_2O; $\Delta\delta_C$ [ppm] = δ_C (288 K) – δ_C (T).

appreciable variation of the ^{13}C-NMR chemical shifts and corroborates the existence of the equilibrium for **A-1 Ba-3** and analogous species. Further proof is provided by adding a MeOH-solution of salt, such as lithium perchlorate to **A-1 Ba-3**, since an ionic species should stabilize the iminium form of **A-1 Ba-3** as shown in *(Scheme 4)*.

Fig. 7 and *Table 9* clearly illustrate the validity of this idea; upon addition of lithium perchlorate the ^{13}C chemical shifts move towards those of the pure iminium form. The dependence of the ^{13}C chemical shifts on the lithium perchlorate concentrations do not obey linear curves and seem to reach a plateau when more than three moles of lithium perchlorate per mole of **A-1 Ba-3** are added to the solution. Conversely, the addition of lithium perchlorate to a solution of the 'pure' iminium zwitterion **A-1 Ba-4**

Scheme 4



does not induce any effect on the ^{13}C-shifts. These experiments have not been extended to the retinal derivative **A-2 Ba-3** because of its high instability. Nevertheless the behaviour of **A-2** derivatives is likely to resemble that of the **A-1** derivatives.

To obtain information about the percentage of the ionic zwitterion species **A-1 Ba-3** and **A-2 Ba-3**, we plotted the variation in chemical shift of the C-atoms of imines

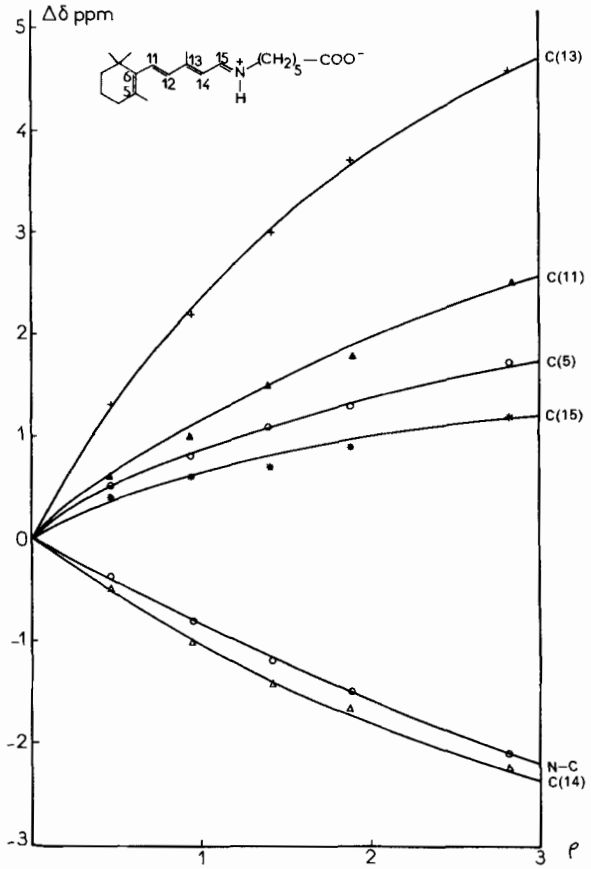


Fig. 7. *^{13}C chemical shift variations* [ppm] vs. *molar fraction of lithium perchlorate for the* **A-1 Ba-3** *zwitterion.* T = −10°, solution: 0.5M in CD_3OD/H_2O. ρ = Number of moles of $LiClO_4$/Number of moles of **A-1 Ba-3**.

Table 9. *The Influence of Lithium Perchlorate on the ^{13}C Chemical Shifts* (in ppm) *of the* **A-1 Ba-3** *Zwitterion.* T = −10°; solvent: CD_3OD (0.5M); ρ^* = Number of moles of $LiClO_4$/number of moles of **A-1 Ba-3**.

