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Solid-State ^{15}N NMR Studies of Tobacco Leaves

ZHIRU MA, DEWEY H. BARICH, MARK S. SOLUM, AND RONALD J. PUGMIRE*

Department of Chemistry and Chemical and Fuels Engineering, University of Utah, Salt Lake City, Utah 84112

Nitrogen-containing compounds are one important class of constituents in tobacco because of various pharmacological and biological properties. Three types of tobacco leaves (burley, bright, and oriental) were studied using solid-state ^{15}N NMR cross polarization with magic-angle spinning, dipolar dephasing and five π replicated magic angle turning (FIREMAT) experiments. The results show that burley tobacco leaves contain significantly more pyridinic nitrogen than that of bright or oriental tobacco leaves. The principal values of ^{15}N chemical shift tensors of nitrogen functional groups were obtained from the FIREMAT data. Possible assignments of solid-state ^{15}N NMR resonances were made using nitrogen chemical shift tensors in some model compounds or isotropic chemical shift values from liquid NMR results. To the best of our knowledge, this is the first solid-state ^{15}N NMR study of tobacco plant material.

KEYWORDS: Solid-state ^{15}N NMR; tobacco leaves; nitrogen compounds; FIREMAT

INTRODUCTION

The tobacco plant is a very complex biomass material, in which more than 2200 compounds have been identified; among these, the nitrogen-containing materials comprise nearly 30% of all reported compounds (*1*, *2*). Nitrogen-containing compounds are an important class of constituents in the tobacco plant because of their various pharmacological and biological properties. These compounds include alkaloids, amino acids, proteins, amines, amides, and other miscellaneous N-containing compounds. The four principal tobacco alkaloids are nicotine, nornicotine, anabasine, and anatabine. Nicotine is the primary alkaloid in commercial tobaccos (*Nicotiana tabacum* and *Nicotiana rustica*).

The isolation and identification of the nitrogen-containing compounds are generally studied by using wet chemical analysis, GC-MS, HPLC-MS and spectroscopy methods (*3*). Jamin et al. (*4*) determined the carbon, nitrogen, and hydrogen stable isotope contents of nicotine extracted from tobacco leaves by using isotope ratio mass spectroscopy and the site-specific natural isotope fractionation (SNIF)-NMR method. Although extensive investigations have been reported on the chemical composition of tobacco material, the formation and conversion of nitrogen-containing compounds, especially during pyrolysis, are not yet well understood.

Solid-state NMR spectroscopy has been increasingly employed to characterize natural and biological materials (*5*−*10*). It is a nondestructive method, which is suitable for complex plant material and insoluble compounds. ^{15}N NMR spectroscopy (*11*, *12*) is hampered by low sensitivity, which results from its very low natural abundance (0.37%), small magnetogyric ratio, and normally long spin−lattice relaxation times. To date, there has been no report on the study of tobacco plant material using solid-state ^{15}N NMR spectroscopy. However, a few papers employing ^{13}C cross polarization with magic-angle spinning (CPMAS) NMR methods were published by Wooten and coworkers (*13*−*15*). Cellulose in tobacco was quantitatively analyzed by ^{13}C CPMAS NMR (*13*), and the ^{13}C CPMAS NMR spectra of cured bright and burley laminae and stems were obtained and interpreted in light of the reference materials including cellulose, hemicellulose, pectin, rutin, chlorogenic acid, etc (*14*).

Chemical shift tensors are very sensitive to electronic environments such as hydrogen bonding, functional group type, and intermolecular interactions, and chemical shift tensor principal values can provide more valuable structural information than the isotropic chemical shifts (*16*). Nitrogen-15 NMR (*17*, *18*) has a large chemical shift range (approximately 1000 ppm), making it a very useful tool for the characterization of molecular structure. In this laboratory, ^{15}N chemical shift tensors of a substantial variety of compounds have been studied using solid-state ^{15}N NMR. Employing modern quantum mechanical methods for interpreting the NMR data, important structural information including intermolecular electrostatic interactions and molecular geometry have been obtained (*19*−*24*). However, the amount of ^{15}N chemical shift tensor data available in the literature is still rather limited (*25*, *26*).

