JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 395–400.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.678

Research Article

Synthesis of ^{14}C-labelled myosmine, [2′-^{14}C] -3-(1-pyrrolin-2-yl)pyridine

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Summary

^{14}C-Labelled myosmine ([2′-^{14}C]-3-(1-pyrrolin-2-yl)pyridine) was synthesized for autoradiography studies starting from [carboxyl-^{14}C]-nicotinic acid by initial esterification of the latter in the presence of 1,1,1-triethoxyethane. Without any purification the ethyl nicotinate formed was directly reacted with *N*-vinyl-2-pyrrolidinone in the presence of sodium hydride, yielding ^{14}C-labelled myosmine. The product was purified by silica gel column chromatography. The radiochemical yield was 15% and the specific activity 55.2 mCi/mmol.

Key Words: [Carboxyl-^{14}C]-nicotinic acid; [2′-^{14}C]-3-(1-pyrrolin-2-yl)pyridine; Synthesis; ^{14}C labelling; One-pot reaction

Introduction

The nicotinoid myosmine is obviously not limited to tobacco because of its widespread occurrence in various edibles and staple foods.[1] *N*-nitrosation of myosmine yields DNA adducts, either directly or via *N*-nitrosonornicotine (NNN), which are associated with oesophageal carcinogenesis in rats.[2,3] In the context of extensive research regarding the biodistribution, activation and metabolism of myosmine the use of

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Received 6 August 2002
Revised 26 September 2002
Accepted 10 October 2002

radiolabelled isotopes is indicated. For whole body autoradiography on rats using an image analyzer, commercially available ^{3}H-myosmine is not appropriate. Image plates have a low sensitivity for ^{3}H and require direct exposure of the slices leading to artefacts and memory effects due to contamination. These problems could be minimized using ^{14}C-labelled myosmine allowing the use of a protective layer between the slice and the plates.

Only one practicable synthesis for ^{14}C-myosmine starting from commercially available [carboxyl-^{14}C]-nicotinic acid has been described by Hu and Bondinell.[4] The main disadvantage is the necessity of removing the intermediate methyl ester by fractional distillation. Therefore an existing method for unlabelled myosmine synthesis, described by Brandänge and Lindblom, was modified.[5] Basically, [carboxyl-^{14}C]-nicotinic acid was esterified[6] to yield ^{14}C-ethyl nicotinate, followed by reaction with *N*-vinyl-2-pyrrolidinone. Using toluene as solvent for the esterification, all further steps could be carried out as a one-pot reaction without purification of the ester. Under acidic conditions the intermediate rearranges under loss of CO_2 to ^{14}C-labelled myosmine. Purification was performed by silica gel column chromatography instead of distillation as suggested by Brandänge and Lindblom but not practicable for micro mass reactions.

Results and discussion

^{14}C-Labelled myosmine was prepared by the route shown in Figure 1.

The radiochemical purity of ^{14}C-labelled myosmine, determined by radio-HPLC was 96.8%. 130 μCi of ^{14}C-myosmine was finally obtained with a specific activity of 55.2 mCi/mmol and a radiochemical yield of 15%. To elucidate the pathway of reaction, preliminary tracer experiments using identical educts (~99% unlabelled nicotinic acid with ~1% [carboxyl-^{14}C]-nicotinic acid instead of 100% [carboxyl-^{14}C]-nicotinic acid), showed yields of 53%. LC/MS measurements of the masses *m/e* 147 (MH^+) and 149 (MH^+) for unlabelled and ^{14}C-labelled myosmine, respectively, indicate their existence as cited in the literature.[7] Identical HPLC-retention times of unlabelled myosmine standard and ^{14}C-myosmine under co-chromatographic conditions indicate a successful synthesis.

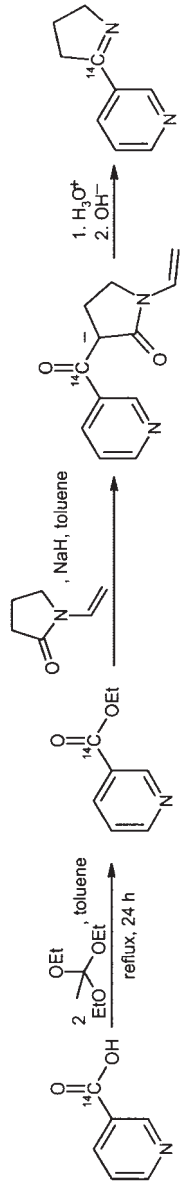

Figure 1. Synthesis of ^{14}C-labelled myosmine

Experimental

General

[Carboxyl-^{14}C]-nicotinic acid (specific activity: 50 mCi/mmol; radiochemical purity: 97.6%) was obtained from Hartmann Analytic (Braunschweig, Germany). l, l, l-triethoxyethane, *N*-vinyl-2-pyrrolidinone and sodium hydride were obtained from Sigma-Aldrich (Deisenhofen, Germany). All other solvents and chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

