Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 927-936 (1999)

Synthesis of ^{13}C-Enriched Pyrrole from 2-^{13}C D-Galactose

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Abstract

The synthesis of ^{13}C-enriched pyrrole, labeled adjacent to the nitrogen atom, is described. A convenient and effective bench-top synthesis is given which uses a readily available enriched pyranose sugar, 2-^{13}C D-galactose, as the starting material. In the first step of the synthesis, D-galactose is oxidized to produce mucic acid in 70-75% yield. Mucic acid is then treated with ammonium hydroxide to give ammonium mucate in 99% yield. In the final step, ammonium mucate is pyrolyzed to form pyrrole with a 35% yield. The pyrrole obtained in this manner is greater than 98% enriched at the α position.

Keywords: ^{13}C enrichment, mucic acid, ammonium mucate, pyrrole synthesis, NMR

Introduction

The five atom heterocycle, pyrrole, has significant importance due to its aromatic properties and its widespread occurrence in nature. The heterocyclic pyrrole ring is found in a number of important biological compounds, including vitamin B_{12}, chlorophyll, and tryptophan.[1,2] Pyrrole is also a precursor to a large number of synthetic compounds,

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CCC 0362–4803/99/100927–10$17.50

Received 21 January 1999
Accepted 1 March 1999

including pyrrolidine and various substituted porphyrins.[3] Among these is also its polymer, poly(pyrrole), an important conjugated macromolecule with excellent environmental stability and uses in several electronic applications, including anti-static coatings, light weight batteries, and thin flexible displays.[4,5] For many of these materials, and particularly for poly(pyrrole), much remains unknown with respect to the synthesis mechanisms and underlying molecular origins of their bulk properties. To these ends, appreciable insight can be gained from solution- and solid-state nuclear magnetic resonance (NMR) spectroscopy studies of these compounds. This is especially true for ^{13}C NMR investigations, which profit form the large (~250 ppm) ^{13}C chemical shift range. In samples containing ^{13}C in natural abundance (1%), however, poor signal sensitivity often makes such experiments infeasible. This difficulty is further exacerbated when the linewidths are large, as is the case for polypyrrole. Appreciable incentive, therefore, exists to increase the concentration, selectively if possible, of ^{13}C in pyrrole and pyrrole-derivatives to permit detailed structural studies of these materials.

Of the few published methods for synthesizing an unsubstituted pyrrole ring, none provide a convenient or facile laboratory pathway for ^{13}C enrichment. Commercially, pyrrole is manufactured by passing furan, ammonia, and steam over an Al_2O_3 catalyst.[1] This process produces high multi-pass yields of pyrrole, however, ^{13}C-enriched furan is not readily available, nor can it be enriched more easily than pyrrole. A more straightforward method has been reported that uses the Paal-Knorr synthesis for making substituted pyrroles with succinaldehyde (which replaces the 1,4-diketone in the usual Paal-Knorr procedure) and ammonia to give pyrrole.[1] However, this approach suffers from the scarce availability of enriched succinaldehyde. Schulte *et al.* have shown that ammonia and diacetylene react in the presence of cuprous chloride to produce pyrrole in 40% yield.[6] In this case as well, the starting material, diacetylene, is not readily available as a ^{13}C-enriched compound, precluding this reaction's use as a convenient enrichment pathway.

