2 mm i.d. glass column packed with 3% OV-101 on 80/100-mesh Supelcoport (Supelco Co.). The air, hydrogen, the high-dose treatment. A dose-related difference showed the majority of the urinary metabolites were recovered as conjugated products under the high-dose treatment.

A significant sex-related metabolic difference was observed under the low-dose treatment. A majority of the urinary metabolites from the female rats were conjugated products, 30% of the administered dose, compared to the 5% observed in the male rats. A similar sex-related difference was also observed in the whole blood residue/time plot, which showed the contribution of biliary recirculation in the disposition of cinmethylin in the female test animals.

ACKNOWLEDGMENT

LITERATURE CITED

Gaughan, L. C.; Unai, T.; Casida, J. E. *J. Agric. Food Chem.* **1977**, 25, 9.
Leffingwell, J. C. Fr. Demande 2 003 498, 1970.
Ohkawa, H.; Kaneka, H.; Tsuji, H.; Miyamoto, J. *J. Pestic. Sci.* **1979**, 4, 143.
Ohloff, G.; Uhde, G. *Helv. Chim. Acta* **1965**, 48, 10.
Peterson, L. W.; Woodward, M. D.; Foster, J. P.; Lee, P. W.; Goss, J. R., Shell Chemical Co., Technical Information, 1983.
Southwell, I. A.; Flynn, T. M. *Xenobiotica* **1980**, 10, 17.
Stearns, S. M.; Lee, P. W. *Lab. Anim. Sci.* **1984**, 34, 395.
Walde, A.; Ve, B.; Scheline, R. R. *Xenobiotica* **1983**, 13, 503.

Fate of [^{15}N]Glycine in Peat as Determined by ^{13}C and ^{15}N CP-MAS NMR Spectroscopy

Laure M. Benzing-Purdie,* Martin V. Cheshire, Berwyn L. Williams, Graham P. Sparling, Christopher I. Ratcliffe, and John A. Ripmeester

Peat samples, nonsterile, sterilized by γ irradiation or autoclaving, were incubated with [^{15}N]glycine for a period of 6 months. The ^{13}C NMR data showed the established trend of increased humification with decreasing particle size and that autoclaving had significantly disturbed the humification–particle size distribution. The ^{15}N CP-MAS NMR spectra showed the presence of [^{15}N]glycine in all fractions after incubation. ^{15}NH$_4^+$, a result of either biological or chemical deamination, was one of the main products in the nonsterile peat series. The ^{15}N spectra also showed resonances corresponding to amine, secondary amide, and pyrrole-type nitrogen and the presence of glycine derivatives and melanoidins. The results presented give the first spectroscopic evidence of the possible involvement of the Maillard reaction in the humification process.

INTRODUCTION

Recent studies (Benzing-Purdie and Ripmeester, 1983; Benzing-Purdie et al., 1983) based on ^{13}C and ^{15}N CP-MAS NMR have shown that natural humic substances resemble very closely melanoidins, the brown, high molecular weight nitrogenous polymers formed upon reaction of carbohydrates and amino acids. These studies lent further support to Maillard's original hypothesis; that this reaction may be at the origin of humic substances in soils (Maillard, 1916).

The objective of the present study is to determine the extent to which this reaction occurs in highly organic peat soils. Natural humic substances take a very long time to form and normally only a very small proportion of any substrate remains after attack by soil organisms. Testing

Chemistry & Biology Research Institute, Agriculture Canada, Ottawa, Canada K1A 0C6 (L.M.B.-P.), Macaulay Institute for Soil Research, Aberdeen, Scotland (M.V.C., B.L.W., G.P.S.), and Division of Chemistry, National Research Council, Ottawa, Canada K1A 0R6 (C.I.R., J.A.R.).

Maillard's hypothesis in a soil environment within a manageable time span therefore requires sterile soil conditions. Unfortunately, all methods of sterilization lead to physical and chemical alterations in the soil (Ramsay and Bawden, 1983). After any sterilization treatment, be it chemical, steam sterilization or γ irradiation, the treated soil will differ from the original. Autoclaving of peat not only damages the physical microenvironment but, due to a combination of high temperature and low pH, results in hydrolysis of organic polymers, particularly polysaccharides, liberating the more labile sugars, e.g. pentoses, which are very reactive in the Maillard reaction. Its one advantage is that it completely destroys enzymatic activity. γ irradiation has been reported to cause the minimum disturbance to the system (Ramsay and Bawden, 1983), although it has been shown to release some nutrients (Brown, 1981; Lynch, 1982; Powlson and Jenkinson, 1976) and certain metabolic activities linked to dead cells and residual extracellular enzymes may still be present. Advantage was taken of the known reactivity of glycine in the Maillard reaction and the data available on the different forms of C and N produced in the reaction of this amino acid with carbohydrates (Benzing-Purdie and Ripmeester,

[¹⁵N]Glycine in Peat

J. Agric. Food Chem., Vol. 34, No. 2, 1986   **171**

1983; Benzing-Purdie et al., 1983) to study in an organic soil the formation of Maillard reaction products from this amino acid. Nonsterile peat and peat samples sterilized by γ irradiation or autoclaving were incubated with [¹⁵N]glycine.

The specific aims of the present investigation were (1) to determine the changes in chemical structures as seen by ¹³C CP-MAS NMR, in different particle size fractions of a control γ irradiated peat sample (series C), an irradiated peat sample incubated in the presence of [¹⁵N]-glycine (series I), an autoclaved peat sample incubated with [¹⁵N]glycine (series A), and a nonsterile peat sample incubated with [¹⁵N]glycine (series P); (2) to determine the nature of the different forms of nitrogen originating from ¹⁵N glycine in these fractions by ¹⁵N CP-MAS NMR; and (3) to assess the potential contribution of the Maillard reaction to the humification process. It is known that particle size fractions of peat differ in their degree of humification and chemical composition (Williams, 1983). Fractionation by particle size should therefore enable the behavior of the more humified parts of the peat to be studied and allow much greater variety of metabolic processes and products to be distinguished.

