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$CH_4/NH_3/H_2O$ Spark Tholin: Chemical Analysis and Interaction with Jovian Aqueous Clouds

GENE D. MCDONALD, BISHUN N. KHARE, W. REID THOMPSON, AND CARL SAGAN

Laboratory for Planetary Studies, Cornell University, Ithaca, New York 14853

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The organic solid (tholin) produced by spark discharge in a CH_4 + NH_3 + H_2O atmosphere is investigated, along with the separable components of its water-soluble fraction. The chemistry of this material serves as a provisional model for the interaction of Jovian organic heteropolymers with the deep aqueous clouds of Jupiter. Intact (unhydrolyzed) tholin is resolved into four chemically distinct fractions by high-pressure liquid chromatography (HPLC). Gel filtration chromatography reveals abundant components at molecular weights ≈600–700 and 200–300 Da. Gas chromatography/mass spectrometry of derivatized hydrolysis products of unfractionated tholin shows about 10% by mass protein and nonprotein amino acids, chiefly glycine, alanine, aspartic acid, β-alanine, and β-aminobutyric acid, and 12% by mass other organic acids and hydroxy acids. The stereospecificity of alanine is investigated and shown to be racemic. The four principal HPLC fractions yield distinctly different proportions of amino acids. Chemical tests show that small peptides or organic molecules containing multiple amino acid precursors are a possibility in the intact tholins, but substantial quantities of large peptides are not indicated. Candidate 700-Da molecules have a central unsaturated, hydrocarbon- and nitrile-rich core, linked by acid-labile (ester or amide) bonds to amino acid and carboxylic acid side groups. The core is probably not HCN "polymer." The concentration of amino acids from tholin hydrolysis in the lower aqueous clouds of Jupiter, about 0.1 μM, is enough to maintain small populations of terrestrial microorganisms even if the amino acids must serve as the sole carbon source.

INTRODUCTION

The atmosphere of Jupiter is dominated by hydrogen and helium (mole fractions $X_{H2} \simeq 0.90$, $X_{He} \simeq 0.10$), with smaller fractions ($\sim 10^{-4}$–10^{-3}) of CH_4, NH_3, H_2O, and H_2S observed or expected on the basis of solar abundances and thermochemical equilibrium. CH_4, NH_3, and H_2O have been observed in the Jovian atmosphere (see, e.g., Gautier and Owen 1989).

Kuiper (1951) was among the first to discuss phase equilibria and the clouds of Jupiter, and predicted the presence of NH_3 cirrus around the 160 K level. Lewis (1969) and Weidenschilling and Lewis (1973) considered the condensation of thermochemical equilibrium species in Jupiter's atmosphere, predicting the existence of H_2O clouds above the altitude at which $p \simeq 8$ bar, $T \simeq 280$ K; NH_4SH clouds above $p \simeq 2.5$ bar, $T \simeq 220$ K; and NH_3 clouds above $p \simeq 0.8$ bar, $T \simeq 160$ K. They showed that the lower part of the H_2O cloud would be liquid—a basic solution containing dissolved NH_3. Telescope and spacecraft observations of Jupiter's clouds reveal a range of yellowish to brown-red hues. These colors may derive from organic or inorganic species (or both) (e.g., West *et al.* 1986). Independent of the nature of the Jovian chromophores, it is certain that (1) lightning and coronal discharge can produce simple (Sagan and Miller 1960) to complex (Woeller and Ponnamperuma 1969, Sagan and Khare 1973) organic products in the regions of the NH_3, NH_4SH, and H_2O + NH_3 (aq) clouds; (2) UV light generates both simple organic molecules such as HCN (Sagan and Khare 1971, Kaye and Strobel 1983, Ferris and Ishikawa 1988) and more complex products including heteropolymers (Sagan and Khare 1973, Ferris and Ishikawa 1988); and (3) UV and magnetospheric electron precipitation produce stratospheric, UV-absorbing hazes that are likely to be organic.

Carlson *et al.* (1987) have recently performed a detailed re-analysis of cloud condensation and chemistry on Jupiter. They also reviewed the spectroscopic determinations of CH_4, NH_3, and H_2O abundances and the H_2S upper limit in the context of the condensate–gas equilibria. While some unexpectedly low abundances derived from spectroscopic models have called into question the existence of thick H_2O clouds, Carlson *et al.* provide arguments supporting deep abundances of H_2O, NH_3, and H_2S about twice solar—that is, $X_{CH4} \simeq 2 \times 10^{-3}$, $X_{NH3} \simeq 3 \times 10^{-4}$, $X_{H2O} \simeq 2 \times 10^{-3}$, and $X_{H2S} \simeq 5 \times 10^{-5}$. Gas-phase abundances are strongly depleted by cloud condensation and chemistry and, at higher altitudes, by UV photochemistry. An H_2O + NH_3 (aq) cloud that is liquid between its base at $p = 4.8$ bar, $T = 275$ K and the $p = 3.7$ bar, $T =$

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253 K level is predicted. Both simple and complex organic compounds produced by the various energy sources mentioned above will diffuse or sediment downward through the atmosphere, encountering this aqueous layer. The possibility of an aqueous organic chemistry on Jupiter thus exists (cf. Sagan 1961). More generally, organic heteropolymers (tholins), produced by a variety of energy sources in the atmosphere, must form a pervasive and deep aerosol layer on Jupiter.

In a simple, first-cut simulation of the formation of tholins in Jupiter's atmosphere, we have subjected equimolar mixtures of CH_4 and NH_3 with a smaller amount of H_2O (~2.5%) to a spark discharge at room temperature and pressure. This gas mixture is, especially because of the absence of H_2 and He, hardly a close simulation of the real Jovian atmosphere. But recent plasma discharge experiments in this laboratory (McDonald *et al.* 1991) in CH_4/NH_3 atmospheres with a 100 : 1 excess of H_2/He also generate a brownish organic solid similar in appearance to the $CH_4/NH_3/H_2O$ spark tholin analyzed here. However, the H_2/He/CH_4/NH_3 solid is produced in much lower yields and is not yet available for detailed analysis. The present study of $CH_4/NH_3/H_2O$ spark tholin is offered as a first step in the understanding of the solid organic products of charge particle chemistry in the atmospheres of Jupiter and the other Jovian planets, and perhaps the early Earth as well.

Eight pairs of electrodes energized by Tesla coils and supplying ~50 kV each were actuated in sequential order (cf. Khare *et al.* 1981). Some chemical analysis of this brownish solid product, using mostly infrared spectroscopy and pyrolytic gas chromatography/mass spectrometry (GC/MS), has been reported (Sagan and Khare 1979, Khare *et al.* 1981). Compounds identified included a wide range of aliphatic and aromatic nitriles, alkanes, alkenes, and aromatic hydrocarbons, pyrrole, and pyridine. There is evidence (Sagan and Khare 1979, Khare *et al.* 1981, Thompson *et al.* 1991) that these compounds are present, either free or bound to other chemical structures, in the intact tholin and are not artifacts of the pyrolysis stage of the analysis. From the sequential vacuum pyrolyses, it was found that many of the nitriles were less strongly bound to the tholin than were most of the hydrocarbons. Except for CO_2, no oxygen-containing compounds were detected among the pyrolyzates, which was taken as evidence for carboxylic acids as the primary repository of O in the tholin. Tentatively, a structure with polynitriles bonded to hydrocarbon moieties was proposed. Jupiter spark tholin hydrolysis products have been analyzed for the presence of protein amino acids (Stoker *et al.* 1990); glycine, aspartic acid, alanine, and glutamic acid were the most abundant, with tyrosine, leucine, isoleucine, and phenylalanine also detected. There is also preliminary evidence that spark tholin hydrolyzates contain at least short peptide-like fractions (Khare *et al.* 1989, Su *et al.* 1989).

In this work we pursue a further chemical analysis of Jupiter spark tholin to understand its structure and composition. Specifically, we are interested in the interaction of this solid with aqueous media as an example of the kinds of chemical species expected to be produced when Jovian organic heteropolymers—produced by UV photolysis, magnetospheric electrons, lightning shocks, and other energetic processes—encounter the H_2O + NH_3 (aq) cloud. We investigate the chemical functional groups present in tholin molecules by spectroscopic techniques as well as wet chemical and chromatographic analyses, and isolate by high-pressure liquid chromatography (HPLC) some chemically distinct fractions of the aqueous solutions of spark tholin. We examine the molecular weight distribution of the water-soluble components of the tholin by gel filtration chromatography, and determine both the amino acid compositions of acid-hydrolyzed samples of the chromatographic fractions and the abundances of other organic molecules released on hydrolysis. Based on these results, we propose candidate molecular structures for the components of $CH_4/NH_3/H_2O$ spark tholin.

METHODS AND MATERIALS

Production of Tholin

The tholin material used in these analyses was prepared earlier (Khare *et al.* 1981). The infrared spectra of the material did not change significantly between production and analysis.

HPLC Separation of Tholin Components

The water-soluble fraction of Jupiter spark tholin prepared by Khare and colleagues (Khare *et al.* 1981) was separated into components by reverse-phase HPLC using a Waters 600 gradient elution system. The column used was a Waters Nova-Pak C_{18}, 3.9 × 300 mm, 4-μm pore size, and the mobile phase was 0–50% 1-propanol in 0.1% trifluoroacetic acid/H_2O. Eluting components were detected using a Waters 990 photodiode array detector over a wavelength range of 200–800 nm. This method separates molecules mainly by polarity: highly polar species do not bond well to the hydrocarbon column surface and are eluted first, while less polar species (more or less regardless of size) are eluted as the propanol percentage is gradually increased.