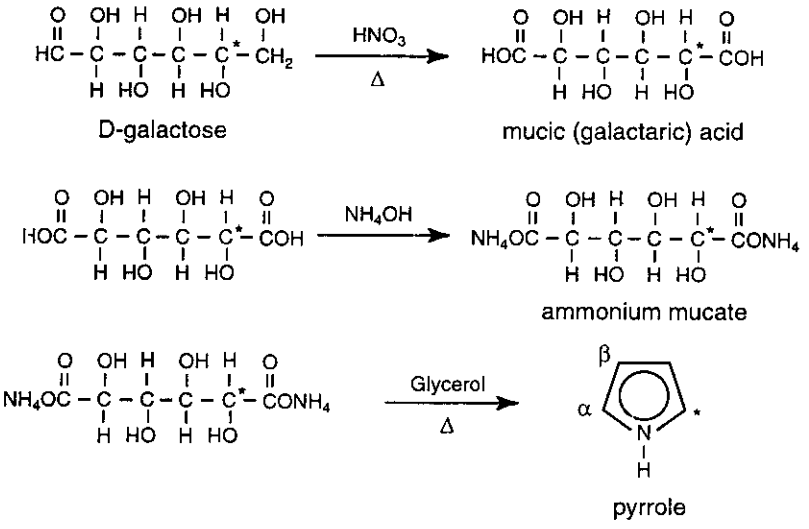

Figure 1. The reaction scheme used in this paper to produce α-^{13}C pyrrole from 2-^{13}C D-galactose. The position of ^{13}C enrichment is indicated on each molecule by an asterisk (*). The more common nomenclature of naming heteroaromatic rings substitutions by the α and β designations are adopted and indicated above.

A significantly more convenient and effective laboratory synthesis of ^{13}C-enriched pyrrole is the pyrolysis of ammonium mucate in the presence of glycerol.[7,8] Isotopically labeled ammonium mucate can be readily synthesized from several of the pyranose sugars, including D-galactose.[9] The synthesis scheme we used to produce α-^{13}C pyrrole is shown in Figure 1 and consists of three steps: the oxidation of D-galactose to mucic acid, the conversion of mucic acid to ammonium mucate, and finally, the pyrolysis of ammonium mucate to yield pyrrole.

Experimental

All experimental details were tested and optimized with non-enriched starting materials before performing experiments on ^{13}C-labeled reactants. Non-enriched compounds and solvents were purchased from Aldrich Chemical and used as received. 2-^{13}C D-galactose with an enrichment level of 99% was obtained from Cambridge Isotope

Laboratories (Andover, Massachusetts). Compound identifications were established for both ^{13}C-enriched and non-enriched products.

Preparation of Mucic Acid

2-^{13}C D-galactose (2.5 g, 1.4×10^{-2} mol, 99% enriched) was placed in a porcelain crucible, after which 26.0 ml of 4.76 N HNO_3 was added. The mixture was stirred over a steam bath for approximately one hour. As the reaction proceeded, NO_2 gas was evolved and a change in color of the solution from clear to the yellow-brown color of NO_2 was observed. Simultaneous with the evolution of NO_2, mucic acid was produced as a milky suspension. The reaction mixture was heated until a thick paste was obtained by evaporation of excess solvent, then removed from the steam bath. Water was added in sufficient quantity (1-5 ml) to restore the paste to a white color and cool the reaction product. The crucible containing the white slurry product was covered and allowed to sit overnight to ensure complete crystallization of the mucic acid. The mucic acid was then filtered and washed 2-3 times with cold water to remove excess HNO_3 and subsequently dried for several hours at 80 °C. After drying, the reaction product was ground into a fine powder and weighed, providing an average yield of 72%. Approximately 23% of the theoretical yield was lost to a competing side reaction that produces the three-ring polysaccharide, raffinose, which is soluble in water and removed during filtration.[9]

Preparation of Ammonium Mucate

Mucic acid (5.1 g, 2.4×10^{-2} mol) was placed in a porcelain crucible, and 3.7 ml of deionized H_2O was added to prevent caking during the subsequent addition of ammonium hydroxide. While stirring, 7.4 ml of concentrated (30 wt%) ammonium hydroxide was added, and the mixture stirred for several minutes. The resulting paste was dried under ambient conditions and then placed under vacuum for one day. Finally, the resulting off-white/beige-colored ammonium mucate was ground into a fine powder resulting in a yield of 99% for this reaction.