EXPERIMENTAL SECTION

Chemicals. [¹⁵N]Glycine (99% ¹⁵N) was purchased from Stohler Isotope Chemicals, Waltham, MA 02154.

Soil. A surface blanket peat bog was sampled from Lon Mor, Inchnacardoch, Highland Region, Scotland. It contained 90–95% water (w/w) of the fresh weight, N, 2.14% of dry matter; C/N = 25 with an ash content of 4% of the dry matter. The pH was 3.5 in 0.01 M $CaCl_2$ (2.5:1 liquid/solid w/w). Vegetation at the sampling site included *Trichophorum caespitosum, Molinia caerula, Calluna, Sphagnum spp.,* and *Myrica gale.*

Soil Sterilization. Lots (15 g) of fresh Lon Mor peat were sterilized by 5 Mrd γ irradiation from a cobalt-60 source (Ethicon Ltd., Edinburgh, UK) or autoclaved at 121 °C for 30 min.

Soil Incubations. [¹⁵N]Glycine (0.45 g) was dissolved in H_2O (15 mL) and sterile filtered through a 0.3-μm Millipore membrane. Portions (5 mL) of this solution were added aseptically to 15-g each of γ-irradiated peat, autoclaved peat, and control peat and the mixtures incubated at 15 °C in the dark for a period of 6 months. The sterility of the γ-irradiated peat and autoclaved peat was checked before and after 6-months incubation by plating onto nutrient agar, PDA agar, and soil extract agar.

Soil Fractionations. These were done by wet sieving as previously reported (Williams, 1983), except that a mechanical sieve shaker was used to separate the fractions smaller than 1 mm. Peat sample (15 g) was dispersed in 150 mL of distilled water either in the presence of $CHCl_3$ or at low temperatures to suppress microbial activity. After shaking, the suspension was passed through a series of seven stainless-steel sieves (British standard 410) of mesh sizes 1, 0.5, 0.25, 0.15, and 0.05 mm, and the residues on each filter were freeze-dried, giving fractions 1–5. The dark suspension containing material <0.05 mm was filtered on sintered-glass funnels (0.005–0.01-mm-diameter pores), and the residue on the filter (fraction 6) and filtrate (fraction S) was freeze-dried.

Cross-Polarization-Magic Angle Spinning (CP-MAS)¹³C NMR. The spectra were obtained at 45.28 MHz, on a Bruker CXP-180 pulsed spectrometer with cross-polarization and magic angle spinning, as previously reported (Benzing-Purdie and Ripmeester, 1983). The cross-polarization time was 1 ms, which was chosen as it gave maximum signal-to-noise. There is probably a slight underestimate of any quaternary and carbonyl carbon intensity. For cross-polarization times greater than ~3 ms, some of the carbon lines had decreased significantly in amplitude. Rf field amplitudes of 42 kHz and magic angle spinning at ≃3.4 kHz in Kel-F rotors of the Andrew type were used with a sweep width setting of 20 kHz. From 2000 up to 30 000, 500 point free induction decays were accumulated and zero-filled to 4K before Fourier transformation. At the spinning rate used, spinning side bands of aromatic and carbonyl carbon resonances occur 75 ppm either side of the main peaks. With the aid of a 40-μs acquisition delay, which suppresses the resonances of carbons strongly coupled to protons, it was possible to simplify the spectrum and assign, without dubiety, most of the spectral features.

The sometimes large variations in the quantities of material in the different size fractions meant that some samples, in particular the soluble fractions, were very small. Consequently, it was not feasible, in both ¹³C and ¹⁵N NMR experiments, to obtain all spectra with identical noise levels within a reasonable time period.

The ¹H relaxation times in the static and rotating frame $(T_1, T_{1\rho})$ were measured for several of the peat samples. Although the relaxation behavior was complex, requiring at least two time constants to characterize the magnetization decays, no great differences between different samples were noted. T_1's were generally less than 100 ms; $T_{1\rho}$'s, several milliseconds. The different types of treatments of the peat are not expected to change these values significantly.

Cross-Polarization-Magic Angle Spinning (CP-MAS) ¹⁵N NMR. The spectra were obtained at 18.25 MHz on the same instrument. A single cross-polarization contact of 1-ms duration was used with matched rf field amplitudes of 28 kHz. Up to 30 000 500 point-free induction decays were coadded at a sweep width setting of 20 kHz. Zero filling to 4K was used before Fourier transformation.

Delrin spinners of the Andrew type were used with spinning speeds of 3.5 kHz. Chemical shifts are referenced to external ammonium nitrate. Assignments were made by comparison with published ¹⁵N NMR data (Levy and Lichter, 1979).

Binding of [¹⁵N]Glycine to γ-Irradiated and Autoclaved Peat. A 30-mg portion of [¹⁵N]glycine in 1.5 mL of distilled water was added to 300 mg each of irradiated and autoclaved Lon Mor peat under sterile conditions. After thoroughly mixing with a glass rod, the mixtures were immediately freeze-dried for ¹⁵N CP-MAS NMR. In a separate experiment, the mixtures of [¹⁵N]glycine with γ-irradiated peat and autoclaved peat were kept at room temperature under aseptic conditions for 1 day and then freeze-dried prior to ¹⁵N CP-MAS NMR analysis.

KCl Treatment. Fraction P_1 (0.28 g) was suspended in 1 M KCl (10 mL) and kept at 70 °C with gentle stirring. After 2.5 h, the suspension was centrifuged, the supernatant discarded, and the procedure repeated a second time. The KCl-treated fraction recovered by centrifugation was washed until the washings were free of chlorine, freeze-dried, and analyzed by ¹⁵N NMR. Sterile conditions were used throughout the experiment.