In the present work, three different types of tobacco leaves were studied using solid- state ^{15}N CPMAS, dipolar dephasing (DD) and five-π replicated magic angle turning (FIREMAT) (*27*) experiments. These ^{15}N enriched tobacco leaves were from burley, bright, and oriental tobacco. The principal values of ^{15}N chemical shift tensors of nitrogen functional groups were obtained by fitting the FIREMAT data. Proposed assignments of solid-state ^{15}N NMR resonances were made using a combination of nitrogen chemical shift tensors in some model com-

* To whom correspondence should be addressed. Tel.: (801)581−7236. Fax: (801)585−6212. E-mail: pug@vpres.adm.utah.edu.

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pounds and isotropic chemical shift values from liquid NMR data.

MATERIALS AND METHODS

Materials. ^{15}N labeled tobacco leaves (burley, bright, and oriental) were provided by Philip Morris USA without documentation of the details of growth conditions, although each of the plants was grown in a similar environment. The burley tobacco leaves were air cured and light brown in color. The bright and oriental tobacco leaves were freeze-dried and green in color. All the samples were stored at ambient conditions and studied as received, that is, at ambient moisture content. The tobacco leaves were crumbled into small pieces when packed into rotor.

NMR Experiments. ^{15}N CPMAS experiments of three different types of tobacco leaves were performed on a Chemagnetics CMX-200 spectrometer with an ^{15}N Larmor frequency of 20.27 MHz, using a Chemagnetics 7.5-mm PENCIL rotor probe. All tobacco samples were run using a standard cross polarization (CP) pulse sequence with the sample spinning at 4 kHz. The proton power during the contact time was reduced to meet the Hartmann−Hahn match condition of nitrogen. The proton 90° pulse was 4.1 μs. The proton T_1 of each of the three types of tobacco leaves was measured by the saturation recovery method and values were in the range of 0.5−1.0 s. The pulse delays were 1 s for burley tobacco and 2 s for bright and oriental tobacco leaves, with a total of 50000 scans for burley and 25000 scans for bright and oriental leaves.

Variable contact experiments were carried out for the three types of tobacco leaves with 21 different contact times ranging from 5 to 25 μs. The values of T_{NH} and $T_{1\rho}$ of some nitrogen functional groups were obtained by fitting the cross polarization curves with the VCL model [28] in which the T_{NH} is fit to one time constant with a Lorentzian function while the $T_{1\rho}$ is fit to a single-exponential decay. The T_{NH} of the pyridine group ($\delta_{iso} = -50$ to -100 ppm) for burley tobacco leaves was 810 ± 80 μs, and the $T_{1\rho}$ value was 5.6 ± 0.5 μs. The T_{NH} and $T_{1\rho}$ values of the pyridine group for the bright and oriental tobacco leaves were not available, due to the very small pyridine nitrogen signal. The T_{NH} values of the large peak ($\delta_{iso} = -220$ to -285 ppm) for the burley, bright, and oriental tobacco leaves were essentially the same (126 ± 7 μs, 117 ± 6 μs, and 135 ± 10 μs, respectively), while the $T_{1\rho}$ values were 4.5 ± 0.3 ms, 6.1 ± 0.3 ms, and 3.0 ± 0.2 ms, respectively. To compare the intensity of different nitrogen components, a contact time of 5 μs was used in the ^{15}N CPMAS experiments, which provides a more reliable measure of the relative amount of nonprotonated pyridinic nitrogen (*29*). The spectral width was 40 kHz. The dipolar dephasing technique (*30*, *31*) was used to distinguish protonated and nonprotonated nitrogens by incorporating twenty seven interrupted decoupling delays ranging from 2 to 1000 μs. The ^{15}N chemical shifts were referenced to glycine at −346.40 ppm on the nitromethane scale.

The 2D FIREMAT (*27*) experiments of burley and oriental tobacco leaves were carried out on a Chemagnetics CMX-200 spectrometer, whereas those of the bright tobacco leaves were performed on a Chemagnetics CMX-400 spectrometer with a ^{15}N frequency of 40.5519 MHz. The spinning speed of the FIREMAT experiment on the burley leaves was 501 Hz. Data points ($n = 96$) were collected during each rotor cycle. The spectral widths were 48078 Hz in the acquisition dimension and 8013 Hz in the evolution dimension. A total of 1200 data points were collected with 16 increments in the evolution dimension. The total number of scans was 11520 for each evolution increment. The FIREMAT experiment of the oriental leaves was obtained at a spinning rate of 451 Hz. The spectral widths were 43290 and 7215 Hz for the acquisition dimension and evolution dimension, respectively. A total of 1250 data points were collected with 16 increments in the evolution dimension. A total of 26680 scans was accumulated for each evolution increment. A shorter contact time (0.8 ms) was used for the oriental leaves because it exhibited the shortest $T_{1\rho}$ value. The short contact time gave an optimum signal-to-noise ratio for this sample.