Synthesis of Pyrrole

Ammonium mucate (5.7 g, $2.3x10^{-2}$ mol) and 3.0 ml of glycerol were added to a 50-ml round bottom flask equipped with a short-path distillation head and a 5-ml collection vessel. While stirring, the reaction mixture was heated in a sand bath to 50 °C over a period of one hour. Once the viscous glycerol had fully wetted the ammonium mucate powder, the heat was slowly increased to 250 °C. During the pyrolysis of ammonium mucate, a change in color from beige to dark-brown was observed, in conjunction with vigorous bubbling of the glycerol as CO_2 was liberated. Pyrrole was formed as an oily phase atop an ammonium-carbonate-saturated aqueous phase in the collection vessel. The reaction was halted by removal of the reaction flask from the sand bath after the glycerol ceased bubbling. Pyrrole was syringed off the top of the aqueous phase and vacuum distilled 2-3 times, resulting in a yield of 35%.

It is interesting to note that glycerol is not necessary to produce pyrrole and does not participate in the reaction. The addition of glycerol, however, does increase the yield by at least 50% as compared to using no solvent, presumably by evenly distributing heat and reactants. Any inert high-boiling solvent, such as propylene carbonate, could enhance the yield for this reaction; however, solvents that are immiscible with pyrrole are best suited for the pyrolysis reaction because no extra purification steps are required.

NMR Characterization

All liquid NMR spectra were acquired on a Bruker Avance-200 spectrometer operating at a ^{1}H frequency of 200.1 MHz and a ^{13}C frequency of 50.3 MHz. The liquid ^{13}C NMR spectra were obtained without proton decoupling in order to elucidate J-couplings. All liquid NMR spectra were taken in $CDCl_3$ and referenced to an internal TMS standard unless otherwise noted. Peak values are recorded in ppm and spectral line shapes are indicated in the following way: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad).

Solid-state NMR spectra were obtained on a Chemagnetics CMX-180 spectrometer operating at a ^{1}H frequency of 180.1 MHz and a ^{13}C frequency of 45.3 MHz. All of the solid-state measurements were conducted under conditions of cross-polarization and magic angle spinning (CPMAS) in order to increase sensitivity and resolution. All resonance assignments were measured in ppm and referenced to TMS with an external standard.

Results and Discussion

Identification of Mucic Acid

Due to its low solubility in common deuterated solvents, the mucic acid product's identity was verified with solid-state ^{13}C NMR. Table 1 contains the resonance assignments for ^{13}C and ^{1}H moieties in both the natural abundance and ^{13}C-labeled mucic acid and ammonium mucate products from our syntheses. The resonance shifts in Table 1 agree with values reported previously.[10,11] These earlier studies, however, have been unable to assign unambiguously the non-carboxylic resonances for mucic acid or for the structurally similar D-glucaric acid.[11,12]

The oxidation of D-galactose with HNO_3 to form mucic acid has a well-understood mechanism.[13] Consequently, the oxidation of 2-^{13}C D-galactose produces mucic acid that is labeled with ^{13}C in the 2 position (adjacent to the carboxylic group), as shown in Figure 1. Thus, the single resonance at 72.2 ppm in the ^{13}C NMR spectrum of the enriched mucic acid product can be assigned to the ^{13}C atoms at the 2-position of mucic acid. We can, therefore, make the following unambiguous ^{13}C resonance assignments for mucic acid in natural abundance: carboxylic ^{13}C atoms (180.8 ppm), ^{13}C atoms in the 3-position (73.4 ppm), and ^{13}C atoms in the 2-position (72.2 ppm).

To verify the purity of the mucic acid, melting temperature data were collected on commercially available (Aldrich) mucic acid and that synthesized in our laboratory. The mucic acid obtained from Aldrich was nominally 97% pure and found to have a melting

temperature range of 212-214 °C. Mucic acid samples prepared in our syntheses by the oxidation of D-galactose were found to have a higher melting point and narrower melting range of 217-218 °C, indicating a purity of better than 97%.