ρ^*	C(5)	C(6)	C(11)	C(12)	C(13)	C(14)	C(15)	C–N
0	133.5	137.4	135.9	136.1	157.0	122.0	163.0	56.0
0.47	134.0	137.4	136.5	136.0	158.3	121.7	163.4	55.6
0.94	134.3	137.3	136.9	136.0	159.2	121.2	163.6	55.2
1.42	134.6	137.2	137.4	135.9	160.0	120.8	163.7	54.8
1.88	134.8	137.2	137.7	135.9	160.7	120.6	163.9	54.5
2.82	135.2	137.1	138.4	135.9	161.6	120.1	164.2	54.3

A-1 Be-3 and **A-2 Be-3** *vs.* acid concentration. This was achieved by gradual addition of TFA until equimolar quantities had been reached. *Fig. 3* and *4* illustrate the linear curves that were obtained for each C-atom. At this temperature the NH H-exchange was fast enough on the NMR time scale to observe the averaged values of chemical shifts, which can be expressed by the following relationship:

$$\delta_c = \chi\ \delta_c\ (\text{iminium}) + (1 - \chi)\ \delta_c\ (\text{imine})$$

χ = molar fraction of iminium form

We verified that stoichiometric amounts of TFA added to **A-1 Be-3** or **A-2 Ba-3** solutions produce NMR spectra identical to those of **A-1 BeH$^+$-3 CF$_3$COO$^-$** or **A-2 BeH$^+$-3 CF$_3$COO$^-$** in the same solvent and at the same temperature, if these salts are prepared directly from the trifluoroacetate salt of ethyl 6-aminohexanoate **BeH$^+$-3**. It is therefore probable that the second term of the above equation is not equal to zero even if the temperature is −50°C. In fact the addition of an excess of TFA produces new shift variations but these are difficult to interpret owing to the partial decomposition of the species present. However, it is possible to obtain some information about the χ value for the **ABeH$^+$-3 CF$_3$COO$^-$** salts by considering the chemical shifts of C(13,11,5) and C(13,11,9,7) of **A-1 BeH$^+$-4 ClO$_4^-$** and **A-2 BeH$^+$-4 ClO$_4^-$** respectively. The latter being tertiary iminium salts, are not subject to H-exchange and furthermore the C-atoms chosen are not subject to α-, β-, or γ-effects due to the additional N-carbon atom. The shift differences observed between **ABeH$^+$-4 ClO$_4^-$** and **ABeH$^+$-4 CF$_3$COO$^-$** for the C-atoms mentioned thus represent the imine participation at −50°C, a hypothesis apparently corroborated by the close fit of the shift values to the curves (see *Fig. 3* and *4*). Calculations show that the complete protonation of **A-1 BeH$^+$-3 CF$_3$COO$^-$** and **A-2 BeH$^+$-3 CF$_3$COO$^-$** occurs at ρ-values of 1.15 and 1.12, respectively (ρ = number of TFA moles/number of imine moles). The curves in *Fig. 4* and *5* may be used as standards in order to estimate the equilibrium constant between the imine and zwitterion for **A-1 Ba-3** (or **A-2 Ba-3**) if the intrinsic ^{13}C shift differences between a 'pure' **ABa** zwitterion and its analogous **ABeH$^+$** derivative can be evaluated.

Comparison of ^{13}C chemical shifts of **A-2 Ba-4** with **A-2 BeH$^+$-4 ClO$_4^-$** and of **A-2 Ba-5** with **A-2 BeH$^+$-5 CF$_3$COO$^-$** (**Ba-5** = *N*-methylaminoacetic acid) indicates that in each case, the electronic and steric differences between the two compounds being compared have an almost identical effect *(Table 10)*. **A-1 Ba-4** and **A-1 BeH$^+$-4** derivatives

Table 10. *The Difference ($\Delta\delta$) in ^{13}C Chemical Shifts Observed between* **ABeH$^+$** *Iminium Ester Salts and the Analogous Zwitterions* **ABa** ((*E*)-structures) (T = −50°). $\Delta\delta_C = \delta_C$ (**ABeH$^+$**) − δ_C (**ABa**) in ppm (see *Tables 4* and *5*).