Amino Acid Analysis of Intact Spark Tholin and of Its HPLC Fractions

Intact spark tholin (3.3 mg) was hydrolyzed by incubation in 6 *M* HCl at 110°C under N_2 for 24 hr and then dried under N_2. Tholin HPLC fractions were collected from the

HPLC effluent, dried under N_2, and hydrolyzed in 6 *M* HCl at 150°C under N_2 for 90 min. Amino acid analysis of both unfractionated and fractionated tholin samples was carried out using the Waters Pico-Tag system at the Biotechnology Division Analytical Facility at Cornell University. This method is capable of detecting both biological and nonbiological amino acids [compounds with both amino (NH_2) and carboxylic acid (COOH) functional groups]. Determination of D/L ratios of hydrolyzed tholin amino acids was carried out by gas chromatography of the *N*-pentafluropropyl isopropyl esters of the amino acids. The column used was a Chirasil-Val capillary column (Alltech), 50 m × 0.25 mm, with He as carrier gas and detection by flame ionization (Abe *et al.* 1983).

UV/VIS Spectroscopy

UV/VIS spectra of intact, unfractionated tholin were obtained with a Cary 14 spectrophotometer, and spectra of individual tholin HPLC fractions with a Waters 990 photodiode array detector. All spectra were obtained in H_2O.

Infrared Spectroscopy

IR spectra were obtained using a Mattson Galaxy 6020 FTIR spectrometer. The spectrum of unfractionated tholin was obtained neat (without solvent) in a KBr pellet, while spectra of HPLC fractions were analyzed as films dried from aqueous solution onto AgCl plates.

Molecular Weight Determination: Gel Filtration

A Bio-Gel P2 gel filtration column was calibrated in H_2O using Blue Dextran 2000, tryptophan, and deoxyguanosine monophosphate as molecular weight standards. The water-soluble fraction of untreated spark tholin was then applied to the column and eluted with H_2O. Material eluting from the column was detected by absorption at 220 nm using a Beckman DB-G spectrophotometer. Fractions containing UV-detectable material were assayed for tholin components by HPLC under the conditions described above. Absorbance at 220 nm was used to plot the elution profile of each component.

Tests for the Peptide Bond

We know that amino acids are released from tholins upon hydrolysis, but we do not know that they are present as amino acids in intact tholins, nor, if they are, how they are bonded in the solid. One possibility is that they are connected in peptide-like chains, as in proteins. To examine this possibility we performed three wet chemistry tests that are routinely used to measure peptides in biological samples:

(*1*) *Biuret peptide assay.* The biuret reagent was prepared using the procedure of Layne (1957). A 1-mg tholin sample was dissolved to the limit of solubility in 1.2 ml H_2O. Four milliliters of biuret reagent was added and the solution incubated at room temperature for 30 min. Absorbance at 550 nm was measured as a standard indicator for this test. The absorbances of both tholin/H_2O and biuret reagent/H_2O were also measured and used to subtract for the native absorbance of both biuret reagent and tholin at 550 nm.

(*2*) *Bradford peptide assay.* The assay was performed using the procedure of Bradford (1976). A calibration curve was constructed using bovine serum albumin standards at 0.5, 1.0, and 2.0 mg/ml. The absorbance of the tholin sample alone at 595 nm was subtracted from the measurements of tholin in the Bradford reagent.

(*3*) *Lowry peptide assay.* The assay was performed using the procedure of Lowry *et al.* (1951). The sample used for the Lowry assay was identical in tholin concentration to that used in the Bradford assay. The native absorbance of the tholin at 750 nm was subtracted from the absorbance measured for tholin in the Lowry reagent.

Functional Group Assays

Samples of unfractionated spark tholin were reacted with hydroxylamine to assay for the presence of aldehyde/ketone and amide/nitrile groups. Samples of 1, 2, and 3 ml saturated aqueous tholin solution as well as a blank containing no tholin were added to a hydroxylamine solution in ethanol. A decrease in the pH of the solution upon addition of the sample would indicate the presence of aldehyde or ketone groups (Ferris *et al.* 1981). In a second test, a 2-mg sample of tholin was added to a hydroxylamine/KOH solution in propylene glycol. The solution was heated to boiling and a few drops of aqueous $FeCl_3$ solution were added. A color change from orange to red-brown would indicate the presence of amide or nitrile groups (Ferris *et al.* 1981).

Trimethylsilyl Derivatization/Gas Chromatography

A 16-mg sample of unfractionated tholin was acid-hydrolyzed as described above and the resulting solution reacted with bis(trimethylsilyl)-trifluoracetic acid in acetonitrile. This procedure produces volatile, trimethylsilyl derivatives of amine and acid functional groups of organic molecules, resulting in a sample that can be analyzed by GC/MS (Gehrke and Leimer 1971). The derivatives produced were separated by gas chromatography using a Hewlett–Packard 5880A gas chromatograph with a CPSIL 5-CB capillary column and a temperature range of 80–325°C. Identification of derivatives was made by obtaining mass spectra with a Hewlett–Packard 5970 mass selective detector and matching those spectra using the

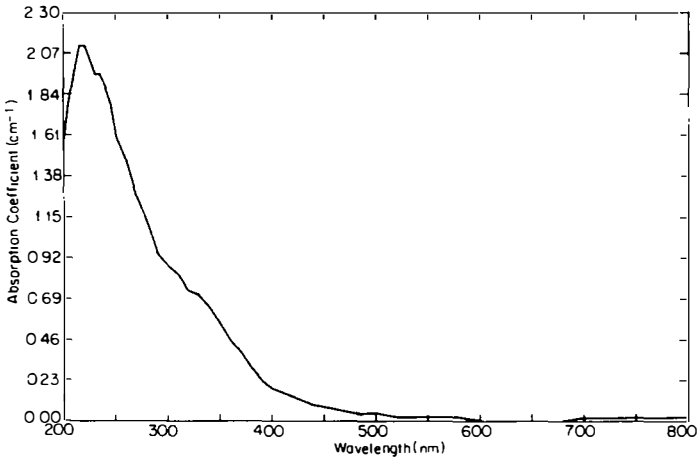


FIG. 1. Absorbance spectrum of unfractionated Jupiter spark tholin from 200 to 800 nm.

computer spectral library. Only spectral matches given better than 80% probability were considered to be likely identifications.

RESULTS AND DISCUSSION

Spectra of Unfractionated Spark Tholin

UV/VIS absorption by organic molecules generally result from bonding- or nonbonding-to-antibonding electronic transitions in some types of double- or triple-bonded functional groups, and/or in conjugated structures (alternating single and multiple bonds) or resonance structures ("aromatic" rings) within the molecule. In the case of Jupiter spark tholin, VIS spectra are also relevant to the possible role such molecules may play as the observed chromophores in the Jovian atmosphere. The UV/VIS spectrum of unfractionated $CH_4/NH_3/H_2O$ spark tholin is shown in Fig. 1. Maximum absorption occurs at approximately 218 nm, with other, much weaker, features at 235 and 325 nm. No significant absorption is observed at wavelengths longer than 500 nm. An absorption maximum near 220 nm with no significant longer wavelength peak is consistent with compounds having one pair of conjugated multiple bonds (Pecsok and Shields 1968), and with α,β unsaturated nitriles or carboxylic acids. We note that the wavelength of maximum UV absorption of Jupiter tholin is very close to that of the incompletely identified 217.5-nm absorption feature in the interstellar medium (e.g., Savage 1975), variously attributed to particles of graphite, silicates, and other materials (Steel and Duley 1987). The peak width at half maximum of the interstellar feature is approximately 48 nm, while that of Jupiter tholin spectra is about 90 nm (Fig. 1), and may be a function of radiation dose.

Infrared absorption in the mid-infrared region (2–20 μm) results from vibrational modes of specific chemical bonds. The mid-infrared spectrum of unfractionated dry spark tholin in a dry KBr pellet is shown in Fig. 2. Prominent features include the O—H/N—H stretching band at 3343 cm^{-1} (2.96 μm), the C—H stretching band at 2934 cm^{-1} (3.4 μm), a probable C≡N band at 2195 cm^{-1} (4.56 μm), a strong C=O band at 1663 cm^{-1} (6.06 μm), a possible N—H bending mode at 1559 cm^{-1} (6.42 μm), and a possible C—H bending mode at 1456 cm^{-1} (6.87 μm). (The apparent feature at approximately 1350 cm^{-1} is an artifact due to baseline correction, while the feature at approximately 2400 cm^{-1} is due to incomplete baseline subtraction of background CO_2 from the air in the spectrometer sample compartment.) The C=O and N—H bending bands are compatible respectively with the so-called amide I and II bonds in peptide spectra, but do not by themselves constitute evidence of peptide bonds. The mid-IR spectrum of spark tholin has some similarities to those of γ-irradiated aqueous NH_4CN and HCN solutions, and to HCN "polymer," as discussed below. Note that the intact spark tholin lacks any significant C—O absorption feature in the 1000–1200 cm^{-1} (10–8.3 μm) range. The absence of this band in the spectrum of unfractionated tholin but its presence in the spectra of tholin HPLC fractions (Fig. 5) is discussed below.