In the above two experiments, all solutions were sterile filtered through 0.3-μm Millipore membranes. The sterility of the peat was checked by plating onto nutrient, PDA, and soil extract agar.
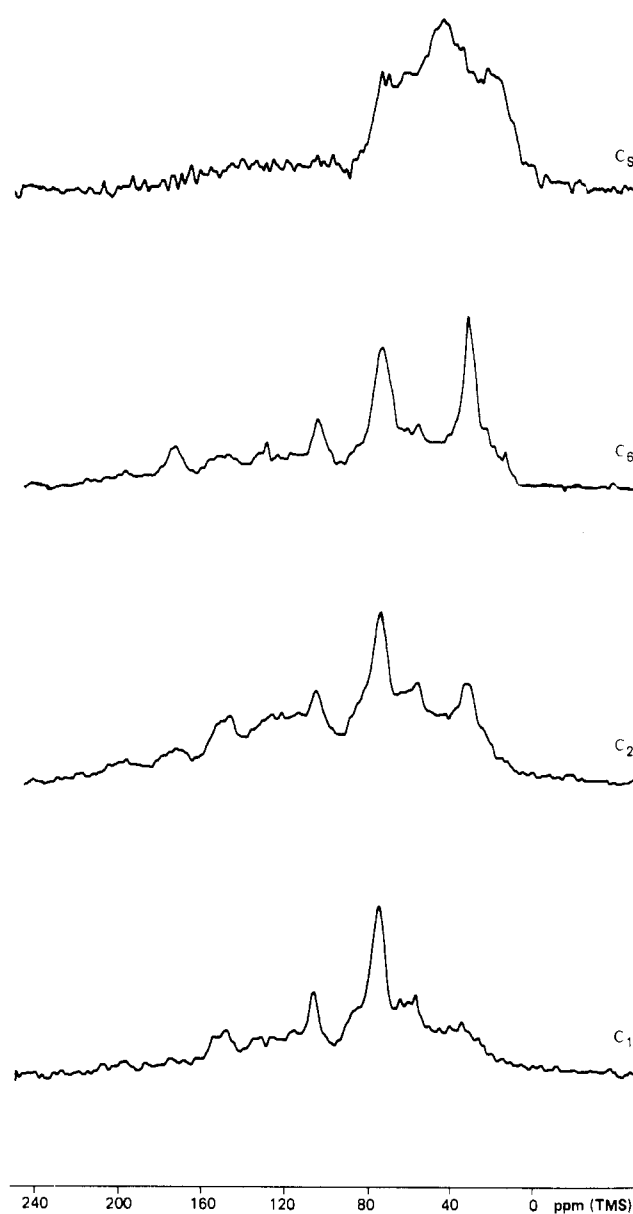
RESULTS AND DISCUSSION

Wet sieving of peat samples incubated for 6 months gave the fractions listed in Table I. The distribution of weight

Table I. Distribution (Percent) of Dry Matter between Particle Size and Water-Soluble (S) Fractions of Incubated Peat

peat treatment		1 (>1)	2 (1–0.5)	3 (0.5–0.25)	4 (0.25–0.15)	5 (0.15–0.05)	6 (0.05–0.005)	S
		fraction no. (size, mm)						
untreated + Gly	P	33	9	13	9	13	16	6
irrad	C	6	14	18	11	20	29	3
irrad + Gly	I	5	4	12	3	27	40	8
autoclaved + Gly	A	4	10	19	12	23	21	12
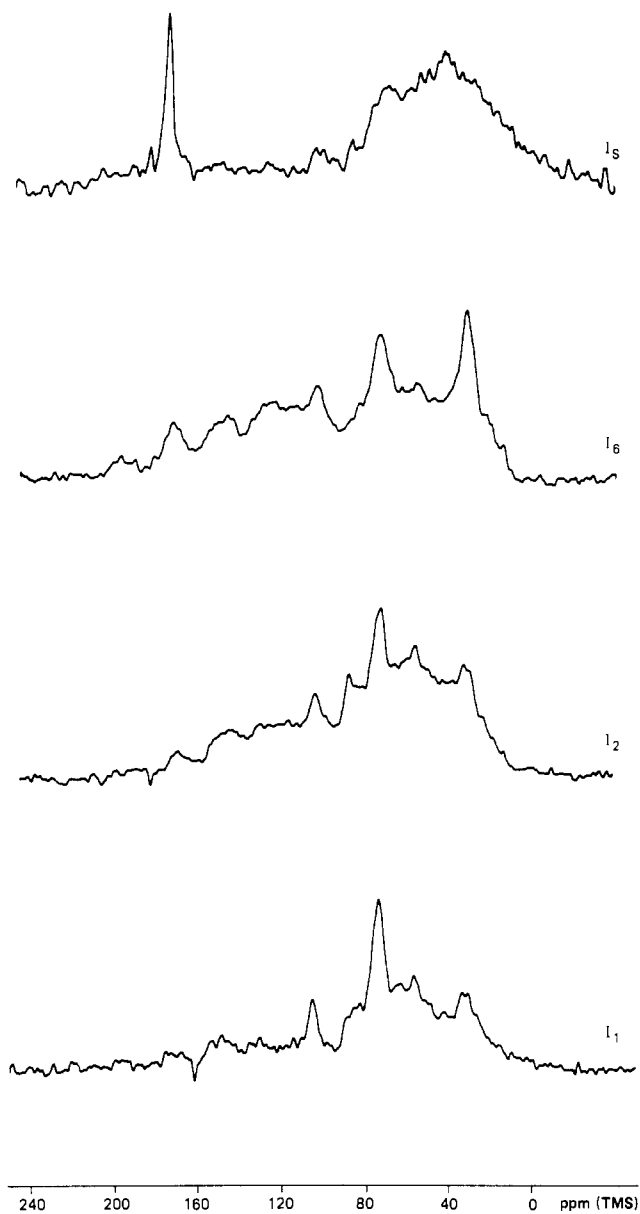
Figure 1. ^{13}C CP-MAS NMR spectra of fractions I_1, I_2, I_6, and I_S (γ-irradiated peat with [^{15}N]glycine).