Compound			Resonance Frequency (ppm)		
Mucic Acid	Nat.-abund.	Solid-State ^{13}C	180.8	73.4	72.2
	^{13}C-enriched	Solid-State ^{13}C	-----	-----	72.2
Ammonium Mucate	Nat.-abund.	Liquid-State ^{1}H	4.27(s)	3.96(s)	2.24(s)
		Solid-State ^{13}C	180.8	74.4	72.8
	^{13}C-enriched	Liquid-State ^{1}H	4.26(s)	9.50(d)	2.23(s)
		Solid-State ^{13}C	-----	74.4	-----
Pyrrole	Nat.-abund.	Liquid-State ^{1}H	7.7(b)	6.6(m)	6.2(m)
		Liquid-State ^{13}C	117.9(d)	107.8(d)	-----
	^{13}C-enriched	Liquid-State ^{13}C	117.4(m)	-----	-----

Table 1. ^{13}C and ^{1}H NMR chemical shift values measured for different moieties in naturally abundant and ^{13}C-enriched mucic acid, ammonium mucate, and pyrrole using liquid- and solid-state NMR.

Identification of Ammonium Mucate

Natural abundance and ^{13}C-enriched samples of ammonium mucate were characterized using solid-state ^{13}C NMR and liquid-state ^{1}H NMR, which yielded the resonance measurements shown in Table 1. Proton resonances were compared to standard mucic acid ^{1}H NMR spectra, because no peak assignments appear to be available in the literature for ammonium mucate. The ammonium mucate proton resonances possessed similar peak positions and intensities to those in standard mucic acid ^{1}H NMR spectra.[10] This is expected, because only the terminal protons of mucic acid have been replaced by ammonium groups to form ammonium mucate. There was no evidence of the ammonium protons which are expected to be too broad for observation.[14] The ^{13}C CPMAS NMR spectrum for the ammonium mucate product indicates that the site of ^{13}C enrichment in the ammonium mucate corresponds to the moiety with the larger chemical shift (74.4 ppm). Since no atomic rearrangement occurs in the acid-base neutralization of mucic acid to form ammonium mucate, the ^{13}C-enrichment site is still the 2 position, as indicated in Figure 1. Spectral assignments for ^{13}C atoms in ammonium mucate are as follows: carboxylic ^{13}C atoms (180.8 ppm), ^{13}C atoms in the 3-position (72.8 ppm), and ^{13}C atoms in the 2-position (74.4 ppm).

Identification of Pyrrole

Liquid-state ^{1}H and ^{13}C NMR spectra of naturally abundant and ^{13}C-enriched samples were used to identify pyrrole as the synthesis product of the ammonium mucate pyrolysis. ^{1}H NMR spectra of unlabeled pyrrole showed resonances at 7.72(b), 6.55(m), and 6.18(m) ppm, corresponding to the N-H proton, the proton attached to the α-carbon atoms, and the proton attached to the β-carbon atoms, respectively. There appeared to be a small amount (< 2%) of impurity present in the pyrrole, as evidenced by a weak signal at 1.37(s) ppm in the proton spectrum; however, the absence of a corresponding signal in the ^{13}C NMR spectrum of natural abundance pyrrole indicates that the impurity is likely inorganic.

The liquid-state ^{13}C NMR spectrum of unlabeled pyrrole contained resonances at 117.9(d) ppm and 107.8(d) ppm, corresponding to the α- and β-carbon resonances, respectively.[15] Each ^{13}C resonance was split into a doublet due to the 184 Hz J-coupling that results from directly bonded ^{13}C-^{1}H moieties. The spectrum of ^{13}C-labeled pyrrole exhibited a multiplet at 117.4 ppm, corresponding to the α-carbon resonances exclusively. This collection of peaks appears to be a doublet of triplets arising from the large ^{13}C-^{1}H J-couplings (184 Hz) and a smaller J-coupling (7 Hz) between directly bonded ^{13}C and quadrupolar ^{14}N. Such ^{13}C-^{14}N J-couplings are expected and confirm ^{13}C enrichment at the α-position in the pyrrole molecule. The absence of any other significant peaks in the ^{13}C enriched pyrrole spectrum indicates an enrichment level of at least 98%.