Solubility and pH of Jupiter Spark Tholin

Jupiter spark tholin is highly soluble in water and dimethylsulfoxide (DMSO), and not significantly soluble in hexane, carbon tetrachloride, or acetone. While tholin is dissolved completely by DMSO in a period of a few hours,

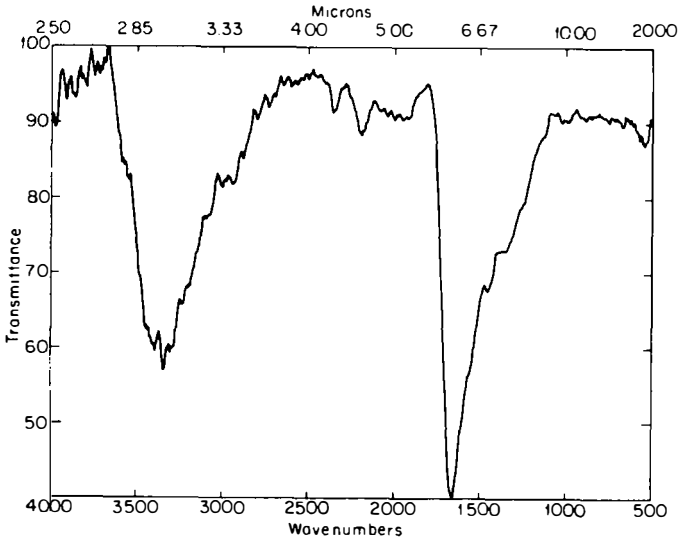


FIG. 2. Infrared transmission spectrum of unfractionated Jupiter spark tholin.

the dissolution of tholin in water is much slower (on the order of days). It is difficult therefore to determine whether all tholin components are water-soluble; however, the bulk tholin is more than 50% water-soluble by weight. The pH of a saturated aqueous solution of intact (i.e., unfractionated and unhydrolyzed) spark tholin is 4.5, indicating the dominance of acidic over alkaline functional groups among the tholin components.

Assays for Peptide and Protein-Like Structures in Aqueous Tholin Solution

In an attempt to assess the degree of occurrence of peptide-like amide bonds or protein-like macromolecules in the spark tholin, samples were subjected to three chemical tests used to quantify biological protein solutions. The biuret reaction (Layne 1957) is a fairly sensitive assay for the presence of peptide bonds. The biuret reagent complexes with peptides (chains of amide-bonded amino acids), with a resultant shift in the absorption maximum of the solution. The biuret reagent/tholin solution showed no increase in absorbance over the linear combination of the native absorbances of tholin and biuret reagent. The negative result obtained upon assay of Jupiter spark tholin indicates that normal peptide bonds between amino acids are not present in substantial quantities. This result does not, however, rule out the presence of modified amide bonds or of single amide bonds within a large molecule (e.g., Fig. 7, discussed below).

The Bradford reagent forms a complex with peptides that absorbs at specific visible wavelengths (Bradford 1976), while in the Lowry assay (Lowry *et al.* 1951) the complex formed reduces a component of the reagent and shifts its absorption wavelength. The Bradford assay on spark tholin gave an apparent concentration of complexing material of 0.3 mg/mg tholin. The Lowry assay gave an apparent concentration of reactive material of 2.3 mg/mg tholin. Both the Bradford and the Lowry assays yielded results that could be interpreted as positive; since these assays are designed only to quantify proteins, however, the meaning of these results is ambiguous. Clearly, the Lowry assay result cannot be the consequence of a simple 1 : 1 reaction, because the tholin is more chemically active in reducing the reagent than is pure protein. It is possible that nonpeptide tholin components can react with both the Bradford and the Lowry reagents in a manner similar to that of peptides or proteins: complexing with Coomassie blue dye or reducing Folin phenol reagent, respectively. The general indication is that spark tholin contains large-scale chemical structures that mimic the reactivity of real proteins toward certain reagents, but are not composed primarily of peptide chains—the amino acids may be attached in some other way to a larger chemical framework. The difference in apparent concentrations obtained from the two assays on tholin samples of identical concentration suggests that different chemical properties of the tholin structure are being measured in the two assays.

The results of the two other functional group assays, performed using hydroxylamine solutions (Ferris *et al.* 1981), were more straightforward. The aldehyde/ketone test showed no decrease in pH of the solution upon addition of tholin samples, thus giving a negative result. In the amide/nitrile test procedure, both the tholin solution and an acetonitrile standard solution exhibited a color change to blue-green, while a blank sample retained the orange color of the $FeCl_3$ reagent. Both the tholin and the acetonitrile samples showed visible absorption maxima at 635 nm against the blank solution. This was interpreted as a clear indication of the presence of amide (CO—NH) and/or nitrile (C≡N) groups in Jupiter tholin—in which abundant nitriles have already been reported (Sagan and Khare 1973, Khare *et al.* 1981).

HPLC Separation of Tholin Components

Aqueous solutions of Jupiter tholin were subjected to reverse-phase HPLC in order to separate any distinct water-soluble components for further analysis. In reverse-phase HPLC the stationary phase (the column surface) is a nonpolar organic material (an 18-carbon chain in the case of a C_{18} column), while the mobile phase is an aqueous or polar organic solvent. The relative strengths of interaction between the sample molecules and the mobile and stationary phases determine the retention time of each compound: In the separation of spark tholin components, an increasing gradient of 1-propanol in acidic water was used to enhance the separation of the components. In this case, molecules with a high content of polar functional groups elute first, while less polar molecules bind more strongly to the nonpolar substrate and elute later, as the organic fraction in the solvent increases. Reverse-phase gradient HPLC separation of water-soluble tholin components resulted in the chromatogram shown in Fig. 3. Four major components were isolated at retention times of 4.8, 5.1, 13, and 23 min, and we designate these fractions W, X, Y, and Z, respectively. Mass balance calculations indicate that approximately 80% by weight of the water-soluble tholin material introduced into the C_{18} column was eluted as the components shown in Fig. 3, approximately 20% remaining unrecovered on the column. It is, however, possible that some of this 20% may have eluded detection due to low absorptivity above 200 nm.

The UV/VIS spectra of the four tholin components isolated were measured by the diode array detector as they eluted from the column (Fig. 4). The four spectra appear to be somewhat different, showing that the overall polarity and UV/VIS-active functional groups are related: a smaller abundance of polar fractions yields greater long-

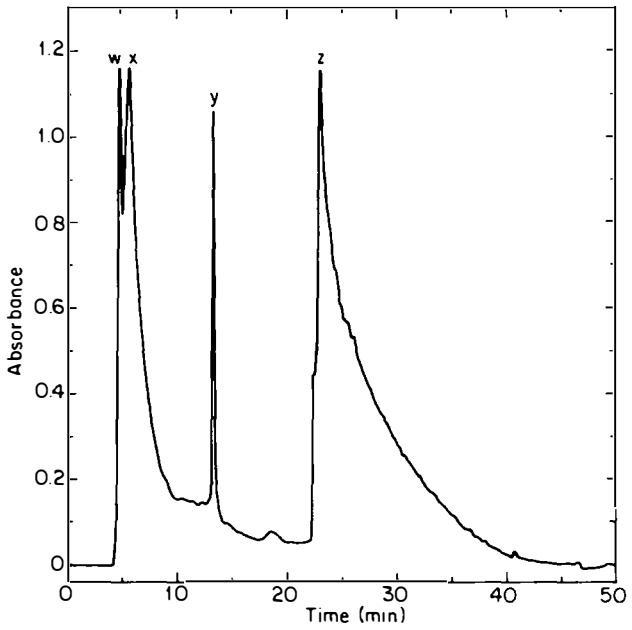


FIG. 3. Reverse-phase HPLC chromatograms of Jupiter spark tholin, H_2O-soluble fraction. Conditions: Waters Nova-Pak C_{18} column, 0–50% 1-propanol in 0.1% TFA/H_2O, detector wavelength 220 nm.

wavelength absorbance. The UV/VIS spectra of the unfractionated tholin (Fig. 1) and the tholin HPLC fractions (Fig. 4) indicate some degree of conjugation (double- or triple-bond resonance) in all fractions by virtue of the observed absorbance maxima at 200–220 nm. Conjugated polyenes with ≥5 carbon atoms were early suggested as the optical frequency chromophore in Jupiter spark tholin (Sagan and Khare 1973); the absorption peak near 200–220 nm indicates that the bulk of tholin conjugation resides in four-atom conjugated systems (i.e., two multiple bonds) (Pecsok and Shields 1968), but some longer chains are still needed to explain the absorption continuing into the visible. Some of the long UV and short VIS absorption may be due to other molecules, e.g., polycyclic aromatic hydrocarbons. The presence of additional lower intensity absorption bands at longer wavelengths in fractions Y and Z suggests a higher degree of conjugation or aromaticity than in fractions W and X.

It is likely that several minor components which are not fully resolved under the HPLC conditions used are present in spark tholin. In particular, various small molecules could coelute with the void volume fraction W. Additionally, separable species could possibly have no UV/VIS-active functional groups, and go undetected in this analysis. Furthermore, the obvious tailing of fraction Z in the chromatogram suggests the possibility that more than one molecular species is present in this fraction. Finally, the 20% by weight which does not elute from the C_{18} column under these conditions represents at least one tholin component and possibly more. Despite the possibility of other undetected or unseparated components, the relative simplicity of the chromatogram compared to the broad range of chemical species that might be imagined is noteworthy. By some mechanism, gas-phase reactions in a high-energy discharge result in solid products that yield distinct and separable chemical fractions in aqueous solution, perhaps indicating a small number of preferentially synthesized products.