	C(5)	C(6)	C(7)	C(8)	C(9)	C(10)	C(11)	C(12)	C(13)	C(14)	C(15)	$^{\Delta\delta}$N–C^a)
A-2 BeH$^+$-4 ClO$_4^-$ / **A-2 Ba-4**	+0.5	0	+0.6	+0.3	+1.3	+0.4	+1.6	+0.4	+3.4	−0.2	+1.4	−3.1
A-2 BeH$^+$-5 ClO$_4^-$ / **A-2 Ba-5**	+0.6	−0.1	+0.5	+0.4	+1.2	+0.5	+1.7	+0.2	+2.9	0	+1.3	−3.2
A-1 BeH$^+$-4 CF$_3$COO$^-$ / **A-1 Ba-4**	+0.4	+0.1					+1.4	+0.2	+3.1	0	+1.8	−3.1

a) $\Delta\delta_{N-C}$ = for the carbon atom in the (*E*)-position.

exhibit similar behaviour. Its seems reasonable to alter the shifts of the zwitterionic species, in order to facilitate comparison of their spectra with those of the iminium ester derivatives **A-1 BeH$^+$-3 CF$_3$COO$^-$** and **A-2 BeH$^+$-3 CF$_3$COO$^-$**. The new shift values thus obtained for the zwitterions are illustrated in *Fig. 3* and *4*. Nearly all the shifts of the C-atoms of **A-1 Ba-3** and **A-2 Ba-3** lie on a straight line. The molar fraction χ of the iminium form can then be easily deduced and the values calculated using this method are approximately 0.68 (T = −50°C) and 0.44 (T = +15°C) for **A-1 Ba-3** and 0.72 (T = −50°C) for **A-2 Ba-3.** These values are only approximate as the reference curves were established in $CDCl_3$, whereas the shift measurements for **A-1 Ba-3** and **A-2 Ba-3** were determined in a mixture of CH_3OH, H_2O and $CDCl_3$.

Conclusion. – These results demonstrate very clearly that the ^{13}C-shifts of the polyene chain are very sensitive, depending particularly on the protonation of imine derivatives and the equilibrium observed in the **ABa** species. We believe that rhodopsin or bacteriorhodopsin exist in similar equilibria and that the presence of an external acid is not a pre-requisite for the iminium binding. This hypothesis has already been used to explain unusual UV-spectral changes of the rhodopsins of *Euphausia superba* [29]. The imine bond may be protonated by the labile protons of the apoprotein. The models compounds synthesized do not provide any further information about the 'opsin shift' since their chemical shifts are not very different from, for example, those of the *N*-butylretinal iminium salts. The result from addition of lithium perchlorate to zwitterionic solutions is supported by the work of *Nakanishi et al.* [19–27] on the influence of a counter-ion on the electronic charge distribution of the polyene chain. The 'opsin shift' could be understood by studying the ^{13}C chemical shift variations of C(13) of rhodopsins upon systematic modifications (pH, presence of a counter anion *etc.*). This C-atom is the most sensitive of all the polyenic chain C-atoms. (The $\delta_{C(13)}$ of a retinylidene amine and the $\delta_{C(13)}$ of its corresponding iminium salt appear 20 ppm apart.) In this case the use of the retinylidene moiety enriched with ^{13}C at the C(13) position is obviously necessary, and is certainly possible [30]. Two similar experiments have been undertaken [9] [10], both involving retinals (embedded in rhodopsin or bacteriorhodopsin) enriched with ^{13}C at C(14) and C(15)-positions. This was probably not the best choice, because these C-atoms exhibit relatively weak variations.

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Experimental Part

Syntheses. – *Starting Materials.* – *Aldehydes.* The all-*trans*-retinal was a gift from *AEC Société de Chimie Organique et Biologique* (Commentry – France). C_{15}-Aldehyde was prepared by the reaction of β-ionone with methyl diethylphosphonoacetate [31] followed by reduction and oxidation reactions [32].