In the FIREMAT experiment of the bright tobacco leaves, the sample spinning rate was 1002 Hz, and a total of 2404 data points was collected. The spectral widths were 96154 Hz in the acquisition dimension and 16026 Hz in the evolution dimension. Sixteen increments were used in the evolution dimension with a total of 12480 scans for each evolution increment. A contact time of 5 ms was used for bright and burley tobacco leaves.

The ^{15}N chemical shift tensors of nitrogen groups in tobacco leaves were extracted by fitting of the 2D FIREMAT data set as previously described.[27] The fitting process and data analysis were carried out on a Sun workstation.

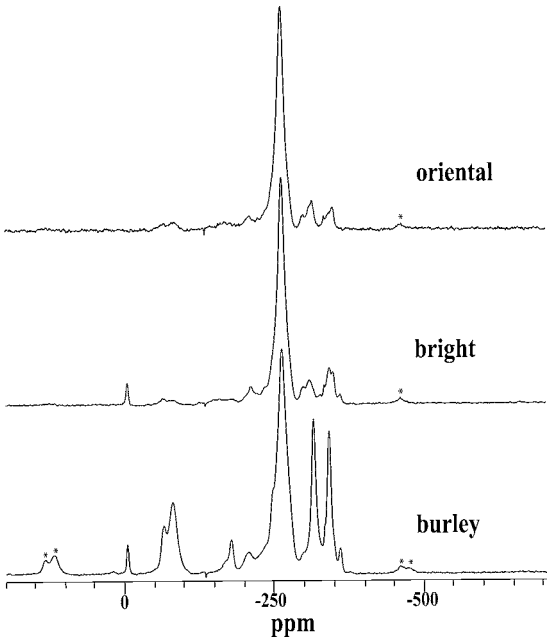

Figure 1. ^{15}N CPMAS spectra of three types of tobacco leaves obtained at a contact time of 5 ms. * = spinning sidebands.

Table 1. Tentative ^{15}N Resonance Assignment in Tobacco Leaves (Chemical Shift Referenced to Nitromethane = 0 ppm)

chemical shift range (ppm)	assignment
–1 to –8	nitrate ion, nitro derivatives
–50 to –100	pyridine and related compounds
–145 to –220	imidazole, pyridinium nitrogens, histidine, purine
–220 to –285	amides, lactams, peptide, pyrrolic-N, indole, carbazole
–285 to –350	amino acids, saturated heterocyclic amines, aniline and its derivatives
–350 to –370	NH_4^+, some amines

RESULTS AND DISCUSSION

The ^{15}N CPMAS spectra of three types of tobacco leaves are shown in **Figure 1**. It can be seen that some nitrogen resonances are seriously overlapped because of the complexity and inhomogeneity of tobacco plant material (e.g., tobacco leaves have the highest content of nicotine, roots have less, and stalks have the least). Within a leaf, the tip and outer area usually have higher alkaloid content than the base and the inner areas, respectively (*32*). The nitrogen resonances in all three types of tobacco leaves are dominated by the signals from −220 to −285 ppm. A tentative assignment of ^{15}N resonances is listed in **Table 1**(*6*, *18*, *33*). Distinct differences are observed from the ^{15}N CPMAS spectra of these three types of tobacco leaves. Strong pyridine compound signals appear between −50 and −100 ppm in the burley tobacco leaves (bottom trace, **Figure 1**), whereas in bright and oriental leaves, this signal is very weak. There are also significant spectral differences in the chemical shift range of −285 to −360 ppm between burley and the other two

Table 2. Relative Contents of Three Nitrogen Groups in Tobacco Leaves[a]

chemical shift range (ppm)	burley	bright	oriental
−50 to −100	31.7	3.4	3.7
−220 to −285	100.0	100.0	100.0
−285 to −350	64.8	25.2	22.6

[a] In each case the intensities of the large peak (−220 to −285 ppm) was normalized to 100 to determine the relative intensities of the peaks in the other major areas of a spectrum.

