THE SYNTHESIS OF ^{14}C-LABELLED N-AMINOOXYACETYL-N'-ISONICOTINOYL-HYDRAZINE DIHYDROBROMIDE

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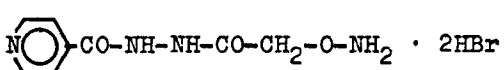
SUMMARY

The N-aminooxyacetyl-N'-isonicotinoylohydrazine dihydrobromide was labelled with ^{14}C in two different positions. In one case we applied a new synthesis route via ^{14}C labelled 4-cyanopyridine and isonicotinic acid. In the other case aminooxyacetic acid was labelled in its carboxyle group.

Key Words: 4-cyanopyridine; isonicotinic acid; aminooxyacetic acid

INTRODUCTION

The aminooxyacetyl-N'-isonicotinoyl-hydrazine (1) is a new, highly potent antituberculotic agent possessing excellent inhibiting properties against Mycobacterium tuberculosis strains in both in vitro and in vivo experiments.[1,2]



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Two isotopic isomers of 1 were prepared for pharmacokinetical and metabolism studies. In one case the ^{14}C atom was placed into the carboxyl group of isonicotinic acid (1a), in the other case into the carboxyl group of aminooxyacetic acid (1b).

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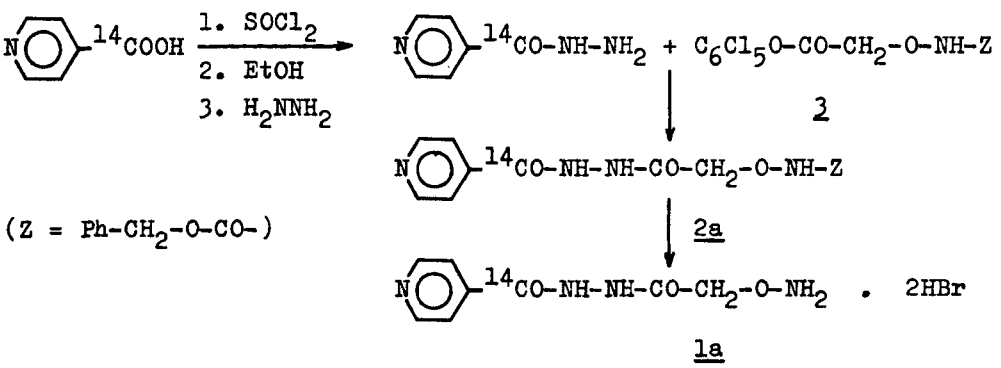
The synthesis of 1a started from 4-cyanopyridine prepared by melting sodium pyridine-4-sulphate with $K^{14}CN$. The resulted 4-cyanopyridine was hydrolyzed to isonicotinic acid which was purified by extraction with ether*. It was transformed to isonicotinic hydrazide by using of the method of Murray and Williams,[3] then it was reacted with the pentachlorophenyl ester of benzyloxycarbonyl-aminooxyacetic acid (3). From the N-(benzyloxycarbonyl--aminooxyacetyl)-N'-isonicotinoyl-^{14}C-hydrazine (2a) formed, the protecting group was removed by HBr in acetic acid (Scheme 1).

SCHEME 1



In the synthesis of 1b, acetic-1-^{14}C-acid was converted into bromoacetic-1-^{14}C acid which was reacted with benzyloxycarbonyl-hydroxyamine (4) to give benzyloxycarbonyl-aminooxyacetic-1-^{14}C acid from which we prepared a pentachlorophenyl ester (6) with DCC; 6 was reacted with isonicotinoyl-hydrazide to afford the isotopic isomer (2b) of 2a from which the protecting group was removed to give 1b (Scheme 2).