Figure 2. ^{13}C CP-MAS NMR spectra of fractions C_1, C_2, C_6, and C_S (control γ-irradiated peat).

between fractions was quite variable because of peat heterogeneity, although the sums of the weights of all fractions recovered from each peat incubation experiment were within 5% of each other. Nevertheless, the weights of fractions 1 in the irradiated series C and I and the autoclaved series A were lower than those of the same fractions in the nonsterile series P, while the weights of fractions 5 and 6 in I, C, and A were well above average. This suggests that both γ irradiation and autoclaving lead to a breakdown of organic matter into smaller particle size fractions. In the case of γ irradiation, this may be caused perhaps by the reaction of radiolysis products of water, $\dot{O}H$, $\dot{H}$, and $H\dot{O}_2$. The large amount of water in the Lon Mor peat favors formation of these free radicals. These results are consistent with an earlier report, indicating that

γ irradiation disrupts the organic material in a soil (Eno and Popenoe, 1964). When polysaccharides are γ irradiated in solution, extensive degradation is observed, resulting in the formation of reducing substances, i.e. sugars. In the solid state, the major chemical changes are chain cleavage and formation of reducing and acid groups (Phillips, 1980). γ irradiation of proteins causes the cleavage of peptide bonds, and with lipids, ester linkages are broken with the formation of acids (Swallow, 1973).

In the present study it was found that autoclaving or irradiating of 15 g of fresh peat released sugars with reducing power equivalent to 2.1 mg of glucose whereas the free sugars in the untreated peat amounted to only 0.3 mg.

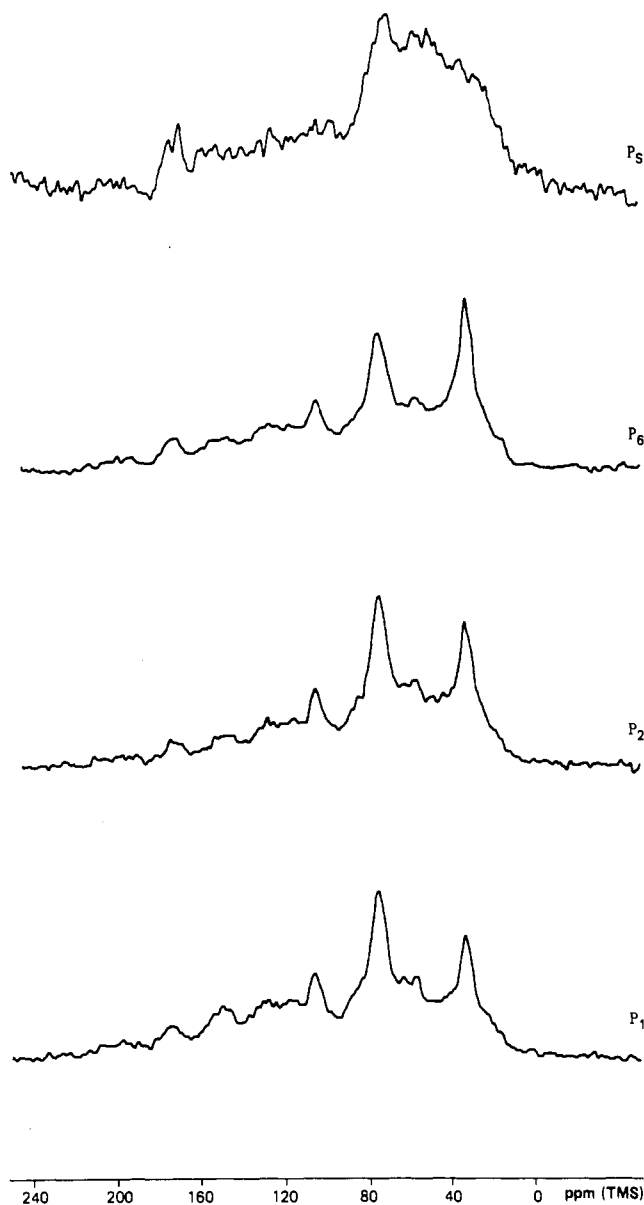^{13}**C CP-MAS NMR Spectra.** Figures 1–4 show the ^{13}C CP-MAS spectra of particle size fractions, 1, 2, 6, and S,

Figure 3. ^{13}C CP-MAS NMR spectra of fractions P_1, P_2, P_6, and P_S (control peat with [^{15}N]glycine).

Figure 4. ^{13}C CP-MAS NMR spectra of fractions A_1, A_2, A_6, and A_S (autoclaved peat with [^{15}N]glycine).

Table II. Relative Integrated Intensity of ^{13}C NMR Spectral Regions Expressed as a Percentage of Total Intensity

sample	size fraction	% areas ^{13}C, ranges in ppm			
		>160	160–110	110–58	58–0
C	6	10.7	17.5	36.5	35.3
I	6	10.3	24.3	33.7	31.7
A	6	5.2	6.6	39.2	49.0
P	6	8.5	19.5	37.0	35.0
C	2	10.0	24.3	37.6	28.1
I	2	4.3	19.7	42.2	33.8
A	2	7.1	10.3	43.1	39.5
P	2	6.5	17.1	42.2	34.2
C	1	6.9	21.5	46.6	25.0
I	1	5.5	15.5	44.3	34.7
A	1	5.4	10.1	49.2	35.3
P	1	9.8	22.9	39.9	27.4

