

Synthesis of 5,5-Dimethyl-1-pyrroline-*N*-oxide-2-¹⁴C

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SUMMARY

5,5-Dimethyl-1-pyrroline-*N*-oxide-2-¹⁴C arrived via coupling of 2-nitropropane with labelled acrylate instead of acrolein. The new alternate step is safer, more convenient and efficient for ¹⁴C-labelling. The radioactive spin trap has a sp. activity of 13 mCi/mmol, >98% purity, and shows no degradation after two months at -80°C in neat form.

Keywords: Methyl acrylate-1-¹⁴C; 5,5-dimethyl-1-pyrroline-*N*-oxide-2-¹⁴C.

INTRODUCTION

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), said to be the most widely used nitron spin trap for oxygen-centered free radicals, has shown some promising pharmacological properties such as reversal of some aging effects, reduction of septic shock fatality, cardioprotection and vasodilatation in ischemia/reperfusion models of myocardial infarction¹. This article describes the synthesis of DMPO-2-¹⁴C which is needed for further disposition and metabolic studies.

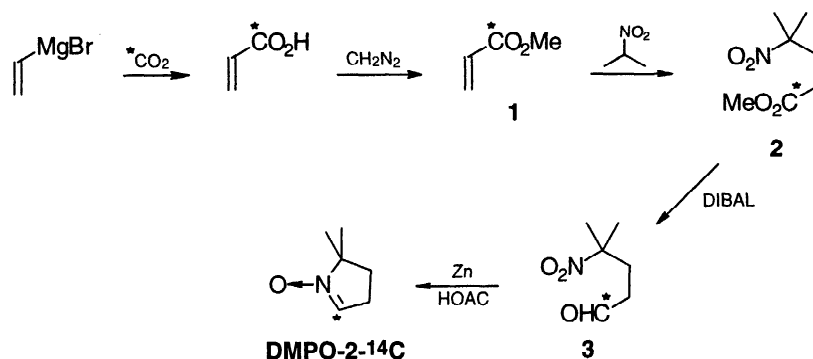
RESULTS AND DISCUSSION

The usual route via coupling of 2-nitropropane^{2,3} and acrolein was considered unsuitable. Acrolein-1-¹⁴C could be prepared from labelled allyl alcohol or acrylate⁴.

However, its isolation and purification were quite difficult due to a combination of the very small quantity, its low boiling point, high solubility in water, and a propensity to polymerize under the conditions.

A new method, which avoided acrolein altogether, was effected as follows (Scheme I). Michael addition of acrylate **1** to 2-nitropropane gave pentanoate **2** in 70 - 92% yield. Reduction of **2** with DIBAL afforded pentanal **3** cleanly (80 - 87%). Reductive cyclization of pentanal **3** with zinc in acetic acid as usual produced the desired DMPO-2-¹⁴C (20 - 51% yield, sp. activity: 13 mCi/mmmole; >98% pure by HPLC). Acrylate **1**, in turn, arrived from carboxylation of vinylmagnesium bromide with Ba¹⁴CO₃ and concentrated H₂SO₄, and subsequent esterification⁵.

Scheme I



Repeated fractionate distillation² produced unlabelled DMPO in high purity suitable for spin trapping. In the case herein, flash column chromatography proved more satisfactory due to the minute quantity. HPLC analysis of the radioactive spin trap after a two-month storage as neat liquid at -80°C detected no sign of degradation.

The alternate acrylate-nitropropane coupling is not only more efficient for ¹⁴C-labeling but also preferable for producing unlabeled DMPO, especially for

commercial purpose. Unlike the existing method where the acrolein-nitropropane coupling gives at best moderate yield from distillation of a complex crude mixture which explodes when overheated, the acrylate-nitropropane addition is clean and high yielding.

EXPERIMENTAL

General. Reactions involving moisture-sensitive reagents were carried out under anhydrous conditions, which signify drying glassware in ovens at 130°C and cooling under N₂, freshly distilling solvents over a desiccating agent under N₂ or using purchased anhydrous-grade materials, and blanketing the reaction mixture with N₂. The structures of all products during developmental cold runs were confirmed via the usual spectroscopic data and physical properties. Melting points were taken with a Mel-Temp II and uncorrected. IR spectra were recorded on a Beckman Acculab 4 or Mattson Galaxy 3000 FT-IR. ¹H and ¹³C NMR were obtained with either a Bruker AC-250 NMR or Varian Unity 300 NMR. GC-MS were recorded with a Hewlett Packard 5995 using the electron impact method unless specified otherwise.

Methyl acrylate-1-¹⁴C. To a THF solution of vinylmagnesium bromide (14 mmole in 20 mL) cooled to -178°C with liquid nitrogen and evacuated to <5 Torr was introduced ¹⁴CO₂ generated from adding 30 mL conc. H₂SO₄ to 100 mCi of Ba¹⁴CO₃ (43.5 mCi/mmole) and 0.985 g cold BaCO₃. The reaction mixture was stirred at -25°C for another 2 hr, diluted with 6 mL THF containing 0.020 g hydroquinone, quenched with 20 mL water, and concentrated under vacuum at 20°C. The resulting aqueous layer was acidified with diluted H₂SO₄ and extracted with Et₂O containing 0.1% hydroquinone. The combined ether layers were dried over MgSO₄, filtered, and then distilled through a 20 cm Vigreux column at bath temperature 35 - 50°C.

To the above crude acrylic acid solution cooled in an ice bath was added an ethereal solution of diazomethane until the yellow color persisted. Most of the solvent

was then removed by distillation through a 20 cm Vigreux column at 35 - 40°C to afford the crude product (24 mCi; 13 mCi/mmole).

Methyl 4-methyl-4-nitropentanoate-1-¹⁴C. To the crude methyl acrylate (24 mCi) in 3 mL THF was added 0.477 g 2-nitropropane (5.4 mmole) and then 0.185 g K₂CO₃ (1.3 mmole). The resulting suspension was stirred at 20°C overnight, treated with Celite, filtered, and then concentrated in a rotorevaporator. Subsequent column chromatography of the crude (silica gel, 4:1 Hex/Et₂O) afforded 16.8 mCi of product (70% yield).

4-Methyl-4-nitropentanal-1-¹⁴C. To the pentanoate obtained above (16.8 mCi) in 20 mL CH₂Cl₂ at -78°C was added 1.14 mmole of DIBAL slowly over 20 min. The resulting mixture was stirred at -75°C for another 3 hr, quenched with diluted HCl. Aqueous layer was extracted with Et₂O. Combined organic layer was washed with sat'd NaCl, dried with MgSO₄, concentrated under vacuum to give the crude product (14.6 mCi; 87% yield).

5,5-Dimethyl-1-pyrroline-N-oxide-2-¹⁴C. To 9 mCi of the pentanal obtained above in 3 mL 95% ethanol at 0°C was added all at once 0.092 g Zn dust followed by 0.158 g glacial acetic acid slowly under vigorous stirring. The resulting mixture was stirred for 5 hr, let stand in a refrigerator for 1 day, filtered to remove the salts, concentrated and dried under vacuum. The residue was redissolved in CH₂Cl₂, washed with water, dried over MgSO₄, and concentrated again to give the crude which, following flash column chromatography (silica gel, 15:1 CH₂Cl₂/MeOH), gave 15.8 mg of the product (1.8 mCi; 20% yield).

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