TFA derivatives on separate runs (see Table II).

Structural Assignment with Respect to Stereoisomers. Immediately following the main fraction of the wine sample, two smaller fractions were eluted which were also present in synthetic **2** derived from a mixture of the diastereomeric 2,3-butanediols. Mass spectra for the three components differed in their abundances at *m/e* 55, 73, 126, and 182 (see Table II). GC–MS analysis of 4,5-dimethyl-1,3-dioxolane-2-propanamine TFA, **2a**, which was synthesized by using essentially pure D(−)-2,3-butanediol, gave only the first (main) fraction. It can therefore be concluded that the main fraction of 4,5-dimethyl-1,3-dioxolane-2-propanamine in wine is an acetal of *threo*-2,3-butanediol and that the two minor fractions are acetals of *erythro*- (*meso*-) 2,3-butanediol.

Stevens (1969) reported the presence of both 2,4,5-trimethyldioxolane and 2,4-dimethyl-5-ethyldioxolane in wine. Muller et al. (1979) also identified a number of cyclic acetals in sherry. This and the present work suggest that cyclic acetals of other aldehydes may be present in wine.

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Registry No. 1-Pyrroline, 5724-81-2; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 1, 85236-72-2; 2-pyrroline trifluoroacetamide, 85236-73-3; 4,5-dimethyl-1,3-dioxolane-2-propane trifluoroacetamide, 85236-74-4; 4-aminobutyraldehyde diethyl acetal, 6346-09-4; [*R*(*R**,*R**)]-2,3-butanediol, 24347-58-8; 1-pyrroline·HCl, 85236-75-5; (*R**,*R**)-butanediol, 35007-63-7; (*R**,*S**)-butanediol, 5341-95-7; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 2, 85236-76-6; 4,5-dimethyl-1,3-dioxolane-2-propanamine isomer 3, 85236-77-7.

LITERATURE CITED

Amerine, M. A.; Ough, C. S. "Methods for Analysis of Musts and Wines"; Wiley: New York, 1980; p 117.
Amoore, J. E. *Chem. Senses Flavour* **1979**, *4*, 153–161.
Daudt, C. E. Ph.D. Dissertation, University of California, Davis, Davis, CA, 1980.
Katritsky, A. S.; Lagowski, J. J. "The Principles of Heterocyclic Chemistry"; Academic Press: New York, 1968; p 135.
Lawless, J. G.; Chadha, M. S. *Anal. Biochem.* **1971**, *44*, 473–485.
Leimer, K. R.; Rice, R. H.; Gehrke, C. W. *J. Chromatogr.* **1977**, *141*, 121–144.
Muller, C. J.; Kepner, R. E.; Webb, A. D. *Am. J. Enol. Vitic.* **1978**, *29*, 207–212.
Ough, C. S. *Vitis* **1968**, *7*, 321–331.
Ough, C. S.; Daudt, C. E.; Crowell, E. A. *J. Agric. Food Chem.* **1981**, *29*, 938–941.
Ough, C. S.; Tabacman, H. *Am. J. Enol. Vitic.* **1979**, *30*, 306–311.
Poisel, H. *Monatsh. Chem.* **1978**, *109*, 925–928.
Schreier, P. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *12*, 59–111.
Stevens, K. L.; Flath, R. A.; Lee, A.; Stern, D. J. *J. Agric. Food Chem.* **1969**, *17*, 1102–1106.
Stille, J. K.; Becker, Y. *J. Org. Chem.* **1980**, *45*, 2139–2145.
Welch, R. C.; Hunter, G. L. K. *J. Agric. Food Chem.* **1980**, *28*, 870–874.

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Elucidation of the Nitrogen Forms in Melanoidins and Humic Acid by Nitrogen-15 Cross Polarization–Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy

Nitrogen-15 labeled melanoidins were synthesized by reaction of xylose and glucose with ^{15}N-labeled glycine and ammonium sulfate and compared by cross polarization–magic angle spinning (CP–MAS) ^{15}N NMR to a ^{15}N-labeled humic acid. The nitrogen in the melanoidins obtained with glycine, like in the humic acid, was mainly in the secondary amide form with some present as aliphatic amines and/or ammonium ions and some as pyrrole and/or indole nitrogen. The pyrrole- and pyridine-like nitrogen appears to exceed the amide nitrogen in the melanoidins obtained from xylose and ammonium sulfate.

