Table I. Observed Slopes[a] of a Plot of Log k *vs.* $(C\Theta)^{1/2}$ for Various Salts in the Reaction of Radiochloride Ion with Methyl Chloride

Salt	Observed slope
LiCl	−8.5
Lithium salicylate	−10.0
$LiClO_4$	−9.1
$NaClO_4$	−7.1
$KClO_4$	−6.3

[a] Theoretical slope = −7.3.

Table II. Calculated[a] and Experimental Rate Constants with Added Salts in the Reaction of Radiochloride Ion and Methyl Chloride in 95% Aqueous Acetone at 60°

	[Salt] = 0.001 M			[Salt] = 0.005 M		
	$k \times 10^4$ l. mol^{-1} sec^{-1}			$k \times 10^4$ l. mol^{-1} sec^{-1}		
Salt	Θ	Exptl	Calcd	Θ	Exptl	Calcd
LiCl	0.494	4.00	4.71	0.325	3.11	3.79
$LiC_7H_5O_3$	0.306	5.10	5.59	0.162	3.94	4.60
$LiClO_4$	0.789	4.03	4.59	0.611	3.63	2.96
$NaClO_4$	0.937	4.55	4.54	0.832	2.65	2.52
$KClO_4$	0.872	4.48	4.49	0.743	3.00	2.67
$RbClO_4$	0.909	3.76	4.41			

[a] Calculated from the equation $\log (k/k_0) = -7.3(C\Theta)^{1/2}$.

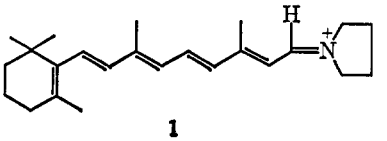
It will be apparent that the phenomenon of a decreased rate constant with increased salt concentration is general and independent of the nature of the salt, whether nucleophilic or nonnucleophilic. It therefore cannot generally be ascribed to the intervention of ion pairs as nucleophiles. Since nucleophilic lithium chloride is in no way unique in its behavior, the phenomenon is most reasonably assumed to reflect the operation of a simple medium effect, consistent with Debye–Hückel theory.

Richard A. Sneen, F. Robert Rolle
Department of Chemistry, Purdue University
Lafayette, Indiana
Received July 22, 1968

Effect of Solvent Polarizability on the Absorption Spectrum of *all-trans*-Retinylpyrrolidiniminium Perchlorate

Sir:

The origin of the anomalous spectroscopic properties of the visual pigments and their photoproducts is one of the intriguing problems confronting research on the physiochemical mechanism of vision. The several models[1-5] to explain the 500- and 478-nm absorption bands of cattle rhodopsin and metarhodopsin I (MRH_{478}), respectively, propose that retinal (11-*cis* and all-*trans* in the case of rhodopsin and MRH_{478}, respectively) bound to opsin as a protonated imine[6-9] is the chromophore responsible for the visible absorption band. Protonated imines of retinal, however, possess absorption maxima in water and alcohol at ~440 nm;[10] to explain the longer wavelength absorption bands of rhodopsin and MRH_{478}, existing theory postulates that the chromophore interacts electrostatically with opsin side-chain groups.[1,11] In this communication we report that the absorption maximum of all-*trans*-retinylpyrrolidiniminium perchlorate[12,13] **1**, which we have chosen for our immediate purposes as a convenient model for the rhodopsin chromophore, shifts from 454 (±4) nm in polar but nonpolarizable solvents to as much as 508 nm in polarizable solvents. We further provide a spectroscopic model which qualitatively explains the unusual phenomenon in both solution and the visual pigments.



1

The remarkable effect of solvent on the absorption maximum of **1** is illustrated in Table I.[14] The table is divided into two sections, one containing solvents that are polar, but *not significantly polarizable*, the other solvents mostly also polar, *but all of them polarizable*. Those solvents described as polarizable are capable of substantial *induced* dipole interactions. As seen in the "nonpolarizable" section, the absorption maximum of **1** (454 ± 4 nm) is not appreciably shifted by either large changes in solvent polarity or the ability of the solvent to hydrogen bond. These data force us to conclude that hydrogen-bond forces and dipole–dipole interactions do not perturb the electronic structure of the iminium salt **1** in a way which significantly alters the λ_{max} of the chromophore.

