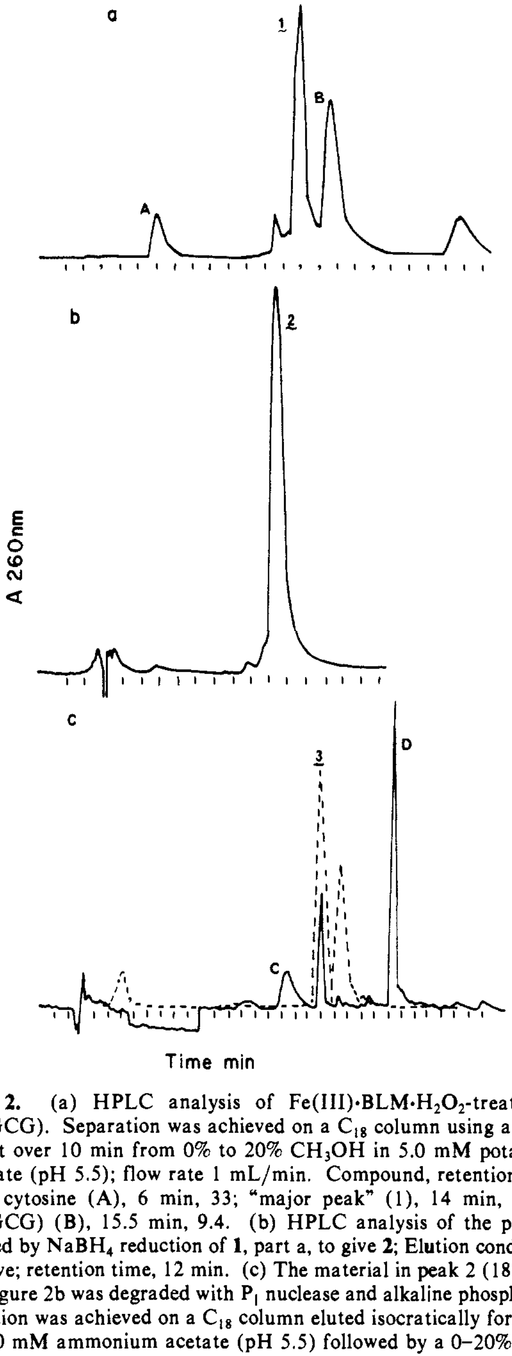

Figure 2. (a) HPLC analysis of Fe(III)·BLM·H_2O_2-treated d-(CGCGCG). Separation was achieved on a C_{18} column using a linear gradient over 10 min from 0% to 20% CH_3OH in 5.0 mM potassium phosphate (pH 5.5); flow rate 1 mL/min. Compound, retention time, nmol: cytosine (A), 6 min, 33; "major peak" (1), 14 min, 12; d-(CGCGCG) (B), 15.5 min, 9.4. (b) HPLC analysis of the product produced by $NaBH_4$ reduction of **1**, part a, to give **2**; Elution conditions, see above; retention time, 12 min. (c) The material in peak 2 (18 nmol) from Figure 2b was degraded with P_1 nuclease and alkaline phosphatase. Separation was achieved on a C_{18} column eluted isocratically for 5 min with 5.0 mM ammonium acetate (pH 5.5) followed by a 0–20% linear gradient in CH_3OH over 20 min. Compound, retention time, nmol: deoxycytidine (C), 17.5 min, 63.5; **3**, 20 min, 19; deoxyguanosine (D), 25.5 min, 64. (—) *A*, 260 nm; (---) ^3H as determined by scintillation counting.

the basis of the known specificity of P_1 nuclease,[5] compound **3** (Figure 1b) is the proposed structure. The material in peak 3 was shown to comigrate[6] with an authentic sample of one diastereomer of **3** prepared by degradation of d(CpG) by Maxam-Gilbert methodology.[7,8]

In addition, cleavage of [^3H]-**3** with snake venom phosphodiesterase resulted in the production of **4** and dGMP. The latter was identified by cochromatography with authentic dGMP by using an ion-pairing reverse-phase system. The carbohydrate moiety **4** eluted with a retention time of 3.5–4.5 min from a reverse-phase column with H_2O elution and was shown to comigrate in two solvent systems with the two diastereomers of **4** prepared by independent chemical syntheses.[9] The overall recovery of **3** from **1** was ~85%.

Similar experiments have also been completed with d-(CGCGCG) and BLM, Fe(II), and O_2 to form activated BLM. The material corresponding to peak **1** has been isolated and identified along with other expected products from the O_2-dependent base propenal pathway.

These results indicate that activated BLM generated by either Fe(II) and O_2 or Fe(III) and H_2O_2 is capable of producing **1** (Figure 1) with concomitant free base release and are consistent with the hypothesis put forth by us[1] that free-base release is the result of 4′-hydrogen abstraction followed by 4′-hydroxylation.