Infrared Spectra of Tholin HPLC Fractions

The infrared spectra at ≈4 cm^{-1} resolution of the four major tholin fractions isolated by HPLC are shown in Fig. 5. Although the spectra are similar, some differences do exist. All fractions show bonds due to C=O groups, but additional bands in the 1600–1750 cm^{-1} (6.3–5.9 μm) range in the W and X fractions indicate the additional presence of C=N, C=C, and/or more than one kind of C=O containing functional groups. Absorptions near 1450 and 1600 cm^{-1} (most prominent in the X and Y fractions) may be aromatic C=C (as in tyrosine and phenylalanine precursors), but a contribution near 1400 cm^{-1} may also be due to ionized carboxyl. The broad O—H/N—H bands near 3000 cm^{-1} also show some shift in wavelength among the four fractions. The 4.6-μm C≡N feature of the bulk tholin is much less evident in the spectra of the individual HPLC fractions. Possibly, nitriles are preferentially present in the ≈20% of bulk tholin not recovered from HPLC during separation. The weak C≡N feature expected near 2200 cm^{-1} is below the noise level in these spectra.

An issue which must be addressed is the appearance of

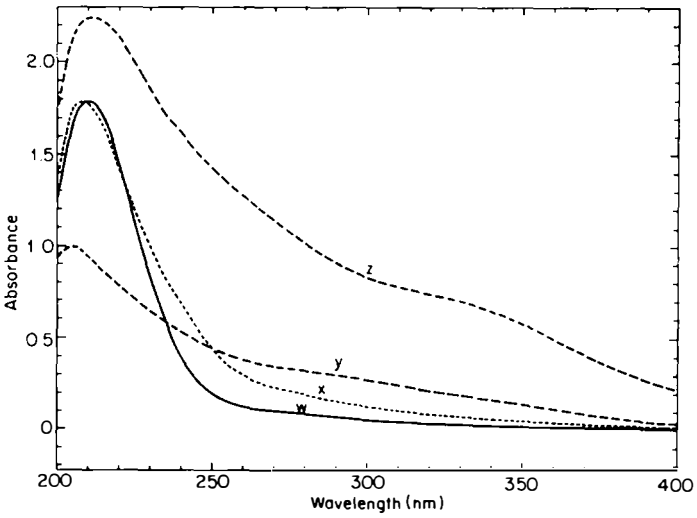


FIG. 4. Absorbance spectra of four major tholin components separated by HPLC: Fraction W, with elution time 4.8 min; *X*, 5.2 min; *Y*, 13 min; and *Z*, 23 min.

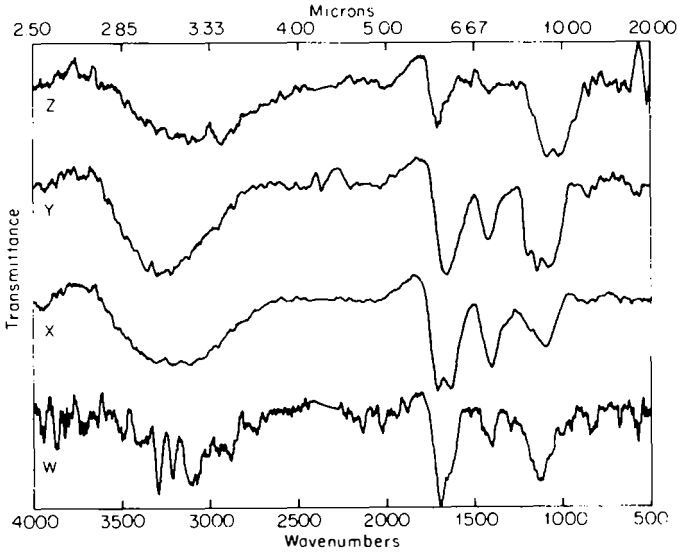


FIG. 5. Infrared transmission spectra of four major Jupiter spark tholin components separated by HPLC.

strong absorption bands in the 1000–1200 cm^{-1} (10–8.3 μm) range in the spectrum of each of the four HPLC fractions. These bands do not appear in the spectrum of the intact, unfractionated spark tholin which has never been dissolved in water (Fig. 2). A tholin sample which has been dissolved in water, then dried without HPLC separation and mixed into a KBr pellet, has an infrared spectrum (data not shown) identical to that in Fig. 2. Thus the change in spectra between the bulk tholin and the tholin fractions is unlikely to be due to reaction with water itself.

This absorption feature is roughly coincident with the silicate reststrahlen feature in common terrestrial rocks and glasses (cf., e.g., Pollack *et al.* 1973). There is also a 218-nm absorption in small silicate particles. We can exclude contamination from such a source, however, because (1) the shape and central wavelength of our 8–10 μm feature vary from fraction to fraction (Fig. 5); (2) the 218-nm and 8- to 10-μm features are uncorrelated (cf. Fig. 2); (3) silicate powders would be sedimented out when the tholin is dissolved and would be filtered out by the column if suspended, so there seems to be no way silicate powder would be present in the fractions and not the intact tholins; and (4) leaching of glasses occurs only in strongly basic solutions, not in the moderate acids used in this experiment.

When unfractionated tholin is incubated in 0.1% trifluoroacetic acid/H_2O (the mobile phase used in the HPLC separation), dried, and mixed into a KBr pellet, two additional IR bands appear at 1200 and 1138 cm^{-1}. These bands occur in the same general wavelength region as, but do not exactly coincide with, the 8–10 μm bands in the HPLC fraction spectra. It seems possible, although it remains undemonstrated, that the hydrolysis of spark tholin components by 0.1% trifluoroacetic acid during HPLC separation could be responsible for the spectral differences observed. (Should this be the case, the reactions involved would not alter the data obtained from acid hydrolysis and amino acid analysis of the HPLC fractions.)

The spectral features in question occur in a wavelength range normally occupied by the C—O bond stretching transitions. Comparisons with spectral indices suggest that the features may be C—O bands of α-amino alcohols or α,β unsaturated alcohols. It is possible that these functional groups are bound as linear or cyclic esters in the tholin, and that the strong C—O band of the alcohols arises only after hydrolysis in the acidic HPLC medium.

Molecular Weight Estimation

An important characteristic of Jupiter tholin molecules is their size or molecular weight distribution. Gel filtration chromatography (a form of size-exclusion chromatography) employs a column of porous gel beads with known pore sizes to estimate the molecular weights of molecules. Longer retention times on a gel filtration column correspond to lower molecular weights (molecules that can enter pores in the gel more readily). The elution profile of spark tholin from a Bio-Gel P2 gel filtration column is shown in Fig. 6A. Two main peaks can be seen at approximately 700 and 300 Da. Shown in Fig. 6B are the gel filtration elution profiles of each of the four major water-soluble tholin components. The estimated molecular weights of these components are given in Table I. Estimated molecular weights range from 200 to 700 g mol^{-1} (Da), with no material eluting in fractions corresponding to molecular weights above approximately 700 Da. Note from Fig. 6B that Component Z may possibly contain two populations of molecules with molecular weights around 300 and 700 Da, and Component Y two populations with molecular weights around 200 and 600 Da. Since the C_{18} HPLC column separates molecules by mean polar/nonpolar properties rather than by molecular weights, such a situation is entirely possible—the HPLC fractions can be thought of as "families" of molecules of different complexities, but with similar ratios of polar-to-nonpolar functional groups.

Acid Hydrolysis of Jupiter Tholin for Further Analysis

At least some of the constituents of tholins can be hydrolyzed to smaller molecules that can be specifically identified using chromatographic techniques. Acid hydrolysis at ~100°C breaks acid-labile bonds, such as amide (CO—NH) and ester (CO—O) bonds, within the structure of the molecule. The products of spark tholin hydrolysis