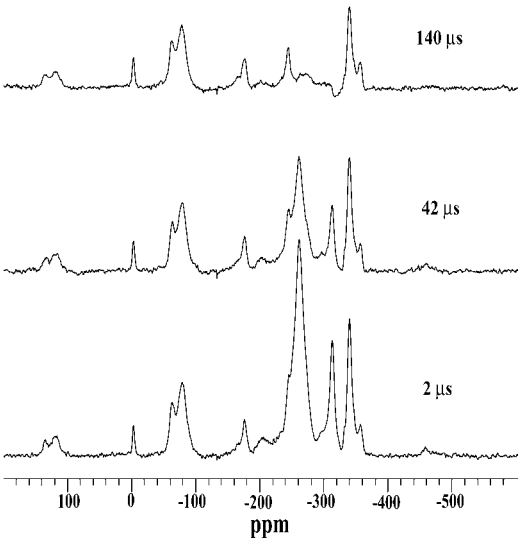

Figure 2. ^{15}N dipolar dephasing spectra of burley tobacco leaves at various dephasing times.

samples. This region is assigned to amino acids and saturated heterocyclic amine nitrogen groups. The approximate relative contents of three main nitrogen groups in tobacco leaves are shown in **Table 2**. The alkaloid (primarily nicotine) pyridinic nitrogen signal appears in the −50 and −100 ppm range. The results show that the burley tobacco leaves have a high alkaloid content compared to that of bright and oriental leaves. The quantity of alkaloids depends not only on the tobacco's genetic constitution and its stage of development but also on the external conditions under which it has grown (*34*). The Maryland and Turkish (Oriental) tobaccos are low in nicotine; the flue-cured, burley, cuban, and Connecticut cigar wrapper are medium; and the Pennsylvania, dark fire-cured, and especially *N. rustica* are high in nicotine content (*32*). Lowe and Sheen (*35*) investigated the pattern of soluble protein (FI and FII) accumulation in bright and burley tobaccos during the growing seasons of 1977 and 1978. Their results revealed that burley contains significantly more FI protein, total proteins, free amino acids, total nitrogen, nitrate nitrogen, and total alkaloids than bright tobacco if averaged over the growing season. From **Table 2**, the amino acids and saturated heterocyclic amine nitrogen contents (−285 to −350 ppm) in burley are much higher than those of bright and oriental tobacco leaves in agreement with Lowe and Sheen's results (*35*).

Figure 2 shows the DD spectra of burley tobacco leaves at dephasing times of 2, 42, and 140 μs. During the dephasing period, the nitrogens that are strongly coupled to protons (e.g., protonated nitrogen) exhibit reduced signal intensity much more rapidly than those weakly coupled to protons (e.g., nonprotonated nitrogen and significant segmental motion or rapid rotation of nitrogen functional groups). The signal intensities at −260 ppm (*36*) and −312 ppm reduced quickly during the dephasing

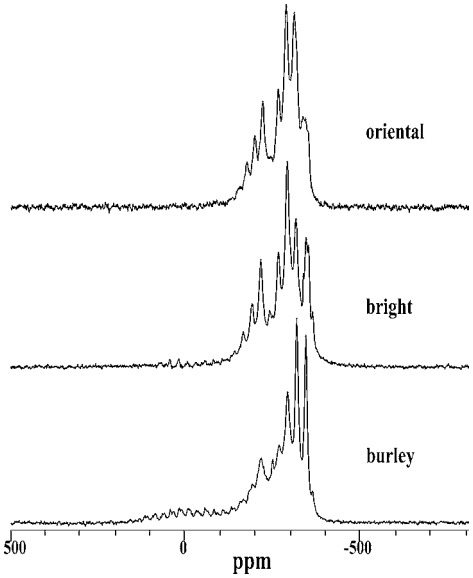

Figure 3. The first increment of the 2D FIREMAT experiment of three types of tobacco leaves.