Acknowledgment. Supported by NIH Grant GM-34454 (to J.W.K. and J.S.). J.W.K. is an American Cancer Society Faculty Research Awardee (1983–1988) and J.S. is an NIH Career Development Awardee (Am 01222) (1983–1988) and recipient of an H.I. Romnes Award, University of Wisconsin.

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[4] Similar experiments have recently been reported by Sugiyama et al.[3g] In these experiments, alkali rather than $NaBH_4$ was used as a trap of **1** or **1a** (Figure 1) and no quantitation of products produced was reported.

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[6] (a) C_{18}-reverse-phase (RP) chromatography: isocratic elution with 5 mM ammonium acetate (pH 5.5) for 5 min, followed by a linear gradient over 20 min to 20% CH_3OH; flow rate, 1 mL/min; retention time, 20 min. (b) Ion-pairing C_{18}-RP chromatography: isocratic elution with 88% 5 mM tetrabutyl ammonium bromide, 50 mM potassium phosphate (pH 4.8), and 12% CH_3OH; flow rate, 1 mL/min; retention time, 19.75–21 min. (c) C_{18}-RP chromatography: isocratic elution with H_2O; flow rate, 1 mL/min; retention time, 2.5 min.

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[9] 2-Deoxy-D-*erythro*-pentitol was prepared by $NaBH_4$ reduction of 2-deoxy-D-ribose. 2-Deoxy-L-*threo*-pentitol was prepared from methyl-β-D-xylopyranose via a five-step synthesis which will be reported elsewhere. ^1H NMR, ^{13}C NMR, and mass spectrometry (EI) of the Me_3Si derivatives are consistent with the proposed structures. Chromatographic separation (system, R_f): (a) cellulose plates impregnated with tungstate developed with acetone/1-butanol/H_2O (5:3:2), erythro isomer (0.58) and threo isomer (0.40); (b) silica gel plates impregnated with tungstate developed with ethyl acetate/isopropyl alcohol/H_2O (2:2:1), erythro isomer (0.41) and threo isomer (0.35).

Increase in the C=N Stretching Frequency upon Complexation of *trans*-Retinylidene-*n*-butylamine with General Lewis Acids

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Received September 30, 1985

Part of the present knowledge on rhodopsin and bacteriorhodopsin photocycle intermediates comes from the resonance Raman behavior of the protein-bound retinal chromophore.[1] In vitro studies of retinal Schiff's bases and their protonated derivatives have been used effectively in showing that a protonated

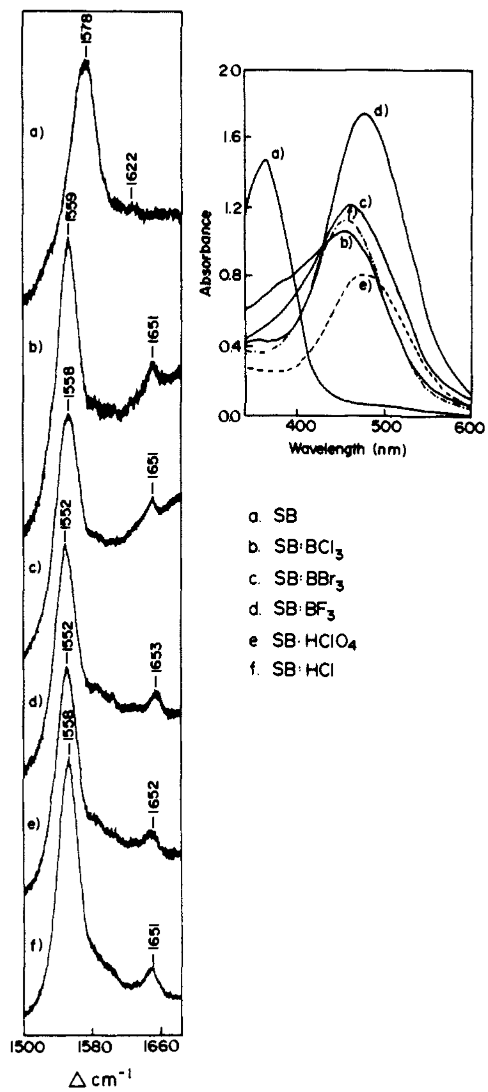

Figure 1. Resonance Raman and absorption (inset) spectra of *trans*-retinal Schiff's base and Lewis acid derivatives in CH_2Cl_2: (a) *trans*-Retinylidene-*n*-butylamine. (b) BCl_3 complex. (c) BBr_3 complex. (d) BF_3 complex. (e) $HClO_4$ complex. (f) HCl complex. Resonance Raman scattering of the samples was obtained by spinning the solutions and using an excitation wavelength of 457.9 nm. A scan speed of 20 cm^{-1}/min, a time constant of 1 s, and a spectral resolution of 5 cm^{-1} were used in recording the Raman spectra on a Spex 1401 monochromator. The absorption spectra were obtained with a 1-mm pathlength cell with concentrations between 1×10^{-3} and 5×10^{-4} M. The short wavelength shoulder in the optical spectrum of the BCl_3 complex is due to incomplete reaction with the Schiff's base.

