

# Chemical Investigation of Titan and Triton Tholins

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We report chromatographic and spectroscopic analyses of both Titan and Triton tholins, organic solids made from the plasma irradiation of 0.9:0.1 and 0.999:0.001 N<sub>2</sub>/CH<sub>4</sub> gas mixtures, respectively. The lower CH<sub>4</sub> mixing ratio leads to a nitrogen-rich tholin (N/C > 1), probably including nitrogen heterocyclic compounds. Unlike Titan tholin, bulk Triton tholin is poor in nitriles. From high-pressure liquid chromatography, ultraviolet and infrared spectroscopy, and molecular weight estimation by gel filtration chromatography, we conclude that (1) several H<sub>2</sub>O-soluble fractions, each with distinct UV and IR spectral signatures, are present, (2) these fractions are not identical in the two tholins, (3) the H<sub>2</sub>O-soluble fractions of Titan tholins do not contain significant amounts of nitriles, despite the major role of nitriles in bulk Titan tholin, and (4) the H<sub>2</sub>O-soluble fractions of both tholins are mainly molecules containing about 10 to 50 (C + N) atoms. We report yields of amino acids upon hydrolysis of Titan and Triton tholins. Titan tholin is largely insoluble in the putative hydrocarbon lakes or oceans on Titan, but can yield the H<sub>2</sub>O-soluble species investigated here upon contact with transient (e.g., impact-generated) liquid water. © 1994 Academic Press, Inc.

## INTRODUCTION

Atmospheres with predominantly N<sub>2</sub> and CH<sub>4</sub> compositions are present on several bodies of the outer Solar System. Titan, Saturn's largest satellite, possesses a 1.5-bar atmosphere of N<sub>2</sub>, with CH<sub>4</sub> mixing ratios ranging from around 0.04–0.10 at the surface, to 0.02 at the tropopause, to 0.06–0.20 in the upper stratosphere (Lellouch *et al.* 1989, Thompson *et al.* 1992, Strobel *et al.* 1992). Triton, Neptune's largest satellite, also possesses a N<sub>2</sub>/CH<sub>4</sub> atmosphere, although the surface pressure is much less (around 16  $\mu$ bar) and the CH<sub>4</sub> mixing ratio is only about 10<sup>–5</sup> at the surface (Broadfoot *et al.* 1989). Pluto seems to be an intermediate case between Titan and Triton. Its atmosphere is mainly N<sub>2</sub> with 0.1 to 10% CH<sub>4</sub> and most likely values of 98% N<sub>2</sub>, 1.5% CH<sub>4</sub>, and 0.5% CO (Yelle 1994, Owen *et al.* 1993); the total surface pressure is  $\geq 3$   $\mu$ bar, and an organic haze layer may be present

(Elliot *et al.* 1989). We would expect N<sub>2</sub>/CH<sub>4</sub> tholins to be synthesized by magnetospheric electrons and/or the solar wind in the atmospheres of Titan, Triton, and Pluto and to be accumulating on their surfaces. Only on Titan is the atmosphere entirely opaque to the electron flux. It seems likely that these three bodies are the first discovered members of a much larger array of worlds, in this and other solar systems, with N<sub>2</sub>/CH<sub>4</sub> atmospheres, on all of which N<sub>2</sub>/CH<sub>4</sub> tholins are being generated.

Our own planet has an N<sub>2</sub>-dominated atmosphere with 1.5 ppm of CH<sub>4</sub>, the latter entirely of biological origin. The CH<sub>4</sub> mixing ratio in the atmosphere of prebiotic Earth is thought to have been comparable to that now seen on Triton (~10–100 ppm) or possibly even greater (Miller 1982, Holland 1984). The chemistry of outer Solar System N<sub>2</sub>/CH<sub>4</sub> atmospheres may therefore be of interest as well for studies of prebiological organic chemistry.

Our laboratory has previously carried out experiments on gas mixtures approximating the compositions of the atmospheres of both Titan and Triton, subjected to plasma discharge approximating the magnetospheric charged particle and solar wind irradiation of the upper atmospheres of these bodies. Experiments using a 90:10 N<sub>2</sub>:CH<sub>4</sub> mixture to simulate the Titanian atmosphere generate a variety of C<sub>1</sub>–C<sub>5</sub> hydrocarbons and nitriles, both saturated and unsaturated (Thompson *et al.* 1991). The total yield of products,  $G_{C+N}$ , in these experiments ranges from 4.0 molecules/100 eV at 17 mbar pressure to 0.79 molecules/100 eV at 0.24 mbar. The most abundant of these products have all been observed on Titan by Voyager's IRIS experiment (e.g., Hanel *et al.* 1981). All of Titan's nitriles and alkynes are found in the abundances predicted by Thompson *et al.* (1991) from experimental results at 0.24 mb and a simple eddy diffusion model. Similar plasma discharge experiments using a rough simulation of Triton's atmosphere (0.1% CH<sub>4</sub> in N<sub>2</sub>,) yield saturated C<sub>2</sub> and C<sub>3</sub> hydrocarbons as well as saturated and unsaturated C<sub>1</sub>–C<sub>5</sub> nitriles (Thompson *et al.* 1989). These experiments gave a  $G_{C+N}$  value of 0.16 molecules/100 eV at 0.56 mbar. Unfortunately, the Voyager 2 IRIS signal level was too low at Neptune to detect such molecules.