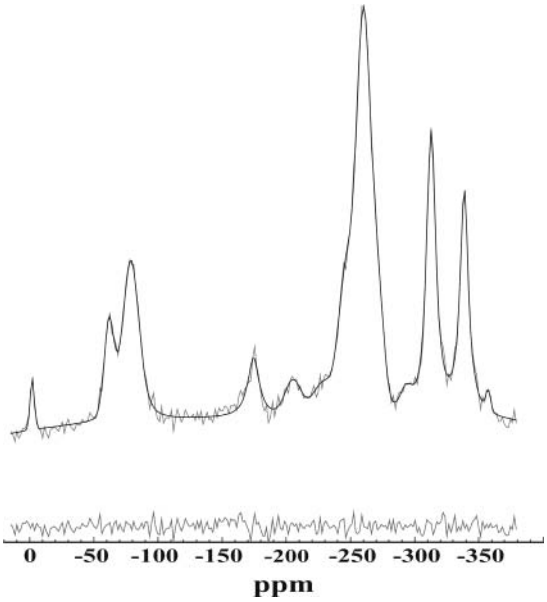

Figure 4. The guide spectrum (containing isotropic chemical shift information only) of burley tobacco leaves. The bottom trace is the residual between the experiment data (light line with noise) and the simulated spectrum (dark line).

period from 2 μs to 42 μs, and almost totally disappeared at 140 μs. These data indicate that these two signals are from protonated nitrogens. For the signals at −2, −62, −77, −175, −244, −338, and −356 ppm, the peak intensities do not reduce dramatically even at the long dephasing time (140 μs); therefore, these peaks belong to nonprotonated nitrogens or nitrogen groups undergoing rapid motion.

To better understand the nitrogen chemical composition in tobacco leaves, 2D FIREMAT experiments were carried out to extract ^{15}N chemical shift tensors of different nitrogen groups. **Figure 3** shows the first increment of the 2D FIREMAT experiment for the three types of tobacco leaves. The guide spectrum of burley tobacco leaves is shown in **Figure 4**. Only isotropic chemical shifts appear in the guide spectrum, and thirteen lines were used for the best simulation. 2D data set analysis was performed to extract the chemical shift tensor

Table 3. ^{15}N Chemical Shift Principal Values of Nitrogen Groups in Burley Tobacco Leaves[a]

N group no.	δ_{iso}[b]	δ_{11}	δ_{22}	δ_{33}	δ_{span}[b]
1	–2.2	82.9	35.2	–124.7	207.6
2	–61.8	207.1	4.6	–397.2	604.3
3	–79.2	158.9	4.2	–400.8	559.7
4	–173.7	–18.6	–137.7	–364.7	346.1
5	–203.0	–82.7	–201.5	–324.7	242.0
6	–240.9	–132.8	–249.3	–340.7	207.9
7	–243.7	–205.4	–242.0	–283.8	78.4
8	–261.5	–155.2	–295.4	–333.8	178.6
9	–267.1	–152.5	–322.6	–326.3	173.8
10	–296.8	–212.1	–339.1	–339.1	127.0
11	–312.7	–272.0	–324.8	–341.3	69.3
12	–338.2	–326.6	–326.7	–361.4	34.8
13	–357.0	–357.0	–357.0	–357.0	0.0

[a] Values are in ppm. [b] $\delta_{iso} = 1/3(\delta_{11} + \delta_{22} + \delta_{33})$, $\delta_{span} = \delta_{11} - \delta_{33}$.

values. The isotropic shifts, principal values, and chemical shift span of nitrogen groups in burley tobacco leaves are given in **Table 3**. These data, in combination with the CPMAS/DD experiment results, provide additional information in identifying nitrogen functional groups. The resonance at −2.2 ppm is assigned to nitrate ion (*25*). The peak at −62 ppm is the pyridine nitrogen in compounds such as nicotine (*22*, *37*) and has a chemical shift span greater than 600 ppm. The signal at −79 ppm can be assigned to hydrogen-bonded pyridinic structures (*12*). The resonance at −174 ppm is nonprotonated nitrogen and can be assigned to substituted pyridinium species (e.g., R−N-substituents) based on its nitrogen chemical shift tensor (*22*). From the nitrogen isotropic and principal values, the δ_{iso} = −203 ppm signal is probably the −NH group in imidazole (*22*). The resonance signal at −240 ppm may be assigned to pyrrolic type functional groups. Some differences in the nitrogen shifts between tobacco leaves and pure compound are possible due to the complex chemical environment in biomaterials. The nonprotonated nitrogen signal at −244 ppm is probably from the amido group in substituted lactams (*12*). Protonated nitrogen resonances in the range of −254 to −267 ppm can be assigned to −C(O)NH− groups in peptides based on their isotropic shift and nitrogen chemical shift tensors (*25*). A small peak at −297 ppm is probably from the arginine δ-NH group (*33*); ω-, ω'-nitrogens in arginine or nitrogen resonances in aniline and substituted aniline compounds (*12*) may appear at −312 ppm. This signal disappeared in the dipolar dephasing experiment because of the strong coupling in the protonated nitrogen group. The resonance signal at −338 ppm can be assigned to the pyrrolidine nitrogen in nicotine (*37*). The nitrogen peak at −357 ppm is assigned to ammonium ion in ammonium nitrate (*12*) because it has no chemical shift anisotropy, due to the symmetric tetrahedral structure of NH_4^+.