of the various incubated mixtures. Several major resonances can be distinguished in the spectra: 30, 55, 72, 105, 155, 175, and 200 ppm. In an attempt to quantify the spectra to some extent, for purposes of comparison, each spectrum was divided into four regions that were then integrated and expressed as a percentage of the total in-
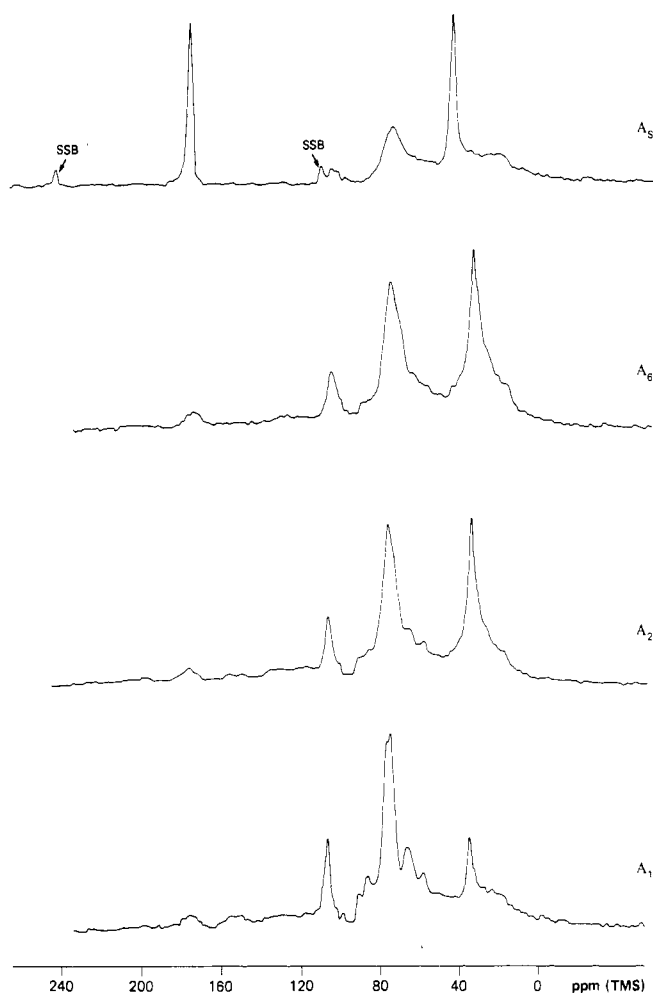
tensity of the spectrum; values are given in Table II. The four regions were defined by making vertical cuts at 160, 110, and 58 ppm, and it should be pointed out that this does not take account of slight overlaps of bands from one region to the next. Admittedly this is crude, but it does permit comparison of relative intensities.

One feature that stands out immediately from the spectra and the relative integrals is that the A series is quite different from the I, C, and P series, which are superficially quite similar. The solid fractions of I, C, and P all show the established trend of increased humification with decreasing particle size (Bracewell et al., 1980; Morita and Levesque, 1980). There is an increase in the 30-ppm resonance on passing from the large particle size fractions to the smaller ones. Conversely, fraction 6 tends to have smaller 72- and 105-ppm resonances than fractions 1 and 2. In other words, there is an increase in aliphatic carbons and a decrease in carbohydrate content in the smaller size fractions. The small variability in the 30-ppm peak of fraction 1 between I, P, and C is not unusual and probably is due to sample heterogeneity. The small size of the I, C, and P soluble fractions made it difficult to obtain good spectra, and it is very probable that a broad probe background feature obscures most of the 0–100-ppm region. These spectra are mainly useful in showing the presence of excess glycine, or carboxyl group carbons.

From a close inspection of the spectra (Figures 1–3) and integrals (Table II) of the solid fractions of I, P, and C, it is apparent that there is an enhanced rate of humification in fraction I_6. Since the I series was irradiated and
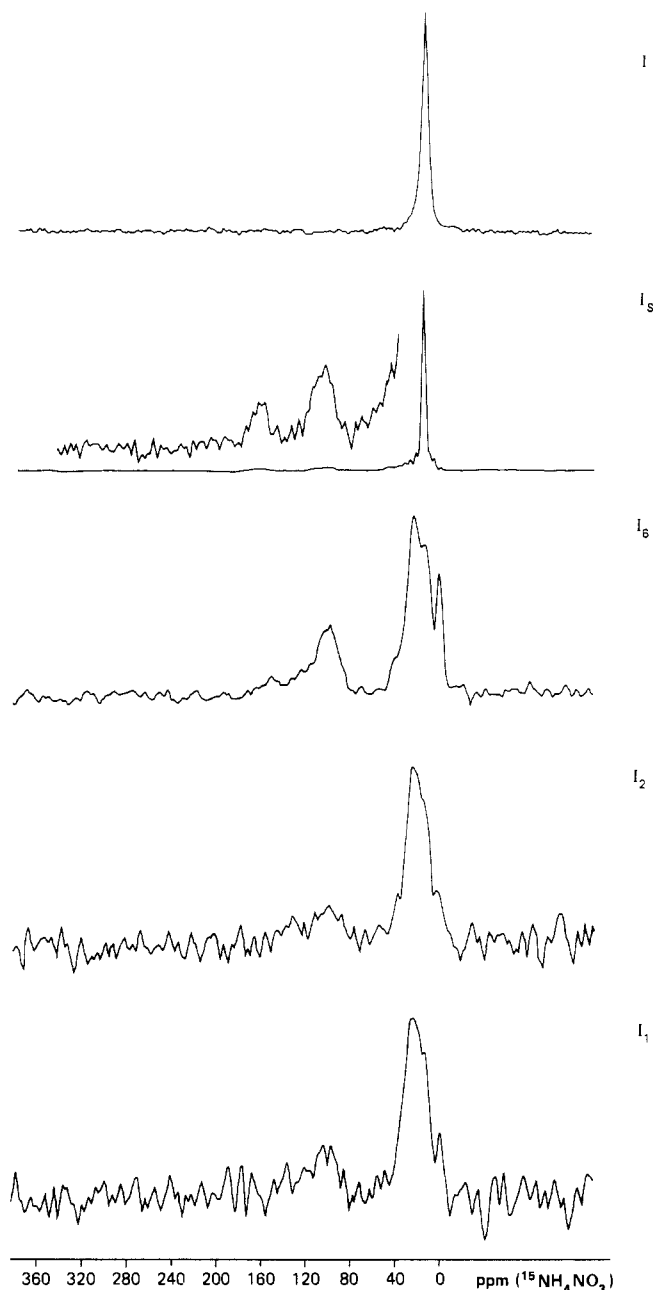
174 J. Agric. Food Chem., Vol. 34, No. 2, 1986

Benzing-Purdie et al.