Synthesis of ^{14}C-labelled myosmine

A mixture of [carboxyl-^{14}C]-nicotinic acid (2.5 mg, 0.02 mmol, 900 µCi) and 1, l, l-triethoxyethane (7.3 µl, 0.04 mmol) in 200 µl dry toluene was refluxed for 16 h (130°C). After cooling, *N*-vinyl-2-pyrrolidinone (4 µl, 0.037 mmol) in 10 µl dry toluene and a solution of sodium hydride (0.26 mmol, 10 mg of a 60% mineral oil dispersion) in 150 µl dry toluene were added dropwise. After 2.5 h of refluxing (130°C) the solution was treated with 90 µl of a mixture of H_2O/concentrated HCl (2:1, v/v) and after stirring for 15 min the solution adjusted with 3 M NaOH to pH 4. After separation from the toluene phase, the water phase was extracted three times with 750 µl $CHCl_3$/MeOH (3:2, v/v) and dried over anhydrous Na_2SO_4. After concentration, the residue was treated with 300 µl concentrated HCl and refluxed for 16 h. The cooled reaction mixture was adjusted to pH 11 and extracted three times with 750 µl $CHCl_3$. The concentrated solution was subjected to silica gel column chromatography (Isolute SPE Columns, 5 g, 25 ml; IST, Grenzach-Wyhlen, Germany) using $CHCl_3$/MeOH (9: 1, v/v) for elution.

Identification of the product

The product was identified by LC/MS as well as by radio-HPLC combined with UV. Mass spectrometric measurements were performed on a Finnigan LCQ (Thermo Finnigan MAT GmbH, Bremen, Germany).

HPLC was performed on a Merck-Hitachi HPLC-UV system combined with a radioactivity monitor (LB 506 C, Berthold, Bad Wildbad, Germany) using a LiChrospher 100 RP-18e 125 × 3 mm 5 µ column (Merck) at a flow rate of 0.7 ml/min with a gradient using

acetonitrile and 15 mM ammonium acetate buffer. After an initial time of 3 min at 0% CH_3CN/100% buffer, CH_3CN was linearly increased over 8 min up to 22% and held for another 16 min. Within 3 min the eluent was changed to 60% CH_3CN/40% buffer, held for 2 min, and returned to 0% CH_3CN/100% buffer within 2 min. The column was reconditioned for at least 10 min.

Unlabelled myosmine standard was synthesised by the method of Brandänge and Lindblom.[5] An HPLC-UV chromatogram was recorded for comparison of the retention times of labelled and unlabelled myosmine (retention time 16.52 min).

Radiochemical purity

The radiochemical purity was determined by radio-HPLC under the same conditions as described above.

Measurement of radioactivity

Aliquots of the ^{14}C-labelled myosmine were mixed with 10 ml of Omni Szintisol scintillator (Merck). The radioactivity was measured in a TriCarb 2300 Liquid Scintillation Analyser (Packard, Frankfurt/Main, Germany).

Specific radioactivity

Determination of the specific radioactivity was performed by LC/MS. Peak areas representing known unlabelled myosmine concentrations were compared with peaks from ^{14}C-myosmine. Identical ^{14}C-myosmine aliquots from LC/MS runs were measured by scintillation counting and balanced with unlabelled myosmine yielding the specific radioactivity.

Conclusion

A one-pot reaction is described for the synthesis of ^{14}C-labelled myosmine. Quantitative esterification of ^{14}C-nicotinic acid in toluene without purification of the ester followed by condensation with *N*-vinyl-2-pyrrolidinone in the same solvent leads to ^{14}C-myosmine.

Acknowledgements

We thank Florian Huber and Hans-Werner Crößmann from Merck KGaA, Institute of Pharmacokinetics and Metabolism, Grafing, Germany for their support and encouragement.

References

1. Tyroller S, Zwickenpflug W, Richter E. *J Agric Food Chem* 2002; **50**: 4909–4915.
2. Wilp J, Zwickenpflug W, Richter E. *Food Chem Toxicol* 2002; **40**: 1223–1228.
3. Zwickenpflug W. *J Agric Food Chem* 2000; **48**: 392–394.
4. Hu MW, Bondinell WE, Hoffmann D. *J Labelled Compd* 1974; **10**: 79–88.
5. Brandänge S, Lindblom L. *Acta Chem Scand* 1976; **30**: 39.
6. Trujillo JI, Gopalan AS. *Tetrahedron Lett* 1993; **34**: 7355–7358.
7. Glenn DF, Edwards WB. *J Org Chem* 1978; **43**: 2860–2870.