The dipolar dephasing spectrum of the bright tobacco leaves is shown in **Figure 5**. The resonance signals centered at −2.5, −208, and −357 ppm are from nonprotonated nitrogens or nitrogen groups undergoing rapid motion (e.g., ammonium ion group). The intensity of the big broad peak in the −220 to −285 ppm range reduced quickly with increasing dephasing time indicating that most resonances in this spectral region are protonated nitrogens. The remaining resonances are probably from a small amount of weakly coupled nitrogens (e.g., nonprotonated or mobile nitrogen groups) or strongly coupled nitrogen (protonated) groups that have not totally decayed during the dephasing time. The 2D FIREMAT experiment was performed to obtain the nitrogen chemical shift tensors. The first increment of the 2D data set is shown in the middle trace

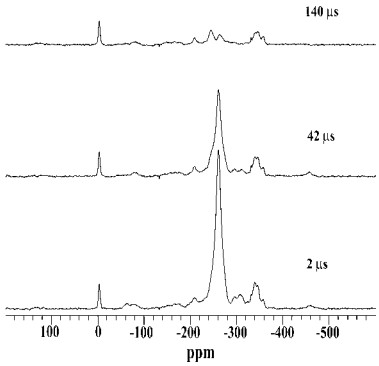

Figure 5. ^{15}N dipolar dephasing spectra of bright tobacco leaves at various dephasing times.

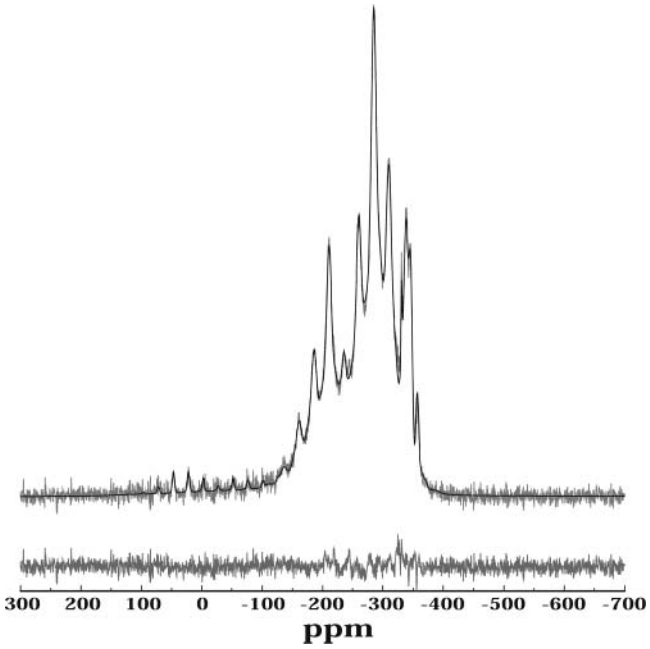

Figure 6. The first increment of the 2D FIREMAT experiment and simulated spectra of bright tobacco leaves. The bottom trace is the residual between the experimental spectrum (light line with noise) and the simulated spectrum (dark line).