Schiff's base occurs in various intermediates in rhodopsin and bacteriorhodopsin photocycles.[1a-e,2] A red-shifted optical absorption spectrum[3] and an increase in the C=N stretching frequency relative to the unprotonated derivative[2] are characteristic of the protonated form of the chromophore. The optical absorption shift has been attributed to increased delocalization of the π-system[1h,4] while the increase in ν(C=N) has been explained by invoking an interaction between the C=N stretching mode and the C=N—H bending motion.[1d-h,2c] The stretch-bend interaction has been postulated to obscure the expected correlation between increased π-delocalization and decreased C=N stretching force constant.[1h]

Table I. λ_{max}[a] and C=C and C=N Stretching Frequencies[b] of *trans*-Retinylidene-*n*-butylamine and Lewis Acid Derivatives

retinal Schiff's base Lewis Acid	λ_{max}	C=C	C=N	solvent
Schiff's base[c]	364	1578	1622	CH_2Cl_2
BCl_3[c]	452	1559	1651	CH_2Cl_2
BBr_3[c]	458	1558	1651	CH_2Cl_2
BF_3[c]	477	1552	1653	CH_2Cl_2
$HClO_4$[c]	476	1552	1652	CH_2Cl_2
HCl[c]	456	1558	1651	CH_2Cl_2
BF_3[d]	441	1561	1656	$(CH_3)_2SO$
HCl[d]	440	1562	1654	$(CH_3)_2SO$
BF_3[e]	456			CCl_4
BF_3[e]	480			$CHCl_3$

[a] λ_{max} in nm. [b] Stretching frequencies in cm^{-1}. [c] This work. [d] From ref 5a. [e] This work (spectra not shown).

Complexation of *trans*-retinal Schiff's base with general Lewis acids, such as BF_3, should remove the C=N—H bending interaction effects on the C=N stretching frequency while maintaining delocalization of the π-system and thus provide a means by which to test this mechanical coupling hypothesis. Our results show that such complexes exhibit optical and vibrational properties similar to the protonated Schiff's base. We interpret the data according to a rehybridization model[5] in which reaction with a Lewis acid alters the electronic properties of the Schiff's base linkage such that the C=N force constant increases to produce the observed increase in ν(C=N).[1,2,5]

A series of *trans*-retinal Schiff's base/Lewis acid complexes (Lewis acid = BCl_3, BBr_3, BF_3) were prepared and characterized.[6] As expected, strong optical absorption red-shifts are observed for these species (Figure 1).[7] The corresponding resonance Raman data indicate that the C=N stretching frequency in the Schiff's base/Lewis acid complexes increases, relative to the free Schiff's base, by an amount similar to that observed upon protonation.

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(6) *trans*-Retinylidene-*n*-butylamine was prepared as the reaction product between *trans*-retinal and *n*-butylamine as described elsewhere.[5a] The retinal Schiff's base was stored under a nitrogen atmosphere at 4 °C prior to its use. The *trans*-retinal Schiff's base/Lewis acid complexes were prepared by adding to the retinal Schiff's base solutions in CH_2Cl_2 an equivalent amount of 0.001 M solution of the corresponding Lewis acid (BCl_3, BBr_3, BF_3) in CH_2Cl_2. The solvent, CH_2Cl_2, was freshly distilled and kept in a dry nitrogen atmosphere over 5-Å molecular sieves. The transfer of the Lewis acids to the solvent or the retinal Schiff's base solution was carried out in a dry nitrogen environment in dry, preheated glassware. (See: Lane, C. F.; Kramer, G. W. *Aldrichimica Acta* **1977**, *10*, 11-18). The Lewis acids, BCl_3, BBr_3, and BF_3, were obtained from Aldrich Chemical Co. and used without further purification.

For example, BF$_3$ (Figure 1d) increases the C=N stretching frequency by 31 cm^{-1}, while in the HClO$_4$ complex (Figure 1e) an increase of 30 cm^{-1} is seen.[8] The other Lewis acid complexes (BCl$_3$, BBr$_3$, and HCl) show comparable increases in the C=N stretching frequency.

