NEW POLYAZAPORPHINE CHEMISTRY FOR THE ORIGIN OF LIFE

A PORPHINE SOLAR ENERGY TRANSDUCER FOR ORIGIN OF LIFE CHEMISTRY: A POSSIBLE SYNTHETIC PATH FROM HCN TO AN OCTAAZAPORPHINE VIA 4H-IMIDAZOL-4-IMINE [(HCN)3] RELATED CHEMISTRY

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Abstract. Molecular orbital spectral predictions suggest that 2,5,7,10,12,15,17,20-octaaza-21H, 23H-porphine has a visible spectral range closely matching that of chlorophyll-a. Since the octaazaporphine is, in its core, a simple derivative of an $(HCN)_{12}$ oligomer, this fact, together with its spectral properties, would suggest that it occupies a high rank as a primordial porphinic solar energy transducer for photochemistry essential to life's formation. The demonstration that the mass 324 hexahydrooctaazaporphine is formed in protic media by the cyclotetramerization of imidazol-4-aminohydroxonium ion or the derived nitrenium ion, and that a mass 318 species consonant with that of the Hückel aromatic octaazaporphine is observed in the course of these studies, strongly supports the proposed octaazaporphine synthesis in a prebiotic hydrocyanic acid milieu.

1. Introduction

Hydrocyanic acid has been shown in the pioneering work of Miller (1953, 1955), and later by many others, to be a key prebiotic reactant in the formation of α-aminocarboxylic acids, aliphatic acids, and RNA-DNA bases – all important life ingredients. In similar Miller-type experiments by Hodgson and Ponnamperuma (1968), and by Simionescu *et al.* (1978), porphinic material with a spectrum similar to etioporphyrin was detected. However, the porphinic yields were a minuscule 100 ppb based on carbon (Hodgson and Ponnamperuma, 1968). Nevertheless, the possible formation of porphines in the primitive earth's oceans is especially significant because of the Mauzerall-Granick hypothesis which links the photochemistry of the prebiotic porphines with that of the highly evolved chlorophylls of the current biomass, and thus proposes that the proto-photosynthetic chemistry of these porphines was incipiently commingled with the origin of life and early biotic evolution (Mauzerall, 1992; Granick, 1957, 1965; Urey, 1952). In model experiments, Mercer-Smith and Mauzerall (1982) demonstrated that free base biogenic porphyrins, through photo-oxidation of an electron donor, formed a radical

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that reduced water to dihydrogen. Thus solar energy was transformed to the large free energy gradient between newly oxidized and reduced species which supported the complex organic chemical reactions required for life's origin and early perseverence.

To explore the seminal features of 'primal photosynthesis' summarized above, Mercer-Smith and Mauzerall (1982) assumed for their studies that the prebiotic porphines were the currently biogenic uroporhyrin and coproporphyrin, derived from the pyrrole porphobilinogen. In this assumption, they extended the Granick Hypothesis (the current biochemical pathway for chlorophyll synthesis recapitulates its biochemical evolution [Granick, 1957, 1965]) from a statement about biotic chemical evolution to one of prebiotic significance. Mauzerall (1992) recognized that a major flaw in this assumption is that these biogenic porphyrins required a prebiotic pyrrole synthesis, with the dilemma that this chemistry has been detected only at low level, and only indirectly by porphine assay in Miller-type experiments. This concern leaves open the question whether uroporphyrin represents only a far advanced stage of biotic porphine development, and thus whether the Granick Hypothesis has any dominion in prebiotic synthesis and evolution of the porphines.

Also, without a plethora of porphinic candidates for the primordial solar energy transducer, little focus has been placed on the match between the visible spectrum of the porphine and the cross section of the visible solar emission. If the conditions required for the formation of life were as energy intensive as its historical sustenance, then by far the greatest energy source available to innovate life was that in the visible range of solar energy, 70 mW cm^{-2}; by comparison, that available in the near UV is only 9 mW cm^{-2} (Mauzerall, 1992; Miller and Orgel, 1973). We would hope that the early porphines would have matched chlorophyll in its efficient spectral overlap with visible solar emission. Yet the porphinic material generated by Hodgson and Ponnamperuma (1968) under primitive earth conditions posssessed the spectral features of a basic etioporphyrin core, which, without the benefit of evolutionary spectral stretch, has no intense absorption maximum beyond 400 nm. We have therefore considered that an all-HCN derived porphine, compatible in its formation with primitive earth conditions, could possible afford the more effective solar spectral match unattainable with the prebiotic and early biogenic porphines.

2. Results and Discussion

2.1. A RETROSYNTHETIC ANALYSIS FOR 2,5,7,10,12,15,17,20-OCTAAZA-21*H*, 23*H*-PORPHINE

A retrosynthesis of the octaazaporphine **1** is shown in Scheme 1. The logic of this retrosynthesis leads to HCN alone as the primordial starting material for the synthesis, and to the acid catalyzed trimerization of HCN (Equation 1) as the retroterminal step in the sequence. HCN has been widely discussed as a primordial reactant in the formation of a host of biologically essential building blocks (Miller, 1955; Ferris
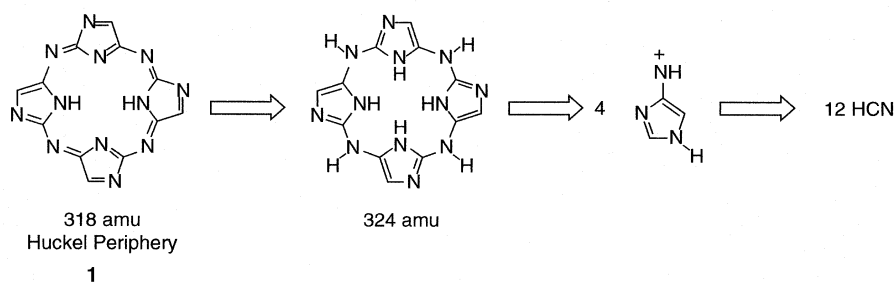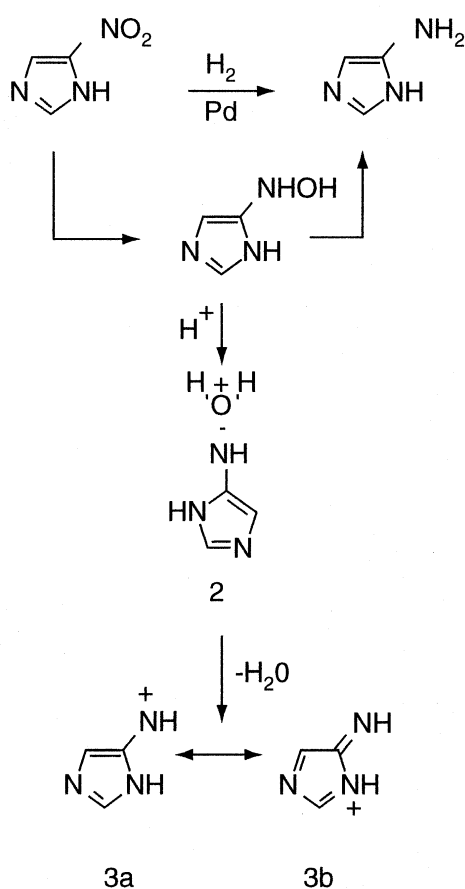
Scheme 1.
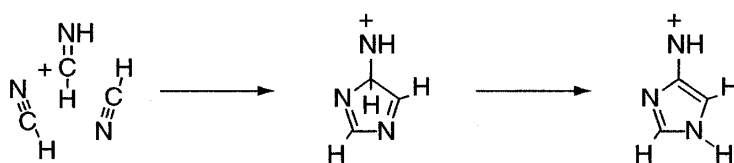
Equation 1.

