^{13}C-LABELLING OF N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

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SUMMARY

N-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP) is converted to a potent neurotoxin by the enzyme monoamine oxidase. Two different ^{13}C-derivatives of the compound have been prepared for detailed n.m.r. studies of the enzyme reaction. Mass spectral analysis of the labelled compounds showed that incorporation of ^{13}C exceeded 90% in each case.

Key words: MPTP, ^{13}C-MPTP, labelled MPTP

INTRODUCTION

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a compound inadvertently produced in attempts by drug users to synthesise analogues of heroin (1,2). This heterocyclic amine serves as a good substrate for the enzyme monoamine oxidase type B (3) which converts it to a potent neurotoxic agent that causes marked degeneration of the substantia nigra, leading to a pathology indistinguishable from Parkinson's disease. It is now believed that the actual toxin is probably the fully oxidised quaternary derivative N-methyl-4-phenyl pyridinium that arises via an intermediate N-methyl-4-phenyl dihydropyridine (4).

The mechanism of the activation of MPTP by monoamine oxidase is not completely understood. A detailed explanation of the process may be valuable not only for providing insights into the mechanism of action of other neurotoxins,

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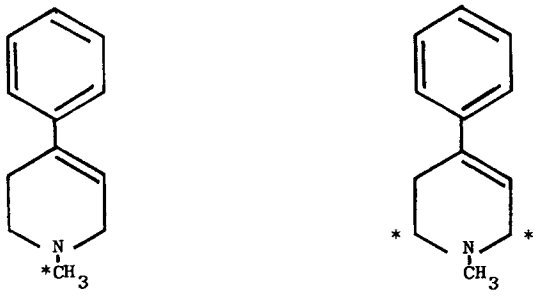
known or suspected, but may also generate new information about the enzyme itself, since heterocyclic amines are not usually oxidised by MAO and tertiary amines are very poor substrates. It was therefore decided to prepare two ^{13}C-labelled versions of MPTP in order to study its transformation by MAO using ^{13}C-nmr techniques. This paper describes the synthesis of (i) N-[^{13}C]-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine and (ii) the compound in which carbon atoms 2 and 6 of the hetero ring of MPTP are labelled with carbon 13.



MPTP, showing positions of ^{13}C-atoms (*)

Materials

4-Phenyl-1,2,3,6-tetrahydropyridine hydrochloride and α-methyl styrene were purchased from Aldrich Chemical Co., Gillingham, U.K. ^{13}C-formaldehyde, 27% w/w in water, isotopic purity 90-99%, was obtained from Amersham International, Amersham U.K.

Experimental

N-[^{13}C]-methyl-4-phenyl-1,2,3,6 tetrahydro pyridine

4-Phenyl-1,2,3,6-tetrahydropyridine was liberated from its HCl salt by dissolving in water, adjustment of the pH to approximately 10 and extraction of the resulting base into ether. The dried extract was evaporated and the base used without further purification. To 1.1 g of this base was added 1 ml of the ^{13}C-formaldehyde solution (ca. 20% excess), followed by 5 ml of 90% formic acid. The solution was heated for 6 hours on a steam bath, after which it was evaporated in vacuo to remove excess formic acid. The residue was

dissolved in water, the pH was adjusted to about 10 and the mixture was extracted with ether. The ether extract was washed with water and dried ($MgSO_4$). The volume of ether was adjusted to 20 ml and 4 ml of iso-propanol was added, followed by a slight excess of a solution of anhydrous HCl in ether. The resulting HCl salt (1.25 g) was collected and crystallised by dissolving in boiling iso-propanol and adding hot ether/acetone (4:1). On cooling, 1.05 g (73%) of the product, m.p. 251-252°C, was obtained. The melting point was unchanged on admixture with an authentic sample of MPTP hydrochloride.

N-methyl-4-phenyl-2,6 [^{13}C]-1,2,3,6-tetrahydropyridine

This was prepared essentially as described by Schmiddle and Mansfield (5). Methylamine hydrochloride (560 mg, 8.3 mmol) and [^{13}C]formaldehyde (500 mg, 16.7 mmoles, as a 27% solution in water) in a 5 ml flask were stirred until homogenous (5 min). α-Methyl styrene (1 g, 8 mmol) was added and the mixture was stirred for 1 hour on a water bath at 90°-95°C. The mixture was cooled and stirred in an ice bath during the dropwise addition of concentrated H_2SO_4 (0.5 ml, 920 mgm, 9.4 mmol). The mixture was then stirred in a water bath at 90-95°C for 2¼ hours, cooled, diluted to 20 ml with water and washed with ether. The aqueous phase was brought to pH 10 (NaOH) and extracted with ether (2 x 25 ml). The extracts were washed with water, dried ($MgSO_4$) and evaporated, leaving 490 mgm (35%) of a pale brown gum. This was dissolved in ether (30 ml) and left for 1 hour before filtering to remove a slight turbidity. The ether was removed by evaporation and the residue (470 mg) was stirred in dry acetone (30 ml) during dropwise addition of a slight excess of dry HCl gas in ether. The suspension was treated with a small crystal of MPTP hydrochloride and left for 3 hours. The product (200 mg, m.p. about 230°C) was collected by filtration and crystallised twice as described above for the ^{13}C-methyl compound. Yield, 80 mg, m.p. 248-250°C, raised to 251° on mixing with authentic MPTP.

Each of the compounds was subjected to mass spectral analysis and compared with the mass spectrum of unlabelled MPTP. Results were as

follows:- MPTP, molecular ion peak of 173 mass units, abundance 100; M + 1 peak, abundance 13.17. [Methyl-^{13}C]MPTP, molecular ion peak of 174 mass units, abundance 100; M + 1 peak, abundance 11.18. 2,6 [^{13}C]MPTP, molecular ion peak 175 mass units, abundance 100; M + 1 peak, abundance 11.28. These data indicate an incorporation of ^{13}C of more than 90% in each case. In the case of the reductive methylation this confirms that all of the carbon of the methyl group is derived from the formaldehyde (6). The other synthesis inevitably produces labelling at 2 positions because formaldehyde is used in formation of carbon atoms 2 and 6 of the heterocyclic ring (5). Either one of these could be a point of attack by MAO, which removes a proton from the α-carbon atom of its amine substrates, though it is more likely that attack will occur at C-6 because the protons at this position will be more acidic than at C-2. Gessner et al. (7) using deuterated MPTP suggested that attack at C-6 of the heterocyclic ring is the rate limiting step in the enzymic reaction. It is also known that open chain β,γ-unsaturated amines, cinnamylamines, are excellent substrates for MAO B (8).

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