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SYNTHESIS OF ^{3}H- AND ^{14}C-LABELED TRACERS FOR STUDIES OF FUNCTIONAL MONOAMINE OXIDASE ACTIVITY

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SUMMARY

The ^{3}H-labeling of clorgyline, L-deprenyl, N,N-dimethylphenethyl amine and N-methylphenethyl amine as well as the ^{14}C-labeling of clorgyline and L-deprenyl are described. Labeling was accomplished by N-alkylation of the free base of the corresponding desmethyl compound, using ^{3}H-methyl iodide, or by a reductive alkylation using ^{14}C-formaldehyde. The crude products were purified by semipreparative HPLC. The total radiochemical yield was 31-65% based on ^{3}H-methyl iodide and 60% based on ^{14}C-formaldehyde. The radiochemical purity was greater than 99 %.

Key Words: ^{3}H-labeling, ^{14}C-labeling, clorgyline, L-deprenyl, N,N-dimethylphenethyl amine, N-methylphenethyl amine

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INTRODUCTION

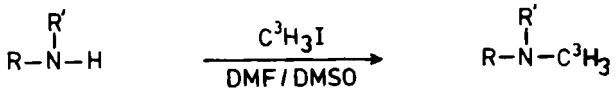
Monoamine oxidase (MAO, EC 1.4.3.4.), which catalyzes the oxidative deamination of neurotransmitter amines, has been subdivided into two types: MAO A and B. The A form is selectively and irreversibly inhibited by clorgyline and the B form by L-deprenyl. Both clorgyline and L-deprenyl act as suicide inhibitors, deactivating the enzyme by covalent bonding to its active site, and these carbon-11 labeled inhibitors have been used to study MAO in vivo in animals [1-3] and in humans.[4]

Another approach for studies of functional MAO activity is to use the substrates for MAO. Dimethylphenethyl amine (DMPA)[5,5a] and methylphenethyl amine (MPA) are two such compounds which, when labeled, might be used for measurements of MAO activity. They are deaminated by MAO to labeled metabolites, some of which are trapped in the brain.

In order to study MAO distribution in vivo in mice and in vitro on post mortem human brain slices by use of autoradiographic techniques, these compounds were labeled with ^{3}H. Clorgyline and L-deprenyl labeled with ^{14}C were also prepared.

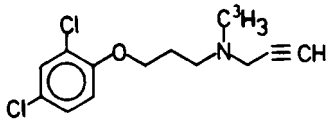
RESULTS AND DISCUSSION

The radioactive label was incorporated by N-alkylation of the free base of the corresponding desmethyl compound with ^{3}H-methyl iodide, by the reactions shown in Scheme 1. Clorgyline (I), L-deprenyl (II), DMPA (III) and MPA (IV) were all labeled in the N-methyl group (Scheme 2).

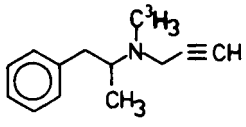


R,R'= H, alkyl

Scheme 1



(I)



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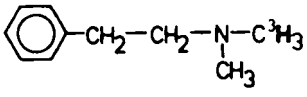
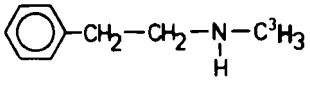


III



IV

Scheme 2

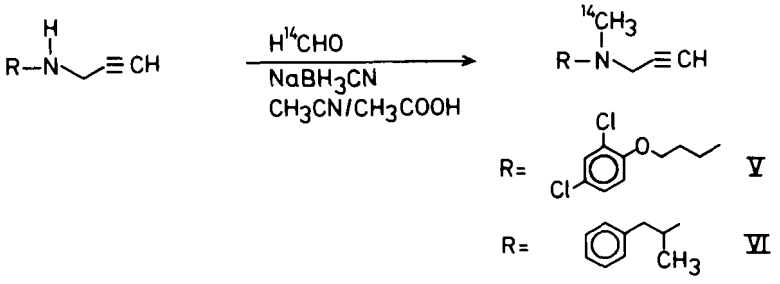
The incorporation of ^{3}H-methyl iodide was accomplished within 5 min in a DMF/DMSO mixture at reaction temperatures ranging between 50-80 °C (Table 1). Compounds I, II, III and IV have previously been labeled with carbon-11 by use of ^{11}C-methyl iodide.[5,6] A statistical method Simplex,[7] was applied to optimize the radiochemical yield of the incorporation of ^{11}C-methyl iodide by varying reaction parameters such as solvent composition, temperature and substrate concentration.[8] An analytical reversed-phase column was used in the HPLC purification. The labeled compounds eluted with the same retention times as standard reference samples. Incorporation of ^{3}H-methyl iodide prior to evaporation ranged between 80-98% for I, II, III and IV. The radiochemical yields given in Table 1

reflect subsequent losses during the work-up procedure. Total radiochemical yields were on the order of 31-65 %, based on ^{3}H-methyl iodide, and the radiochemical purity was better than 99% after HPLC-purification. The experimental details are summarized in Table 1. The specific radioactivities obtained were essentially the same as that of the starting ^{3}H-methyl iodide used (85 Ci/mmol).