Plasma discharge experiments with these gas mixtures

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produce, in addition to gas-phase products, higher-molecular-weight organic solids known as tholins. Elemental analysis of bulk Titan tholin yields an empirical formula of  $C_3H_5N_2$  (Sagan *et al.* 1984). Titan tholin has been characterized to some extent: pyrolytic/GC/MS analysis reveals many compounds, including alkanes, alkenes, nitriles, aromatic hydrocarbons, and a range of nitrogen heterocyclic molecules (Khare *et al.* 1984b, Ehrenfreund *et al.* 1994), while acid hydrolysis generates up to 16 amino acids, with glycine, aspartate, alanine, and  $\beta$ -alanine the most abundant (Khare *et al.* 1986). A UV-vis-IR spectrum calculated from Titan tholin optical constants gives a good fit to the observed spectrum of Titan atmospheric haze (Sagan *et al.* 1992). No other candidate materials do.

These previous studies provide substantial evidence that continuous flow plasma discharge laboratory experiments produce both gases and solids that well represent those found in Titan's atmosphere, and give some confidence that the physical and chemical properties of these products apply to the organic gas, haze, and surface sediment on Titan. Presumably, analogous results for Triton and Pluto will have some relevance there, although observational limitations have not yet allowed this to be verified directly.

In the present paper, we further characterize Titan tholin and perform an initial characterization of Triton tholin. These analyses include high-resolution infrared and ultraviolet spectra, liquid chromatographic separation of the  $H_2O$ -soluble components, and yields of amino acids upon tholin hydrolysis.

## EXPERIMENTAL METHODS AND RESULTS

An inductively coupled plasma discharge driven by a Tesla coil producing approximately  $6.3 \times 10^{20}$  eV/hr at 2 torr and  $1.8 \times 10^{20}$  eV/hr at 1 torr was employed on appropriate gas mixtures to produce both Titan and Triton tholins. In order to obtain quantitative yields, the discharge vessel used was a Pyrex tube of approximately 0.4 cm interior diameter by 30 cm length connected by Cajon fittings to a vacuum system in a continuous flow configuration. Both gas mixtures (10%  $CH_4$  in  $N_2$  and 0.1%  $CH_4$  in  $N_2$ ) were purchased from, premixed, and analyzed by Matheson. Details of the apparatus and procedures have been reported elsewhere (Thompson *et al.* 1991, McDonald *et al.* 1991). The experiments were carried out at room temperature. The plasma was applied in the Titan simulation for 24 hr, which—crudely allowing also for solar UV—corresponds to <1000 years of irradiation by saturnian magnetospheric electrons (solar UV excluded) in the Titan stratosphere (Thompson *et al.* 1994).

The empirical formulae of bulk Titan and Triton tholins (as determined through elemental analysis by

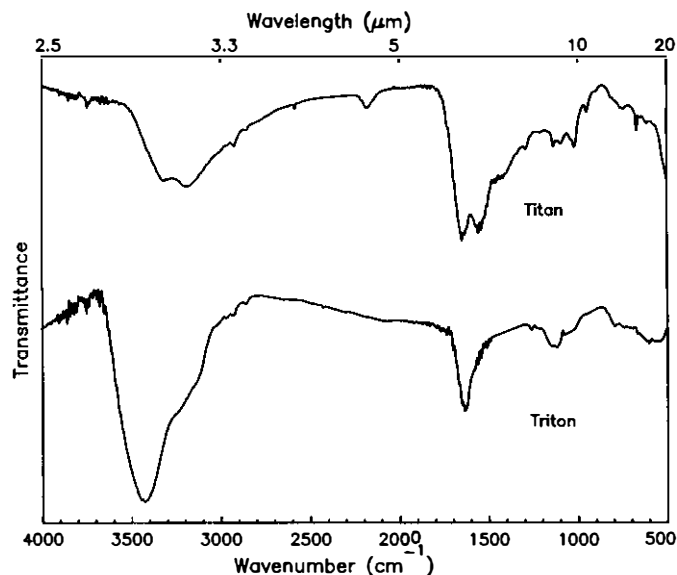


FIG. 1. FTIR spectra of bulk Titan (top) and Triton (bottom) tholin.

Schwartzkopf Microanalytical Laboratories, Woodside, NY) are  $C_3H_5N_2$  and  $C_3H_5N_4$ , respectively. More nitrogen is incorporated into Triton tholin during the plasma discharge synthesis than into Titan tholin. Because of the prominent role of nitrogen chemistry in both Titan and Triton tholin chemistry, it is clearly wrong to describe these tholins as hydrocarbons. Indeed, Triton tholin has more N than C. Quantitative yields of auroral electron-derived Titan tholin are calculated from these experiments, and are shown to account for the derived sedimentation rate of Titan stratospheric haze in Thompson *et al.* (1994).

The N/C ratios of Titan and Triton tholins determined by elemental analysis (0.67 and 1.33, respectively) do not show a direct correlation with the initial N/C ratios (18 and 1998) of the starting mixtures. Chang *et al.* (1983) reported a direct correlation between the reactant and nonvolatile product N/C ratios in spark discharge experiments on  $CH_4/N_2/H_2O$  mixtures in which initial N/C = 0.06 and 2.0, and product N/C = 0.004 and 0.05. The differences in parameters in these experiments—spark discharge vs plasma discharge, initial N/C  $\leq 2$  vs initial N/C  $\gg 2$ , presence vs absence of  $H_2O$ —may all have contributed to the observed differences in product vs reactant N/C correlations between the two data sets.