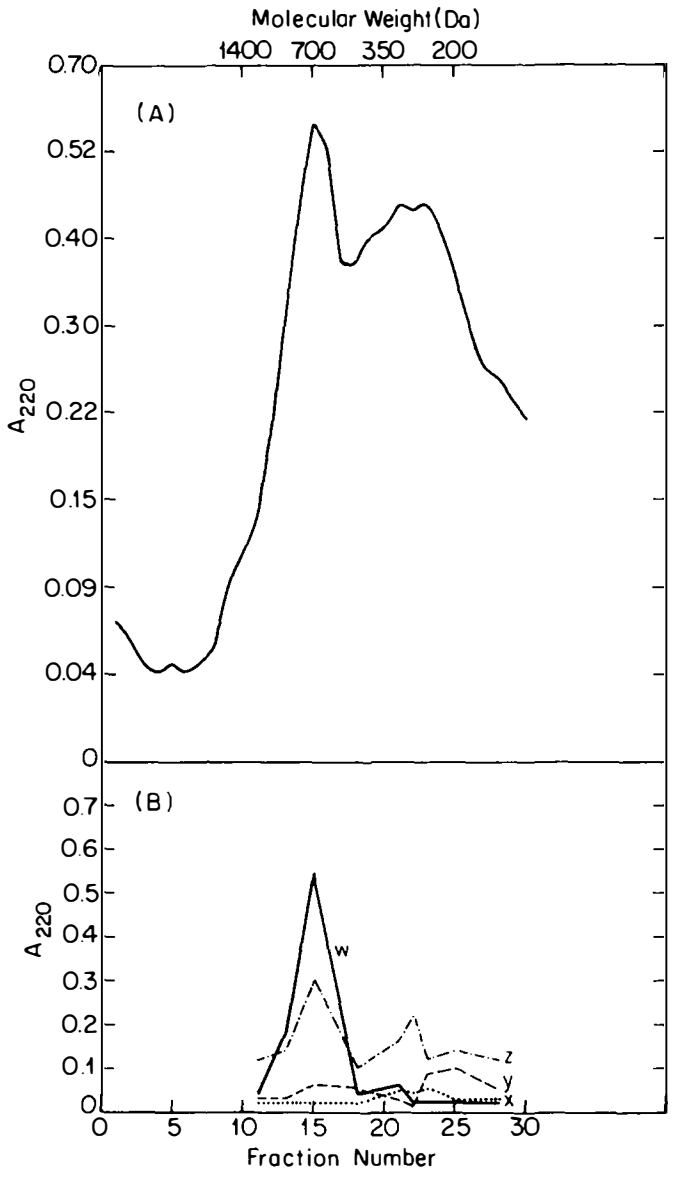


FIG. 6. Elution profile of Jupiter spark tholin on Bio-Gel P2 gel filtration column in H_2O. (A) P2 column effluent profile at 220 nm. (B) Profile of four tholin components determined by HPLC assay of fractions.

were analyzed both by GC/MS (via their volatile derivatives) and by liquid-phase amino acid analysis:

GC/MS of derivatized hydrolysis products. Involatile organic acids and amines produced by acid hydrolysis of spark tholin can be derivatized by reaction with a reagent such as bis(trimethylsilyl)-trifluoroacetic acid to render them volatile and subject to analysis by GC/MS. The compounds identified in Jupiter tholin by this method are listed in Table II, and include the amino acids glycine, alanine, and aspartic acid, as well as several organic hydroxy acids and diacids. While not as much discussed as amino acids, these are expected components of extraterrestrial organic matter; e.g., similar carboxylic acids and hydroxy acids, and in particular hydroxyacetic acid, have been found in samples of the Murchison meteorite (Peltzer and Bada 1978).

The amount of each hydrolysis product present in the original tholin sample was estimated by assuming that the GC/MS ion count per mole of derivatized molecule was approximately equal to the GC/MS ion count per mole determined using an acetonitrile standard. Conversion to mg/mg tholin was based on a 16-mg initial sample, with calculated weights of hydrolysis products corrected for water gained upon hydrolysis. Some error in these mass balance calculations may arise from the use of acetonitrile as the standard for ionization efficiency. Other functional groups do not ionize with exactly the same efficiency as the nitrile group, although extreme variations are not generally seen. The efficiency factor is on the order of 10^{-15} mole per MS ion count for this system.

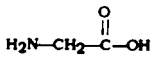
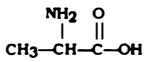
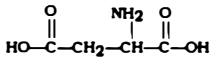
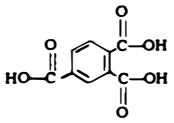
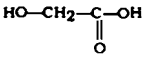
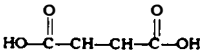
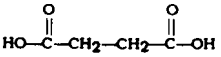
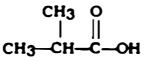
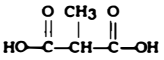
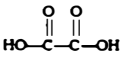
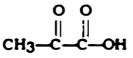
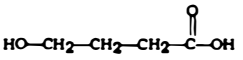
Amino acids. Amino acids obtained upon acid hydrolysis of intact tholin and of the four major HPLC fractions are listed in Tables III and IV. The most abundant amino acids in these analyses are glycine (Gly), alanine (Ala), aspartate (Asx), β-alanine (β-Ala), and β-aminobutyrate (β-ABA). These are some of the amino acids most commonly found in discharge experiments in reducing atmospheres (e.g., Miller 1953, Khare *et al.* 1986). On the other hand, the nonprotein amino acids β-aminoisobutyric acid, sarcosine, and γ-aminobutyric acid, which are also common discharge products, were not detected. Determination of the abundances of norleucine, α-aminoisobutyric acid, and isovaline was not possible due to an inadequate length of run time during analysis or a lack of reactivity with the derivatizing reagent, phenylisothiocyanate.

The isolated HPLC fractions of spark tholin, upon acid hydrolysis, differ significantly from one another in their amino acid compositions, indicating the probable generation in reducing atmospheres of families of molecules with distinctly different amino acid compositions. However, the same five amino acids—Gly, β-ABA, β-Ala, Asx,

TABLE I
Estimated Molecular Weights of Tholin Components

Elution time (min)	Molecular weight
4.8, W	700
5.1, X	300
13, Y	200
	600
23, Z	300
	700

TABLE II
Jupiter Spark Tholin Acid Hydrolysis Products

		g/g tholin (±0.0005)
Glycine		0.070
Alanine		0.003
Aspartic acid		0.006
1,2,4-Benzenetricarboxylic acid		0.010
Hydroxyacetic acid		0.001
2-Butenedioic acid		0.033
Butanedioic acid		0.006
2-Methylpropanoic acid		0.004
Methylpropanedioic acid		0.004
Ethanedioic acid		0.014
Pyruvic acid		0.048
4-Hydroxybutanoic acid		0.002
Total		0.201
Total (minus amino acids)		0.122

Ala—plus glutamate (Glx) are dominant in each of the four fractions.

Comparison of the amino acid analysis of intact tholin with those of tholin HPLC fractions reveals that the sum of the amino acid abundances over the four fractions is in most cases quite close to that seen in the intact tholin. However, threonine (Thr), valine (Val), isoleucine (Ile), arginine (Arg), and α-aminobutyric acid (α-ABA) are all present in somewhat higher abundances in the hydrolysis products of the bulk tholin than in the sum of the hydrolysis products of the HPLC fractions. This remaining mass of amino acids may be contained in any tholin components not captured in the four detected HPLC fractions. In contrast, the levels of Gly and phenylalanine in the sum of the HPLC fractions are somewhat higher than those seen in the unfractionated tholin analysis. The levels of Gly, Ala, and Asx in intact tholin determined by trimethylsilyl derivatization (Table I) are reasonably consistent with those obtained by amino acid analysis of unfractionated tholin (Table II).

Glycine has no optical activity. The simplest protein amino acid with two enantiomers is alanine. The results of the determination of the D/L ratio of alanine from the hydrolysis of Jupiter tholin are shown in Table III. This determination was carried out by gas chromatography of the *N*-pentafluoropropyl isopropyl ester of the amino acid using a capillary chromatography column with a chiral stationary phase. This column resolves the two optical isomers of each derivatized amino acid into two distinct peaks. These peaks can then be integrated to determine the ratio of the D-isomer to the L-isomer.

The alanine present in the hydrolysis products of tholin is clearly racemic within the accuracy of the analysis. Terrestrial organisms use only L-amino acids in proteins, while the amino acids produced in prebiotic synthesis experiments should be racemic (containing equal amounts of both isomers). Since alanine is the most commonly occurring amino acid in terrestrial proteins, this result

TABLE III
Amino Acid Composition of Unfractionated Tholin (±0.2 μmole/g)

Amino acid	μmole/g	μg/g	D/L[a]
Asx[b]	54.3	6244.5	
Thr	7.3	737.3	
Ser	8.4	730.8	
Glx	35.5	4579.5	
Gly[b]	833.4	47503.8	
Ala[b]	51.6	3663.6	0.96 ± 0.13
Val	8.8	871.2	
Ile	44.5	5028.5	
Leu	3.5	395.5	
Tyr	11.8	1923.4	
Phe	2.6	382.2	
Lys	3.0	384.0	
β-Ala	50.0	3550.0	
Arg	14.8	2308.8	
β-ABA	352.0	36256.0	
α-ABA	6.4	659.2	
Total	1487.9	115218.3 = 0.1 g/g tholin	

Note. Asx, aspartate/asparagine; Glx, glutamate/glutamine; β-ABA, β-aminobutyric acid; α-ABA, α-aminobutyric acid.

[a] Determined as pentafluoropropyl isopropyl ester on Chirasil-Val GC column.

[b] Also detected by GC/MS.

TABLE IV
Amino Acid Composition of Tholin HPLC Fractions

Amino acid	Elution time (min) 4.8, W	5.1, X	13, Y	23, Z	Total
	μmole/g original tholin sample (±0.2 μmole/g)				
Asx	7.2	17.0	2.9	33.4	60.5
Thr	0.1	<0.1	0.2	<0.1	0.3
Ser	1.3	1.4	1.0	6.9	10.6
Glx	<0.1	5.4	2.6	28.8	36.8
Gly	157.8	571.5	44.7	478.2	1252.2
Ala	3.5	13.1	3.5	31.4	51.5
Val	0.7	1.3	<0.1	<0.1	2.0
Ile	0.6	1.0	0.3	5.8	7.7
Leu	<0.1	2.9	<0.1	<0.1	2.9
Tyr	1.5	1.8	0.3	9.4	13.0
Phe	0.3	7.0	0.4	8.8	16.5
Lys	0.2	<0.1	0.1	1.3	1.6
β-Ala	2.2	24.8	3.2	36.0	66.2
Arg	<0.1	<0.1	0.8	1.6	2.4
β-ABA	40.2	228.3	19.0	69.8	357.3
α-ABA	0.2	<0.1	0.1	<0.1	0.3

Note. Asx, aspartate/asparagine; Glx, glutamate/glutamine; β-ABA, β-aminobutyric acid; α-ABA, α-aminobutyric acid.

demonstrates that major contamination by terrestrial microorganisms of the tholin samples tested has not occurred.