Figure 5. ^{15}N CP-MAS NMR spectra of fractions I$_1$, I$_2$, I$_6$, and I$_S$ (γ-irradiated peat with [^{15}N]glycine) and I: ^{15}N CP-MAS NMR spectrum of mixture of [^{15}N]glycine and γ-irradiated peat.

Figure 6. ^{15}N CP-MAS NMR spectra of fractions P$_1$, P$_2$, P$_6$, and P$_S$ (control peat with [^{15}N]glycine).

contains glycine, this observation is not inconsistent with the formation of melanoidin polymers.

There are a number of obvious differences in the series A spectra. It is quite clear that autoclaving has significantly disturbed the humification–particle size relationship. This is also reflected in the distribution of material among the different size fractions (Table I). (It was mentioned earlier that autoclaving is known to be a fairly harsh sterilization treatment for the peat.) All solid A fractions, and especially A$_2$ and A$_6$, show much less intensity in the aromatic and phenolic chemical shift region. In the A$_1$ spectrum, which represents a small fraction of the total, the lines of carbohydrate are quite sharp, suggesting that this fraction is much more crystalline than in the I, P, and C series; perhaps only large particles are left that are more resistant to degradation. The A$_1$ spectrum in fact looks very much like that of a wood (i.e., largely cellulose). From the A$_S$ spectrum it is also apparent that a large amount of glycine has passed through the process
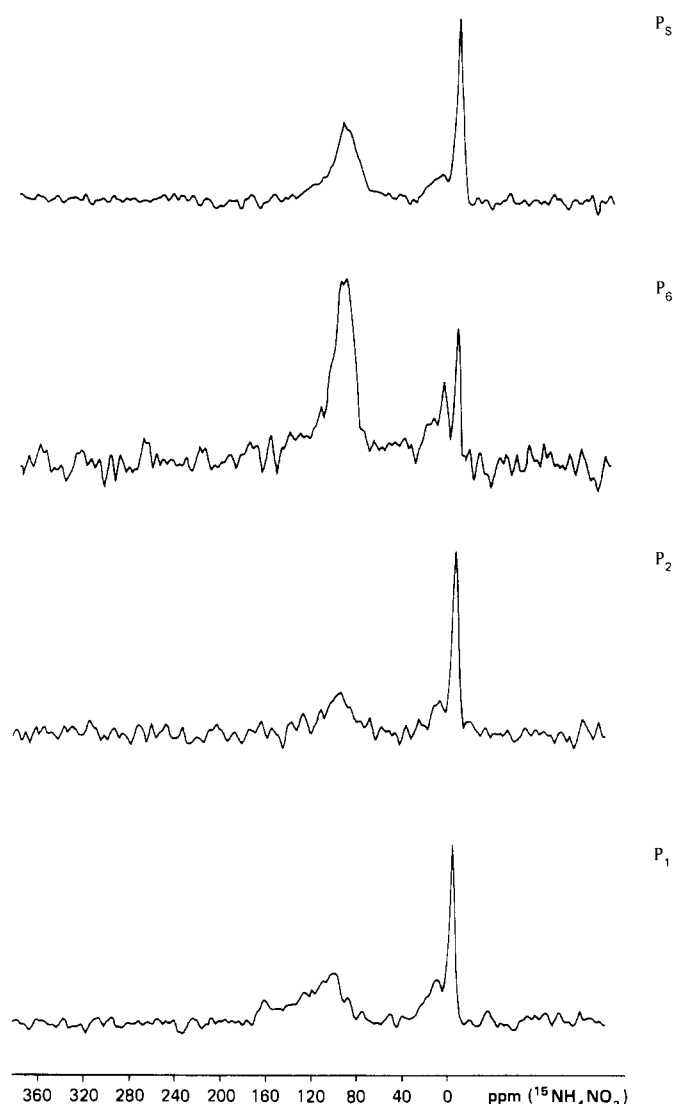
unchanged. This result is not too surprising if one considers the relative low temperature (15 °C) and short time of incubation.

^{15}N CP-MAS NMR Spectra. The ^{15}N CP-MAS spectra of the particle size fractions are shown in Figures 5–7.

The ^{15}N spectra of fractions 1, 2, 6, and S of the I series show several peaks in each fraction. I$_6$ shows a signal at 0 ppm, attributed to ^{15}NH$_4^+$. Earlier reports (Eno and Popenoe, 1964) had shown that γ irradiation of Everglades muck increases the release of extractable N and exchangable NH$_4^+$. The absence of this peak in the ^{15}N NMR spectrum of the mixture of γ-irradiated peat and ^{15}N glycine, kept under sterile conditions for 1 day, eliminates the very slight possibility that the 0 ppm peak may be due to natural-abundance ^{15}NH$_4^+$ and not ^{15}NH$_4^+$ formed as a result of [^{15}N]glycine addition. Furthermore, irradiated peat alone shows no signal here. Thus, peaks at 0 ppm seen in the ^{15}N CP-MAS spectra of all fractions may be presumed to arise from the added [^{15}N]glycine. The fact that ^{15}NH$_4^+$ appears in the solid fractions and not in the soluble suggests that this ion may be held on the exchange sites of the solid organic matter. Deamination of glycine can theoretically occur enzymatically or chemically. After a radiation dose of 5 Mrd, enzymatic activity of a waterlogged peat may be reduced considerably. It has
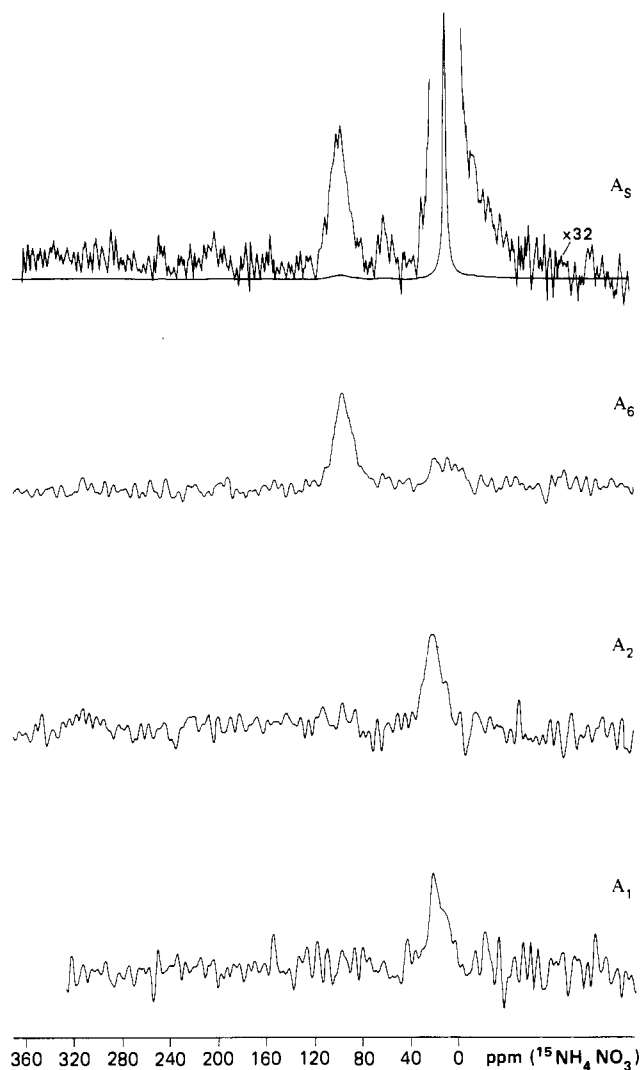
Figure 7. ¹⁵N CP-MAS NMR spectra of fractions A_1, A_2, A_6, and A_S (autoclaved peat with [¹⁵N]glycine).