**Bulk infrared spectra of tholins.** In order to obtain infrared spectra, Titan tholin was extracted from the discharge tube with dimethylsulfoxide, DMSO. The solution was dried onto an AgCl optical window and spectra obtained with a Mattson Galaxy 6020 FTIR spectrometer at  $2\text{ cm}^{-1}$  resolution. The spectrum of Titan tholin obtained in this way is shown in Fig. 1 (top). It exhibits several

C—H stretching absorption bands. The bands at 2854 and 2924  $\text{cm}^{-1}$  (3.13 and 3.02  $\mu\text{m}$ ) are probably due to single-bonded  $\text{CH}_2$  and/or  $\text{CH}_3$  groups, while the 2960  $\text{cm}^{-1}$  band (3.38  $\mu\text{m}$ ) is due to C—H functional groups. The feature at 3005  $\text{cm}^{-1}$  (3.33  $\mu\text{m}$ ) may be due to double-bonded or aromatic C—H groups. The dominance of the 2854 and 2924  $\text{cm}^{-1}$  bands suggests that most of the C—H bonds in Titan tholin are located on single-bonded carbons.

The Titan tholin spectrum contains two N—H stretching features, at 3196 and 3316  $\text{cm}^{-1}$  (3.13 and 3.02  $\mu\text{m}$ ). These bands are probably due mainly to primary and secondary amino ( $\text{NH}_2$ ) groups, although the 3316  $\text{cm}^{-1}$  band may in part represent imine ( $\text{C}=\text{N}-\text{H}$ ) bonds. The contribution of any adsorbed water to these bands should be minimal, since the water O—H feature normally occurs at higher wavenumbers.

As suggested by Khare *et al.* (1984), the 2181  $\text{cm}^{-1}$  (4.59  $\mu\text{m}$ ) feature is probably due to nitrile ( $-\text{C}\equiv\text{N}$ ) [and/or, less probably, isocyano ( $\text{C}\equiv\text{N}-$ )] groups. The small shoulder at about 2200  $\text{cm}^{-1}$  (4.55  $\mu\text{m}$ ) could be due either to  $\text{C}\equiv\text{C}$  or to conjugated nitriles. The two bands at 1650 and 1560  $\text{cm}^{-1}$  (6.06 and 6.41  $\mu\text{m}$ ) are probably due mostly to  $\text{C}=\text{C}$ , although some contribution from  $\text{C}=\text{N}$  and  $\text{N}=\text{N}$  is also to be expected. The N—H bending mode of amino groups may also add to the strength of the absorption in this region. The 1450 and 1300  $\text{cm}^{-1}$  (6.90 and 7.69  $\mu\text{m}$ ) features can be assigned to various C—H bending modes, while the bands in the 1000–1150  $\text{cm}^{-1}$  (10 to 8.7  $\mu\text{m}$ ) region are probably due to C—C and C—N stretching.

In order to obtain sufficient quantities of Triton tholin for analysis, a plasma discharge system (LFE Model PDS-504) of greater capacity than we have employed in previous experiments was used. This system is equipped with four discharge chambers of approximately 13 cm interior diameter and 30 cm length. The RF generator is capable of delivering 100–200 W to the system. Triton tholin was generated, scraped from the walls of the discharge chambers, and pressed into a KBr pellet for infrared spectral analysis. Spectra were obtained as above on a Mattson Galaxy 6020 FTIR spectrometer at 2  $\text{cm}^{-1}$  resolution. The infrared spectrum of Triton tholin is also shown in Fig. 1 (bottom).

Surprisingly, the Triton tholin spectrum shows no apparent  $\text{C}\equiv\text{C}/\text{C}\equiv\text{N}$  absorbances. Weak C—H stretching bands are visible at 2856, 2930, and 2965  $\text{cm}^{-1}$  (3.50, 3.41, and 3.37  $\mu\text{m}$ ). Alkene C—H bands are probably present but hidden by the large 3249  $\text{cm}^{-1}$  (3.08  $\mu\text{m}$ ) N—H band. The N—H stretching bands at 3424 and 3249  $\text{cm}^{-1}$  (2.92 and 3.08  $\mu\text{m}$ ) are probably due to amino groups, although the 3424  $\text{cm}^{-1}$  band may be enhanced to some extent by adsorbed water. The 1636  $\text{cm}^{-1}$  (6.11  $\mu\text{m}$ ) feature is probably a combination of  $\text{C}=\text{C}/\text{C}=\text{N}$  stretching and

N—H bending modes, while the bands at 1140 and 1118  $\text{cm}^{-1}$  (8.77 and 8.94  $\mu\text{m}$ ) can be identified as due to C—C/C—N stretching.

From this analysis of the bulk infrared spectra of Titan and Triton tholins, we can conclude that most of the hydrogen atoms in Titan tholin are present in single-bonded C—H and N—H groups. Nitrile and/or isocyano groups are detectable, and several kinds of carbon-carbon and carbon-nitrogen double bonding, as well as perhaps some nitrogen-nitrogen double bonding, appear to be present. Triton tholin, on the other hand, seems to be composed mostly of a more limited suite of double-bonded carbon/nitrogen functional groups and abundant amino/imino groups, with relatively few nitrile groups.

Since nitrogen heterocyclic compounds are known to be components of Titan tholin (Khare *et al.* 1984b, Ehrenfreund *et al.* 1994), and since the N/C ratio is larger in Triton than in Titan tholin, it is plausible that such compounds are constituents of Triton tholin as well. The moderate-strength absorption features at 600–900 and 1000–1200  $\text{cm}^{-1}$  are consistent with this conclusion. However, the heterocyclics of greatest interest, purines and pyrimidines, have not yet been identified.