Gas-Phase Precursors in H_2-Rich Atmospheres

As mentioned above, discharge experiments using $CH_4/NH_3/H_2O$ gas mixtures are a very crude simulation of the gas-phase chemistry which occurs in the Jovian atmosphere. A more realistic simulation would employ at least a 10^{-2} dilution of CH_4 and NH_3 in H_2 and He. Until experiments can be completed using such starting mixtures, some sense of the products that would be generated can be obtained by examining the equivalent fully reduced homologues of $CH_4/NH_3/H_2O$ spark tholin hydrolysis products (Table I). For example, hydroxyacetic acid and ethanedioic acid would yield ethane if fully reduced. Pyruvic acid, methylpropanedioic acid, butanedioic acid, 2-methylpropanoic acid, 2-butenedioic acid, and benzenetricarboxylic acid would yield propane, 2-methylpropane, butane, 2-methylpropane, butene, and trimethylbenzene, respectively. Glycine, if fully reduced, would give ethylamine, while alanine and aspartic acid would give branched amines. These reduced homologues are all plausible minor constituents of the Jovian atmosphere.

Aspects of the Molecular Structure of Jupiter Tholin Inferred from the Data

To suggest aspects of the molecular structure of tholin molecules, we summarize the foregoing data:

(1) Jupiter spark tholin is substantially water-soluble and has a pH of 4.5 in aqueous solution.

(2) Four major fractions which constitute distinct families of molecules can be obtained from liquid chromatography of aqueous solutions of spark tholin.

(3) The water-soluble tholin molecules range in molecular weight from approximately 200 to 700 Da, and at least two of the HPLC fractions may be composed of molecules with at least two subgroups of molecular weights.

(4) Functional group assays have determined the presence of amide and/or nitrile groups, but not aldehyde or ketone groups, in spark tholin.

(5) Infrared spectra of intact tholin indicate the presence of C—H, C=O, N—H, C≡N, and probably conjugated and/or aromatic C=C groups, but no C—O single bonds of CH_xOH groups. Infrared spectra of HPLC fractions of tholins show additional features possibly due to the C—O bonds of α-amino or α,β unsaturated alcohols.

(6) Upon acid hydrolysis of tholin samples, both amino acids and simple carboxylic acids are obtained. From the mass balance calculations presented in Tables II and III, the hydrolysis products that are detectable by gas chromatography/mass spectrometry of trimethylsilyl (TMS) derivatives or amino acid analysis constitute approximately 22% of the total mass of tholin. Amino acids or their nonhydrolyzed precursors appear to account for about 10% (0.10 g/g tholin) of the total mass, while small carboxylic acids and hydroxy acids (or their precursors) account for about 12% (0.12 g/g tholin). Thus, the remaining 78% by mass of the tholin is not acid-labile, does not derivatize, or is involatile as TMS derivatives under the chromatographic protocol employed.

The data from acid hydrolysis of spark tholin do not indicate whether amino acids or simple carboxylic acids are present in tholin as the acids themselves or as precursors such as aminonitriles or imides in which at least some oxygen atoms are replaced by nitrogen atoms. The acidic pH of aqueous spark tholin solutions (see above) suggests that some of the acid groups are either present in the original tholin or produced readily by oxidation or hydrolysis from nitrogenous precursors (imidines, etc.) in the solid. For the remainder of this discussion of possible structures the term "amino acid" is used to mean either amino acids or their precursors, and the term "organic acid" either simple carboxylic acids or their precursors.

The average molecular weight of both amino acids and the small organic acids identified as tholin hydrolysis products (Table II) is around 100 Da. If amino acids represent approximately 10% by weight and organic acids 12% by weight of tholin molecules, then for a 700-Da tholin molecule the average mass of amino acid contained would be around 70 Da and of organic acid around 100 Da. This would correspond to approximately one amino acid

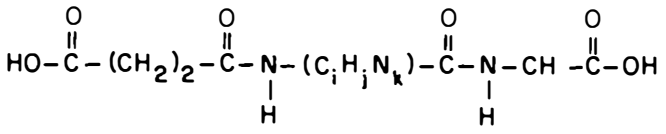


FIG. 7. A generalized molecular structure which incorporates the features postulated for Jupiter spark tholin molecules. The central core, $C_iH_jN_k$, is highly unsaturated.

molecule and one 2- to 4-carbon organic acid molecule per 700-Da tholin molecule. These molecular groups would have to be linked to the remainder of the tholin molecule by acid-labile bonds in order to be released upon acid hydrolysis. These acid-labile bonds are probably ester (—COO—) or amide (—CONH—) linkages.

The remainder of the "average" 700-Da tholin molecule must be about 500 Da in mass and resistant to acid hydrolysis. The empirical formula of the bulk tholin, as calculated from previous analyses (Sagan and Khare 1979), is $C_5H_7N_3O$—suggesting that this remainder consists almost entirely of carbon, hydrogen, and nitrogen atoms, since most of the oxygen present is accounted for by the amino and organic acids. The empirical formula of spark tholin also indicates a high C/H ratio ($\simeq 1$) and thus a significant degree of unsaturation (carbon–carbon double and triple bonds) in this major ($C_iH_jN_k$) core portion of the molecule. A heuristic molecular structure which incorporates the foregoing characteristics of Jupiter tholin molecules is shown in Fig. 7. The detailed structure of the core portion remains to be determined, but may contain conjugated double-bond groups which contribute significantly to the UV/VIS spectrum of spark tholin.

Such a structure also seems consistent with the major pyrolytic products of Jupiter spark tholin (Khare *et al.* 1981)—CO_2 from carboxyl and carbonyl groups, NH_3 from amino and imino groups, nitriles, and, especially at higher temperatures, alkanes, alkenes, pyrroles (and aromatic compounds) from the cores of the tholin molecules. The hydrocarbons tend to be more strongly held; indeed, Jupiter tholin is only half-dissociated at 950°C (Khare *et al.* 1981), and the composition of this refractory component is the least tractable part of the study of tholin chemistry.

The water-soluble tholin fractions with apparent molecular weights of 200–300 Da may lack the acid-resistant structure mentioned above. These smaller molecules may consist simply of two or three amino or organic acids linked by an amide or ester bond. In HPLC fractions Y and Z, molecules of both 200–300 and 600–700 Da appear to be present in the same fraction. This situation could occur if the lower molecular weight molecules consisted of two amino acids or organic acids alone, while the higher molecular weight fraction consisted of molecules in which two, more polar acids were linked to the larger, presumably nonpolar acid-stable structure described above. In other words, in order to exhibit similar retention times on the C_{18} HPLC column, which separates compounds only by net polarity and not by molecular weight, two molecules need only have approximately the same ratio of polar-to-nonpolar functional groups. The tailing observed in the chromatogram of fraction Z could be explained by the presence of a range of molecular species in that fraction, each with similar or identical polar functional groups but differing slightly in the composition of the nonpolar core. The approximately 20% by weight of tholin which does not elute from the C_{18} HPLC column may represent that portion of the acid-stable, nonpolar component which is not linked to amino acids or organic acids.

The possible occurrence of short peptides in Jupiter spark tholin is neither necessitated by nor inconsistent with the observations reported herein. The presence of a possible amide II or N—H bending mode at 1559 cm^{-1} (6.42 μm) leaves open the possibility of short peptides in spark tholins, since this band is a characteristic of peptide infrared spectra. Positive Bradford and Lowry tests indicate some complexing and chemical reducing properties similar to those of proteins, although the biuret test was negative. In addition, amides and/or nitriles were indicated by the chemical functional group tests, by IR spectroscopy, and by previous work. Small nitriles may have been hydrolyzed to free amino acids; large polynitriles may have been hydrolyzed only at their peripheries.

The above discussion of possible chemical structures is of course highly speculative. Future mass spectrometry and elemental analyses of the individual tholin HPLC fractions should permit a more accurate determination of the structure of each family of tholin molecules. The foregoing model for tholin structure suggests that solids are formed in the plasma discharge by the initial synthesis of several types of small (100–200 Da) molecules, which then react to form larger structures. [Some of these structures, including the amino acid precursors, may derive from free-radical gas-phase reactions of nitriles and alkynes (Thompson and Sagan 1989).]