As seen in the polarizable solvent section of the table, aromatic solvents such as benzene and pyridine moderately red shift the λ_{max} to 464 and 471 nm, respectively. The effect of organic halides on the absorption maximum is in the expected order: I > Br > Cl, although we wish to emphasize at the outset that we are *not* dealing with intermolecular charge-transfer transitions.[14] Another important factor is the bulk geom-

(1) For reviews of the spectral properties of the visual pigments, their photoproducts, and some physical models proposed to explain them, see: (a) R. Hubbard, D. Bownds, and T. Yoshizawa, *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 301 (1965); (b) C. D. B. Bridges, "Photobiology, Ionizing Radiations; Comprehensive Biochemistry," Vol. 27, Elsevier Publishing Co., New York, N. Y., 1967, p 31; (c) E. W. Abrahamson and S. E. Ostroy, *Progr. Biophys.*, **17**, 181 (1967).

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(3) C. S. Irving and P. A. Leermakers, *Photochem. Photobiol.*, **7**, 665 (1968).

(4) I. G. Galindo, *Bull. Math. Biophys.*, **29**, 677 (1967).

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(12) Prepared by the method of N. J. Leonard and J. V. Paukstelis, *J. Org. Chem.*, **28**, 3021 (1963).

(13) The difference between the respective spectral properties of *cis*- and *trans*-retinal isomers is relatively insignificant with respect to the magnitude of the anomolous red shifts observed for both isomers in the opsin environment.

(14) We should note, as do Bayliss and McRae,[15] that the most easily measured and recorded spectral data are the absorption maxima, rather than the more theoretically relevant O–O bands. Such are extremely difficult to determine energetically in structureless spectra, thus the usual reliance on values of λ_{max}. Since the long-wavelength band changes neither in shape nor approximate oscillator strength with solvent variation for the chromophore of interest (**1**), use of values of λ_{max} is physically reasonable for our purposes; also on this same basis *the spectra cannot result from intermolecular charge transfer transitions.*

(15) N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002 (1954), and related references therein.

Table I. Absorption Maxima and Extinction Coefficients of the Pyrrolidiniminium Perchlorate in Various Nonpolarizable and Polarizable Solvents[a]

Nonpolarizable Solvents	Dipole moment	λ_{max} of **1**, nm	ϵ
CH_3OH	1.70	453	
C_2H_5OH	1.69	458	35,000
CH_3COOH	1.74	454	37,100
$(CH_3)_3COH$		451	
CH_3CN	3.84	452	35,400
$CH_3(SO)CH_3$	3.90	452	35,000
CH_3COCH_3	2.89	450	38,000
Dioxane	0.3	450	37,600
$C_2H_5OC_2H_5$	1.15	451	
Polarizable solvents			
Benzene	0.0	464	
Pyridine		471	
$C_6H_5SCH_3$	1.28	474	37,100
C_6H_5Cl	1.70	477	35,800
C_6H_5Br	1.70	480	34,900
CH_3I	1.60	480	35,800
C_2H_5Br	2.03	477	36,700
C_2H_5I	1.92	495	
CH_2Cl_2	1.54	496	37,100
CH_2Br_2	1.43	496	
CH_2I_2	1.09	496	
$CHCl_3$	1.02	481	38,900
$CHBr_3$		487	35,800
$ClCH_2CH_2Cl$	1.19	497	38,900
$ClCH_2CHClC_2H_5$		467	37,600
$ClCH_2CH_2CHClCH_3$		469	
CHCl=CHCl (*cis*)	1.90	508	37,100
CHCl=CHCl (*trans*)	~0.0	474	40,200
$CHCl{=}C(CH_3)_2$		485	
o-Dichlorobenzene	2.52	503	
m-Dichlorobenzene	1.72	485	

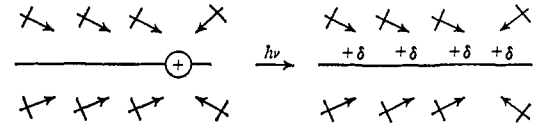
[a] Half-height band widths are virtually constant.

etry of the solvent molecule; note 1,2-dichloroethane (497 nm) *vs.* 1,2-dichlorobutane (467 nm), *trans*-dichloroethylene (474 nm) *vs. cis*-dichloroethylene (508 nm), and *o- vs. m*-dichlorobenzene (503 *vs.* 485 nm).