*Chromatographic analyses of tholins.* Neither Titan nor Triton tholin contains any easily detectable component that is soluble in common organic solvents such as hexane, methylene chloride, benzene, or acetone. Both tholins, however, contain a component, estimated to be a few percent by mass of the total tholin, which is  $\text{H}_2\text{O}$ -soluble. Since aqueous chemistry is of interest from an exobiological and early Earth perspective, and since liquid  $\text{H}_2\text{O}$  is likely to be generated episodically over significant areas of Titan (and, less likely, Triton) by impact events (Thompson and Sagan 1992), we have concentrated our analyses in the remainder of the present paper on this component. It is of possible relevance to future *in situ* measurements on Titan by the Huygens entry probe of the Cassini mission.

Figure 2 shows the reverse-phase liquid chromatograms of both Titan and Triton tholins. These chromatograms are obtained by elution of the  $\text{H}_2\text{O}$ -soluble fraction of each tholin on a Waters Nova-pak  $\text{C}_{18}$  HPLC column, with a mobile phase of 0–50%  $\text{CH}_3\text{CN}$  in 0.1% trifluoroacetic acid/ $\text{H}_2\text{O}$ . Eluting compounds were detected using a Waters 990 photodiode array detector at a wavelength of 208  $\mu\text{m}$ . Each water-soluble fraction of tholin is composed of four major HPLC peaks, at approximately 4, 5, 6, and 7 min retention time. In the Titan tholin chromatogram, the 7-min peak is the largest, while the 6-min peak is the largest in the Triton chromatogram. In both cases the fractions corresponding to these peaks have been labeled A, B, C, and D, although in the case of Triton tholin the 6 min peak is resolved into two components,  $\text{C}_1$  and  $\text{C}_2$ . Other peaks are seen in the 15–25-min region of both

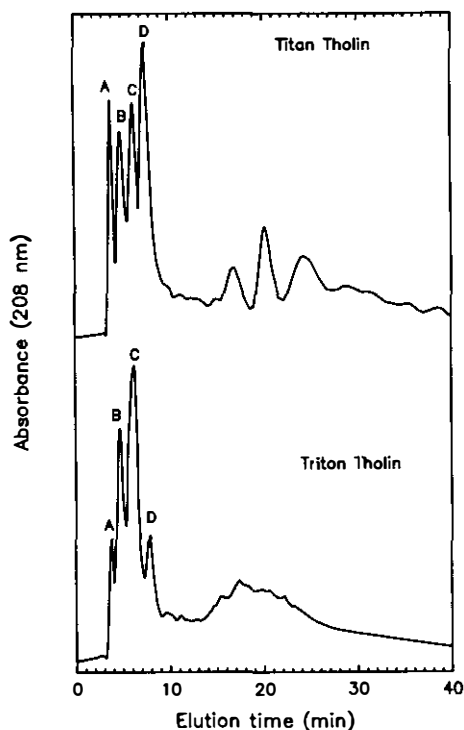


FIG. 2. HPLC chromatograms of water-soluble components of Titan (top) and Triton (bottom) tholins.

chromatograms. The 17–18-min peak in both cases is due to a HPLC mobile phase component, while the other peaks have yet to be fully investigated.

Figures 3 and 4 show the ultraviolet spectra of the Titan and Triton tholin HPLC fractions collected as they eluted

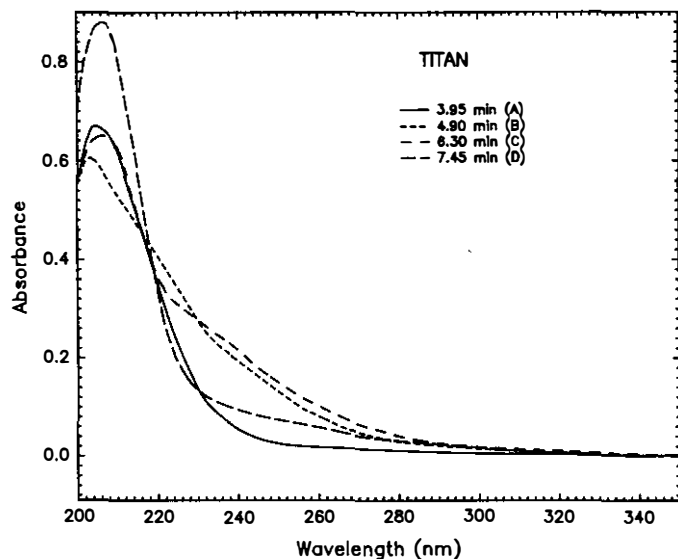


FIG. 3. Ultraviolet spectra of four Titan tholin water-soluble HPLC fractions.

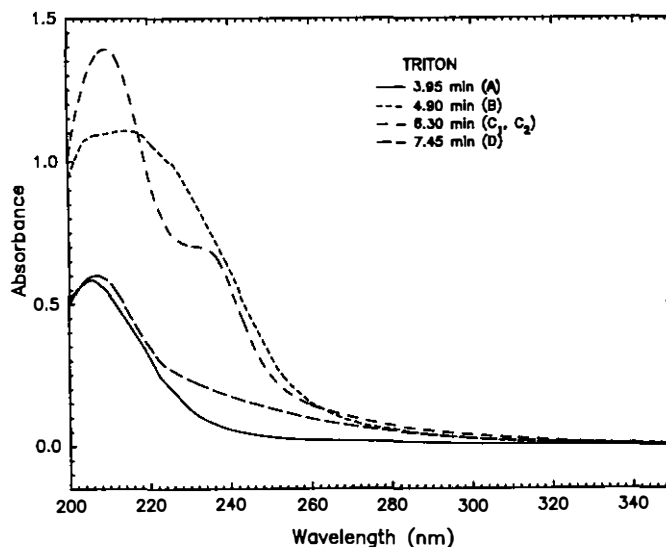


FIG. 4. Ultraviolet spectra of four Triton tholin water-soluble HPLC fractions.