Comparisons with Other Simulations

We compare our results on the composition of spark tholin with those obtained for a different, but possibly chemically related, material formed by the γ-irradiation of aqueous solutions of inorganic cyanides or organic nitriles. Draganić *et al.* (1976) demonstrated the formation of NH_3, H_2CO, and seven amino acids from the γ-irradiation of 0.1 *M* HCN in aqueous solution, and found a strongly positive biuret test for amide bonds indicating a yield $G_{amide} = 0.4$–2.1 depending on pH (radiation yields G are in units of molecules/100 eV). Acid hydrolysis re-

leased more amino acids (G_{aa} = 0.2), and peptide-like materials were postulated. Draganić and Draganić (1977) reported the results for γ-irradiation of 0.1 *M* NH_4CN and NaCN solutions, and found up to 18% of the total product with amide bonds (G = 0.5–0.8) and 14% as amino acids (G = 0.3–0.6). For NH_4CN irradiation, amino acid abundances followed the order glycine ≫ aspartate > serine > alanine. Draganić *et al.* (1977) presented the infrared spectra of these materials—which show general similarities to the intact spark tholin spectra. The HCN- or NH_4CN-derived polymers were further characterized by Niketic *et al.* (1982), who found about eight fractions with molecular weights ranging from 1500 to ~20,000; all these fractions yielded amino acids, typically in different abundance distributions, upon hydrolysis. All the fractions gave a positive biuret test, and many fractions were acted upon by pronase, a collection of enzymes able to hydrolyze both amide and ester linkages. (However, some NH_4CN fractions were pronase inhibitors.) None of the fractions from NH_4CN irradiation could be detectably hydrolyzed by aminopeptidase M, an enzyme which hydrolyzes peptides having free amino-termini; on the other hand, most of the fractions from HCN irradiation were acted on by this enzyme, demonstrating the presence of at least short peptide segments. On average, about 10% of each fraction was released as amino acids on hydrolysis, while up to 30% of the peptide-like bonds were acted on enzymatically.

Similarly, we find that several fractions can be isolated from the solid Jupiter tholin heteropolymer produced by gas-phase sparking of CH_4 + NH_3 + H_2O, and each yields, on acid hydrolysis, amino acids in differing relative abundances. However, the molecular weights produced from γ-irradiated solutions are much higher. Our smaller molecules, ≲700 Da, are more similar in size to the "HCN oligomers" formed in ≳0.1 *M* HCN solutions without irradiation. Although "HCN oligomers" release amino acids upon hydrolysis and have been suggested to contain peptides (e.g., Matthews *et al.* 1977), much evidence against peptide bonds in such materials has been accumulated (see review by Ferris *et al.* 1981).

The amino acid composition of spark tholin and its fractions is unlike that of any of the aqueous XCN polymers in having detectable quantities of aromatic amino acids (phenylalanine, tyrosine), arginine, and lysine. Because Draganić *et al.* did not report any analysis for carboxylic or hydroxy acids, comparisons cannot be made for other small organic molecules.

We are pursuing further work with chiral ion-exchange chromatographic techniques to investigate the indications of some peptide-like fractions in the soluble fraction of spark tholin. In addition, we have begun additional simulations that more nearly approximate conditions in the Jovian atmosphere and clouds.

Tholins and HCN "Polymers"

The possible existence of a $C_iH_jN_k$ core to the Jupiter tholin chromatographic fractions (Fig. 7) with, at least overall, $i/j \simeq j/k \simeq 1$ raises the prospect that this core is some variant of HCN oligomer. Moreover, the infrared spectrum of HCN "polymer" (Woeller and Ponnamperuma 1969, Fig. 9) and that of Jupiter spark tholin (Sagan and Khare 1973) are superficially quite similar, as has been recently stressed by Cruikshank *et al.* (1990); and among the abundant amino acids found in hydrolysis products of HCN "polymer" (Ferris and Hagen 1984), several appear in our list of hydrolysis products of Jupiter spark tholin (Asp, Ser, Glx, Gly, β-Ala, Leu, Ile, α-ABA).

There are, in fact, several different categories of HCN "polymer," including (1) a true polymer $(HCN)_n$, the repetition of the same unit n times; (2) HCN heteropolymer or oligomer, which has the same rough abundance of organic functional groups (and therefore roughly the same IR spectrum), but is not repetitious, especially in its hydrocarbon and other side chains; and (3) HCN *solution* polymer or oligomer, which displays a more oxygenated composition. A heteropolymer prepared from NH_3/CH_4 or N_2/CH_4 may be quite different from (1), (2), and (3) while preserving similarities in the gross IR features.

Our expectation that spark tholin is dissimilar to HCN "polymers" is borne out by

(a) the variety and complexity (not just a few simple nitriles and hydrocarbons) of the gas-phase precursors of tholins (McDonald *et al.*, 1991, Thompson *et al.* 1991, Yung *et al.* 1984);

(b) the variety and complexity of the pyrolytic GC/MS products of spark tholin (Khare *et al.* 1981);

(c) the marked difference in UV spectra (results in this paper compared with, e.g., those of Woeller and Ponnamperuma 1969, Mitzutani *et al.* 1975);

(d) the differences in aromatic and other amino acids, remarked above; and

(e) the differences in relative band strengths between HCN "polymer" and tholin as seen in the compilation of Cruikshank *et al.* (1990).

We conclude that the IR similarities can be explained by a similar distribution of functional groups rather than a detailed structural identity, and that it is undemonstrated and unlikely that Jupiter spark tholins are predominantly HCN "polymer."

Amino Acids in the Jovian Clouds

Lewis (1980) estimated the production rate of organic compounds by lightning-produced shocks in Jupiter's atmosphere. His quenched thermodynamic equilibrium cal-

culations are only partially accurate for shock waves, and not relevant to other nonthermodynamic equilibrium processes (e.g., UV, magnetospheric e^- and other charged particle radiation, and coronal discharge). Bar-Nun *et al.* (1984) performed laboratory shock-wave experiments with $H_2/CH_4/NH_3/H_2O$ + Ar in a 2 : 1 : 1 : 6 ratio, and found approximately linear dependence of amino acid yield (in hydrolysis products) on $H_2:CH_4$ ratio. Integrating the results of several experiments, they estimate a yield $\sim 1 \times 10^7$ molec erg^{-1} ($G \simeq 2 \times 10^{-3}$ molec/100 eV) for amino acids. Correcting the lower value given by Lewis, they arrived at a total lightning energy dissipation rate of ~ 2 erg cm^{-2} sec^{-1}, and estimated small (~ 0.1 μM) steady-state amino acid concentrations in the Jovian clouds.

In the process we envision here, solid particulates are formed by any of several (UV, e^-, coronal, shock) processes and interact with the liquid after sedimenting to the water cloud, producing amino acids in the H_2O + NH_3 solution. However, we have also found the nitrile forms of the amino acids alanine and glycine in the gas phase of irradiated H_2 + He + CH_4 + NH_3 in simulation experiments, in yields $G \simeq 10^{-3}$ for $X_{CH4} = 10^{-2}$ (McDonald *et al.* 1991). Both the solid tholins and the gaseous amino nitriles contribute to the amino acid inventory; in any case, sources are not limited to thunder shocks. As on Uranus and Neptune (Thompson *et al.* 1987), magnetospheric electron precipitation can produce substantial amounts of organic products; for Jupiter we estimate roughly 0.1 erg cm^{-2} sec^{-1} (2×10^{10} dissoc cm^{-2} sec^{-1}) from precipitating electrons (global average). As shown in the model of Kaye and Strobel (1983) and the laboratory work of Ferris and Ishikawa (1988), UV photochemistry can also produce nitrogenous organics and N-containing heteropolymers. The direct photolysis rate of CH_4 is $\sim 1.5 \times 10^{10}$ dissoc cm^{-2} sec^{-1}, and the rates for both C_2H_2-catalyzed and NH_3 photolysis are at least an order of magnitude higher. If H_2S is present, longer wavelength UV photons can be harvested and the amino acid production rate will greatly increase (Sagan and Khare 1971).

Without analyzing UV-produced tholins, we cannot confidently estimate the (possibly large) UV contribution to amino acids. However, for other energy sources we believe that the estimates of Bar-Nun *et al.* (1984) are reasonable, and are generally in accord with the observations of Thompson *et al.* (1987), who showed that for H_2 + He + CH_4 atmospheres, overall yields of organic products do not fall precipitously, but approximately linearly, with X_{CH4}. A concentration of amino acids of 0.1 μM in the Jovian clouds is significant: Small populations of terrestrial microorganisms can be maintained in 0.1 μM solutions of amino acids, with the amino acids as the sole carbon source (Mitchell and Slaughter 1989). Judging from the results of the present paper, concentrations of metabolizable organic matter larger than those calculated from amino acids alone should be present in the deep aqueous clouds of Jupiter.

Tholins and Jovian Chromophores

It is more difficult to evaluate the possibility that organic tholin molecules are important chromophores in the Jovian atmosphere. The yellow to dark brown colors exhibited by spark tholin are vaguely consistent with some colors observed in the clouds of Jupiter. However, the extraction of the spectra of chromophores from the overall scattering and spectra properties of belt, the Great Red Spot, brown "barges," and other cloud features has not yet been achieved. Until it has, it will not be possible to assess quantitatively the relationship between spark tholins and Jovian chromophores.

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REFERENCES

ABE, I., S. KURAMOTO, AND S. MUSHA 1983. Heliflex Chirasil-Val: GC of amino acid enantiomers. *J. High Res. Chromatogr. and Chromatogr. Commun.* **7**, 366–370.

ATREYA, S. K. 1986. *Atmospheres and Ionospheres of the Outer Planets and Their Satellites*. Springer-Verlag, Berlin.

BAR-NUN, A., N. NOY, AND M. PODOLAK 1984. An upper limit to the abundance of lightning-produced amino acids in the jovian water clouds. *Icarus* **59**, 162–168.