Table I summarizes λ_{max}, ν(C=N), and ν(C=C) data for several Schiff's bases, protonated Schiff's bases, and Lewis acid complexed Schiff's base species in various solvents. The similarities in these properties for the latter two classes of compounds, as well as their solvent dependencies (see also ref 3), demonstrate that the absorption red-shift, the increase in ν(C=N), and the decrease in ν(C=C) are general properties of Lewis acid/retinal Schiff's base reactions. In agreement with previous results,[1h,2c] the ethylenic (C=C) frequency of the retinal Schiff's base/Lewis acid complexes shows a stronger correlation with the magnitude of the absorption red shift than the C=N stretching frequency. For example, the difference between the λ_{max} values for the BF$_3$ and BBr$_3$ complexes is 19 nm. The corresponding differences in their C=C and C=N[9] stretching frequencies are 7 and 2 cm^{-1}, respectively, which indicates that the C=C stretching force constant is more sensitive to changes in the π-system than is the C=N stretching frequency. This suggests, in turn, that the changes associated with the C=C and the C=N stretching frequencies upon reaction with Lewis acids are regulated by more than a single mechanism.

The fact that protonated retinal Schiff's base and retinal Schiff's base complexes with BCl$_3$, BBr$_3$, and BF$_3$ show similar values for ν(C=N) suggests that electronic rearrangement occurs upon protonation which strengthens the C=N stretching force constant. These effects are more important, apparently, than stretch–bend mechanical coupling in determining ν(C=N). These conclusions are supported by work on aromatic Schiff's bases and ketimines,[5a,10] in which analogous frequency increases for ν(C=N) upon reaction with general Lewis acids were noted, by ab initio calculations,[5b] which show that the C=N stretching force constant in the simple Schiff's base, methylimine, increases by 0.51 mdyn/Å upon protonation, and by normal coordinate analysis on model Schiff's bases, which show that the stronger force constant calculated for the protonated species leads to an increase in ν(C=N) comparable to the experimental increase.[5a] In this model, the behavior of the C=N bond upon protonation is analogous in many respects to that postulated for nitriles where an increase in the C$\equiv$N stretching frequency upon reaction with general Lewis acids has been attributed to a decrease in the C$\equiv$N bond length and to an increase in the C$\equiv$N stretching force constant.[11]

The ab initio calculations[5b] provide further insight into the nature of the electronic effects invoked above. Upon protonation of methylimine, we calculate that a charge redistribution that involves shifts in both the σ-system and the π-system of the Schiff's base linkage occurs. As a result, the electron density distribution increases for nitrogen and decreases for carbon.[5b] This is in agreement with ^{13}C and ^{15}N NMR studies which show that the carbon resonance of the Schiff's base linkage shifts downfield whereas the nitrogen shifts upfield upon protonation of aromatic[12] and retinal Schiff's bases.[13] This suggests, as previously indicated,[5a,12b,14] that there is an increase in the electron-withdrawing character of the C=N group upon protonation or reaction of Lewis acids with Shiff's bases.

Acknowledgment. This research was supported by grants from the McKnight Foundation (G.T.B.), NIH (GM25480) (G.T.B.), and NSF (CHE 8519752) (J.F.H.).

Registry No. *trans*-Retinylidene-*n*-butylamine, 36076-04-7; *trans*-retinylidene-*n*-butylamine borontrichloride complex, 104241-63-6; *trans*-retinylidene-*n*-butylamine borontribromide complex, 104215-82-9; *trans*-retinylidene-*n*-butylamine borontrifluoride complex, 104215-83-0; *trans*-retinylidene-*n*-butylamine perchlorate, 28448-69-3; *trans*-retinylidene-*n*-butylamine hydrochloride, 28448-64-8.

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(7) The λ_{max} for protonated Schiff's base complexes is solvent-dependent, as has been pointed out by Blatz and co-workers.[3,4a] Similar solvent dependencies are observed for the Schiff's base/general Lewis acid complexes (compare, for example, the extent of the red-shift in the BF$_3$ and HCl adducts in CH$_2$Cl$_2$ and in Me$_2$SO in Table I). This supprorts the idea that the physical phenomena underlying the behavior of protonated and general Lewis acid complexed Schiff's bases are similar.

(8) The possibility that a retinal Schiff's base/HF complex was formed instead of the retinal Schiff's base/BF$_3$ complex can be ruled out since the HF complexes in CCl$_4$ and CHCl$_3$ solutions have λ_{max} at 447 and 468 nm, respectively.[3a] In these solvents, λ_{max} for the BF$_3$ complexes appears at 456 and 480 nm (Table I), respectively.

(9) The small increase in ν(C=N) for the Schiff's base/BF$_3$ complex, relative to the Schiff's base/BCl$_3$ complex, is similar to the observed trend[11a] for aromatic nitriles upon complexation with BF$_3$ and BCl$_3$.

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