SYNTHESIS OF [4,6-^{13}C]NICOTINIC ACID

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SUMMARY

A simple, economic synthesis of [4,6-^{13}C]nicotinic acid is described.

Key words: Nicotinic acid, Carbon-13, Glycerol.

INTRODUCTION

Nicotinic acid (1) labelled with ^{13}C in C(4) was required for nuclear magnetic resonance experiments with NAD^+-dependent enzymes, and for biosynthetic tracer experiments. Degradation of quinoline (2) is known to give good yields of nicotinic acid [1,2], and a synthetic approach based on quinoline was sought. The modified Friedländer synthesis used to prepare [5,6-$^{13}C_2$]nicotinic acid [2] was considered, but, because of the difficulties associated with sythesising 2-aminobenzaldehyde labelled in the aldehyde moiety, this approach was abandoned in favour of a Skraup quinoline synthesis.

The Skraup synthesis of quinoline [3] involves condensation of aniline with glycerol, which is normally present in large excess. In the current synthesis quinoline must be labelled at C(2) and C(4) (see scheme) and hence glycerol is the required labelled component.

RESULTS AND DISCUSSION

[1-^{13}C]Glycerol was prepared as previously described [4]; an alternative starting material, particularly suitable for very small scale work when expense is less important, is the commercially available [1,3-$^{13}C_2$]glycerol. The Skraup synthesis was performed with aniline in slight excess and the resulting quinoline was labelled equally in C(2) and C(4). This was degraded to

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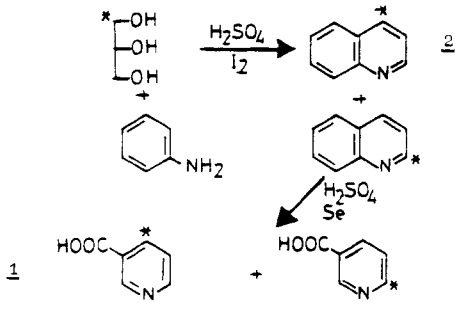
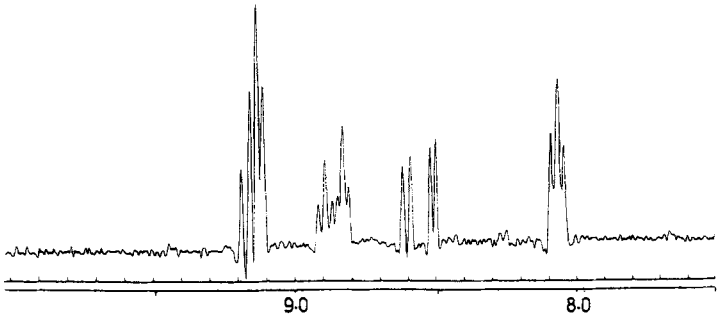
Scheme



nicotinic acid by the procedure of Leete [2], using selenium and sulphuric acid.

Nicotinic acid labelled equally in C(4) and C(6) was thus obtained in 45% yield over two steps, although on a scale using less than 200 mg glycerol the yield in the Skraup reaction is reduced. When synthetic glycerol is used the overall yield is 10% from K ^{13}CN.

The proton NMR spectrum of labelled nicotinic acid is interesting in that three bond ^{1}H-^{13}C coupling is clearly visible (figure 1). C(4)H



^{1}H NMR spectrum of [4,6-^{13}C]Nicotinic Acid

(centred at δ8.89) therefore gives rise to a broad triplet due to the species in which the label is at C(6), flanked by two doublets due to the species in which the label is at C(4). A slight upfield shift (0.02 ppm) of the two doublets relative to the triplet is also discernible at 300 MHz. C(6)H gives a similar pattern, although the downfield doublet overlaps with the signal due to C(2)H. C(5)H at δ8.08 appears as a broad triplet. The two-bond ^{1}H-^{13}C coupling with C(4) and C(6) is much smaller than the three bond coupling constants, and so results only in broadening of the signal.

