SYNTHESIS OF ^{14}C-LABELLED INHIBITORS OF MONOAMINE OXIDASE

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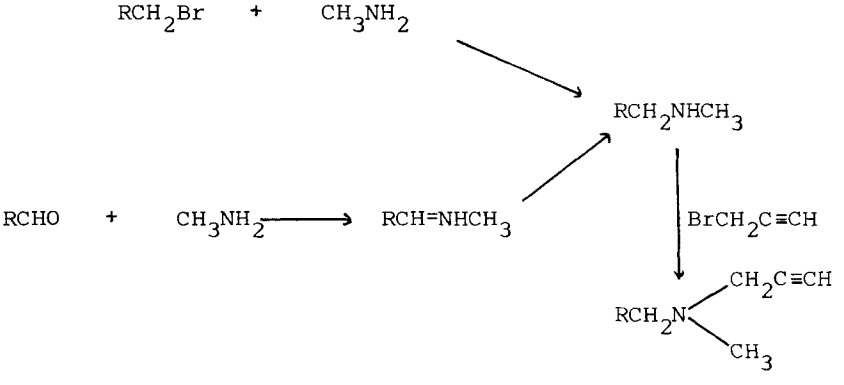
SUMMARY

The monoamine oxidase inhibitors pargyline and clorgyline have been synthesised by methylation of the appropriate secondary propargylamines, using [^{14}C]dimethyl sulphate. This simple method should be generally applicable to other compounds of this class,

Key words: [^{14}C] pargyline, [^{14}C] clorgyline, monoamine oxidase inhibitors.

A number of aralkylamines substituted on the N atom with a propargyl group are irreversible inhibitors of monoamine oxidase (MAO) (1). Such derivatives are widely used in studies of the enzyme. These compounds include N-benzyl-N-methyl prop-2-ynylamine (pargyline), N-[3-(2,4-dichlorophenoxy)propyl]-N-methyl prop-2-ynylamine (clorgyline) and N-1-(phenylisopropyl)-N-methyl prop-2-ynylamine (deprenil). The last two named are particularly interesting because they are selective inhibitors respectively of the two forms, A and B, of MAO (2,3). This type of inhibitor is most usually synthesised by one or other of the following routes:-



0362-4803/81/101545-04$01.00

Received December 8, 1980
Revised January 16, 1981

It is possible to reverse the order of introduction of the methyl and propargyl groups by reacting the appropriate primary aralkylamine with propargyl halide, followed by N-methylation. This route usually gives lower overall yields because of the formation of by-products in the first step, but has the distinct advantage that in a radioactive synthesis the label can be introduced at the last stage. There are a number of ways of inserting [^{14}C]-methyl into these compounds. Use of methyl halides poses the problem of reagent volatility as does reductive alkylation with formaldehyde, which could also lead to reduction of the triple bond in the propargyl group. It was decided that [^{14}C] dimethyl sulphate would be a useful reagent despite the fact that the specific activity of the product would be only half that of the methylating agent. We have used this reagent to label pargyline and clorgyline.

Our studies with these two inhibitors, labelled in this way, show that the label is incorporated into the enzyme on reaction with MAO (4). Hence N-methylation seems to be a convenient means of labelling these compounds. A further advantage of this method is that it should be generally applicable to other MAO inhibitors of this class, of which a large number are known. Although we have used ^{14}C as label, the commercially available [^{3}H] dimethyl sulphate could be used should a higher specific activity be desired.

N-benzyl-N-[^{14}C]methyl prop-2-ynylamine. N-benzyl propargylamine was prepared using a published procedure (5). It was purified by crystallisation of its hydrochloride salt. The free base was liberated from this salt and distilled under reduced pressure. It had a boiling point of 54°C/2 torr.

[^{14}C]Dimethyl sulphate, specific activity 64 μCi/μmol, was obtained from The Radiochemical Centre, Amersham, England.

To [^{14}C] dimethyl sulphate (50μCi) was added 270μg (approx. 2μmoles) of N-benzyl propargylamine in 0.5ml of ether freshly distilled from sodium. The reaction mixture was sealed and left for 16 hours at room temperature, after which the solvent was carefully evaporated at room temperature. The reaction mixture was dissolved

in a small quantity of dry redistilled methanol and applied as a streak to four thin layer chromatography plates (20x5cm) coated with silica gel. Chromatography in n-butanol, acetic acid, water (4:1:1) for a distance of 18cm separated excess starting amine from the product which was located by means of a Packard radiochromatogram scanner. The appropriate area of silica gel was removed and the radioactivity eluted with methanol. A drop of methanolic HCl was added to the extract and the solvent was evaporated . The residue was dissolved in 0.5ml of water. At this stage the product was contaminated with [^{14}C]methyl hydrogen sulphate which was removed as follows. The aqueous solution was applied to a column of QAE-Sephadex, Cl^- form (Pharmacia, Sweden), (0.24x2.8cm) in water and the eluate was collected. The column was washed with 1.5 ml of water and the washings were added to the first eluate. The material thus obtained from the column was isographic with authentic pargyline and produced a single radioactive spot on paper electrophoresis at pH 5 in acetate buffer, This radioactivity co-migrated with pargyline. The yield was 12μCi (48% of theoretical based on [^{14}C]dimethyl sulphate).

N-[(2,4-dichlorophenoxy)propyl]-N-[^{14}C]methyl prop-2-ynyl-amine. 1-bromo-3-(2,4-dichlorophenoxy)propane was prepared as described by Gagnon et al. (6). This was converted to the 1-amino derivative using the Gabriel synthesis. N-propargylation of this product was exactly as described for N-benzyl propargylamine. It had a boiling point of 129°C/0.8 torr. The labelling with ^{14}C was as described above, using 50μCi of [^{14}C]dimethyl sulphate and 450μg of amine. Purification of the product was as follows. The reaction mixture was applied as a streak to three separate strips of Whatman 3MM paper (28x7.5cm) previously soaked in 0.05M Na/K phosphate buffer, pH 7.1. Prior to application of the sample, which was placed approximately 8cm from the anodic end, the surplus moisture was removed by blotting between two filter papers. The sample was then subjected to electrophoresis in the buffer mentioned above for 3 hours at a constant current of 25mA. The strips were dried in air at room temperature and radioactivity was located as previously described. Two bands of radioactivity were found, one which had migrated 3cm towards the anode, [^{14}C]methyl hydrogen sulphate,

another which had moved less than 0.5cm towards the cathode. This latter band was the desired product, behaving as authentic clorgyline on chromatography and on electrophoresis at pH 5 in acetate buffer. The excess of starting amine was found to have travelled about 4cm towards the cathode, as would be expected, since it has a higher pK_a than clorgyline, which itself has a pK_a below 7. (4). After drying in air the product was eluted from the paper with dry methanol. The yield was 10μCi (40% of theoretical based on [^{14}C]-dimethyl sulphate).

These syntheses were also carried out twice on the same scale using unlabelled, freshly distilled dimethyl sulphate, with similar results. A repeat of the labelling synthesis gave poor yields.

Recently, Parkinson and Callingham have shown that N-3-[^{3}H]-benzyl prop-2-ynylamine can be methylated with unlabelled dimethyl sulphate to give [^{3}H]pargyline (7). The use of [^{14}C]formaldehyde to label the methylene carbon of the propargyl group of both clorgyline and deprenil has been described by Fowler (8).

Acknowledgements: I wish to thank Ms J. Lawson for excellent technical assistance.

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