Melanoidins, the dark brown nitrogenous polymers resulting from the interaction of carbohydrates and amino acids, have been part of man's diet since fire was first used for food preparation (Mauron, 1981). These polymers may also be at the origin of humic substances, as suggested by Maillard (1912, 1916). Until recently, however, this hypothesis was difficult to evaluate. A recent study (Benzing-Purdie and Ripmeester, 1983) using solid-state ^{13}C NMR revealed striking similarities between synthetic melanoidins and a humidified soil. These results, which lend support to Maillard's hypothesis, led us to pursue this comparison further. In spite of the importance of nitrogen to soil fertility (Cooke, 1981), almost half of the soil nitrogenous components remain unidentified (Greenfield, 1979; Ivarson and Schnitzer, 1979), mainly because when chemical degradation is attempted, some are unhydrolyzable and others may be decomposed as a result of the necessary strong hydrolytic conditions. Since melanoidins also contain nitrogen, and as no direct information about the nitrogen in these substances can be obtained from ^{13}C CP–MAS NMR, the ^{15}N solid-state NMR technique was applied. Solid-state ^{15}N NMR spectroscopy offers several advantages over solution NMR. No solubilization of material is necessary, thereby eliminating possible errors due to partial solubility of products. Also, as in ^{13}C CP–MAS NMR, cross polarization and magic angle spinning ^{15}N NMR increases the sensitivity and gave, for example, a line width of 0.5 ppm in the case of polycrystalline glycine (Opella et al., 1981). ^{15}N CP–MAS NMR has been used recently in studies of protein turno-

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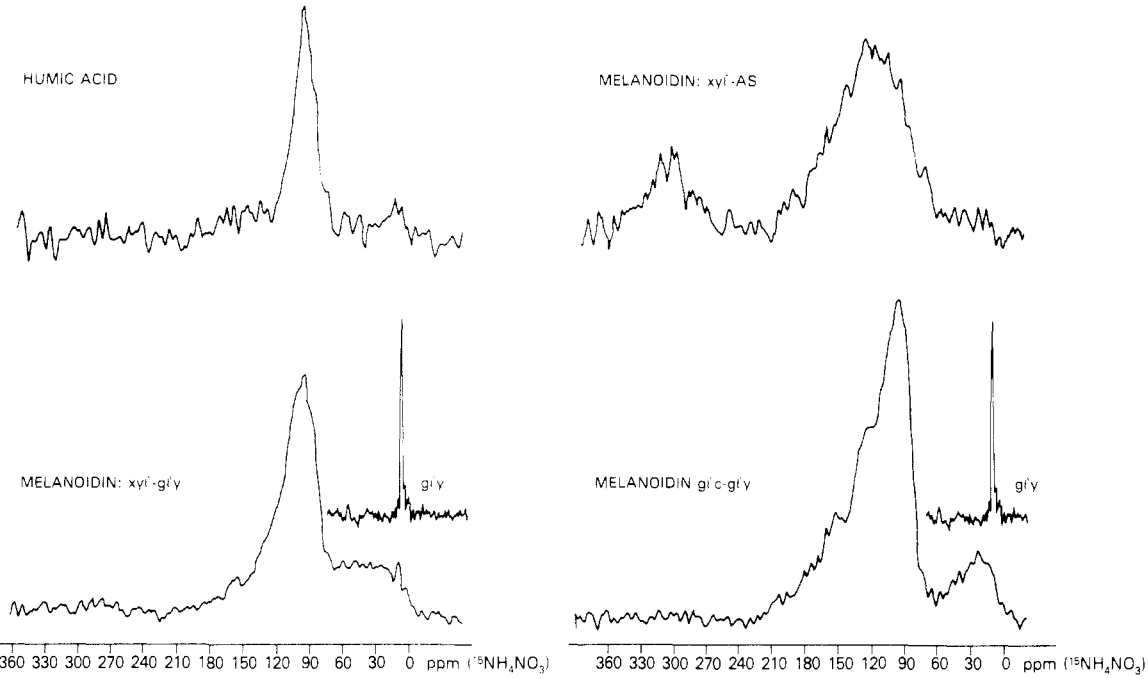

Figure 1. ^{15}N CP–MAS spectra of ^{15}N-labeled humic acid and melanoidins **1** (Xyl–Gly), **2** (Glc–Gly), and **3** (Xyl–AS). Spectrum of glycine-^{15}N is shown as an insert.

vers (Schaefer et al., 1981) and in the structural elucidation of biomolecules (Cross et al., 1982) but has never been applied to melanoidins or soil humic acid. This communication presents the first ^{15}N CP–MAS NMR spectra of melanoidins and a humic acid from a mineral soil.

EXPERIMENTAL SECTION

Chemicals. D-Xylose and D-glucose were purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL 60085. Glycine-^{15}N (99% ^{15}N) and ammonium sulfate (99% ^{15}N) were purchased from Stohler Isotope Chemicals, Waltham, MA 02154.