and Hagan, Jr. 1984; Zahnle, 1986; Stribling and Miller, 1987; Kasting and Brown, in press), yet the chemistry of Equation 1 is unprecedented for the oligomerization of HCN. The latter lacuna in studies of prebiotic organic chemistry has likely had its origin in the speculation that the primordial oceans were slightly basic, and thus by far the largest body of HCN oligomerization chemistry has focused on base catalyzed processes which claim diaminomaleonitrile (DAMN) as the major synthon. Despite the impressive success of the former studies, acid catalyzed processes are not perforce precluded. In fact, 'density function' theoretical calculations (Parr and Yang, 1989; Labanowski and Andzelm Eds., Dixon, Andzelm, Fitzgerald, Wimmer and Jasien, 1990) have been performed in our laboratory and predict that Equation 1 is highly favored thermodynamically: ΔG –75.88 kcal/mole, ΔS –25.86 cal/°K/mole, and ΔG –68.17 kcal/mole at 298 °K.

Despite the favorable energetics for the formation of the conjugate monoprotic acid of 4*H*-imidazol-4-imine, this chemistry may have been hidden from experimental verification because of exceptional reactivity in building higher polymers. We have therefore set the goal of an HCN independent synthesis of the imidazole-4-nitrenium ↔ 1-hydro-4-imino-4*H*-imidazolium ion (the former name is used in this paper), and an assessment of its possible role as a synthon in the polyazaporphine synthesis of Scheme 1. Any success attained in the latter study would be preamble to future studies starting from HCN directly.

2.2. Direct formation of imidazole-4-nitrenium ion [(HCN)$_3$·H$^+$] or imidazol-4-aminohydroxonium ion [(HCN)$_3$·H$_3$O$^+$]

In the catalytic hydrogenation of 4,4'-dinitrophenyl ether to 4.4'-diaminophenyl ether in 1-butanol, <u>N</u>-butylated by-products were shown to arise only from an intermediate formed on the nitro to amino sequence, but not from the monoamine or diamine itself. Pedrotti and Neumer (unpublished results) proposed that the reaction of an aryl nitrenium ion, derived from the intermediate aryl hydroxylamine, gave alkylated product by an initial nitrenium ion insertion on the butanol (no tests were performed to discriminate between the intermediacy of the nitrenium ion or its hydroxonium ion precursor). We therefore thought possible the engagement of similar chemistry *via* the hydrogenation of 4-nitroimidazole (Scheme 2).


Scheme 2.

The hydrogenation of 4-nitroimidazole in methanol over palladium on charcoal catalyst gave an intensely blue solution with $\lambda_{max} = 585$ nm in methanol. The

latter absorption falls well short of the range expected for the blue component of porphinic spectra. Reduction of the blue solution with sodium dithionite gave a yellow-tan solution which, upon shaking in air, returned the blue color. This was again inconsistent with porphine behavior, and suggested instead the Würster type, one-electron redox chemistry of a nitrogen-centered radical cation (Michaelis *et al.*, 1939). It was further demonstrated that only red-tan solutions were observed when the hydrogenation bomb contents were discharged directly to a container inerted with nitrogen; on exposure to air, the latter solution turned intensely blue.

Although there was early evidence that the blue component, *nom de recherche* Nitrenium Blue, was not indefinitely stable in methanol, and with time deposited a blue black solid which remained virtually unchanged at 690 °C, the blue component could be isolated by immediate double chromatography on silica gel that had been pre-eluted with a 1:10/water:methanol mixture, followed by compound elution with methanol. The visible spectrum (Figure 1) of the chromatographed blue component, **4**, was virtually unchanged in its visible maximum (588 nm, methanol) relative to that of the crude hydrogenate. The beginning, middle, and end eluates of triply chromatographed **4** all possessed spectra identical to Figure 1.