BRADFORD, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

CARLSON, B. E., M. J. PRATHER, AND W. B. ROSSOW 1987. Cloud chemistry on Jupiter. *Astrophys. J.*, 559–572.

CRUIKSHANK, D. P., W. K. HARTMANN, D. J. THOLEN, L. J. ALLAMANDOLA, AND R. H. BROWN 1990. Solid C≡N bearing material in the outer solar system. *Bull. Am. Astron. Soc.* **22**, 1098.

DRAGANIĆ, Z. D., I. G. DRAGANIĆ, AND M. BOROVICANIN 1976. The radiation chemistry of aqueous solutions of hydrogen cyanide in the megarad dose range. *Radiat. Res.* **66**, 42–53.

DRAGANIĆ, I. G., Z. D. DRAGANIĆ, S. JOVANOVIĆ, AND S. V. RIBNIKAR 1977. Infrared spectral characterization of peptidic material produced by ionizing radiation in aqueous cyanides. *J. Mol. Evol.* **10**, 103–109.

DRAGANIĆ, Z. D., AND I. G. DRAGANIĆ 1977. The radiation chemistry of aqueous solutions of ammonium and sodium cyanides in the megarad dose range. *Radiat. Res.* **69**, 223–229.

FERRIS, J. P., E. H. EDELSON, J. M. AUYEUNG, AND P. C. JOSHI 1981. Structural studies on HCN oligomers. *J. Mol. Evol.* **17**, 69–77.

FERRIS, J. P., AND W. J. HAGAN, JR. 1984. HCN and chemical evolution: The possible role of cyano compounds in prebiotic synthesis. *Tetrahedron* **40**, 1093–1120.

FERRIS, J. P., AND Y. ISHIKAWA 1988. Formation of HCN and acetylene

oligomers by photolysis of ammonia in the presence of acetylene: Applications to the atmospheric chemistry of Jupiter. *J. Am. Chem. Soc.* **110,** 4306–4312.

GAUTIER, D., AND T. OWEN 1989. The composition of outer planet atmospheres. In *Origin and Evolution of Planetary and Satellite Atmospheres* (S. K. Atreya, S. K., J. B. Pollack, and M. S. Matthews, Eds.), pp. 487–512. Univ. of Arizona Press, Tucson.

GEHRKE, C. W., AND K. LEIMER 1971. Trimethylsilylation of amino acids: Derivatization and chromatography. *J. Chromatogr.* **57,** 219–238.

GURNETT, D. A., R. R. SHAW, R. R., ANDERSON, W. S. KURTH, AND F. L. SCARF 1979. Whistlers observed by Voyager 1: Detection of lightning on Jupiter. *Geophys. Res. Lett.* **6,** 511–514.

ITZHAKI, R. F., AND D. M. GILL 1964. A micro-biuret method for estimating proteins. *Anal. Biochem.* **9,** 401–410.

KAYE, J. A., AND D. F. STROBEL 1983. HCN formation on Jupiter: The coupled photochemistry of ammonia and acetylene. *Icarus* **54,** 417–433.

KHARE, B. N., C. SAGAN, J. E. ZUMBERGE, D. S. SKLAREW, AND B. NAGY 1981. Organic solids produced by electrical discharge in reducing atmospheres: Tholin molecular analysis. *Icarus* **48,** 290–297.

KHARE, B. N., C. SAGAN, H. OGINO, B. NAGY, C. ER, K. H. SCHRAM, AND E. T. ARAKAWA 1986. Amino acids derived from Titan tholins. *Icarus* **68,** 176–184.

KHARE, B. N., C. SAGAN, W. R. THOMPSON, L. FLYNN, AND M. A. MORRISON 1989. Amino acids and their polymers in the lower clouds of Jupiter? Preliminary findings. *Origins Life* **19,** 495–496.

KUIPER, G. P. 1951. In *The Atmospheres of the Earth and Planets* (G. P. Kuiper, Ed.), Chap. 12. Univ. of Chicago Press, Chicago.

LAYNE, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. In *Methods in Enzymology* (Colowick, S. P., and Kaplan, N. O., Eds.), Vol. 3, pp. 447–454, Academic Press, San Diego.

LEWIS, J. S. 1969. The clouds of Jupiter and the NH_3–H_2O and NH_3–H_2S systems. *Icarus* **10,** 365–378.

LEWIS, J. S. 1980. Lightning synthesis of organic compounds on Jupiter. *Icarus* **43,** 85–95.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

MATTHEWS, C., J. NELSON, P. VARMA, AND R. MINARD 1977. Deuterolysis of amino acid precursors: Evidence for hydrogen cyanide polymers as protein ancestors. *Science* **198,** 622–625.

MCDONALD, G. D., W. R. THOMPSON, AND C. SAGAN 1991. Laboratory Studies of Radiation Chemistry in the Jovian Atmosphere. *Bull. Am. Astron. Soc.*, in press.

MILLER, S. L. 1953. A production of amino acids under possible primitive Earth conditions. *Science* **117,** 528–529.

MITCHELL, W. J., AND J. C. SLAUGHTER 1989. *Biology and Biochemistry for Chemists and Chemical Engineers.* Ellis Horwood, Chichester.

MITZUTANI, H., H. MIKUNI, M. TAKAHASI, AND H. NODA 1975. Study on the photochemical reaction of HCN and its polymer products relating to primary chemical evolution. *Origins Life* **6,** 513–525.

NIKETIC, V., Z. DRAGANIC, S. NESKOVIC, AND I. DRAGANIC 1982. Enzymatic characterization of peptidic materials isolated from aqueous solutions of ammonium cyanide (pH 9) and hydrocyanic acid (pH 6) exposed to ionizing radiation. *J. Mol. Evol.* **18,** 130–136.

PARKER, F. S. 1983. *Applications of Infrared, Raman and Resonance Raman Spectroscopy in Biochemistry.* Plenum, New York.

PECSOK, R. L. AND L. D. SHIELDS 1968. *Modern Methods of Chemical Analysis,* pp. 191–194. Wiley, New York.

PELTZER, E. T., AND J. L. BADA 1978. α-Hydroxycarboxylic acids in the Murchison meteorite. *Nature* **272,** 443–444.

POLLACK, J. B., O. B. TOON, AND B. N. KHARE 1973. Optical properties of some terrestrial rocks and glasses. *Icarus* **19,** 372–389.

SAGAN, C., AND S. L. MILLER 1960. Organic synthesis in reducing planetary atmospheres. *Astron. J.* **65,** 499.

SAGAN, C. 1961. On the origin and planetary distribution of life. *Radiat. Res.* **15,** 174–192.

SAGAN, C., AND B. N. KHARE 1971. Experimental jovian photochemistry: Initial results. *Astrophys. J.* **168,** 563–570.

SAGAN, C., AND B. N. KHARE 1973. Red clouds in reducing atmospheres. *Icarus* **20,** 311–321.

SAGAN, C., AND B. N. KHARE 1979. Tholins: Organic chemistry of interstellar grains and gas. *Nature* **277,** 102–107.

SAVAGE, B. D. 1975. Ultraviolet photometry from the orbiting astronomical observatory XX. The ultraviolet extinction bump. *Astrophys. J.* **199,** 92–109.

STEEL, T. M., AND W. W. DULEY 1987. A 217.5 nanometer absorption feature in the spectrum of small silicate particles. *Astrophys. J.* **315,** 337–339.

STOKER, C. R., P. J. BOSTON, R. J. MANCINELLI, W. SEGAL, B. N. KHARE, AND C. SAGAN 1990. Microbial metabolism of tholin. *Icarus* **85,** 241–256.

SU, Y.-L., Y. HONDA, P. E. HARE, AND C. PONNAMPERUMA 1989. Search of peptide-like materials in electric discharge experiments. In *Origins of Life: The Sixth ISSOL Meeting and the Ninth International Conference,* p. 18. Prague, Czechoslovakia. [Abstracts]

THOMPSON, W. R., T. HENRY, B. N. KHARE, L. FLYNN, J. SCHWARTZ, AND C. SAGAN 1987. Light hydrocarbons from plasma discharge in H_2–He–CH_4: First results and uranian auroral chemistry. *J. Geophys. Res.* **92,** 15,083–15,092.

THOMPSON, W. R., AND C. SAGAN 1989. Atmospheric formation of organic heteropolymers from N_2 and CH_4: Structural suggestions for amino acid and oligomer precursors. *Origins Life* **19**(3–5), 503–504.

THOMPSON, W. R., T. J. HENRY, J. SCHWARTZ, B. N. KHARE, AND C. SAGAN 1991. Plasma discharge in N_2 and CH_4 at low pressures: Experimental results and applications to Titan. *Icarus* **90,** 57–73.

WEIDENSCHILLING, S. J., AND J. S. LEWIS 1973. Atmospheric and cloud structures of the Jovian planets. *Icarus* **20,** 465–476.

WEST, R. A., D. F. STROBEL, AND M. G. TOMASKO 1986. Clouds, aerosols, and photochemistry in the Jovian atmosphere. *Icarus* **65,** 161–218.

WOELLER, F., AND C. PONNAMPERUMA 1969. Organic synthesis in a simulated Jovian atmosphere. *Icarus* **10,** 386–392.

YUNG, Y. L., M. ALLEN, AND J. PINTO 1984. Photochemistry of the atmosphere of Titan: Comparison between model and observations. *Astrophys. J. Suppl. Ser.* **55,** 465–506.