Synthesis of Melanoidins. ^{15}N-Labeled melanoidins, **1**, **2**, and **3**, were synthesized by a method previously described (Benzing-Purdie and Ripmeester, 1983) from a 1 molar solution (10 mL) of xylose and glycine (20% ^{15}N), glucose and glycine (40% ^{15}N), and xylose and ammonium sulfate (25% ^{15}N). The reaction mixtures were kept at 68 °C for 30 days. The melanoidins from xylose–glycine and xylose–ammonium sulfate were recovered by filtration, washed with water, and dried. The melanoidin from the glucose–glycine reaction mixture was recovered after exhaustive dialysis and evaporated to dryness at 38 °C.

Soil Humic Acid. An Orthic Humic Gleysol, moistened to and maintained at two-thirds field capacity, was incubated with 300 ppm of $Na^{15}NO_3$ and 5000 ppm of $^{13}CH_3COONa$ at 25 °C for 7 months. During this period, the soil was subjected to one dry–wet and two freeze–thaw cycles. The ^{15}N-labeled humic acid (2.7% ^{15}N excess) was isolated from the soil by standard procedures (Preston and Ripmeester, 1982).

Cross Polarization–Magic Angle Spinning (CP–MAS) ^{15}N NMR Spectra. The spectra were obtained at 18.25 MHz on a Bruker CXP-180 pulsed spectrometer with cross polarization and magic angle spinning. A single cross polarization sequence was used with matched rf field amplitudes of 28 kHz. The cross polarization time was 1 ms. Up to 30 000 500-point free induction decays were coadded at a sweep width setting of 20 kHz. Zero filling up to 4K was used before Fourier transformation. Delrin spinners of the Andrew type were used with spinning speeds of ≃3500 Hz. Chemical shifts are referenced to external ammonium nitrate. Assignments were made by comparison with published ^{15}N NMR data (Levy and Lichter, 1979).

Table I. Yields and Microanalytical Data[a] of Melanoidins and Humic Acid

substance	C	H	N	yields, g
melanoidin 1 (Xyl-Gly)	56.51	6.70	3.61	0.690
melanoidin 2 (Glc-Gly)	64.20	5.66	4.10	0.290
melanoidin 3 (Xyl-AS)	57.50	4.36	4.60	0.100
humic acid	49.88	5.73	2.85	

[a] Data are reported on an ash-free basis.

RESULTS AND DISCUSSION

The reaction of xylose with glycine gives a higher yield of melanoidins than glucose with glycine, and glycine is more reactive than ammonium sulfate (Table I). These results are not surprising since it had been shown previously that the pentoses, in particular xylose and arabinose, are the most reactive sugars and glycine is the most reactive amino acid (Manskaya and Drozdova, 1968). The microanalytical data of the melanoidins and humic acid are similar (Table I), even though the melanoidins are simple polymers arising from the interaction of one reducing sugar and one amino acid or ammonium salt.

The three ^{15}N CP–MAS NMR spectra of melanoidins **1** and **2** and the soil humic acid show a predominant peak at 97 ppm (Figure 1), characteristic of a secondary amide type nitrogen. In addition, the three spectra show an absorption band at 155 ppm due to a pyrrole-type nitrogen and one at 128 ppm, attributed to either a secondary amide nitrogen or a pyrrole and an indole nitrogen. The band at 128 ppm is more pronounced in the melanoidins than in the humic acid. The ^{15}N CP–MAS NMR spectra obtained by using cross polarization times of 2, 3, or 5 ms show more clearly the bands at 128 and 155 ppm. The spectrum of melanoidin **1** (Figure 2) is given as an example. Thus, the broadness of the major peak at 97 ppm (melanoidin **1**, Figure 1) is mainly due to the overlapping of three peaks with maxima at 97, 128, and 155 ppm. Amide groups in peptide linkages give a signal at 97 ppm with a broadness of about 50 ppm (Schaefer et al., 1979; Cross et al.,

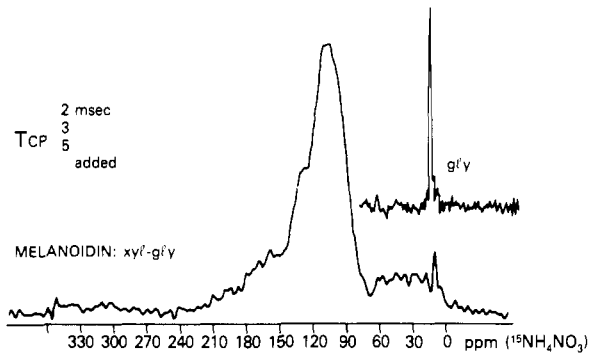

Figure 2. ^{15}N CP–MAS spectrum of ^{15}N-labeled melanoidin 1 (Xyl–Gly): cross polarization times 2, 3, and 5 ms spectra added. Spectrum of glycine-^{15}N is shown as an insert.

