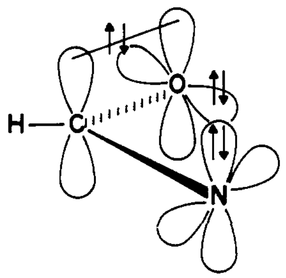
The "SCF" energies are indicated in Figure 5. We list as the SCF energy of the ${}^1A''$ state the energy of ${}^3A''$ plus twice the appropriate exchange integral (computed with ${}^3A''$ orbitals). For the ${}^1A'$ state we take the two by two CI result using the configurations $|p_N{}^2\rangle$ and $|\sigma_N{}^2\rangle$ with the orbitals as determined by the INO technique described previously.

To construct the final CI function for each of the four states we reduced the number of orbitals from 29 to 22 by eliminating highly antibonding functions localized on a single center while maintaining the first three orbitals doubly occupied. We then generated all configurations for the first order wave function,[5,11] ordered them on the basis of a two by two CI with the "SCF" structure, and selected the best 100 (spin and symmetry adapted) structures. The resulting energies are displayed in Figure 5 along with the corresponding STO-3G results.

Note that although the total energy in the more elaborate basis is more than two atomic units (~1250 kcal/mol) lower that that computed in the STO-3G basis the relative separations are only slightly affected. In going from the SCF to the CI the energy level pattern remains the same, the relative separation changing very little. Further a detailed analysis of the natural orbitals substantiates our minimal basis set conclusions presented previously, the main exception being the occupancies of the σ_N and p_N orbitals in the ${}^1A'$ state. In this calculation these orbitals host 0.61 and 1.38 electrons, respectively, while our previous result was 0.74 and 1.26. Although it increased the asymmetry in the N charge distribution this result concurs with the minimal basis in predicting a significant σ_N occupancy in this state.

Previous Work

The only previous study which we are aware of is by Alewood, Kazmaier and Rauk[12] who studied HCNO, CH_3CNO, and OHCNO using a minimal basis of Slater orbitals and assuming the geometry of the nitrene was that of the corresponding amide. They constructed the molecular orbitals for the configuration corresponding to



and estimated the energies of various excited states within the virtual orbital approximation.

Although we prefer a larger S-T separation we concur with their conclusion that HCON and CH_3CON have triplet ground states (they estimate by 15 and 12 kcal/mol, respectively). While we have not studied HOCON, which they predict to have a singlet ground state, some 23 kcal/mol below the triplet, our prediction that CH_3OCON should possess a triplet ground state would seem to be at variance with their result.

We believe the differences in the predictions of the two calculations are a result of Alewood, Kazmaier, Rauk's use of the virtual orbital approximation which leads to less than an optimal description of the various electronic states.

References and Notes

(1) Camille and Henry Dreyfus Teacher–Scholar, 1972–1977.
(2) W. Lwowski, Ed., "Nitrenes", Wiley-Interscience, New York, N.Y., 1970.
(3) (a) G. Herzberg, "Spectra of Diatomic Molecules", Van Nostrand, Princeton, N.J., 1950; (b) S. V. O'Neil and H. F. Schaefer III, *J. Chem. Phys.*, **55**, 394 (1971).
(4) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).
(5) See, for example, H. F. Schaefer III, "The Electronic Structure of Atoms and Molecules", Addison-Wesley, Reading, Mass., 1972.
(6) T. H. Dunning, Jr., *J. Chem. Phys.*, **53**, 2823 (1970).
(7) S. Huzinaga, *J. Chem. Phys.*, **42**, 1293 (1965).
(8) C. C. J. Roothann, *Rev. Mod. Phys.*, **32**, 179 (1960).
(9) D. B. Neumann, H. Basch, R. L. Kornegoy, L. C. Snyder, J. W. Moskowitz, C. Hornback, and S. P. Liebmann, Polyatom (Version 2), Program 199, Quantum Chemistry Program Exchange, Indiana University.
(10) C. F. Bender and E. R. Davidson, *J. Phys. Chem.*, **70**, 2675 (1966).
(11) H. F. Schaefer, R. A. Klemm, and F. E. Harris, *Phys. Rev.*, **181**, 137 (1969).
(12) P. F. Alewood, P. M. Kazmaier, and A. Rauk, *J. Am. Chem. Soc.*, **95**, 5466 (1973).