With the evidence to follow, it will be shown that the nitrenium blue fraction **4** is an unseparated mixture of an hexahydrooctaazaporphine and its corresponding Würster salt type radical cation formed *via* the cyclotetramerization of the conjugate acid of the mass 81 HCN trimer, Equation 2.
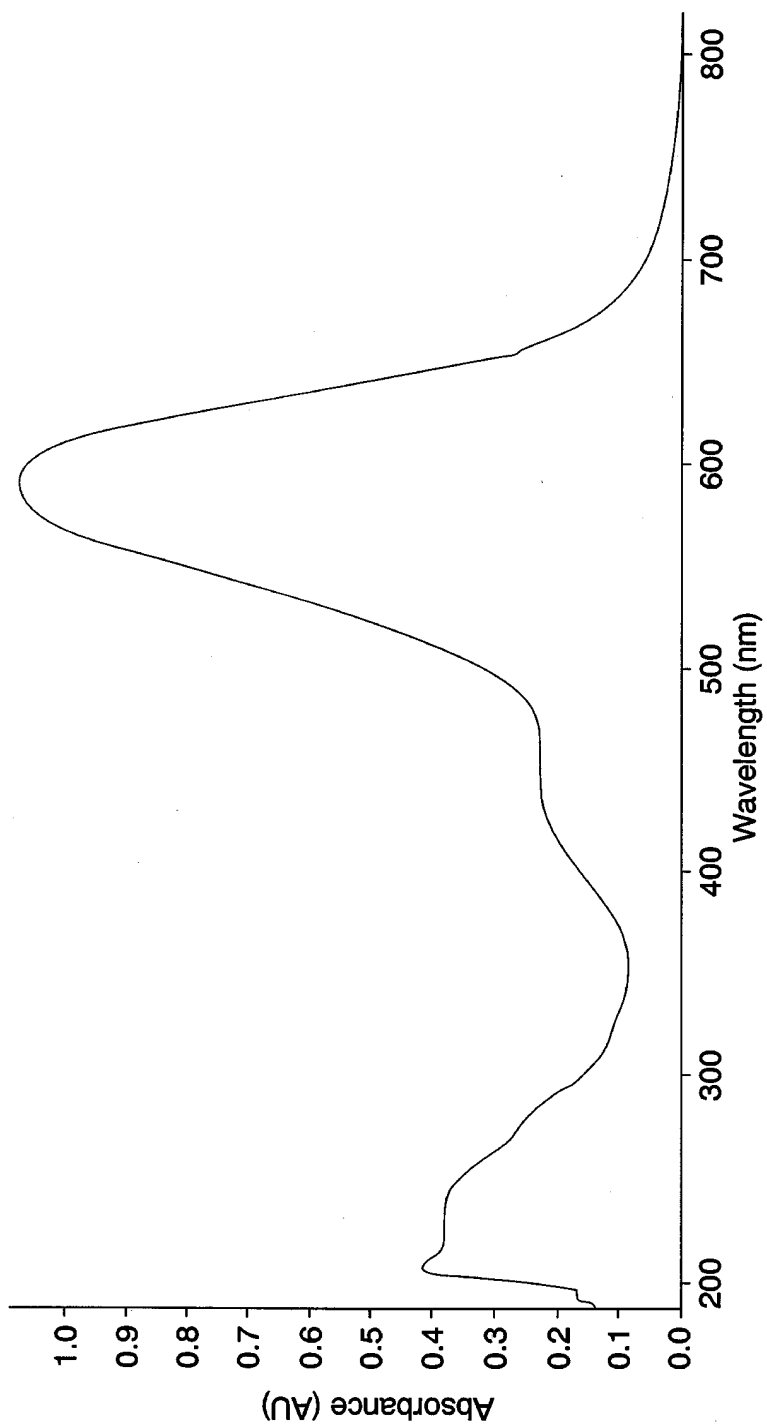

$(HCN)_{12}$

Equation 2.
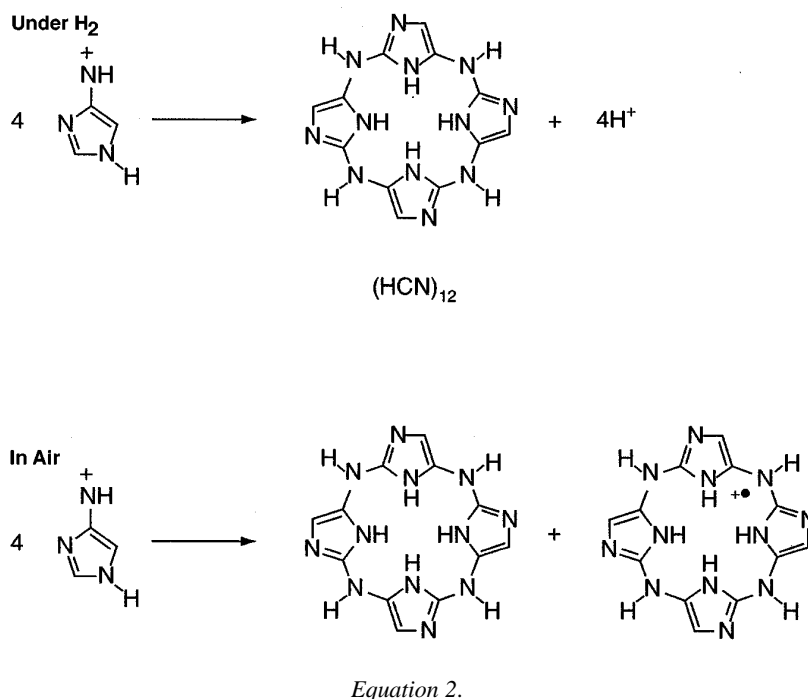
Figure 1. The UV-Vis spectrum of **4** in methanol; a = 27.7 L/g · cm at 588 nm.

2.3. MALDI MASS SPECTRA

MALDI mass spectra of the chromatographed nitrenium blue (**4**) gave an unusual molecular ion cluster with ions observed at m/z 324 (5%), 325 (85%), 326 (100%), 327 (35%), and 328 (4%), Figures 2a and 2b.

The same multiplicity of peaks was duplicated faithfully in the corresponding masses of the parent sodium ion adduct. Sequential single hydrogen atom reduction of hexahydrooctaazaporphine or its radical cation in the matrix during the laser-generated desorption/ionization event could account for the cluster of molecular ion peaks. In contrast to the multiple mass cluster seen in the MALDI spectrum, electrospray mass spectrometry revealed only one molecular ion signal at m/z 325, plus its ^{13}C isotope, which is consistent with $(HCN)_{12}$, MW = 324. This confirms that all MALDI mass peaks in the cluster arise from parent molecules of 324 amu, and that the interpretation given above is correct.

Both the electrospray and MALDI mass spectra of the blue compound revealed significant additional masses. Thus, in the MALDI spectrum, a mass of 243, $(HCN)_9$, was represented by the cluster m/z 244, 245, 246, and 247. The latter mass cluster was absent in the MALDI mass spectrum of highly chromatographically refined **4**, and thus the 243 mass is not a fragment of the higher MW = 324 species (Figure 2c).

Electrospray mass spectrometry, when run at higher than normal voltages, revealed a species of 318 amu, and, of somewhat lesser intensity, 320 amu. While the latter masses do not compel belief in the oxidation sequence of Equation 3
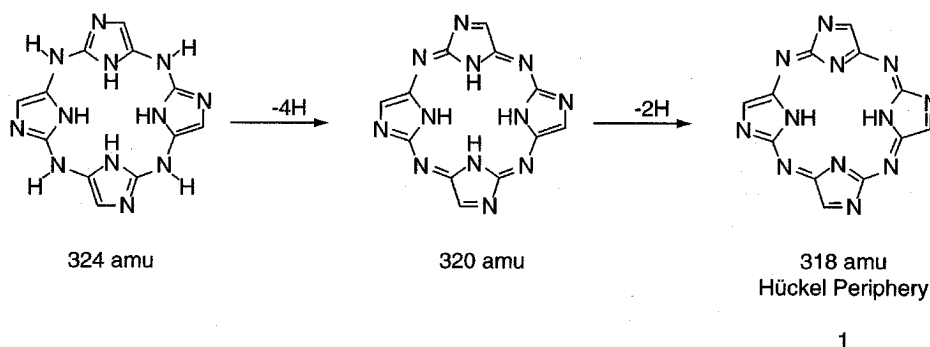