using a Waters 990 photodiode array detector. All fractions have  $\lambda_{\max} = 200\text{--}220\text{ nm}$ . We note in passing the major interstellar UV absorption peak in this range. The Titan tholin fractions all have  $\lambda_{\max} = 200\text{--}210\text{ nm}$ , while in two Triton tholin fractions  $\lambda_{\max}$  is in the 210–220-nm range. Fractions B and C of both tholins have secondary bands in the 220–250-nm range, with the Triton B and C fraction secondary bands being more pronounced. There is no significant absorbance at  $>340\text{ nm}$  in any of the tholin HPLC fractions.

The primary UV bands in all the HPLC fractions are attributed to either conjugated double-bond or amino group  $n \rightarrow \sigma^*$  transitions. The fact that the azo group ( $-\text{N}=\text{N}-$ )  $n \rightarrow \pi^*$  transition at around 340 nm is not seen in these spectra seems to rule out the presence of significant nitrogen–nitrogen double bonding in the HPLC fractions. The absence of a strong band in the 250–260-nm region excludes purines as a *major* constituent.

Infrared spectra of Titan fractions A, B, C, and D, as well as Triton fractions A, B, C<sub>1</sub>, C<sub>2</sub>, and D, were obtained by collecting each fraction from the effluent of multiple HPLC runs, pooling the samples of each fraction, and drying onto AgCl windows. The spectra of these fractions at  $2\text{ cm}^{-1}$  resolution are shown in Figs. 5 and 6.

The spectrum of the A fraction of Titan tholin shows little C–H stretch or double-bond absorbance, but significant N–H and C–C/C–N stretching bands. The B fraction shows a large C=C/C=N absorbance, as well as two C–H bending bands and three weak but identifiable C–H stretching features. The C fraction spectrum resembles that of fraction A with the addition of three C–H stretch bands and possibly some C=C/C=N absorbance. Finally, the D fraction of Titan tholin also dis-

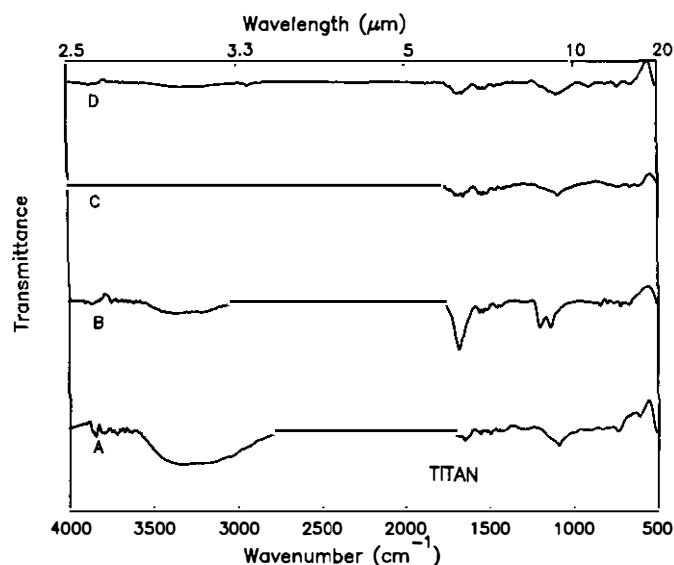


FIG. 5. FTIR spectra of four Titan tholin water-soluble HPLC fractions. Fine structure near 1500 and 3750  $\text{cm}^{-1}$  is due to laboratory background water vapor and should be ignored.

plays significant C—H, C—C/C—N, and N—H functional groups. Some C=C/C=N absorbance may also be present, although gaseous  $\text{H}_2\text{O}$  (readily apparent from its rotational fine structure) in the spectrometer beam partially obscures this region.

In comparing the spectrum of the bulk Titan tholin with those of its water-soluble HPLC fractions, the most interesting observation is that the nitrile absorbance at

around 2200  $\text{cm}^{-1}$  (4.55  $\mu\text{m}$ ) seen in the bulk spectrum is absent in all fractions. The C=C/C=N band in the bulk spectrum also seems to be proportionally larger than in the fractions, indicating that the nitrile-containing components, and to some extent the double-bond containing components, of Titan tholin are not  $\text{H}_2\text{O}$ -soluble (or do not elute from the  $\text{C}_{18}$  HPLC column under the conditions used).

The Triton tholin fraction A spectrum is complex, with N—H, C—H, C—C/C—N, and double-bond stretching bands. The B fraction is dominated by N—H, C—H, and C=C/C=N stretching as well as C—H bending bands. The  $\text{C}_1$  spectrum is similar to fraction B. Fraction  $\text{C}_2$  appears to be quite similar to  $\text{C}_1$ , although the N—H stretching band is more intense. Fraction D shows C—H, N—H, and C—C/C—N bands, but no detectable C=C/C=N absorbance.

Comparison of the infrared spectrum of bulk Triton tholin with spectra of the HPLC fractions reveals that the C—H stretching features at 2800–3000  $\text{cm}^{-1}$  are more prominent in the fractions than in the bulk tholin. No detectable nitrile absorption appears in either the Triton bulk tholin spectrum or in the spectra of any of the fractions.