In parallel with this work Oberfrank, Hull and Rétey developed an elegant synthesis of a mixture of [4-^{13}C]nicotinic acid and nicotinamide from [^{13}C]formic acid [5], in which C(4) is labelled with complete regio-specificity. However, the synthesis described here is much simpler when commercial glycerol is used, and is less expensive when synthetic [1-^{13}C]glycerol is employed. The distribution of the label in C(6) and C(4) may be advantageous in biosynthetic experiments but disadvantageous in experiments to determine enzyme mechanisms, because the crucial position, C(4), is only 50% labelled. This synthesis therefore complements that of Oberfrank, Hull and Rétey.

EXPERIMENTAL

Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded on Bruker WH300 and WM250 instruments operating at 300 MHz and 63MHz respectively. Mass spectra were recorded on a VG Analytical 30F spectrometer.

[2,4-^{13}C]Quinoline

[1-^{13}C]Glycerol (204 mg, 2.2 nmole), aniline (0.3 ml, 307 mg, 3.3 nmole) and iodine (15 mg) were stirred together. Sulphuric acid (0.5 ml) was added slowly and the mixture was then heated to 170° and stirred at this temperature for 2 hours. The mixture was cooled and sodium hydroxide solution (10%) was added until it was basic. The solution was diluted with water (to 20 ml) and extracted with chloroform (3x20 ml). The combined chloroform extracts were dried over sodium sulphate and the solvent was removed *in vacuo*. Flash

chromatography on silica with chloroform as eluent separated excess aniline from quinoline (169 mg, 59%). ^{1}H δ($CDCl_3$) 8.95 [0.5 H, bs, C(2)H, label at C(4)], 8.93 [0.5H, ddd, J=175Hz, 4.3Hz, 1.8Hz, C(2)-H, label at C(2)], 8.18 [0.5H, m, C(4)H, label at C(2)], 8.16 [0.5H, dd, J=160Hz, 8.5Hz, C(4)H, label at C(4)], 8.14 [1H, d, J=7.5Hz, C(8)H], 7.85 [1H, d, J=7.5Hz, C(5)H], 7.75 [1H, t, J=7.5Hz, C(7)H], 7.67 [1H, t, J=7.5Hz, C(6)H], 7.43 [1H, m, C(3)H]. ^{13}C δ($CDCl_3$) 150.1 [strong C(2)], 148.1 [C(8a)], 135.6 [strong, C(4)], 129.1 [broad, unresolved C(7), C(8), C(4a)], 127.5 [C(5)], 126.2 [(C(6)], 120.7 [S+2d, J=54Hz, 63Hz, C(3)].

[4,6-^{13}C]Nicotinic Acid

[4,6-^{13}C]Quinoline (18 mg), concentrated sulphuric acid (0.8 ml) and selenium (25 mg) were heated together with stirring at 290° for 20 hours. Heat was supplied by a Leister-Kombi Electric Hot Air Welding Tool Type "Triac". The mixture was then cooled, the pH adjusted to 3 with potassium hydroxide solution, and the resulting solution was continuously extracted with ether overnight. Evaporation of the solvent and sublimation gave [4,6-^{13}C]nicotinic acid (13 mg, 75%), m.p. (uncorrected) 228-231° (lit [6] 236-237°). ^{1}H δ(D_2O) 9.12 [1H, bs, C(2)H], 8.89 [0.5H, t, J=8Hz, C(4)H, label at C(6)], 8.87 [0.5H, dd, J=171Hz, 8Hz, C(4)H, label at C(4)], 8.83 [0.5H, t, J=6Hz, C(6)H, label at C(4)], 8.81 [0.5H, dd, J=183Hz, 6Hz, C(6)H, label at C(6)], 8.07 [1H, broad t, J+7Hz (average of coupling to C(4)H and C(6)H), C(5)H]. m/e (chemical ionisation, ammonia) 125 (100%), 124 (15%).

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