Equation 3.

which leads to the octaazaporphine, they are at least consistent with it. In addition to the MS evidence for the 243 amu species cited above, molecular masses of 241 and 240 amu have also been observed; each of the three masses was singularly portrayed in electrospray analyses which were conducted independently of the other two. The latter sequence of masses suggests the patrimony of structures shown below.
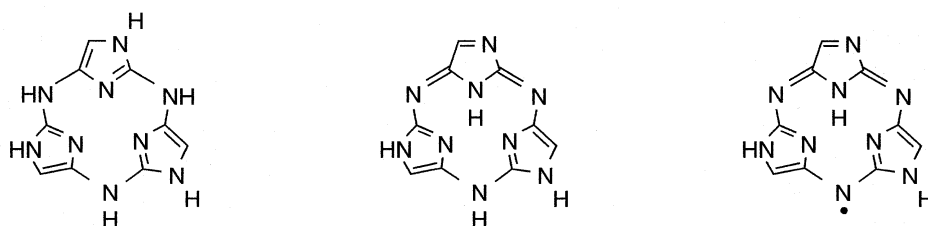
2.4. THE NITRENIUM ION TETRAMER IS CYCLIC

Urea was added to the nitrenium blue in 1 L of methanol eluate from double-chromatography to confer stability to the compound during concentration to solid. The 300 MHz ^{1}H NMR spectrum of this solid solution, **4a**, in DMSO-d_6, after exchange with trifluoroacetic acid-d, is shown in Figure 3.

The single imidazole C-proton resonance at $\delta = 7.573$ ppm indicates that the self-condensations of the imidazole nitrenium ion to give the 324 amu species have all occurred at a single C-ring position, and that the tetramerization has been cyclic. The atomic orbital composition of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) calculated for **3a** suggest that the oligomerization occurs via the intermolecular binding of the 2- and 6′- positions. We conclude that the 324 amu species is, at least in part of its existence, the hexahydrooctaazaporphine, **4-Parent Base**. This substantiates that the intended chemistry of ancillary nitrenium ion formation as discussed above is effective, and that, if the same intermediate is generated by HCN trimerization,
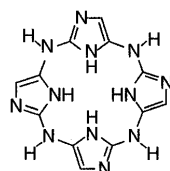
324 amu

4–Parent Base
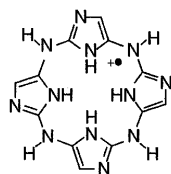
4-Radical Cation

it can be expected to cyclocondense to an octaazaporphine core skeleton without crippling interference from protic solvent. The formation of nitrenium blue was equally effective in aqueous solution with a 4-nitroimidazole substrate level of 0.05 *M*; of course, the oligomerizing nitrenium ions had to be at still lower concentration.

All attempts to obtain a high resolution mass molecular weight of **4** by FAB-MS analysis were unsuccessful in our hands.

2.5. THE IDENTITY OF THE SPECTRALLY BLUE COMPONENT OF NITRENIUM BLUE

While the 324 mass species was always seen to be a correlative property of the nitrenium blue double-chromatographic isolates, the structure **4-Parent Base**, by intuition as well as molecular orbital spectral prediction, has no absorption in the 588 nm region. The ease of decoloration of the blue color by sodium dithionite, and equally facile recovery of color by reaction with air, suggest that the blue component of nitrenium blue is the simple, one-electron oxidation product of the **4-Parent Base**, namely hexahydrooctaazaporphine **4-Radical Cation**. This cyclic Würster salt type radical cation has unusual stability for one centered at an *N*-H position (Michaelis *et al.*, 1939); this is a consequence of having a large number of equi-energy populations of canonical resonance structures by virtue of the ion's cyclic symmetry. In our hands, a solution ESR spectrum of **4** was detectable, but not impeccably resolved from noise in solvents sufficiently polar to dissolve the blue solid residue. An insoluble salt of nitrenium blue was therefore prepared with the possible templating acid, 1,2,4,5-benzenetetracarboxylic acid. The salt, **4b**, in methyl sulfoxide solution, possessed a λ_{max} of 663 nm. To demonstrate that the basic ligand was unchanged by salt formation, the salt was treated with basic IRA-68 ion exchange resin in methanol, the blue component was returned to soluble form, and the spectral maximum was that of the original nitrenium blue, λ_{max} 589 nm.

A solid sample of the salt **4b** exhibited the ESR spectrum shown in Figure 4. There was concern that the templating acid could act as an electron transfer oxidant, and thus generate the radical detected above. The ESR spectrum of the urea solid solution, **4a**, of nitrenium blue mentioned above confirmed a single resonance ESR spectrum with g = 2.0056; thus nitrenium blue contains a radical species irrespective of possible oxidation by the templating acid.