in **Figure 3**, which is quite different from the spectrum in the lower trace in **Figure 3**. **Figure 6** is the 2D FIREMAT data fitting for bright tobacco leaves. Fifteen peaks were used to obtain the best statistical fit of the data. The nitrogen isotropic shifts, principal values, and spans of different nitrogen groups in bright leaves are listed in **Table 4**. From **Figure 5** and the data in **Table 4**, the signals centered at −2.7 and −357 ppm can be identified as nitrate and ammonium ion, respectively. While the nitrogen source used for growing the N-15 labeled plants is not known, these data strongly suggest that the material was uniformly labeled ammonium nitrate. In this sample, the alkaloid content is very small. A very weak signal at about −76 ppm can be assigned to hydrogen-bonded pyridinic compounds. The small peaks in the range of −140 to −208 ppm are probably from π- and τ-nitrogens in histidine and its isomers or pyrrollic nitrogen (*12*). Most nitrogen resonances in the large peak (−220 to −285 ppm) are protonated nitrogen from −CONH group in peptides or amides (*12*, *25*), and the signal decreased quickly in the dipolar dephasing experiment. The signal at −295 ppm is probably from the δ-nitrogen in arginine, and the ω− and ω'−nitrogens in arginine appear at −308 ppm (*12*, *38*). Free amino acids and saturated heterocyclic amine resonance signals

Table 4. ^{15}N Chemical Shift Principal Values of Nitrogen Groups in Bright Tobacco Leaves[a]

N group no.	δ_{iso}[b]	δ_{11}	δ_{22}	δ_{33}	δ_{span}[b]
1	−2.7	79.4	41.4	−129.0	208.4
2	−75.7	166.0	−6.3	−386.9	552.9
3	−149.6	−11.5	−152.9	−284.6	273.1
4	−175.0	80.2	−236.3	−369.1	449.3
5	−207.5	−120.0	−201.9	−300.7	180.7
6	−237.2	−152.4	−278.8	−280.5	128.1
7	−254.7	−150.0	−289.2	−324.9	174.9
8	−260.3	−154.9	−304.1	−321.7	166.8
9	−267.1	−163.7	−302.5	−335.1	171.4
10	−294.5	−223.6	−327.7	−332.3	108.7
11	−308.4	−249.1	−337.1	−338.9	89.8
12	−330.7	−310.3	−324.8	−356.9	46.6
13	−338.5	−321.8	−334.5	−359.3	37.5
14	−346.0	−332.9	−342.9	−362.3	29.4
15	−356.7	−356.6	−356.6	−357.0	0.4

[a] Values are in ppm. [b] $\delta_{iso} = 1/3(\delta_{11} + \delta_{22} + \delta_{33})$, $\delta_{span} = \delta_{11} - \delta_{33}$.

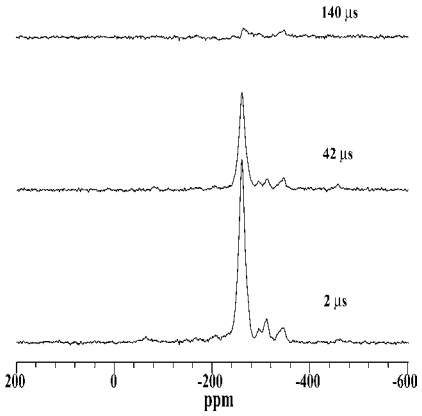

Figure 7. ^{15}N dipolar dephasing spectra of oriental tobacco leaves at various dephasing times.

fall into the −330 and −350 ppm range. The signal intensity reduced slowly in the dipolar dephasing experiment which may be due to some motion of $-NH_3^+$ groups.

Figure 7 is the dipolar dephasing spectra of oriental tobacco leaves at dephasing times of 2, 42, and 140 μs. The nitrogen resonance signals in this sample essentially decayed at 140 μs, indicating that most of the nitrogen groups in the leaves of this tobacco are composed primarily of protonated nitrogens. Compared with the burley and bright tobacco leaves, no ammonium nitrate was found in oriental tobacco leaves. The pyridine signal in alkaloids was very weak and almost indiscernible. Considering the sensitivity of most nitrogen resonances in the oriental leaves, a contact time of 0.8 ms was used in the dipolar dephasing and 2D FIREMAT experiments. This value may slightly reduce the pyridine signal in the oriental sample, but it is beneficial for the observation of most other nitrogen resonances. The first increment of the 2D FIREMAT spectra is shown in the top trace in **Figure 3**. **Table 5** lists the nitrogen isotropic shifts, principal values and chemical shift spans of nitrogen groups in oriental tobacco leaves. A small resonance signal at −205 ppm (see **Figure 1**) can be assigned to the −NH group in imidazole (*22*). The big strong peak including resonances from −250 to −270 ppm includes contributions from the −CONH− groups in peptides or amides (*25*); the isotropic shifts and nitrogen chemical shift tensors are characteristics of these types of nitrogen functional groups. Two resonance signals at about −298 ppm and −311 ppm are assigned to δ-nitrogen, and ω- and ω'-nitrogens in arginine, respectively. Most signals