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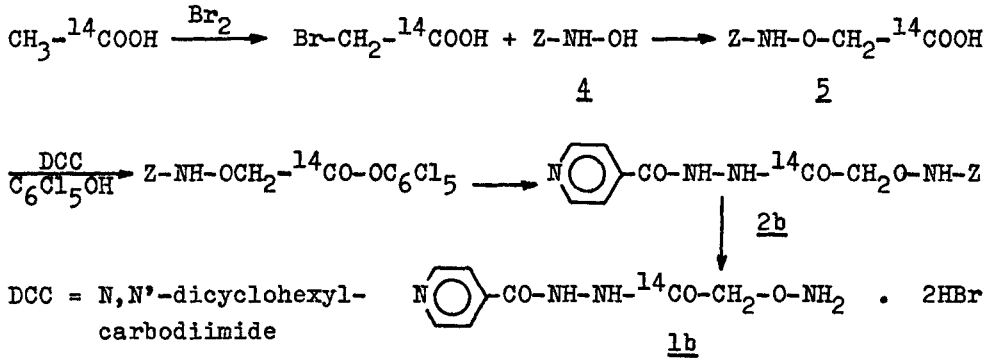
* The ^{14}C labelled isonicotinic acid has already been synthetised by Murray and Williams.[3,4] We have chosen our synthesis route since we needed labelled 4-cyanopyridine for other purposes, too.

SCHEME 2



EXPERIMENTAL

Melting points are uncorrected. One-dimensional thin layer chromatography was carried out on 5x20 cm plates using 0.2 mm thick Kieselgel $PF_{254+366}$ (MERCK). The activity was measured by a Packard TRI-CARB liquid scintillation system.

4-^{14}C-Cyanopyridine

179.1 mg (2.77 mmoles, 155.4 mCi) of $K^{14}CN$ and 510.0 mg (2.82 mmoles) of sodium pyridine-4-sulphate[*] were thoroughly mixed and sublimated with open flame under 40 Torr. The sublimation was finished when an orange colour appeared on the surface of the cooler. The material was dissolved in $CHCl_3$, the solvent removed and an orange-coloured substance was obtained (216.7 mg) which was dissolved in a mixture of 5 ml of benzene and 5 ml of ethyl acetate and purified by chromatography on a 15 mm diameter column with 20 g of Kieselgel (MERCK, 70-135 mesh) using benzene:ethyl acetate (1:1) as solvent. In this way a white crystalline material was obtained which gave only one spot by TLC (benzene:ethyl acetate 1:1). Yield: 161.5 mg (86.0 mCi) = 56%.

[*] The sodium pyridine-4-sulphate was prepared by Ochiai's method,[5] then it was freed from NaCl on an ion-exchange resin. In the presence of NaCl, the yield of 4-cyanopyridine strongly decreased.

Carboxyl-^{14}C-isonicotinic acid

161.5 mg (1.55 mmoles, 86.0 mCi) of 4-cyanopyridine in 10 ml of distilled hydrochloric acid were refluxed for 24 hours and allowed to stand for two days (in this case, most part of isonicotinic acid precipitated as white crystals). The pH of the solution was adjusted to 3.5 and the isonicotinic acid was extracted with ether in a continuous extractor for 24-48 hours. In this case, the time of the extraction was 36 hours and 130 mg of isonicotinic acid were obtained. After addition of inactive isonicotinic acid to the solution, the rest of isonicotinic acid was extracted, too. According to non-radioactive runs, the yield is about 90-95%.

Carboxyl-^{14}C-isonicotinic hydrazide

130 mg (1.06 mmoles, 58.6 mCi) ^{14}C-isonicotinic acid were refluxed in 10 ml of thionyl chloride for 25 minutes, until the solution became clear. Then the excess of tionyl chloride was evaporated under reduced pressure and the residue was refluxed in 10 ml of ethanol for one hour. The solution became clear again. The excess of ethanol was evaporated under reduced pressure, then the residue was taken up with 15 ml of 5% $NaHCO_3$ solution and extracted with ether in a continuous extractor for 4 hours. After the removal of ether, the residue was refluxed with 0.2 ml of 98% hydrazine hydrate in 10 ml of ethanol for 30 hours. After evaporation of ethanol, 143.4 mg of carboxyl-^{14}C-isonicotinic hydrazide were obtained. Yield: 99% (1.05 mmoles, 58.0 mCi).

N-(Benzyloxycarbonyl-aminooxyacetyl)-N'-carboxyl-^{14}C-isonicotinoyl-hydrazine (2a)

414 mg (3 mmoles, 58.0 mCi) of carboxyl-^{14}C-isonicotinic hydrazide were added to 1.45 g of 3 dissolved in 15 ml of dimethylformamide and the mixture was kept at room temperature for 3 days. Then the dimethylformamide was evaporated under vacuum and the

residue was recrystallized from 6 ml of 70% of ethanol; 1.429 g of 2a were obtained. Yield: 78%, mp 160-162°C. Adding 2 to the mother liquor, another portion (912 mg) of 2a was obtained with low specific activity.