In conclusion, the material identified as nitrenium blue throughout this paper has been shown to be a mixture of the colorless base 5,10,15,20,22,24-hexahydro-2,5,7,10,12,15,17,20-octaaza-21*H*,23*H*-porphine and its highly colored, one-electron oxidation product, the corresponding radical cation. An associative affinity of this type of redox couple is not uncommon in the literature of Würster salts (Michaelis, 1939). The gegen ion of the radical cation is unidentified.
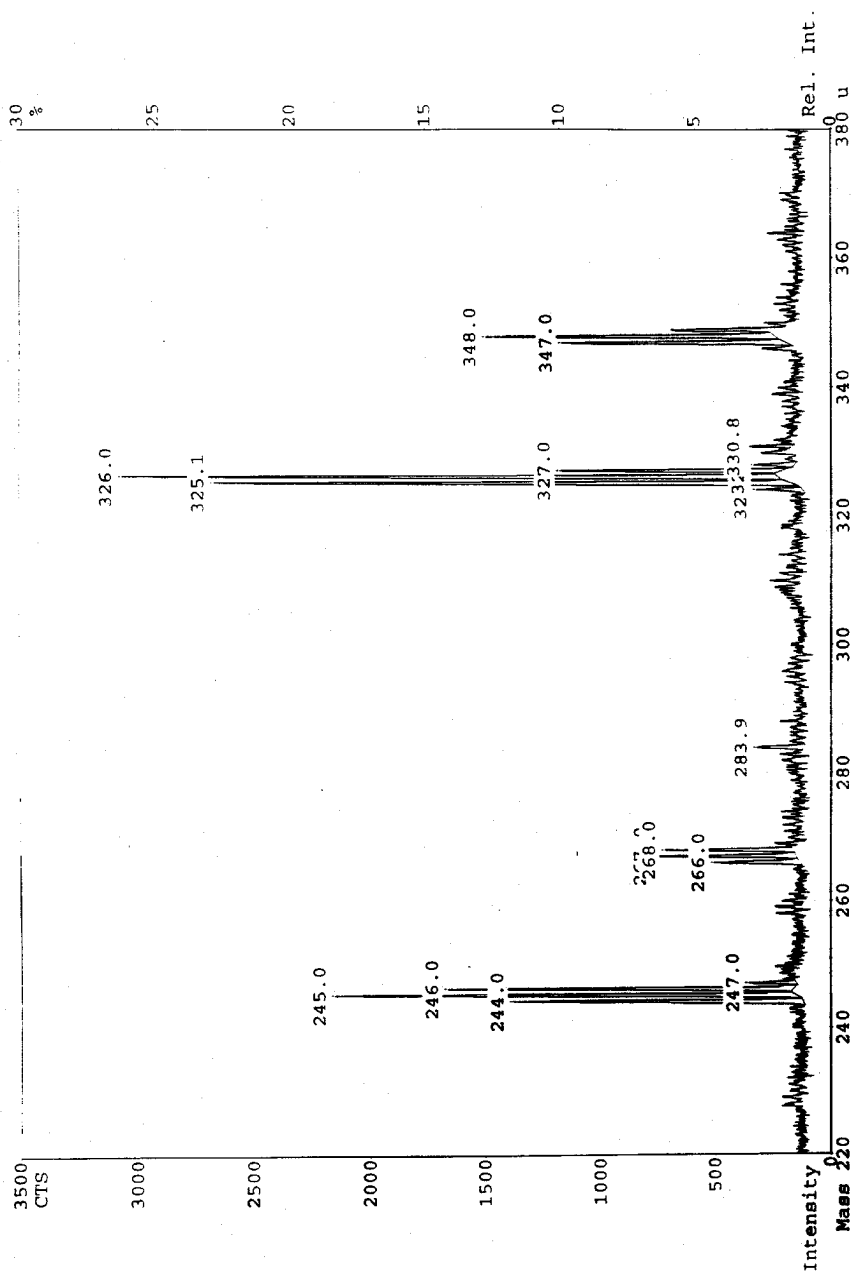
Figure 2a. The MALDI mass spectrum of Nitrenium Blue, (HCN)₁₂, **4** (hexahydrooctaazaporphine, 324 amu), with the molecular mass 243, (HCN)₉, attending. Evidence for the oligomerization of **3a**↔**3b**.
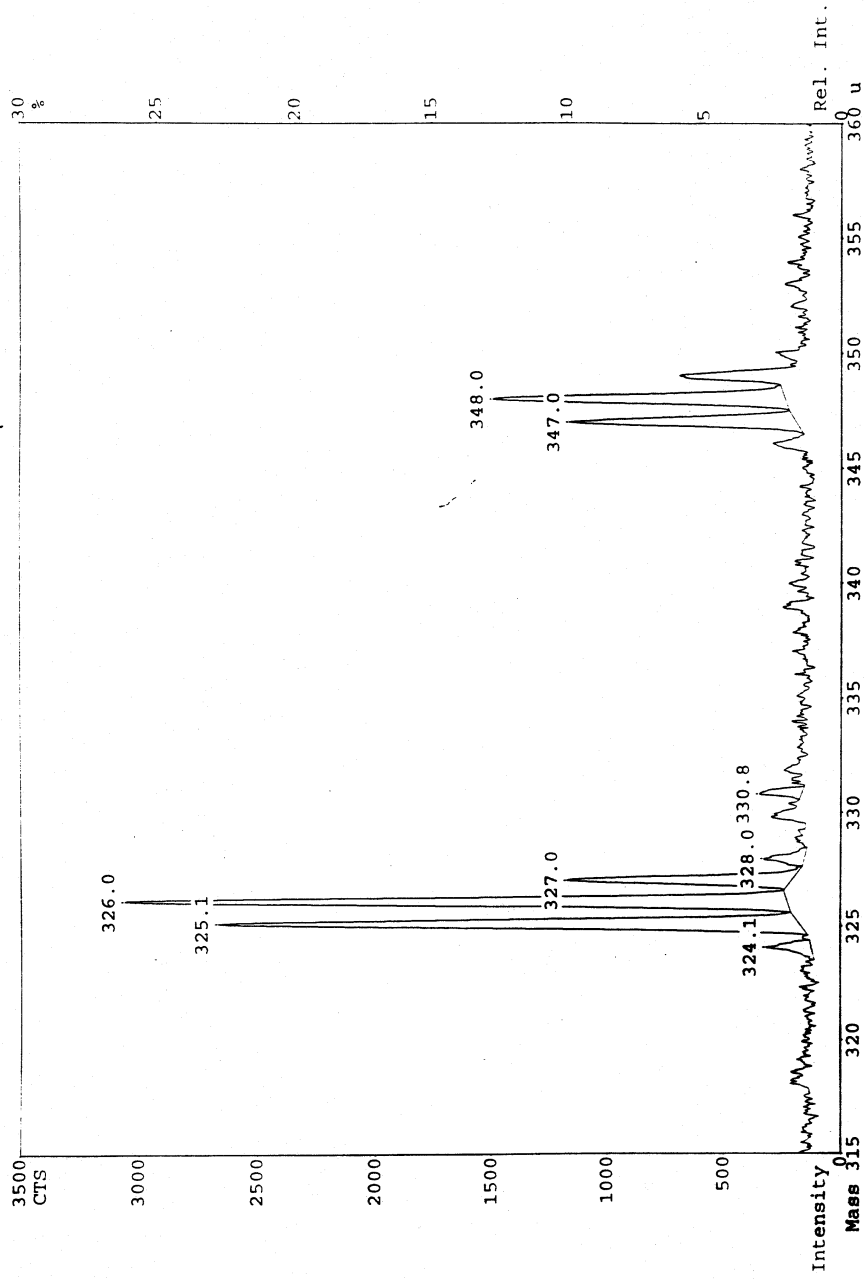
Figure 2b. The MALDI mass spectrum of Nitrenium Blue; blowup of the mass 324 region in Figure 2a.
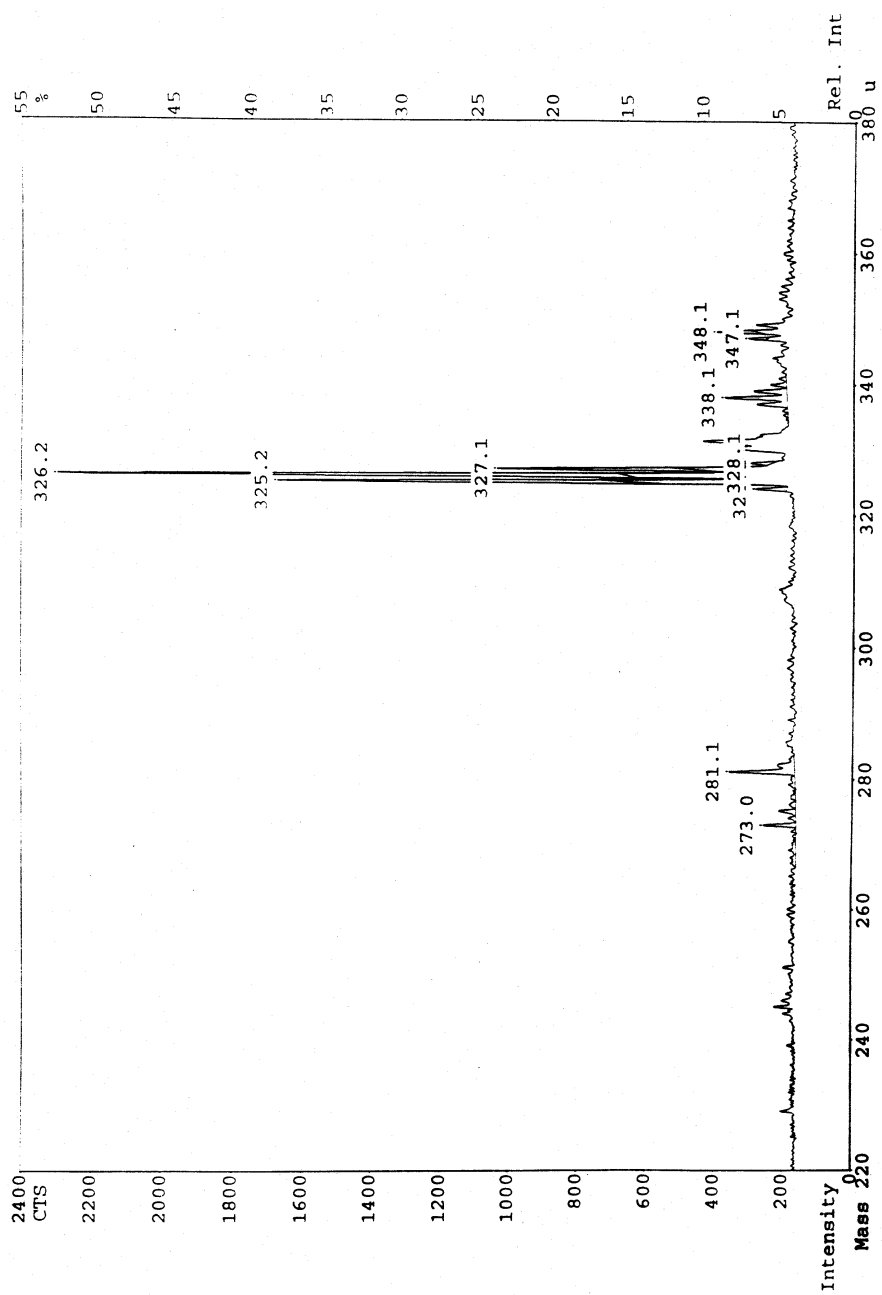
Figure 2c. The MALDI mass spectrum of Nitrenium Blue, $(HCN)_{12}$, chromato-graphically free of $(HCN)_9$ mass 243.