been reported that enzymes in peat are more radiosensitive than those of soil because of the absence of protection afforded by adsorption on clay minerals, the nature of the humic–colloid enzyme complex, or the greater indirect damage caused by the products of radiolysis of water (Brown, 1981). However, the absence of a resonance at 0 ppm in any of the autoclaved peat + [¹⁵N]glycine fractions (series A) as seen later indicates that the $^{15}NH_4^+$ observed in the I series does result from the deamination through residual enzyme activity.

The four fractions I_1, I_2, I_6, and I_S show a resonance at 10 ppm. This signal is the predominant one in I_S and is attributable to [¹⁵N]glycine. From the fractionation study using ¹⁴C-labeled substrate (Williams and Cheshire, unpublished results) glycine would be expected in all fractions. When [¹⁵N]glycine was added to irradiated or autoclaved peat, the ¹⁵N CP-MAS spectrum of the mixture at time zero, and after 1 day under sterile conditions, showed only one signal, at the same chemical shift as free glycine (10 ppm) (Benzing-Purdie et al., 1983), though broader (I, Figure 5).

Fractions I_1, I_2, and I_6 show a major resonance at 28 ppm, which can be attributed to aliphatic amines, e.g. derivatives of glycine. Compounds having ¹⁵N resonances in the same region are found in the early stages of the Maillard reaction (Adrian, 1982). The presence of the 28 ppm peak in the autoclaved peat fraction indicates a chemical rather than an enzymatic reaction.

All I fractions show a peak at 97 ppm attributable to secondary amide N. Here again its presence in the autoclaved peat (A_6 and A_S) suggests a chemical reaction. This resonance may also be attributed to products of the Maillard reaction. When glycine and glucose react to form melanoidins, most of the N is in secondary amide form, but the reaction also shows the formation of small amounts of pyrrole type N, as seen in the ¹⁵N CP-MAS spectra of both I_6 and I_S at 155 ppm. These results support the contention that, during incubation with peat, the [¹⁵N]glycine reacted with the reducing sugars present before or produced as a result of the irradiation, to give products having N in the same form as in the Maillard reaction products.

In the P series, one of the most significant resonances in terms of intensity is the $^{15}NH_4^+$ signal at 0 ppm, resulting from deamination of [¹⁵N]glycine. This cation seems to be fixed on the organic matrix of all solid fractions. (A repeated treatment of P_1 with KCl resulted in the complete exchange of $^{15}NH_4^+$ by K^+, as evidenced by the absence of the 0 ppm signal in the ¹⁵N NMR after treatment.) Fractions P_1, P_2, and P_S show a small resonance in the [¹⁵N]glycine region (10 ppm), while P_6 has a more pronounced one. The presence of a relatively larger amount of [¹⁵N]glycine in fraction P_6 may perhaps be the result of protection from microbial or enzymatic attack conferred by humic substances. The amounts of glycine used in these incubation experiments were relatively large, which might explain its presence after 6 months. All fractions show a signal in the secondary amide N region of the NMR spectra (97 ppm), the latter resonance being the most important one in P_6 and P_S. There is also a small resonance in the aliphatic amine N region of the spectrum (28 ppm).

With the autoclaved peat, the particulate fractions show resonance peaks at 28 and 97 ppm, the major difference from the P and I series being in the relative signal to noise ratio. The spectra of A_1 and A_2 are very similar to each other, showing a resonance at 28 ppm attributable to primary amine N. Fraction A_6, on the other hand, shows a signal in the secondary amide region (97 ppm) with relatively little N in other forms. The soluble fraction A_S shows a strong signal for [¹⁵N]glycine (10 ppm), with little or no glycine present in A_1, A_2, and A_6. A broad signal in A_S at 97 ppm was attributed to secondary amide N. Pyrrole type N could not be detected.