Linear Dichroic Spectra and Fluorescence Polarization of Biliverdin

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Abstract: The ratio of the oscillator strengths of the near uv (Soret) and visible band of biliverdin is 3.2. The angle between the transition moments of these two bands, which are assigned to $S_4 \leftarrow S_0$ (analog of $B_x \leftarrow A$ in porphyrins) and $S_1 \leftarrow S_0$ (analog of $Q_y \leftarrow A$ in porphyrins) transitions, respectively, is estimated to be 68° from PVA linear dichroism and approximately 55–60° from fluorescence polarization measurements. These values are consistent with the theoretically predicted angle of 56° (SCF-MO-CI PPP data). Analysis of these data suggests that the likely conformation of the π-electron system is neither fully linear nor circular as in the case of porphyrin.

The bile pigment, biliverdin, is spectroscopically interesting because its structure and absorption spectra are similar to the P_{fr} form of the plant photoreceptor pigment, phytochrome. Furthermore, analysis of the electronic spectrum of biliverdin is of theoretical interest in ascertaining the effect of opening of the cyclic polyene ring on porphyrin spectra. In order to analyze the absorption spectrum of biliverdin we have utilized linear dichroism and fluorescence po-

Table I. Oscillator Strengths (f) of the Absorption Bands of Biliverdin at 77° K

λ_{max}	f	λ_{max}	f
707	0.19	~435 sh	~0.09
660	0.14	380	1.06

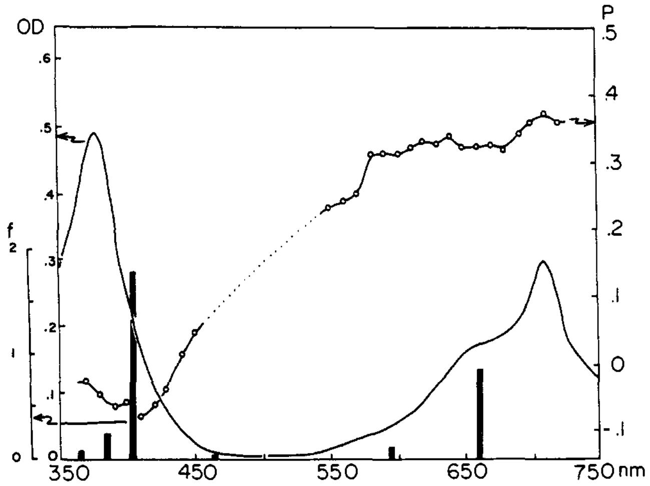

Figure 1. Absorption spectrum of biliverdin in ethanol at 77°K. Polarized fluorescence excitation (O) is shown, along with predicted energy and oscillator strengths (vertical bars) calculated by the SCF-MO-CI PPP method.

larization. Interpretation of results is also aided by the Pariser-Parr-Pople SCF-MO-CI calculations with 30 singly excited configurations.

Experimental Section

Biliverdin was obtained from Sigma Chemical Co. and was purified by chromatography on a polyamide layer with methanol:water (3:1, v/v), as described previously.[1,2] Biliverdin dimethyl ester was synthesized and purified by Lightner.[2] Since results on biliverdin and its dimethyl ester were identical, biliverdin was chosen for description in this communication.

The absorption spectra were recorded on a Cary 118C spectrometer, which was equipped with special cell holders to accommodate the optical dewars for low-temperature measurements. The absorption spectra were resolved by a Du Pont curve resolver, where appropriate. The fluorescence spectra were recorded on a high-resolution spectrofluorometer with a single-photon-counting system.[2] The polarized fluorescence spectra were obtained using Glan-Thompson polarizers, as described previously.[3]

The linear dichroism of biliverdin was measured using the modified method of Tanizaki and Kubodera.[4] First, the poly(vinyl alcohol) (PVA) sheet was allowed to swell in distilled water overnight. The swollen sheet was then immersed in the pigment solution (ethanol:water, 50:50, v/v) for 3-4 hr. The dyed PVA sheet (PVA gift from Tanizaki) was washed with solvent and dried at room temperature. The dyed sheet (OD > 3) was then stretched as much as 10 times its original length using a homemade film stretcher. An appropriate section of the stretched film was then mounted on a special cell holder, along with a similarly treated film without pigment on another holder to be used for a reference. The linear dichroic spectrum of the PVA film was recorded on the Cary 118C spectrophotometer in parallel and perpendicular orientations for Polarcoat uv polarizers with respect to the stretching axis of the film.