An attempt to match the ultraviolet and infrared spectra of the HPLC fractions from Titan tholin with those of Triton tholin yields a good match between the two D fractions in both the infrared and ultraviolet. Titan fraction C also resembles the two D fractions in the infrared, but its ultraviolet spectrum contains a secondary band at around 230 nm not seen in the other two spectra. The Titan B fraction and the Triton  $\text{C}_1$  and  $\text{C}_2$  fractions appear similar in the infrared, but are mediocre matches at best in the ultraviolet. It is apparent from these spectra that the  $\text{H}_2\text{O}$ -soluble fractions of Titan and Triton tholins are not identical, either in relative abundance or in spectral signatures.

**Molecular weight estimation.** The molecular weight distributions of the  $\text{H}_2\text{O}$ -soluble fractions of both Titan and Triton tholins were estimated by gel filtration chromatography, using Bio-Gel P2 (Bio-Rad) in a gravity-fed column with  $\text{H}_2\text{O}$  as the mobile phase. Bio-Gel P2 is a polyacrylamide gel filtration matrix with a fractionation range of approximately 100–1800 Da. The column was calibrated with Blue Dextran 2000, methyl red, and Coomassie Blue as molecular weight standards. The fractions were assayed by absorbance at 210 nm. The gel filtration chromatograms are shown in Fig. 7. The Triton tholin chromatogram shows a small peak at the void volume (fraction 10) as well as prominent peaks at fractions 18 (MW  $\approx$  600 Da), 28 (MW  $\approx$  300 Da), and 34 (MW  $\approx$  200 Da). The Titan tholin chromatogram, in addition to a large peak at fraction 18, has a broad peak eluting in fractions 24–46—which may be due to a suite of molecules differing

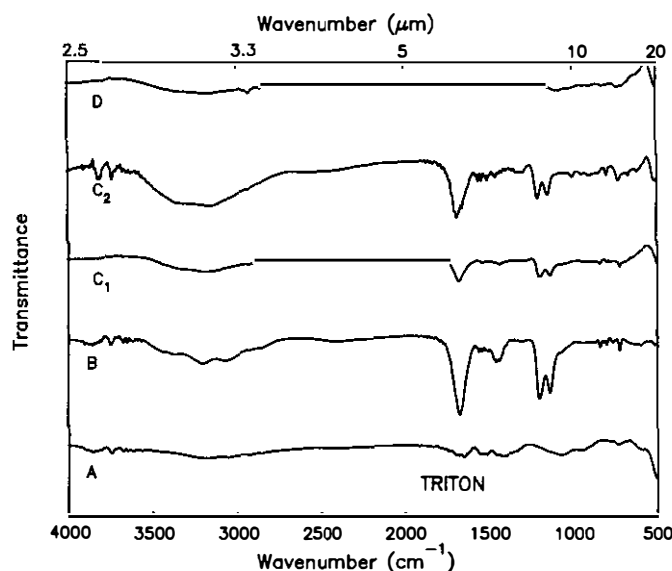


FIG. 6. FTIR spectra of five Triton tholin water-soluble HPLC fractions.

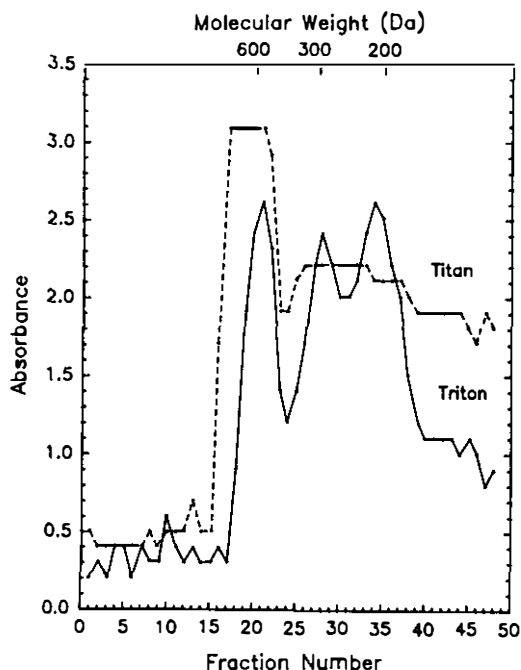


FIG. 7. Gel filtration chromatograms of water-soluble components of Titan (dashed line) and Triton (solid line) tholins.

in molecular weight by only 1–2 carbon or nitrogen units (12–14 to 24–28 Da). The gel filtration chromatogram of  $\text{CH}_4/\text{NH}_3/\text{H}_2\text{O}$  spark (Jupiter) tholin (McDonald *et al.* 1991) resembles the Titan tholin chromatogram more than that of Triton tholin, as the spark tholin chromatogram also contains the broad peak at 200–300 Da. The water-soluble components of Titan and Triton tholins, like those of spark tholin, consist of polar molecules with only ~10 to 50(C + N) atoms—molecules of more modest size than might have been expected.

**Amino acid compositions.** Table I shows the yield of various amino acids upon hydrolysis of Triton tholin in HCl/propionic acid at 150°C for 85 min. The amino acids were detected using the Waters Pico-Tag HPLC method at the Biotechnology Analytical Facility at Cornell University. Also shown in Table I are the yields of amino acids from previous analyses of Titan tholin hydrolysate (Khare *et al.* 1986). Gas chromatography of pentafluoropropyl isopropyl ester derivatization of Triton tholin hydrolysate on a Chirasil-Val column produced *D/L* ratios of 1.20 for Ala and 0.93 for Leu, within the error bars for a racemic mixture after integration of the gas chromatogram. Clearly, biological contamination of the Triton tholin samples did not occur on a significant scale. Gas chromatographic analysis of derivatized samples of Titan tholin hydrolysate on a Chirasil-Val column has also shown that the amino acids produced on hydrolysis are

racemic and not due to biological contamination (Khare *et al.* 1986).