Infrared absorption spectra of pyrrole showed a characteristic N-H stretching band at 3410 cm^{-1}. In addition, characteristic stretching bands for aromatics were observed at 1075 cm^{-1}, 1047 cm^{-1}, and 1016 cm^{-1}. The spectrum also showed a very strong out-of-plane C-H bending band at 740 cm^{-1}. The infrared absorption peaks were consistent with standard IR spectra of pyrrole in the literature.[16]

Conclusion

We have synthesized ^{13}C-enriched pyrrole using a convenient and effective bench-top approach to label the product selectively at the α-carbon position. The three-step procedure given uses a readily available enriched pyranose sugar, 2-^{13}C D-galactose, as the starting material and subsequent oxidation to produce mucic acid in 70-75% yield. Mucic acid is then treated with ammonium hydroxide to give ammonium mucate in 99% yield. In the final step, ammonium mucate is pyrolyzed to form pyrrole with a 35% yield. The pyrrole obtained in this manner is greater than 98% ^{13}C-enriched at the α-carbon atom.

Acknowledgements

The authors thank H. Zimmerman, Prof. F. Wudl, and N. Melosh for synthetic advice and helpful discussions. This work was supported in part by the U.S. National Science Foundation under grants DMR-9632716 and the David and Lucile Packard Foundation.

References and Notes

1. Acheson, R.M., *An Introduction to the Chemistry of Heterocyclic Compounds*. Wiley: New York, 1976.
2. Badger, G.M., *The Chemistry of Heterocyclic Compounds*. Academic Press: New York, 1961.
3. Jackson, A.H., *Pyrroles*, in *4. Heterocyclic compounds*, 4. Heterocyclic compounds, D. Barton and W.D. Ollis, Editors. Pergamon Press: Oxford, 1979.
4. Skotheim, T.A., *Handbook of Conducting Polymers*. M. Dekker: New York, 1986.

5. Skotheim, T.A., Elsenbaumer, R.L., and Reynolds, J.R., *Handbook of Conducting Polymers*. M. Dekker: New York, 1998.

6. Schulte, K.E., Reisch, J., and Walker, H., Chemische Berichte, **98**: 98-103 (1965).

7. McElvain, S.M. and Bolliger, K.M., *Pyrrole*, in *Organic Syntheses, Collective Volume 1*, Organic Syntheses, Collective Volume 1, H. Gilman and A.H. Blatt, Editors. J. Wiley & Sons: New York, 1941.

8. Blicke, F.F. and Powers, J.L., Industrial and Engineering Chemistry, **19**: 1334-1335 (1927).

9. Lewis, B.A., Smith, F., and Stephen, A.M., *Galactaric Acid and Its Derivatives*, in *Methods in Carbohydrate Chemistry*, Methods in Carbohydrate Chemistry, R.L. Whistler, Editor. Academic Press: New York, 1963.

10. Pouchert, C.J. and Behnke, J., *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*. Aldrich Chemical Co.: Milwaukee, 1993.

11. Nagels, L., Von Dongen, W., and Parmentier, F., Phytochemistry, **21**(3): 743-746 (1982).

12. Horton, D. and Zbigniew, W., Carbohydrate Research, **105**: 95-109 (1982).

13. Streitwieser, A. and Heathcock, C.H., *Introduction to Organic Chemistry*. Macmillan: New York, 1985.

14. Silverstein, R.M., Bassler, G.C., and Morrill, T.C., *Spectrometric Identification of Organic Compounds*. Wiley: New York, 1981.

15. Elguero, J., Marzin, C., and Roberts, J.D., Journal of Organic Chemistry, **39**(3): 357-363 (1974).

16. Pouchert, C.J., *The Aldrich Library of FT-IR Spectra*. Aldrich: Milwaukee, 1997.