Table 1. Experimental conditions and results of tritium labeling.

Name	Solvent mixture (DMF/DMSO)	Temp. (^{o}C)	Substrate conc. (mg)	Reaction time (min)	Radiochem. yield (%)
Clorgyline	85/15	50	4.0	5	65
L-Deprenyl	75/25	70	7.2	5	38
Dimethylphenethyl amine	84/16	70	6.0	5	59
Methylphenethyl amine	80/20	80	4.0	5	31

Clorgyline and L-deprenyl were also labeled in the N-methyl group with ^{14}C by the reductive methylation of the corresponding desmethyl compound with ^{14}C-formaldehyde and sodium cyanoborohydride (Scheme 3).[9,10] The radiochemical yields were 60% and the radiochemical purity, as determined by liquid scintillation counting of sections of thin layer chromatographs, was greater than 99%. Comparison of the HPLC UV chromatographs with calibration standards for clorgyline and deprenyl was used to determine that the specific activities of the ^{14}C products were 50 mCi/mmol.



Scheme 3

EXPERIMENTAL

General. ^{3}H-Methyl iodide was purchased from Amersham (10 mCi, 1mL toluene solution, 85 Ci/mmol). ^{14}C-Formaldehyde was purchased from New England Nuclear (0.5 mCi, 53 mCi/mmol). The syntheses of desmethyl-L-deprenyl and desmethylclorgyline have been described elsewhere.[11] β-Phenethyl amine, N-methylphenethyl amine and N,N-dimethylphenethyl amine were all purchased from Aldrich. LC was performed on a Hewlett-Packard 1084 B chromatograph equipped with a 250 x 4.6 mm Spherisorb C-18 10 μm column, a variable wavelength detector in series with an LKB Wallac 1208 Betacord or a Ramona scintillator flow detector. Aqueous 0.005 M ammonium formate, pH 3.5 (A), and methanol (B) were used as the mobile phase. The LC programs used were the following: (compound I): flow 3.0 mL/min, UV 262 nm, column temperature 60°C, LC time 0-12.0 min, B=35%; time 12.0-13.0 min, gradient B 35-80%; time 13.0-21.0 min, B=80%; time 21.0-22.0 min,gradient B 80-35%. (Compound II): flow 3.0 mL/min, UV 258 nm, column temperature 60°C, LC time 0-11.0 min, B=15%; time 11.0-12.0 min, gradient B 15-80%; time 12.0-19.0 min, B=80%; time 19.0-20.0 min, gradient B 80-15%. (Compounds III and IV): flow 3.0 mL/min, UV 254 nm, column temperature 60°C, LC time 0-2.0 min, B=5%; time 2.0-6.0 min, gradient B 5-40%; time

6.0-7.0 min, gradient B 40-80%; time 7.0-12.0 min, B=80%; time 12.0-13.0 min, gradient B 80-5%. Radioactivity in collected samples was measured with an LKB Wallac 1214 Rackbeta liquid scintillation counter. N-(Methyl-^{14}C)-labeled clorgyline and L-deprenyl were isolated using a 10 x 250 mm Sperisorb S5 ODS 2 LC column and a solvent mixture of methanol and aqueous 0.05 M ammonium formate (80:20 for clorgyline and 70:30 for L-deprenyl) with UV detection at 254 nm.

Alkylation procedure (I, II, III, and IV, Scheme 1, 2 and details in Table 1). ^{3}H-Methyl iodide (150 μL toluene solution) was added to a 2 mL reaction vial containing 4.0-7.2 mg of the free base of the corresponding desmethyl-compound of I, II, III or IV in 800 μL DMF/DMSO solvent mixture. The vessel was sealed and heated at 50-80°C for 5 min. The reaction solution was injected into the HPLC column in 100-200 μL portions. The appropriate HPLC fractions were collected by use of the radioactivity flow monitor and evaporated. (In the case of L-deprenyl and clorgyline hydrochloric acid (2 M, 50 μL) was added before evaporation). The residue was dissolved in 2 mL water and analyzed for radioactivity in the liquid scintillation counter.

Alkylation procedure (V and VI, Scheme 3).Carbon-14 formaldehyde (0.5 mCi, 9.4 μmol), sodium cyanoborohydride (43 μmol) and 30 μmol of the desmethyl compound of I or II in 500 μL of acetonitrile containing 2 μL of glacial acetic acid were reacted at room temperature for one hour. The products were isolated by HPLC. After addition of hydrochloric acid, the fraction containing the product was evaporated. The residue was dissolved in 2 mL water and analyzed.

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