Results and Discussion

Absorption and polarized fluorescence excitation spectra are shown in Figure 1, along with oscillator strengths evaluated by the SCF-MO-CI PPP method. The oscillator strengths in Table I have been evaluated from eq 1, where

$$f = 4.33 \times 10^{-9} \int \epsilon(\bar{\nu}) d\bar{\nu} \qquad (1)$$

Table II. The Calculated $S_n \leftarrow S_0$ Transition Energies (eV) and Oscillator Strengths (f) by SCF-MO-CI PPP Method

Conformation	$S_n \leftarrow S_0$	Energy, eV	f	θ,[a] deg	Remarks
1	$S_1 \leftarrow S_0$	1.93	2.48	34	Linear
	$S_2 \leftarrow S_0$	1.98	0.19		
	$S_3 \leftarrow S_0$	2.83	0.014		
	$S_4 \leftarrow S_0$	3.06	0.06		
	$S_5 \leftarrow S_0$	3.19	0.13		
2	$S_1 \leftarrow S_0$	1.63	0.25	108	Cyclic
	$S_2 \leftarrow S_0$	2.12	0.04		
	$S_3 \leftarrow S_0$	2.42	0.021		
	$S_4 \leftarrow S_0$	3.22	1.20		
3	$S_1 \leftarrow S_0$	1.98	2.52	37	Linear
	$S_2 \leftarrow S_0$	2.06	0.06		
	$S_3 \leftarrow S_0$	2.98	0.035		
	$S_4 \leftarrow S_0$	3.08	0.056		
4	$S_1 \leftarrow S_0$	1.85	0.83	56	$^2/_3$ cyclic
	$S_2 \leftarrow S_0$	2.07	0.10		
	$S_3 \leftarrow S_0$	2.68	0.11		
	$S_4 \leftarrow S_0$	3.04	1.79		
5	$S_1 \leftarrow S_0$	1.87	0.23	92	Linear
	$S_2 \leftarrow S_0$	2.00	2.12		
	$S_3 \leftarrow S_0$	2.95	0.02		
	$S_4 \leftarrow S_0$	3.05	0.46		
	$S_5 \leftarrow S_0$	3.20	0.54		
6	$S_1 \leftarrow S_0$	1.83	0.24	72	$^1/_2$ cyclic
	$S_2 \leftarrow S_0$	1.88	0.64		
	$S_3 \leftarrow S_0$	2.72	0.006		
	$S_4 \leftarrow S_0$	3.02	1.01		
	$S_5 \leftarrow S_0$	3.21	0.66		
	$S_6 \leftarrow S_0$	3.26	1.18		
7	$S_1 \leftarrow S_0$	1.95	0.68	86	Close to linear
	$S_2 \leftarrow S_0$	2.00	1.15		
	$S_3 \leftarrow S_0$	2.90	0.067		
	$S_4 \leftarrow S_0$	3.08	1.27		
8	$S_1 \leftarrow S_0$	1.84	1.06	73	Cyclic
	$S_2 \leftarrow S_0$	2.16	0.05		
	$S_3 \leftarrow S_0$	2.72	0.15		
	$S_4 \leftarrow S_0$	3.12	0.53		
	$S_5 \leftarrow S_0$	3.15	1.96		

[a] θ = angle between $S_1 \leftarrow S_0$ (visible) transition and $S_4 \leftarrow S_0$ (near uv) transitions.

$\epsilon(\bar{\nu})$ is the molar extinction coefficient as a function of wave number (in cm^{-1}).

From Figure 1 and Table I, it is apparent that the bilinoid chromophore assumes a conformation more similar to that of a nonsymmetric cyclic polyene (e.g., chlorophylls) than to that of a linear polyene (e.g., all-trans carotenoids). Such a qualitative analogy is a useful guide in assigning approximate conformation(s) for a given bilinoid chromophore of the open tetrapyrrole system. Further analysis, described below, indeed suggests that a nonlinear, nonspherical conformation is most likely in resolving the observed absorption spectra.