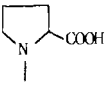
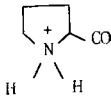
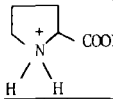
Amino Acids and Derivations. Ethyl amino esters were prepared from amino acids with EtOH in the presence of $SOCl_2$ [33]. *t*-Butyl amino esters were used in some cases because they are more stable than the ethyl esters. After protecting the amine function with the phthalimido group, the resulting amino acid was condensed on isobutylene in acidic media [34] [35]. The phthalimido group was then removed in the presence of aqueous $MeNH_2$ [16].

Salts **BaH$^+$** *and* **BeH$^+$**. Equimolar amounts of the amino acid (or ester) and the acid HX were successively introduced into a flask containing H_2O (30 ml for 0.1 mol). The mixture was stirred for ½ h at r.t. H_2O was eliminated under reduced pressure and the resulting salt was recrystallized in EtOH, EtOH/$CHCl_3$ or in MeCN. *Polyenic Imino Esters and Iminium Salts* **ABe, ABeH$^+$, ABaH$^+$, ABa**. – *Preparation of Imines* **ABe**. A solution of the amino ester (10^{-2} mol) in 5 ml of Et_2O was slowly added to a solution of the polyenic aldehyde (10^{-2} mol) in 15 ml of Et_2O at r.t. under a stream of dry N_2. Molecular sieves (3A), 20 g were introduced into the aldehyde solution prior to the addition of **Be** to ensure the completion of the reaction. The mixture was stirred for 2 h and filtered. The solvent was eliminated under reduced pressure and the imines thus prepared were used without further purification. Owing to their instability the solutions required were prepared *immediately* and *stored* in the refrigerator.

Preparation of the Iminium Salts. – Treatement of the Imines **ABe** *with Acid.* The **ABeH$^+$** salts were prepared by the addition of a 1M solution of acid in the required solvent ($CHCl_3$, Et_2O, *etc.*) to the same volume of a 1M imine solution (in $CHCl_3$, Et_2O or MeOH) at –40°. NMR spectra of the resulting solutions were run without further purification.

Preparation of Iminium Salts **ABeH$^+$, ABaH$^+$** *and* **ABa**. Because of their instability, these salts were prepared immediately prior to running their NMR spectra as follows: preparation of a 1.0M solution of the polyenic aldehyde (in $CDCl_3$) in a NMR tube; preparation of a 1.1M solution of the **Ba, BaH$^+$**, or **BeH$^+$** in a second tube, (in MeOH or MeOH/H_2O, details in *Table 11*). It was necessary to dissolve the amino acid in H_2O first and then to introduce MeOH for the less soluble compounds. In each case these solutions were added to the aldehyde solution as quickly as possible. The reactions were complete in ½ to 24 h *(Table 11)*.

Table 11. *Conditions Allowing Complete Reactions between Compounds* **ABa**, **ABaH$^+$** *and* **ABeH$^+$** (1 ml of 1.10M solutions) *and the Polyenic Aldehydes* **A-1** *and* **A-2** (1 ml of 1M solutions in $CDCl_3$). Y^- = 10-camphorsulfonate anion.