Proposal. In the ground state the iminium nitrogen of **1** carries a formal and predominantly nondelocalized (based on nmr data[16]) positive charge. Upon excitation to the Franck-Condon (F–C) state the positive charge is delocalized and redistributed along the polyene chain.[1a]

Bayliss and McRae[15] have defined in general terms the ways in which dipole–dipole, dipole–induced-dipole, hydrogen bonding, and dispersion forces affect the relative energy of ground and F–C excited states, and thus the absorption spectra. As an extension of these solvent–solute forces or interactions, we intend to emphasize the influence of static charge–induced-dipole interactions, which in qualitative terms will be a very sensitive function of solvent electronic polarizability. In the ground state of **1**, for relatively polar solvents, the most important solvent–solute interactions are dipole–dipole and static charge– (or ion–) dipole. Thus both polarizable and nonpolarizable solvents possessing permanent dipole moments will be moderately effective at solvating the solute, such solvation being more a function of the solvent dipole moment than solvent "type" (polarizable *vs.* nonpolarizable), in the absence of significant steric effects.

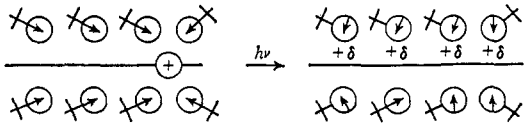
With absorption of a photon, the F–C state is generated in 10^{-15} sec; this time span is two to four powers of ten shorter than that required for solute–solvent relaxation. Thus the orientations of all atoms in the chromophore and solvent cage are *identical* in both ground and F–C states. Consider, then, the situation in nonpolarizable solvents; let us choose ethanol (or an equivalent solvent) specifically, with a dipole moment in the vicinity of 1.5 in order to compare with a polarizable solvent of similar dipole moment (such as methylene chloride or methyl iodide). Equation 1 schematically represents the ground → F–C state transition. Nonpolarizable dipoles are represented by conventional hatched-arrow notation. The π system of the polyene is represented by a line with indicated charge densities. The anion is ignored in this approximate scheme. Since no solvent–solute reorganization



(1)

is allowed, the F–C state greets a relatively hostile environment, raising the energy of the latter state so that absorption maxima are higher in energy than in the following case with polarizable solvents. Equation 2 presents the situation with permanently polar, polarizable solvents, represented as

where the small arrow within the circle represents the induced-dipole moment and is free to rotate within the



(2)

lifetime of the F–C state, implying polarizability.

The following conclusions, fully consistent with the data in Table I but appreciably condensed, seem intuitively reasonable. (1) The ground states for the processes represented by eq 1 and 2 are equivalently stabilized if the dipole moments and sizes of the respective solvent molecules are roughly equal. (2) The F–C state in the latter case is considerably more stabilized than in the former due to the ability of the polarizable solvents to repolarize within the lifetime of generation of the F–C state (without requiring relaxation, which the nonpolarizable solvents must, but cannot, do to achieve the same energetically stabilizing result). The net result is that the static charge–induced-dipole interactions, permissible with polarizable solvents, result in absorption spectra at longer (and more variable) wavelengths than spectra observed in nonpolarizable solvents capable of only dipole–dipole or static charge– (permanent) dipole interactions with solute. The variations in λ_{max} with variation in chemical structure of the polarizable solvents, as composite functions of permanent dipole moment, size, and

(16) Upon protonation of the *n*-butylimine derivative of *all-trans*-retinal in $CDCl_3$ by D_2SO_4 the chemical shift of the C_{13} methyl group, measured on a Varian A-60A, shifted −16 cps and the α-methylene protons, −14 cps, whereas the C_9 methyl group shifted −4 cps and the C_2 and C_6 methyl groups, −1 cps. These shifts indicate that the charge is predominantly localized in the vicinity of the nitrogen atom. In acetone solvent localization appears even more complete.

polarizability, are predictable, and will be subsequently discussed.[17]

The relatively large fraction of aromatic amino acids[5] contained in rhodopsin leads us to speculate that the protonated imine chromophore (analogous to **1**) bound in opsin has, in its immediate environment, aromatic side-chain groups oriented in such a way as to make the binding site highly polarizable, resulting in significant red shifts of the λ_{max} of the visual pigments and initial photoproducts. Changes in the conformation of opsin would certainly change the orientation of the polarizable groups and hence alter the local polarizability of the binding site. Thus genetic differences (with animal species) in the absorption maxima of the visual pigments, and the spectral shifts observed upon their photolysis[1] may be rationalized on the basis of the proposed model for the spectral behavior of the pyrrolidiniminium perchlorate **1**.

Acknowledgments. The authors are indebted to several of our Wesleyan colleagues and to Drs. R. S. Cooke and E. M. Kosower for stimulating discussion. Financial support was provided by the National Institutes of Health, the National Science Foundation, the Connecticut Research Commission, and the Petroleum Research Fund, administered by the American Chemical Society.

