THE SYNTHESIS OF NICOTINIC ACID-7-^{13}C

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Received August 18, 1977

SUMMARY

Nicotinic acid, an intermediate in the pyridine nucleotide cycle, was labeled with ^{13}C at the 7 position. The reaction of CuCN-^{13}C (prepared from NaCN-^{13}C) with 3-bromopyridine, followed by hydrolysis to the acid and purification by ion exchange chromatography, gave an overall yield of 72% nicotinic acid-7-^{13}C. This greater than 3-fold increase in yield over previous methods is the result of *in situ* hydrolysis of the nitrile intermediate.

Key Words: Nicotinic Acid

INTRODUCTION

Although recent advances in methodology and instrumentation have minimized experimental difficulties in nmr of natural abundance carbon-13, specific carbon-13 enrichment still greatly facilitates ^{13}C-nmr studies of biological molecules (1) and biochemical processes (2). Nicotinic acid-7-^{13}C was synthesized in order to examine its metabolism in castor bean seedlings. To conserve NaCN-^{13}C, a synthesis was developed by which a three-fold yield increase over previously published procedures is obtained (3).

DISCUSSION

Nicotinic acid-7-^{14}C has been prepared (4) by treating 3-bromopyridine with butyllithium, followed by carboxylation with CO_2-^{14}C. This method is unsuitable for micropreparations, requires at least four days to complete and yields only 55% of labelled nicotinic acid based on $BaCO_3$-^{14}C, the CO_2-^{14}C source. An alternate method (3) involving hydrolysis of 3-cyanopyridine, prepared from the reaction of 3-bromopyridine with CuCN, though less time-consuming, results in a yield of 23%

Abbreviations used are: nmr, nuclear magnetic resonance spectroscopy; NAD, nicotinamide-adenine dinucleotide; NADH, reduced nicotinamide-adenine dinucleotide; NMN, nicotinamide mononucleotide; NMNH, reduced nicotinamide mononucleotide; TMS, tetramethyl silane.

0362-4803/78/0614-0893$01.00

when NaCN-^{13}C is used as the source of carbon-13. Modification of the method of McElvain (3) has increased the yield of nicotinic acid to 72% based on NaCN-^{13}C, the starting material. This approximately 3-fold increase in yield results from the *in situ* hydrolysis of the nitrile intermediate to the acid product. In addition, rather than employing the method suggested by Supniewski (5) for CuCN-^{13}C preparation from NaCN-^{13}C in which 50% of the label is immediately lost in the form of cyanogen gas, the alternate method of Barber (6) was used which resulted in an almost quantitative yield of CuCN-^{13}C.

The method presented here for synthesis of nicotinic acid-7-^{13}C, coupled with the method of Blumenstein, *et al.* (7) for the enzymatic preparation of NAD-carbonyl-^{13}C, NADH-^{13}C, NMN-^{13}C, NMNH-^{13}C, and N-Methylnicotinamide-7-^{13}C from nicotinic acid-7-^{13}C, make these biologically important molecules readily available for ^{13}C-nmr studies.

EXPERIMENTAL AND RESULTS

The method of Barber (6) for CuCN-^{13}C production from NaCN-^{13}C was modified as follows. The following solutions were prepared: $CuSO_4$ (13.2 g; 0.0526 moles) in 42 ml H_2O at 40-50°, $NaHSO_3$ (3.66 g; 0.0265 moles) in 10 ml H_2O at 50-60° and NaCN (1.78 g; 0.0363 moles) in 7 ml H_2O at 50-80°. In a ventillated hood, the $NaHSO_3$ solution was slowly added with stirring to the $CuSO_4$ solution. Immediately and quickly, this resultant solution was added to the NaCN solution and allowed to stand for ten minutes. The precipitate formed was filtered, washed with boiling water followed by cold EtOH, and dried at 120° overnight to yield a beige powder. The synthesis of nicotinic acid from the reaction of CuCN-^{13}C with 3-bromopyridine was based upon that presented by McElvain et al. (3). To 2.7 g (0.030 moles) CuCN-^{13}C in a 100-ml round bottom flask with a magnetic stirring bar, set for refluxing, 4.4 ml (0.0456 moles) 3-bromopyridine was added. This mixture was heated in an oil-bath (165-170°) for one hour. (The mixture should go into a

black viscous solution upon heating at 165°-170° and may begin to solidify within the hour's heating time.) The solidified tar was completely resuspended upon addition of 4 g NaOH in 40 ml EtOH and refluxed for three hours. The hydrolysate was evaporated to dryness, redissolved in 80 ml 2N NaOH and filtered. The filtrate, if opaque, was filtered again to give a clear greenish brown solution. The filtrate volume was reduced and applied to a water washed Dowex 1-X8 formate column (50-100 mesh; 41 x 1.7 cm). The elution was begun with 120 ml H_2O followed by 500 ml 0.25 N HCOOH. The UV absorbing fractions were pooled and the reduced volume was applied to a H_2O washed Chelex column (50-100 mesh; 20 x 2.1 cm) in order to remove residual copper ions. The column was then eluted with water and the UV absorbing fractions again pooled. The reduced volume was applied to a Dowex 1-X8 formate column (100-200 mesh; 22 x 2.0 cm). The column was washed with 100 ml water and the nicotinic acid eluted with 150 ml 0.25 N HCOOH. The UV absorbing fractions were pooled and evaporated to complete dryness to yield a white powder, mp 235° (lit (4) value 228-229°), 72% recovery. The 15.08 MHz, proton decoupled ^{13}C-nmr spectrum obtained at alkaline pH shows peaks at 174.3, 151.7, 150.4 (d, J = 2.8 Hz), 138.9, 133.5 (d, J = 66.7 Hz) and 125.2 (d, J = 2.8 Hz) ppm from TMS. These peaks were assigned to C-7, C-6, C-2, C-4, C-3 and C-5, respectively, which is in agreement with those assignments made for nicotinamide (8). A vicinal coupling constant greater than the geminal coupling constant has also been observed in substituted benzene rings (9). These chemical shifts are identical with those obtained from an authentic sample of nicotinic acid.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. Barker for the NaCN (^{13}C) and Dr. Richard Fuentes for technical assistance.

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