Inspection of Table II suggests that neither perfectly cyclic nor essentially cyclic conformations match the observed spectrum with respect to relative intensities of the visible and near uv bands. Thus, the observed oscillator strength ratio of the visible (sum of Q_x and Q_y bands) to near uv (Soret) bands is 3.2 from Table I, and conformation 4 in Table II and Figure 2 yields the ratio closest to the observed value. This comparison is more clearly displayed in Figure 1. Other conformations in Figure 2 and Table II are not as satisfactory as conformation 4 in matching the observed spectrum, although conformation 8 yields the visible to near uv band ratio similar to conformation 4. The choice of the latter over the former can be made because the calculated f values of conformation 8 are too large and its polarization angle θ (73°) between the visible and near uv bands is significantly larger than that of conformation 4. Conformation 8 is sterically less likely than conformation 4. Similarly, sev-

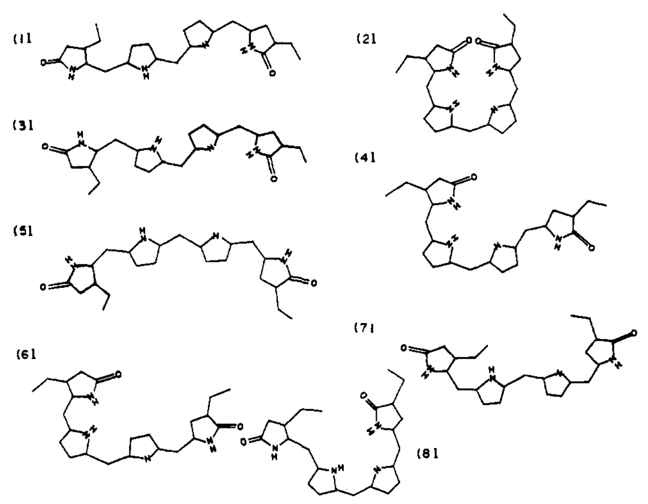

Figure 2. π-Electron systems of isomeric conformations of biliverdin.

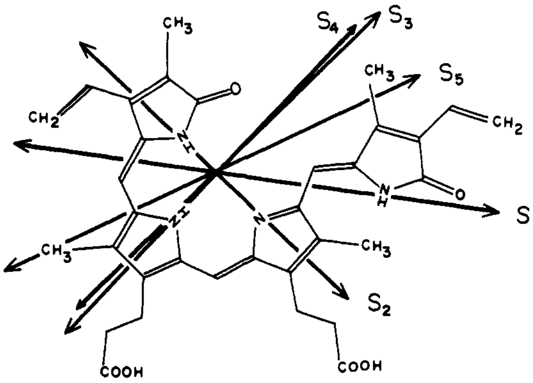

Figure 3. Polarization diagram for biliverdin (conformation 4, Figure 2).

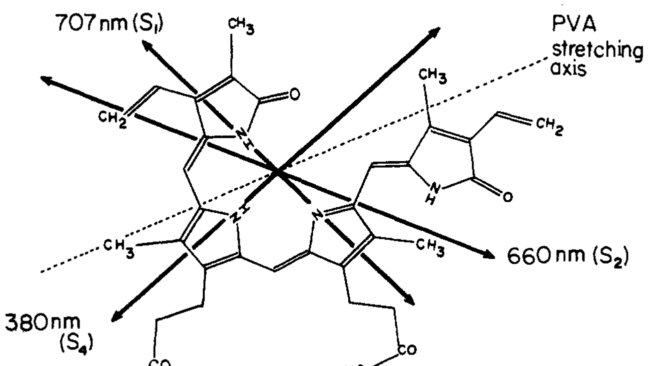

Figure 4. Relative orientations of the visible and near uv transition moments deduced from linear dichroic data. The stretching axis coinciding with the preferential molecular orientation axis is assumed as indicated upon inspection of the molecular dimension.

Table III. Dichroic Data for Biliverdin in PVA Film

Band	λ_{max}, nm	d_0	α, deg
$S_1 \leftarrow S_0$ $(Q_y)^b$	675 (707)^a	0.8	68
$S_2 \leftarrow S_0$ (Q_x)	625 (660)	1.0	45
$S_3 \leftarrow S_0$?	440 (435)	1.75	21
$S_4 \leftarrow S_0$ (B_x)	370 (380)	1.8	18

[a] At 77°K (Figure 1). [b] Based on the porphyrin notation.