(17) Because of space limitations, this discussion is very compact. A more quantitative discussion of this general phenomenon will appear at a later date: C. S. Irving, G. W. Byers, W. C. Pringle, and P. A. Leermakers, to be published.

(18) National Science Foundation Undergraduate Research Participant, summer 1968.

(19) Alfred P. Sloan Fellow.

Charles S. Irving,[18] Gary W. Byers, Peter A. Leermakers[19]
Hall-Atwater Laboratories, Wesleyan University
Middletown, Connecticut 06457
Received December 12, 1968

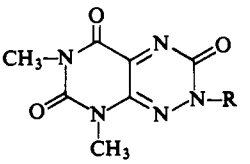
Structure and Total Synthesis of the Pyrimido[5,4-*e*]-*as*-triazine Antibiotic, 2-Methylfervenulone[1]

Sir:

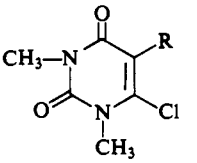
We wish to report the structure and total synthesis, by two independent routes, of the antibiotic 2-methylfervenulone (MSD-92) (2,6,8-trimethylpyrimido[5,4-*e*]-*as*-triazine-3,5,7(2H,6H,8H)-trione, **1**).

2-Methylfervenulone, previously designated as MSD-92, was isolated from the fermentation broth of an unidentified actinomycete and shown to have broad *in vitro* antibiotic activity.[2] It was correctly identified as a trimethylpyrimidotriazinetrione, although the specific structures proposed have proven to be incorrect.[2] Our results show this antibiotic to be the third member of a triad of pyrimido[5,4-*e*]-*as*-triazine antibiotics which includes toxoflavin[3] and fervenulin.[4] Both of the latter compounds have been synthesized[5-9] by routes which unequivocally establish their structures. We now report the unequivocal synthesis of **1**.

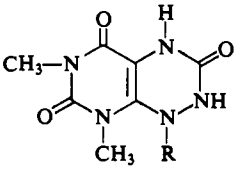
Heating 5-carbethoxyamino-1,3-dimethylbarbituric acid[10] with phosphorus oxychloride in the presence of 4–5% of added water, followed by concentration *in vacuo*, addition of ice, and filtration, gave 6-chloro-1,3-dimethyl-5-isocyanatouracil (**2**),[11] mp 145–146° (65%). Addition of methylhydrazine in acetonitrile solution then gave 4-(6-chloro-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-methylsemicarbazide (**3**), mp 199–200° (46%) (benzylidene derivative, mp 182–183°), which upon stirring in aqueous solution at 65–70° with 1 equiv of sodium acetate over a 7-hr period, while the reaction solution was diffused with air, gave 2-methylfervenulone (MSD-92) (**1**), mp 181–182° (41% yield). The product was identical with the naturally occurring antibiotic both in physical (melting point, mixture melting point; nmr, uv, and ir spectra; tlc) and in biological properties.[12]



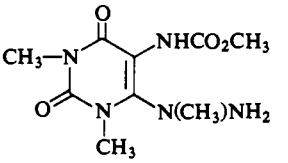
1, R = CH_3
4, R = H



2, R = N=C=O
3, R = $NHCON(CH_3)NH_2$
5, R = $NHCONHNH_2$
8, R = $NHCO_2CH_3$



6, R = H
7, R = CH_3



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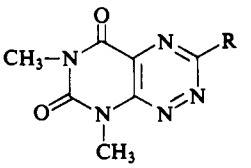
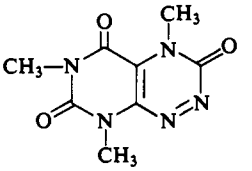


10, R = Cl
11, R = OCH_3



12

An alternate and more convenient synthesis of **1** was achieved as follows. Addition of diethyl azodicarboxyl-

(1) This investigation was supported by the U. S. Army Medical Research and Development Command (Contract No. DA-49-193-MD-2777) and is Contribution No. 530 in the Army research program on malaria. We also acknowledge partial support of this work by a grant from Eli Lilly and Co.

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(11) Satisfactory microanalytical and spectral data were obtained for all compounds reported.

(12) We are indebted to Dr. Frank J. Wolf of the Merck, Sharp and Dohme Research Laboratories, Rahway, N. J., for an authentic sample of the naturally occurring antibiotic and for the bioassay of synthetic **1**.