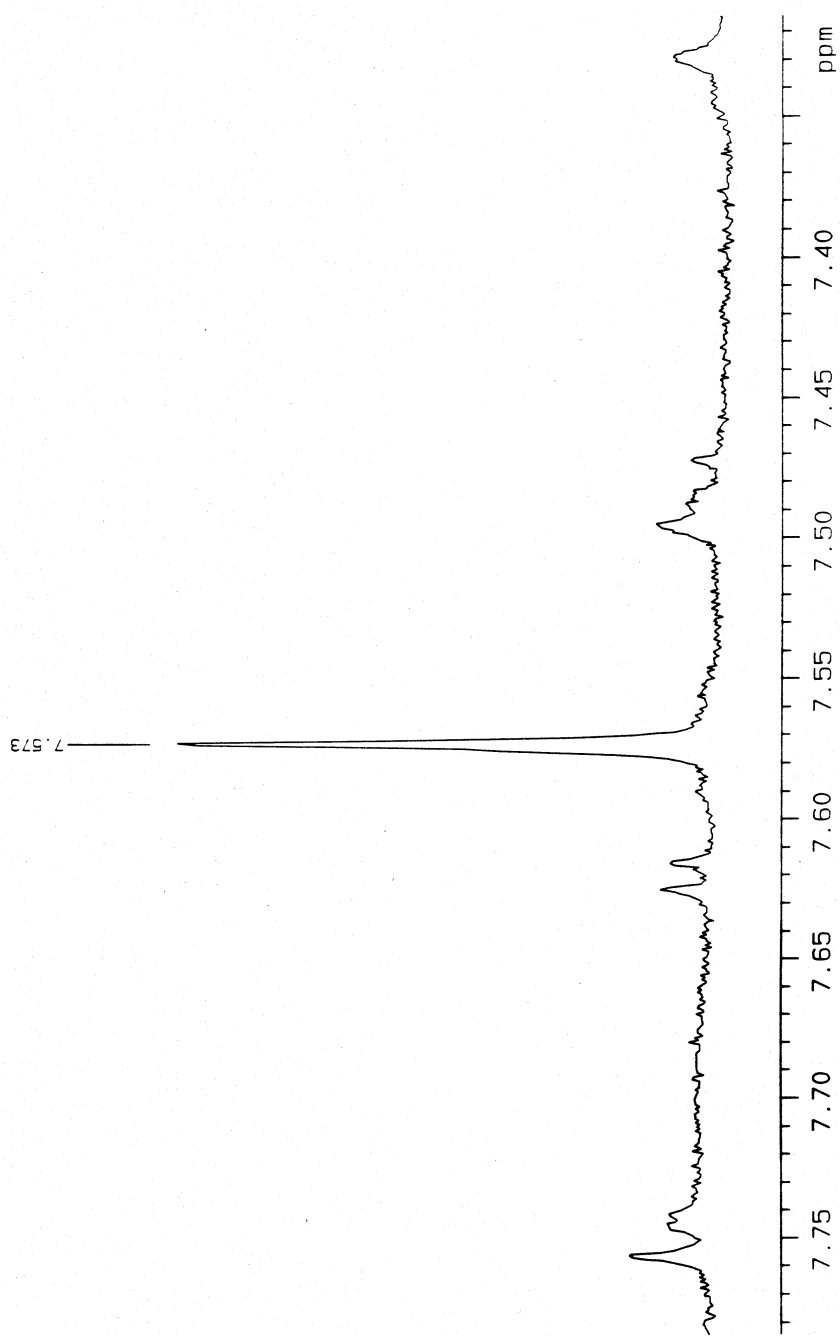*Figure 3.* The 300 MHz ^{1}H NMR spectrum of the urea solid solution of **4a** in DMSO-d_6: hexahydrooctaazaporphine/radical cation **4** with trifluoroacetic acid-d added for proton exchange, $\delta = 7.573$ ppm.
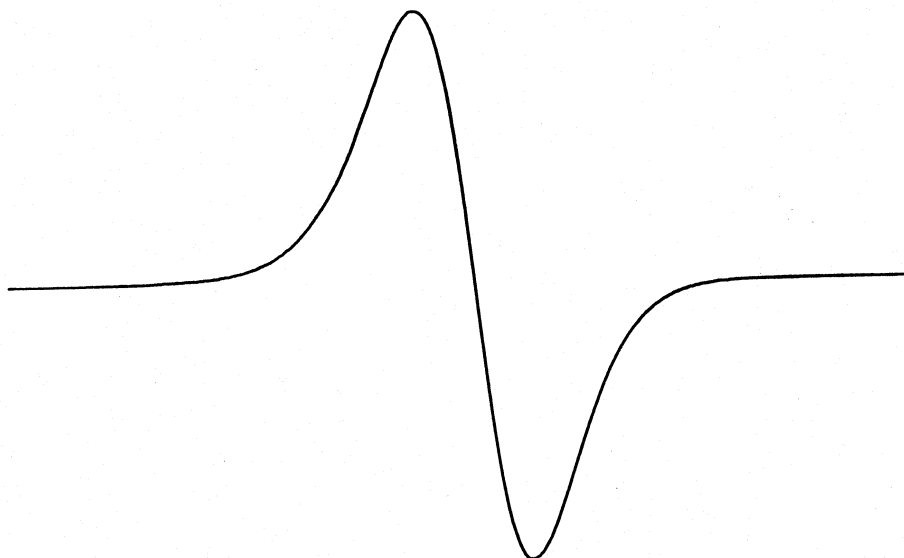
Figure 4. The ESR spectrum of solid **4b**, the template acid salt of **4**, hexahydro-octaazaporphine/radical cation, g = 2.0053, ΔH = 9.7 Gauss.