1982). The presence of a secondary amide nitrogen and a pyrrole or pyrrole-type nitrogen in the melanoidins corroborates postulated structures for these polymers (Kato and Tsuchida, 1981). Melanoidin 1 shows a peak at 10 ppm that is assigned to the nitrogen of glycine as glycine run under the same conditions gives a signal at the same chemical shift. This is further corroborated by our previous results (Benzing-Purdie and Ripmeester, 1983), based upon acid hydrolysis, showing that the polymer contained about 1% of the starting amino acid. This peak is not resolved in melanoidin 2 obtained from glucose and glycine, whose spectrum shows a broad band centered at 24 ppm. This band is also found in the soil humic acid spectrum but cannot be assigned with certainty, as nitrogen of a variety of functional groups, e.g., aliphatic amines, ammonium ions, anilines, and guanidines, absorb in this region. The ^{15}N CP–MAS spectrum of melanoidin 3, obtained from xylose and ammonium sulfate, has many different features. The spectrum shows a large band centered around 115 ppm which may be attributed to secondary amide nitrogen but more likely is due mainly to an indole or a pyrrole nitrogen based on the high aromaticity shown by the ^{13}C CP–MAS NMR. Another less important band around 315 ppm may be assigned to pyridine or pyridine-like nitrogen. The presence of this type of nitrogen is not too surprising, as more than 20 pyridine derivatives have been characterized as products in Maillard reactions (Vernin and Parkanyi, 1982).

The results reported here reveal striking similarities between the melanoidins synthesized from reducing sugars and amino acids and a humic acid from a mineral soil. The nitrogen in the melanoidins, like in the humic acid, is mainly in the secondary amide form with some present as aliphatic amines and/or ammonium ions and some as pyrrole and/or indole nitrogen. These findings may explain in part the difficulties encountered in the identification of the nitrogen in the soil and the elucidation of the structures of melanoidins. Sterically hindered secondary amides are very resistant to acid hydrolysis. The ^{15}N NMR evidence also corroborate our previous findings based on ^{13}C solid-state NMR and lend further support to Maillard's hypothesis. Our studies clearly show that ^{15}N CP–MAS NMR is effective in elucidating the various forms of nitrogen in soil, which is of obvious importance to soil science. Knowledge gained through this approach should help understand and manage soil nitrogen toward more optimum agronomic results. This is especially so, as the evidence indicates that much of the soil nitrogen is in one form, i.e., secondary amides. The results obtained are also relevant to food chemistry, as melanoidins are important constituents of many cooked foods and elucidation of their structures is an important goal of this branch of science (Fujimaki et al., 1981).

Registry No. Nitrogen, 7727-37-9.

LITERATURE CITED

Benzing-Purdie, L.; Ripmeester, J. A. *Soil Sci. Soc. Am. J.* **1983**, *47*, 56.
Cooke, G. W. "The Chemistry of Soil Processes"; Greenland, D. J.; Hayes, M. H. B., Eds.; Wiley: New York, 1981; p 563.
Cross, T. A.; DiVerdi, J. A.; Opella, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 1759.
Fujimaki, M.; Hodge, J. H.; Theander, O. "Progress in Food and Nutrition Science"; Eriksson, C., Ed.; Pergamon Press: Oxford, 1981; Vol. V, p 157.
Greenfield, L. G. *Mauri Ora* **1979**, *7*, 25.
Ivarson, K. C.; Schnitzer, M. *Can. J. Soil Sci.* **1979**, *59*, 59.
Kato, H.; Tsuchida, H. "Progress in Food and Nutrition Science"; Eriksson, C., Ed.; Pergamon Press: Oxford, 1981; Vol. V, p 147.
Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley: New York, 1979; p 28.
Maillard, L. C. *C. R. Hebd. Seances Acad. Sci.* **1912**, *154*, 66.
Maillard, L. C. *Ann. Chim. (Paris)* **1916**, *5* (Ser. 9), 258.
Manskaya, S. M.; Drozdova, T. V. "Geochemistry of Organic Substances"; Shapiro, L.; Breger, J. A., Eds.; Pergamon Press: Oxford, 1968; p 28.
Mauron, J. "Progress in Food and Nutrition Science"; Eriksson, C., Ed.; Pergamon Press: Oxford, 1981; Vol. V, p 5.
Opella, S. J.; Hexem, J. G.; Frey, M. H.; Cross, T. A. *Philos. Trans. R. Soc. London, Ser. A* **1981**, *299*, 665.
Preston, C. M.; Ripmeester, J. A. *Can. J. Spectrosc.* **1982**, *27*, 99.
Schaefer, J.; Skokut, T. A.; Stejskal, E. O.; McKay, R. A.; Varner, J. E. *J. Biol. Chem.* **1981**, *256*, 11574.
Schaefer, J.; Stejskal, E. O.; McKay, R. A. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 274.
Vernin, G.; Parkanyi, C. "Chemistry of Heterocyclic Compounds in Flavours and Aromas"; Vernin, G., Ed.; Horwood: Chichester, U.K., 1982; p 151.

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