In conclusion, the use of ¹⁵N CP-MAS NMR allowed us to determine the fate of the ¹⁵N of glycine in γ-irradiated, autoclaved, and nonsterile peat after 6-month incubation. The ¹⁵N spectra showed almost the same ¹⁵N resonances, but with different intensities in all solid peat samples. The major difference was noted in the water-soluble fractions, where, as expected, in the sterile γ-irradiated and autoclaved peat, a large portion of the original glycine could still be recovered, while little could be found in the nonsterile fraction. The latter soluble fraction, P_S, showed instead a strong resonance at 0 ppm due to $^{15}NH_4^+$. The presence of this peak in P_S may be considered an indication of nonsterility. (This was corroborated by results obtained following a 6-month incubation of autoclaved peat with [¹⁵N]glycine that did not remain sterile). Resonances, at 10, 28, and 97 ppm attributed respectively to [¹⁵N]glycine, aliphatic amines, and secondary amide linkages could be identified. In the nonsterile peat incubation experiment, the presence of aliphatic amines and secondary amide linkages could be the result of microbiological activity, extracellular enzymatic activity, or chemical reaction, whereas their presence in the sterile γ-irradiated and au-

176 *J. Agric. Food Chem.*, Vol. 34, No. 2, 1986

Benzing-Purdie et al.

toclaved peats suggests that chemical reaction such as the Maillard reaction is the most likely explantation. On the other hand, the occurrence of $^{15}NH_4^+$ in the γ-irradiated series appears to be dependent on a residual enzymatic activity.

As a general conclusion, ^{13}C CP-MAS NMR has allowed us to answer the first aim of our investigation; to determine the changes in chemical structures in different particle size fractions of the I, C, P and A series. The results obtained confirm spectroscopically the trend of increased humification with decreasing particle size and the detrimental effect of autoclaving on the humification–particle size relationship. ^{15}N NMR spectroscopy has shown that glycine added to an organic soil is not only transformed to give NH_4^+ but has also reacted to form compounds having N in the same form as in the low and high molecular weight Maillard reaction products. Such products will also contribute to the ^{13}C spectra, but it is not possible to distinguish them from the ^{13}C spectrum of the rest of the peat on the basis of the present data. Humification is a very slow process, and therefore one would expect, even under ideal conditions, the formation of only small amounts of Maillard products during a 6-month incubation period at 15 °C, making their detection difficult. Nevertheless, the reaction could become significant because of the very long periods of time involved in the humification process.

The above data were obtained with [^{15}N]glycine as the amino compound. It is to be expected that in a natural soil environment any compound bearing a free amino function (free amino acids, NH_2 groups of protein-bound amino acids, e.g. lysine) or ammonia can react with any carbonyl group to form Maillard or amino carbonyl reaction products. The discovery of early Maillard reaction products in mammalian tissue (Lamport, 1980), implies the possibility that similar products may occur in plants even before degradation takes place. This has also been suggested because of the ease with which arabinose and xylose, two hemicellulose components, react with lysine at a temperature of 40 °C (Knipfel et al., 1983). The long-term yellowing of paper (Pernemalm, 1978) has also been attributed to the Maillard reaction. In 1973, Stevenson reported several studies in agreement with the supposition that nonbiological reactions, the Maillard reaction in particular, are of considerable importance in the transformation of amino acids in soils. More recently (Mal et al., 1982; Hayes et al., 1984) it has been shown that melanoidins have many properties similar to that of humic acids. These include infrared and UV absorption and DTA and DTG characteristics as well as elemental composition. It has also been stated recently that carbohydrates readily react with amino acids to form humic substances and then probably protokerogens (Nagy, 1982). Our findings based

on peat incubation studies in the presence of [^{15}N]glycine give the first spectroscopic evidence of the possible involvement of the Maillard reaction in the humification process.

LITERATURE CITED

Adrian, J. In "Handbook of Nutritive Value of Processed Food"; Rechcigl, M., Jr., Ed.; CRC Press: Boca Raton, FL, 1982; Vol. 2, p 529.

Benzing-Purdie, L.; Ripmeester, J. A. *Soil Sci. Soc. Am. J.* **1983**, *47*, 56.

Benzing-Purdie, L.; Ripmeester, J. A.; Preston, C. M. *J. Agric. Food Chem.* **1983**, *31*, 913.

Bracewell, J. M.; Robertson, G. W.; Williams, B. L. *J. Anal. Appl. Pyrolysis* **1980**, *2*, 53.

Brown, K. A. *Soil Biol. Biochem.* **1981**, *13*, 469.

Eno, C. F.; Popenoe, H. *Soil Sci. Soc. Am. Proc.* **1964**, *28*, 533.

Hayes, M. H. B.; Dawson, J. E.; Mortensen, J. L.; Clapp, C. E.; Hausler, M. J. International Conference on Structures and Complexes of Humic Materials; International Humic Substance Society: Birmingham, England, 1984.

Knipfel, J. E.; McLeod, J. G. McCaig, T. N. In "The Maillard Reaction in Foods and Nutrition"; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; p 361.

Lamport, D. T. A. In "The Biochemistry of Plants"; Preiss, J., Eds.; Academic Press: New York, 1980; Vol. 3, p 501.

Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley: New York, 1979; p 28.

Lynch, J. M. *J. Gen. Microbiol.* **1982**, *128*, 405.

Maillard, L. C. *Ann. Chim. (Paris)* **1916**, *5* (Ser. 9), 258.

Mal, S. S.; Slivka, E. M.; Povarkova, P. S.; Yurkevich, Ye. A. *Pochvovedenie* **1982**, *9*, 66.

Morita, H.; Levesque, M. *Can. J. Soil Sci.* **1980**, *60*, 285.

Nagy, B. *Naturwissenschaften* **1982**, *69*, 301.

Pernemalm, P. A. Unpublished Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 1978.

Phillips, G. O. In "The Carbohydrates, Chemistry and Biochemistry"; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. 1B, p 1217.

Powlson, D. S.; Jenkinson, D. S. *Soil Biol. Biochem.* **1976**, *8*, 179.

Ramsay, A. J.; Bawden, A. D. *Soil Biol. Biochem.* **1983**, *15*, 263.

Stevenson, F. J. Advances in Organic Geochemistry, VIth International Meeting on Organic Geochemistry, Rueil-Malmaison, France, 1973, p 701.

Swallow, A. J. "Radiation Chemistry. An introduction"; W. Clowes & Sons Ltd.: Longman Group: London, 1973.

Williams, B. L. *J. Soil Sci.* **1983**, *34*, 113.

Williams, B. L.; Cheshire, M. V. Unpublished results, Macaulay Institute for Soil Research, Aberdeen, Scotland, 1984.