The most striking difference between the amino acid compositions of the two tholins is the rather high yield of leucine from Triton tholin. (Leucine is not a common terrestrial contaminant in, e.g., meteorites, is not one of the so-called fingerprint amino acids, and is present here, within the error bars, in racemic proportions.) It is found in the Triton tholin hydrolysate in amounts comparable to those of aspartate, glutamate, and alanine. In contrast, Khare *et al.* (1986) detected no leucine in the hydrolysate of Titan tholin. Since leucine has a hydrocarbon side chain, it might be expected to occur at higher levels in tholins made from gas mixtures with higher concentrations of  $\text{CH}_4$  or other hydrocarbons. This is apparently not the case with  $\text{N}_2/\text{CH}_4$ -produced tholins. The presence of phenylalanine is also of interest in this context. Amino acids with N-containing side chains (arginine and lysine) are found in Triton tholin—as might be expected for a highly N-dominated starting mixture.

Other than the difference in leucine levels, the amino acid compositions of the major components of both tholin hydrolysates are rather similar. The yields of amino acids from both Titan and Triton tholins are as much as an order of magnitude less for some amino acids (glycine, alanine, glutamate, and  $\beta$ -aminobutyrate) than those obtained from  $\text{CH}_4/\text{NH}_3/\text{H}_2\text{O}$  tholins (McDonald *et al.* 1991).

TABLE I  
Amino Acid Yields from Titan and Triton Tholins  
(mg/g Tholin)

Amino acid	Triton tholin	Titan tholin*
Glycine	6.90	5.30
Alanine	1.16	0.70
$\beta$ -Alanine	0.50	1.20
$\alpha$ -aminobutyric acid	0.08	0.10
Aspartic acid	1.91	1.10
Serine	0.35	n.d.
Valine	0.28	trace
Leucine	1.93	n.d.
Isoleucine	0.17	n.d.
Glutamic acid	1.47	0.40
Threonine	0.22	trace
Proline	0.17	n.d.
Phenylalanine	0.16	n.d.
Lysine	0.21	n.d.
Arginine	0.53	n.d.

Note. n.d., not detected.

\* From Khare *et al.* 1986.

## DISCUSSION

*Similarity of Titan and Triton tholins.* Our plasma discharge experiments show that the chemistry which produces tholins from a 1:9  $\text{CH}_4:\text{N}_2$  mixture is different from that which leads to tholin production in a 1:1000  $\text{CH}_4:\text{N}_2$  mixture. The empirical formulae of the resulting tholin samples show a marked difference in C/N ratios. The infrared spectra of the bulk tholins from the two experiments are not identical (Fig. 1), although roughly the same suite of functional groups appears. The major exception is the nitrile group, which is clearly present in the spectrum of bulk Titan tholin but not in the spectrum of bulk Triton tholin. The IR spectra show no strong aromatic C—H stretching ( $3000\text{--}3100\text{ cm}^{-1}$ ) or C—H bending ( $700\text{--}900\text{ cm}^{-1}$ ) modes, indicating that there is not a large degree of aromaticity in either tholin. Polycyclic aromatic hydrocarbons have, however, been detected in Titan tholin at a level of about  $10^{-4}\text{ g/g}$  (Sagan *et al.* 1993).

Further analysis of the  $\text{H}_2\text{O}$ -soluble fractions by HPLC reveals that, while the number of chromatographic fractions is about the same in both tholins, there are definite differences in the infrared and ultraviolet spectra. All the data on both bulk and fractionated Titan/Triton tholins, taken together, lead us to suggest that the organic residue produced in the atmosphere and present on the surface of Titan has only a limited similarity to that on Triton. It would be wise, therefore, to exercise caution when using Titan data (either observational or laboratory) to model the atmospheric hazes or surface material of Triton, Pluto, or other bodies in the outer Solar System.

The decrease in  $\text{CH}_4$  mixing ratio with altitude in Titan's troposphere from about 0.1 to 0.02 (Lellouch *et al.*, 1989, Thompson *et al.* 1992) and the increase in the stratosphere from 0.02 to  $>0.06$  (Strobel *et al.* 1992) raise the interesting possibility that the actual tholin chemistry on Titan may be some combination of the chemistry active in our two experiments, which cover the range 0.1–0.001 in  $\text{CH}_4$  mixing ratio. An important future task will be to determine the optical constants for Triton tholin, as has been done for Titan tholin (Khare *et al.* 1984a), and to use combinations of both optical constant data sets to model organic hazes present in the outer Solar System.

*Mechanism of tholin formation.* The mechanism of  $\text{N}_2/\text{CH}_4$  tholin formation has not been investigated experimentally in any detail; however, a general mechanism has been suggested (Thompson and Sagan 1989) involving the polymerization of gas-phase nitriles ( $\text{R—CN}$ ) with transient species such as  $\text{RCNH}^+$  and  $\text{HCNH}$ . A linear polymer having the general structure  $\text{R}_1\text{C}=\text{NR}_2$ , probably with some degree of cross-linking, would be the result. The nitrile band present in the infrared spectrum of Titan

tholin suggests the existence of terminal nitrile groups, while the absence of that band in the spectrum of Triton tholin indicates a lack of such groups. The differences in chemical and spectroscopic properties between Titan and Triton tholins point to differences in mechanisms of formation for the two tholins, but this question is clearly in need of additional study.