N-Aminooxyacetyl-N'-isonicotinoyl-carboxyl-^{14}C-hydrazine (1a)

2.34 g (3.84 mmoles, 45.2 mCi) of 2a were stirred in 24 ml of 4N HBr in acetic acid for 50 minutes. Then 50 ml of ether were added. The precipitated crystals were separated by filtration and washed with 2x8 ml of ether. The crystals were dissolved in 25 ml of methanol which contained 0.01 moles of HBr, treated with Norite, filtered and 40 ml of ether was added to the solution. Pale yellow crystals precipitated which were separated by filtration and washed with ether. Weight: 1.18 g (3.19 mmoles, 30.0 mCi). Yield: 83%, mp 166-168°C. The purity of 1a was checked by TLC, using 1 as standard (solvent:benzene:ethanol 1:1).

Bromoacetic-1-^{14}C acid

Acetic-1-^{14}C acid was prepared from 410 mg (5 mmoles, 50.0 mCi) of dried sodium acetate-1-^{14}C by means of dry HCl obtained from 330 mg (5.5 mmoles) of NaCl. The acetic-1-^{14}C acid was distilled into a reaction flask on a vacuum line at 140°C. Weight: 279 mg (4.7 mmoles); 0.27 ml (0.85 g, 5.1 mmoles) of bromine and a catalytical amount of red phosphorus were added. The mixture was slowly heated to 110-120°C (about 30 minutes) and kept at this temperature for one hour. The solution became yellow and after cooling, it solidified. The solid was dissolved in 3 ml of carbon tetrachloride, treated with Norite, filtered, the solvent was removed and 577 mg (4.15 mmoles, 41.5 mCi) of bromoacetic-1-^{14}C acid were obtained as white crystals. Yield: 83%.

Benzyloxycarbonyl-aminooxyacetic-1-^{14}C acid (5)

577 mg (4.15 mmoles, 41.5 mCi) of bromoacetic-1-^{14}C acid and 570 mg (10 mmoles) of KOH were dissolved in 10 ml of 80% ethanol

and 0.72 g of 4 was added. After heating, the solvent became clear. It was refluxed for 20 minutes, then the solvent was removed under reduced pressure. The residue was treated with 20 ml of water, the mixture was acidified to pH 3, then extracted with 3x10 ml of $CHCl_3$. The extracts were washed with 10 ml of 25% NaCl solution, dried with $MgSO_4$ and the solvent was removed. The residue is a colourless oil. Weight: 820 mg, yield: 77%. Total activity: 38.4 mCi.

N-Benzyloxycarbonyl-aminooxyacetic-1-^{14}C acid pentachlorophenyl ester (6)

To the solution of 820 mg (3.65 mmoles, 38.4 mCi) of 5 in 15 ml of dioxane, 980 mg (3.82 mmoles) of pentachlorophenol and 850 mg (4.1 mg) of DCC were added. The mixture was stirred at room temperature for 3.5 hours. The precipitated dicyclohexyl urea was removed by filtration and washed with 3x5 ml of dioxane, then the dioxane was evaporated under reduced pressure and the residue was recrystallized from 12 ml of ethanol-dioxane (85:15) mixture. Weight: 200 mg (12%).* By adding inactive 6 to the mother liquor we obtained another 300 mg of 6 with low specific activity.

N-Aminooxyacetyl-1-^{14}C-N'-isonicotinoyl-hydrazine dihydrobromide (1b)

150 mg (1.08 mmoles) of isonicotinic hydrazide were added to 503 mg (1.06 mmoles) of 6 dissolved in 10 ml of absolute dimethylformamide and the mixture was kept at room temperature overnight. The solvent was removed and the solid residue was dissolved in 2 ml of acetic acid and 5 ml of 4N HBr in acetic acid, stirred for 40 minutes, then 15 ml of ether were added. A solid mass precipitated, the solvent was decanted, the residue was dissolved in 10 ml of methanol and 0.2 ml of 4N HBr in acetic acid, and precipi-

* In this case the quality of DCC was not satisfactory. The yields of inactive runs were about 40-50%.

tated with 10 ml of ether. Weight: 260 mg (3.1 mCi, 63%; mp 164-166°C. The purity was checked by TLC (see 1a).

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