Table 5. ^{15}N Chemical Shift Principal Values of Nitrogen Groups in Oriental Tobacco Leaves[a]

N group no.	δ_{iso}[b]	δ_{11}	δ_{22}	δ_{33}	δ_{span}[b]
1	−205.3	−83.1	−206.5	−326.2	243.1
2	−252.8	−147.0	−283.3	−328.0	181.0
3	−260.1	−153.3	−301.8	−325.3	172.0
4	−267.5	−163.2	−304.3	−334.9	171.7
5	−298.0	−230.6	−331.1	−332.3	101.7
6	−311.5	−269.6	−321.4	−343.6	74.0
7	−339.7	−325.4	−327.8	−365.9	40.5
8	−345.7	−344.3	−346.1	−346.8	2.5

[a] Values are in ppm. [b] $\delta_{iso} = 1/3(\delta_{11} + \delta_{22} + \delta_{33})$, $\delta_{span} = \delta_{11} - \delta_{33}$.

between −330 and −350 ppm are probably from free amino acids and some amines. However, for the signal at −346 ppm, it is also possible that the ammonium ion in ammonium chloride (*12*, *25*) appears in this area because it has equivalent principal values (within experimental error) due to the symmetric structure of NH_4^+.

From the chemical shift span values ($\delta_{span} = \delta_{11} - \delta_{33}$) in three types of tobacco leaves (**Tables 3**, **4**, and **5**), the pyridine related compounds have the largest chemical shift span (more than 500 ppm) and this unusual property has been explained by Solum et al. (*22*) The ammonium ion has the smallest span (essentially zero) because of its symmetric structure. The chemical shift span of nitrate is around 200 ppm, and the span of amide groups in peptides ranges from 160 to 180 ppm. The chemical shift tensor principal values of the same type of nitrogen group may fluctuate a bit with the small differences in the structural environment. However, the characteristics are similar for the same kind of nitrogen group. Therefore, different nitrogen groups can be identified by a combination of isotropic shifts and chemical shift tensor principal values.

The tobacco plant is a very complicated material that includes thousands of chemical compounds; among these, more than 600 are nitrogen-containing compounds (*2*). It is difficult to make a total plant analysis by solid-state NMR methods because of insufficient spectral resolution. Therefore, some of the spectral assignments to the tobacco leaves are probably not unique due to the overlap of the spectra. However, because the chemical shift tensors are very sensitive to the electronic/structural environment, they are very useful as a guide for spectral assignment. Some resonance signals (e.g., ammonium nitrate, pyridine related compounds, etc.) can be clearly identified by comparing the ^{15}N chemical shift tensors with the principal values obtained from model compounds. Unfortunately, only a limited amount of nitrogen chemical shift tensors are available due to the difficulties associated with observation of the nitrogen signal at natural abundance and the normally long T_1 values in nitrogen compounds.

In the present work, three kinds of tobacco leaves were investigated using solid-state ^{15}N NMR methods. This is the first time these methods have been applied to tobacco material. The results from ^{15}N CPMAS experiments indicate peptide and amide resonances (−220 to −285 ppm) are the dominating signals in three types of tobacco leaves. Burley tobacco leaves contain more alkaloids (primarily nicotine) than that of bright and oriental leaves. In combining the dipolar dephasing experiment with 2D FIREMAT data, possible spectral assignments were made based on the nitrogen chemical shift tensors of model compounds and isotropic shifts in liquid NMR data taken from the literature. Hence, the data presented in this paper offer a glimpse of the presence of the major nitrogen functional groups and their variations in these tobacco leaves. These data will

form a basis for interpreting the changes produced by pyrolysis reactions in future studies of tobacco chars and tars.

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(36) Some of the chemical shift values used in the text are slightly different from the isotropic shift values given in **Tables 3**−**5**. Isotropic chemical shifts and shift ranges obtained from 1D CPMAS spectra are used in the text, whereas the isotropic shift values in the tables are obtained from fitting the 2D FIREMAT data. The values of δ_{iso} obtained from the 2D data are the average of the three principal values of the chemical shift tensor. The experimental error in each of these principal values is of the order of 1 ppm. Hence, the averages of the three principal values

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