2.6. THE PUTATIVE OCTAAZAPORPHINE, MW = 318

When the benzenetetracarboxylic acid salt of nitrenium blue was treated with methanolic diethylamine, and the resulting solution allowed to stand in air overnight, a bright blue solid was found to coat the wall of the glass container. The latter solid, contrary to the properties of nitrenium blue, was insoluble in either methanol or methyl sulfoxide, even at the boil. However, the precipitate was soluble in boiling methyl glycolate, and remained soluble on cooling. Electrospray MS of the latter solution revealed a signal at m/z 319, the $[M+H]^+$ of the Hückel aromatic octaaza-porphine (MW = 318), yet none at m/z 325. It will be recalled that m/z 318 was seen in high voltage, in-source collision electrospray MS studies of **4** in which m/z 325 was observed. While the latter detection of mass 318 was likely associated with fragmentation of a high mass species by a collision process, the former was that of a chemically generated species. Studies of the overt oxidation of **4** to the octaazaporphine are in progress.

2.7. PPP π-ORBITAL SPECTRAL ANALYSIS OF OCTAAZAPORPHINE **1**

The application of Pariser-Parr-Pople (PPP) π-molecular orbital calculations to successfully predict spectra of heteroaromatic and functionally substituted aromatic species has been described in the works of Gordon and Neumer (1974), Fukanaga *et al.* (1976), and Gordon (1980). The spectral predictions for the octaazaporphine

and the heme based congeneric porphine, and for the corresponding conjugate monoprotic acids are compared in Table I. Also included is the visible spectrum of chlorophyll-*a*.

Table I

PPP π-orbital spectral comparison of octaazaporphine **1** and porphine, and their conjugate acids

	Porphine			Octaazaporphine **1**	
	λ_{max}, nm	$\varepsilon \times 10^{-2}$		λ_{max}, nm	$\varepsilon \times 10^{-2}$
S1	640	6	S1	621	165
S2	570	29	S2	556	33
S3	384	265	S3	420	167
S4	359	253	S4	386	38
S5	344	674	S5	363	32

	Porphine $\cdot H^+$			Octaazaporphine $\cdot H^+$	
	λ_{max}, nm	$\varepsilon \times 10^{-2}$		λ_{max}, nm	$\varepsilon \times 10^{-2}$
S1	617	41	S1	641	302
S2	593	12	S2	471	34
S3	413	25	S3	471	3
S4	376	17	S4	449	16
S5	343	1050	S5	427	10

Chlorophyll-*a*[a]	
(Obs. in ether)	
λ_{max}, nm	$\varepsilon \times 10^{-2}$
662	902
615	143
578	80
534	36
430	1179
410	759

[a] *Porphyrins and Metalloporphyrins*, Ed. Smith, K. M., Elsevier Scientific Publishing Company, New York, 1975, p. 880.

Albeit that the calculated spectral maxima are not corrected for solvation (typically a correction to longer wavelength), the octaazaporphine has the major portion of its absorption envelope above 400 nm, whereas that of porphine falls below 400 nm. In the latter case, the observed Soret band of porphine at 385 nm is consistent with the calculated features.

The photon absorption disparity that favors the octaazaporphine as the superior match with solar emission is yet more accentuated in the case of the conjugate acids; in this case, almost the entire absorption of the protonated octaazaporphine is in the blue 640 nm band, consistent with the merocyanine features of this species,

whereas by far the major absorption of the protonated porphine is deep in the near UV. So, whether unprotonated or protonated, the octaazaporphine has visible spectral assets that were attained by chlorophyll and other biogenic porphines only after eons of biochemical evolution. As befits a prebiotic solar visible energy transducer, octaazaporphine, more so than its porphine counterpart, would have been ready to operate with high solar spectral absorptivity from the moment of its chemical inception. If the octaazaporphine were the primordial agent of prebiotic photosynthesis, then upon the waning of the prebiotic HCN chemical era, the enzymatic synthesis of contemporary, pyrrole-based porphines evolved to supplant the imidazole-based catalyst.