*Amino acids from tholins.* The yields of amino acids from both these tholins are substantial, constituting 1–2% by weight of the tholin mass. Some other tholins give even higher yields (McDonald *et al.* 1991). The fact that the amino acid yield of Triton tholins (with no discernible  $\text{C}\equiv\text{N}$  feature) exceeds that of Titan tholin (with a modest  $\text{C}\equiv\text{N}$  feature) suggests that the amino acids derive from molecular structures other than aminonitriles. This result supports the mechanism proposed by Thompson and Sagan (1989) for the formation of tholin from abundant gas-phase free radicals and uncharged molecules and their proposal that amino acids derive from resulting polyimino chains in the tholin, substituted with functional groups derived from abundant gas-phase radicals that form the side chains of amino acids upon hydrolysis. The polyimino model is also supported by the species found in recent pyrolytic GC/MS studies (Ehrenfreund *et al.* 1994) of Titan tholin performed in preparation for the Cassini ACP experiment.

*Aqueous organic chemistry on Titan and Triton.* The presence of a detectable  $\text{H}_2\text{O}$ -soluble component in both Titan and Triton tholins indicates that aqueous organic chemistry on these two bodies is possible if liquid water is present for significant periods of time. Amino acids are released from Titan tholin upon acid hydrolysis (Khare *et al.* 1986). For  $\text{CH}_4/\text{NH}_3/\text{H}_2\text{O}$  spark tholin, a significant fraction of amino acids are released upon short exposure to basic  $\text{NH}_3 + \text{H}_2\text{O}$  solutions or even pure  $\text{H}_2\text{O}$  (Khare *et al.* 1989, McDonald *et al.* 1991). Thompson and Sagan (1992) have shown that impacts into a Titan surface rich in  $\text{H}_2\text{O}$ -ice and organic sediments would have (1) mixed the surface, producing a regolith of  $\sim 80\%$   $\text{H}_2\text{O}$  and 20% organic solids, and (2) produced large quantities of partially ( $\sim 10\text{--}20\%$ ) liquid impact ejecta; over 3.5 Gyr, some 60% of the surface would have been blanketed one or more times by ejected slurry which requires several hundred years to freeze. The impact events are violent enough to hydrolyze tholin completely in the well-heated portion of the ejecta volume, while residual  $\text{H}_2\text{O}$  melt pools in the crater floor and ejecta blanket-covered topography (which Thompson and Sagan primarily discuss) provide a transient aqueous environment in which simple nitriles and tholins can hydrolyze and interact at  $\leq 0^\circ\text{C}$  under neutral to basic conditions (depending on the fraction of  $\text{NH}_3$  in the regolith). The rates of amino acid production in cold neutral to basic conditions are poorly known.

*Interaction with hydrocarbon lakes or oceans.* Neither Titan nor Triton tholin is soluble in organic solvents, particularly nonpolar solvents (as described above). This suggests that any liquid hydrocarbon oceans or lakes which may exist on Titan (e.g., Lunine *et al.*, 1989) do not have large concentrations of dissolved high-molecular-weight organics. Substantial submarine organic deposits, however, may be present (Sagan *et al.* 1986).

*Relevance of  $N_2/CH_4$  discharge experiments to early Earth.* Although the atmosphere of prebiotic Earth was probably dominated by carbon dioxide and nitrogen, a  $CH_4$  mixing ratio of  $10^{-3}$ – $10^{-5}$  is considered reasonable by some (e.g., Miller 1982, Holland 1984). Therefore, discharge experiments using  $N_2/CH_4$  mixtures similar to our Triton mix have some relevance to the possible atmospheric organic chemistry on early Earth, and thus to the origin of life. In particular, our experiments show that complex gaseous organic molecules and tholins can be produced in an atmosphere with a  $CH_4$  mixing ratio as low as  $10^{-3}$ . Tholins on early Earth could—assuming the surface was above  $0^\circ\text{C}$ —hydrolyze to produce amino acids, plus a range of other polar organic species (particularly if  $H_2O$  is also present) that have not been fully characterized. Such aqueous interactions of tholins on Titan have previously been investigated (Thompson and Sagan 1992).

Nucleic acid bases (purines and pyrimidines) have also been identified as pyrolysis products of Titan tholin (Khare *et al.* 1984b) and may be released in aqueous environments (Ehrenfreund *et al.* 1994). The strong double-bond functional groups evident in the infrared spectra of both tholins have been noted above, as has the lack of an  $-N=N-$  (azo) ultraviolet spectral signature in either tholin. These data, combined with the higher N/C ratio in Triton tholin (1.33 vs 0.67 for Titan tholin), suggest that carbon–nitrogen double bonds should be major features of the structure of Triton tholin components. These structures may well include nitrogen heterocycles such as imidazoles, pyrazines, pyridines, purines, and pyrimidines. The high N/C ratio of Triton tholin indicates that tholins produced from atmospheres of  $N_2$  with small  $CH_4$  mixing ratios could be one of the richest extraterrestrial sources of nitrogen heterocycles and related compounds that have been modeled to date. Discharge experiments with small amounts of  $CH_4$  in  $N_2/CO/CO_2$ -based atmospheres would not only serve as reasonable models of early terrestrial atmospheric chemistry, but the results for  $10^{-3}$   $CH_4$  in  $N_2$  suggest that such experiments may yield substantial yields of molecules important for early chemical evolution.

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