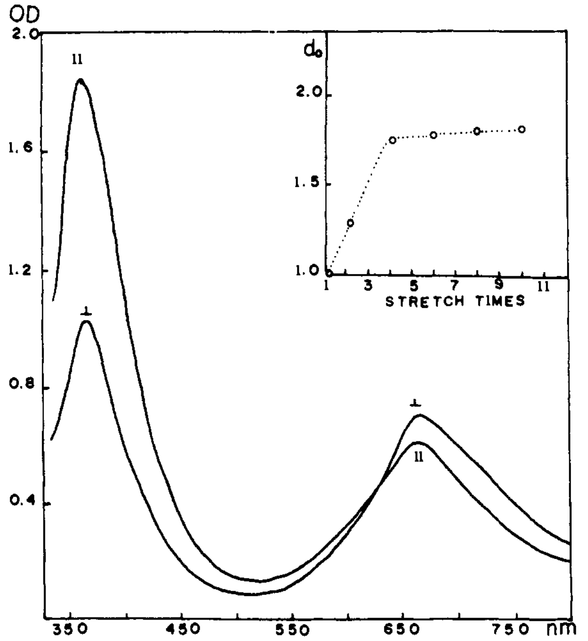

Figure 5. Linear dichroic spectra of biliverdin in a stretched PVA film at room temperature. Insert shows the dichroic ratio at 375 nm as a function of stretching.

eral other conformations related to the phytochrome chromophore[5] have been considered, but the results are not as satisfying as conformation 4.

Figure 3 shows polarization directions of visible and near uv transitions for conformation 4. Figure 4 shows the linear dichroic spectra of biliverdin in a stretched PVA film. Analysis of the linear dichroic spectra yields polarization assignments shown in Figure 4, vide infra. In general, agreement between the calculated and experimental (vide supra) polarization directions is reasonably good, except for the calculated absolute orientation of the long wavelength transition (Q_y) (cf. Table III and Figure 4).

The dichroic ratio at 600-650 nm (Figure 5) is about unity ($d_0 \simeq 1$), corresponding to the $S_2 \leftarrow S_0$ absorption band (cf. Table I and Figure 1). The first absorption band at 670-800 nm shows negative dichroism ($d_0 \simeq 0.8$) with respect to the stretching axis. On the other hand, the near uv band possesses the highest dichroic ratio, indicating that the angle between the transition dipole of this band and the effective molecular orientation axis (stretching axis) is smallest among the absorption bands in biliverdin. It should be noted that the dichroic ratio (d_0) recorded at the tenfold stretching in Figure 5 represents a maximum polarization attainable from the stretching of the PVA film, as illustrated by the insert in Figure 5. It is, therefore, apparent that the polarization direction of the long wavelength and near uv bands is not parallel. This is further confirmed by the polarized fluorescence excitation spectrum (Figure 1), which shows a negative polarization degree for the near uv band with respect to the first band ($S_1 \leftarrow S_0$ absorption $\parallel$ to $S_1 \rightarrow S_0$ fluorescence). From the Levshin-Perrin relation between the absorption and emission oscillators,[6,7] the angle θ between the visible and near uv transitions is estimated to be 55-60°. The dichroic ratio of unity for the $S_2 \leftarrow S_0$ band is also independent of the degree of stretching to a large extent. This is possible only if $\alpha = 45°$ (angle between the transition dipole and stretching axis) for this band. Calculation of the F value (effective fractional orientation factor of the molecules) is necessary to determine polarization axes of other bands. However, a unique F value cannot be calculated using the traditional eq 2 of Fraser,[8] Beer,[9] and Yogev et al.[10] and $\alpha = 45°$ for the $S_2 \leftarrow S_0$ band

$$d_0 = \frac{OD_\parallel}{OD_\perp} = \frac{F\cos^2\alpha + 0.33(1 - F)}{0.5F\sin^2\alpha + 0.33(1 - F)} \qquad (2)$$

To avoid this difficulty, eq 3 following Gangakhedkar et al.[11] can be used

$$d_0 = \frac{OD_\parallel}{OD_\perp} = \frac{2\cos^2\alpha + K}{\sin^2\alpha + K} \qquad (3)$$

where K is a stretch-dependent orientation parameter defined by eq 4, where θ is the angle which the molecular orientation axis makes with the stretching direction. Rearranging eq 3, we have

$$K = \frac{2\int_0^{\pi/2} F(\theta)\sin^2\theta \, d\theta}{\int_0^{\pi/2} F(\theta)(2 - 3\sin^2\theta)\, d\theta} \qquad (4)$$

$$\cos^2\alpha = \frac{d_0 + K(d_0 - 1)}{d_0 + 2} \qquad (5)$$

Integration of eq 4 yields $K = 2$. Substituting this value and $d_0 = 1.8$ for the $S_4 \leftarrow S_0$ band (near uv band) into eq 5 and solving for α, we obtain $\alpha = 18°$. Similarly, α's for $S_1 \leftarrow S_0$ ($d_0 = 0.8$) and $S_3 \leftarrow S_0$ ($d_0 = 1.75$), etc., can be calculated (Table III).