2.8. Conclusions

The Mauzerall and Mauzerall-Granick Hypotheses that the prebiotic synthesis of organic compounds necessary for life's origin required a porphinic visible solar energy transducer are now supported by the key demonstration that the formal HCN trimer, as the imidazole-4-nitrenium ion, undergoes cyclic tetramerization to the hexahydrooctaazaporphine [$(HCN)_{12}$] precursor 2,5,7,10,12,15,17,20-octaza-21H,23H-porphine. This cyclocondensation occurs at ambient temperature and at low concentration of the $(HCN)_3 \cdot H^+$ intermediate in protic media, including water. Since the visible absorption spectrum of the polyazaporphine was shown by calculation to be similar to that of chlorophyll-a, the existence of early, pyrrole-based porphines may not have been an essential requirement for efficient use of solar enery in the abiogenesis of life on Earth.

The novel intermediate in the above polyazaporphine chemistry is imidazole-4-nitrenium ion $\leftrightarrow$ 1-hydro-4-imino-4H-imidazolium ion, $(HCN)_3 \cdot H^+$, the conjugate monoprotic acid of a formal HCN trimer. Consistent with the proposed retrosynthesis for the octaazaporphine, the nitrenium ion has the correct atom connectivity for an HCN origin. Although this intermediate was prepared independently of acid catalyzed HCN oligomerization, this 5-membered ring trimerization has been shown to be highly thermodynamically favored by calculation. In transforming HCN to the strongly basic 4H-imidazol-4-imine core, a new opportunity is created for further aggregation and oligomerization of HCN trimer units on acidic clays or on polybasic acid backbones. Thus, the overall oligomerization of twelve HCN molecules to an $(HCN)_{12}$ porphinic core would not necessarily be kinetically limited by low HCN concentrations in the primordial oceans. Because of the unique structure and reactivity of 4H-imidazol-4-imine revealed in these studies, it is readily envisioned that the possible complicity of this intermediate in the origin of life goes well beyond simply being the source of the first photosynthetic porphine.

3. Experimental Section

Matrix assisted laser desorption/ionization (MALDI) mass spectra were obtained on a Thermo Bioanalysis Vision 2000 time-of-flight mass spectrometer (Hemel Hempstead, England) operated in the reflectron mode with 5 kV ion acceleration and 1 kV postacceleration. A nitrogen laser (337 nm), Laser Science, Inc. (Newton, MA), was used with the beam attenuated to just above threshold for observing analyte ions. Mass spectra were obtained by summing the ions obtained from 25 laser shots. 2,5-Dihydroxybenzoic acid (Sigma), as obtained from the manufacturer, was dissolved in methanol (10 mg/ml) and used as a matrix solution. A 0.5 μL solution of a 2:1 mixture of matrix solution and a concentrated methanol solution of the analyte was added to the MALDI target and allowed to air dry at ambient temperature.

Electrospray mass spectra were obtained on a MicroMass Trio 2000 quadrupole mass spectrometer (Manchester, England) fitted with a combination electrospray/APCI ion source. A saturated methanol solution of the analyte was diluted with an equal volume of methanol and electrosprayed at a flow rate of 5 μL/min. The voltage difference between the atmosphere-vacuum aperture and the first skimmer was maintained at 26 V as a typical condition for generation of only molecular ions and at 61 V for generation of in-source collision-induced fragment ions.

^{1}H NMR analyses were performed on a 300 MHz Varian VXR-300 spectrometer, references to internal TMS ($\delta = 0$), and the ESR spectrometer was a Bruker ESP-300. A Hewlett Packard 8452A Diode Array Perkin spectrophotometer was used for UV-Visible spectral determinations, and a Brinkman 682 titrator equipped with a glass pH electrode was used in titrations with 0.1 N aqueous HCl. Hydrogenations in glass were run on a Burke-Harper SK-137 shaker reactor, and the 10% Pd on charcoal catalyst was Alfa Products lot number 042480. All other hydrogenations were run in a 400 mL Hastelloy C-276 shaker bomb.

All chemical starting materials were of commercial origin, and used without further purification. The packing for column chromatography was silica gel, Davisil^{tm} grade 634, 100–200 mesh.

5,10,15,20,22,24-Hexahydro-2,5,7,10,12,15,17,20-octaaza-21H, 23H-porphine, Parent and Radical Cation (4). A mixture of 4-nitroimidazole (5.00g, 44.2 mmol), 10% Pd on charcoal (0.250g, suspended in 10 mL 2-propanol to prevent autoignition with methanol vapor), and methanol (200 mL) was loaded in a 400 mL shaker bomb, and inerted with nitrogen sweep and evacuation. The mixture was hydrogenated at 80 °C and 500 psi dihydrogen pressure for 6 h with shaking. The reaction mass was typically discharged in air, in which case it was intensely blue; replicate hydrogenations gave the same result, and the hydrogenation was run in glass to demonstrate that color formation was independent of materials of bomb contruction. Chromatography columns were 6.35×20 cm silica gel that were pre-eluted with 1 L 90% methanol-10% H_2O, followed by 1.4 L methanol. The bomb contents

were added directly to the column, and the blue component was eluted in 1.5 L methanol. The latter eluate was concentrated *in vacuo* on a rotary evaporator to 100 mL, and the concentrate was re-chromatographed as above. Concentration to dryness as above, avoiding excessive heating, gave 0.148 g (4.13% if pure) of **4**, mass 324 by electrospray MS; mass 243, $(HCN)_9$ was also detected in chromatographically less refined isolates. The NMR spectrum of **4** showed total absence of 4-nitroimidazole, the major component in the forefractions. The results of the panoply of spectroscopic analyses of **4** are discussed in the text.

The preparation of **4** was equally successful when run in water without external heating (33°C).

The Urea Solid Solution of 4 (4a). To a doublychromatographed fraction of **4** in 1 L of eluate, prepared and isolated as above, was added 2.60 g of urea. The solution was concentrated to solid *in vacuo* on a rotary evaporator, 2.78 g.

The 1,2,4,5-BenzeneTetracarboxylic Acid Complex of 4 (4b). A doublychromatographed fraction of **4** in 1 L of eluate, prepared and isolated as above, was titrated to pH 4.7 with 0.1 N aqueous HCl, and 12.5g of 1,2,4,5-benzene tetracarboxylic acid was added to the homogeneous solution, stirred at room temperature for 0.5 h, and the precipitated complex collected by vacuum filtration, washed copiously with methanol and dried in air, 0.328 g.

Acknowledgements

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