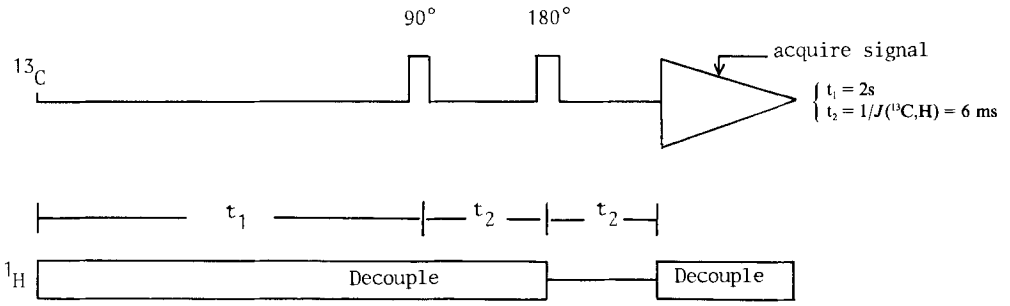
Type of amino acid			Relative solvent volumes	Reaction times [h at r.t.]	
				C_{15}-Aldehyde **A-1**	all-*trans*-retinal **A-2**
NH_2–$(CH_2)_3$–COOH		**Ba-2**	CH_3OH/H_2O (0.8/0.2)	1.5	2.0
NH_2–$(CH_2)_5$–COOH		**Ba-3**	CH_3OH/H_2O (0.8/0.2)	1.5	2.0
		**Ba-4**	CH_3OH	0.75	0.75
CH_3–NH–CH_2–COOH		**Ba-5**	CH_3OH/H_2O (0.8/0.2)	4.0	3.0
NH_3^+–CH_2–COOH	CF_3COO^-		CH_3OH/H_2O (0.8/0.2)	4.0	4.0
	Y^-	**BaH$^+$-1**	CH_3OH/H_2O (0.8/0.2)	8.0	8.0
NH_3^+–$(CH_2)_3$–COOH	CF_3COO^-		CH_3OH	4.0	4.0
	Y^-	**BaH$^+$-2**	CH_3OH	8.0	8.0
NH_3^+–$(CH_2)_5$–COOH	CF_3COO^-		CH_3OH	10.0	10.0
	Y^-	**BaH$^+$-3**	CH_3OH	15.0	15.0
	ClO_4^-		CH_3OH	0.5	1.0
	Y^-	**BaH$^+$-3**	CH_3OH	24.0	24.0
NH_3^+–$(CH_2)_3$–COOEt	CF_3COO^-	**BaH$^+$-3**	CH_3OH	4.0	4.0
	ClO_4^-	**BaH$^+$-4**	CH_3OH	0.5	0.5

NMR Experiments. – *^{1}H-NMR Spectra.* The ^{1}H-NMR spectra were run in the continuous wave mode at 100 MHz *(Varian XL 100-A)* and at 250 MHz (*Cameca* spectrometer); 0.5M solutions were used in the solvents indicated *(Table 11)*. NOE measurements were carried out on solutions which had been carefully filtered and degassed under an Ar. The irradiation power required was 90 dB on the XL 100 spectrometer.

^{13}C-NMR Spectra. The ^{13}C-NMR spectra were recorded in the Fourier transform mode on a *Bruker WH 90* spectrometer at 22.635 MHz. The solutions were the same as those used for ^{1}H-NMR spectra. Parameters: SW 6,000; AT = 0.679 s; D.E. = 4 ms; number of scans = 3000 to 5000.

The non-decoupled spectra were recorded with the aid of the gated-decoupling technique (delay = 1 s). Several off-resonance spectra were run for the same compound at different values of the irradiation frequency (irradiation power = 0.5 W).

Parameters used for the attached proton test spectra:



The t_2 time $1/J$ was chosen as a mean value corresponding to $^{1}J(^{13}C,H)$-values of 125 to 160 Hz.

The ^{13}C-shift variations of **A-1 Be-3** or **A-2 Be-3** imines observed upon the addition of TFA were measured by studying the ^{13}C-NMR shifts of each of the solutions described in *Table 12*. The mixtures were prepared at $-30°$.

Table 12. *Preparation of the Solutions Used for the Study of the ^{13}C-Shift-Variation of Imines* **A-1 Be-3** *and* **A-2 Be-3**. (The volumes described above were added to 1 ml of a 1M solution of the relevant imine in $CDCl_3$ in each case, to obtain the desired ρ-value.) ρ = Number of moles of TFA/Number of moles of **ABe** imine.

ρ	Volume [ml] of the TFA-solution 1M in $CDCl_3$	Volume [ml] of $CDCl_3$ added
0	0	1
0.1	0.1	0.9
0.2	0.2	0.8
0.3	0.3	0.7
0.4	0.4	0.6
0.5	0.5	0.5
0.6	0.6	0.4
0.7	0.7	0.3
0.8	0.8	0.2
0.9	0.9	0.1
1	1	0

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