Conclusion

In conclusion, it is suggested that the results of linear dichroism, fluorescence polarization, and relative intensities of the visible vs. "Soret" bands of biliverdin can best be fit to an open, semicircular conformation (e.g., Figure 4). Neither stretched linearly nor fully circular conformations are consistent with the experimental and theoretical analyses described in this report. We further note that the chromophore conformation of phytochrome is likely to be similar to that of biliverdin on the basis of spectral analogies.

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References and Notes

(1) Z. M. Petryka and C. J. Watson, *J. Chromatogr.*, **37**, 76 (1968).
(2) P. S. Song, Q. Chae, D. A. Lightner, W. Briggs, and D. Hopkins, *J. Am. Chem. Soc.*, **95**, 7892 (1973).
(3) M. Sun, T. A. Moore, and P. S. Song, *J. Am. Chem. Soc.*, **94**, 1730 (1972).
(4) Y. Tanizaki and S. Kubodera, *J. Mol. Spectrosc.*, **24**, 1 (1967).
(5) M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry*, **11**, 4025 (1972).
(6) V. L. Levshin, *Z. Phys.*, **32**, 307 (1925).
(7) F. Perrin, *Ann. Phys. (Leipzig)*, **12**, 169 (1929).
(8) R. D. B. Fraser, *J. Chem. Phys.*, **21**, 1511 (1953).
(9) M. Beer, *Proc. R. Soc. London, Ser. A*, **236**, 136 (1956).
(10) A. Yogev, L. Margulies, and Y. Mazur, *J. Am. Chem. Soc.*, **92**, 6059 (1970).
(11) N. S. Gangakhedkar, A. V. Namjoshi, P. S. Tamhane, and N. K. Chaudhuri, *J. Chem. Phys.*, **60**, 2584 (1974).

Energetics, Stabilities, and Possible Structures of $CH_5^+(CH_4)_n$ Clusters from Gas Phase Study of Equilibria $CH_5^+(CH_4)_{n-1} + CH_4 = CH_5^+(CH_4)_n$ for $n = 1-5$

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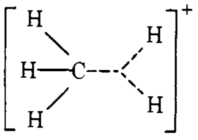
Abstract: The temperature dependence of the equilibria ($n - 1,n$) $CH_5^+(CH_4)_{n-1} + CH_4 = CH_5^+(CH_4)_n$ was measured in a pulsed electron beam mass spectrometer with a high-pressure ion source for n = 1 to 5. The $-\Delta H$ values obtained were (0,1) 7.4, (1,2) 5.9, (2,3) 4.1, (3,4) 3.9 kcal/mol. The observed enthalpy and free energy changes are compatible with the three-center bond structure for CH_5^+ proposed in the literature. The first two methane molecules interact with the two hydrogen atoms with lowest electron densities forming bridged three-center bonds. Similar structures are also proposed for the higher clusters. An analysis of deviations of the experimental van't Hoff plots permits one to establish the quality of the experimental measurement and the fraction of ions dissociated by the sampling process.

The process $CH_4^+ + CH_4 = CH_5^+ + CH_3$ occurring in electron irradiated methane was among the first ion-molecule reactions observed in mass spectrometers with elevated ion source pressure.[1] However, the existence of protonated methane, protonated ethane $C_2H_7^+$, and attendant ion chemistry in the gas phase observed subsequently in ion-molecule mass spectrometers[2] did not attract wide attention. The few early theoretical calculations on the structure of CH_5^+ used drastically empirical approaches.[3,4] Two more elaborate SCF-MO considered only a limited number[5,6] of structures which later were proved to be of high energy. Strong interest in the CH_5^+ or methonium ion and alkane protonation was created by the work of Olah[7] who investigated protonation of alkanes in super acid (magic acid) liquid solutions. These studies suggested a three-center bond structure I with C_s symmetry. Several theoretical calculations[8-11] have provided independent evidence that this structure is the one with the lowest energy.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Some time ago a program of study of ion-solvent molecule interactions in the gas phase was initiated in this labo-