LABELLED COMPOUNDS OF POTENTIAL BIOLOGICAL INTEREST

VI. Preparation of some labelled local anaesthetic aminoethers.[+]

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SUMMARY

2'-(2-Diisopropylaminoethoxy)-butyrophenone (ketocaine) and 1-butoxy-2-(2-diisopropylaminoethoxy)-benzene show a good anaesthetic effect on topical application. The synthesis of both of these compounds specifically labelled with ^{14}C is described. The preparation of ketocaine generally labelled with tritium and specifically labelled with deuterium is also reported.

Key words: Tritium, Deuterium, Carbon-14, Exchange labelling, Ketocaine, Percutaneous anaesthetics.

Factors governing the skin penetration of various local anaesthetics in order to achieve percutaneous anaesthesia has been extensively studied in our laboratories (1-3). It was found that 2'-(2-diisopropylaminoethoxy)-butyrophenone (I, ketocaine, Recordati, Milan, Italy) was the percutaneously most efficacious of the known local anaesthetics investigated.

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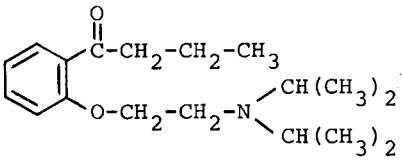
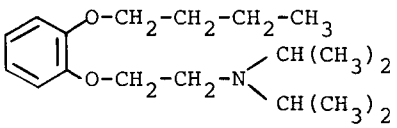
On the basis of correlations between physicochemical properties and pharmacological action, a number of aminoethers of catechol were prepared and tested (4). One of these new compounds, 1-butoxy-2-(2-diisopropylaminoethoxy)-benzene (II) was found to merit further studies. In order to facilitate pharmacological and toxicological investigations, different ^{3}H- and ^{14}C-labelled forms of compounds I and II were prepared.



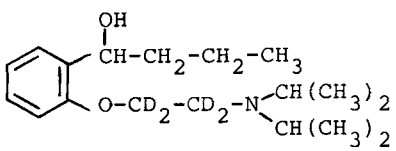
I, ketocaine



II

For studies of metabolism, both I and II labelled with ^{14}C at the methylene carbon adjacent to the oxygen in the diisopropylaminoethoxy group were synthesized. Ketocaine generally labelled with tritium was also prepared and used in skin permeation and distribution studies. Furthermore, mass fragmentographic investigations required the preparation of ketocaine-d_4 in which the four hydrogen atoms at the two methylene carbons in the diisopropylaminoethoxy group were substituted by deuterium. By reduction of ketocaine-d_4 with lithium aluminium hydride, one of the possible metabolites of ketocaine (III) labelled with deuterium was obtained.

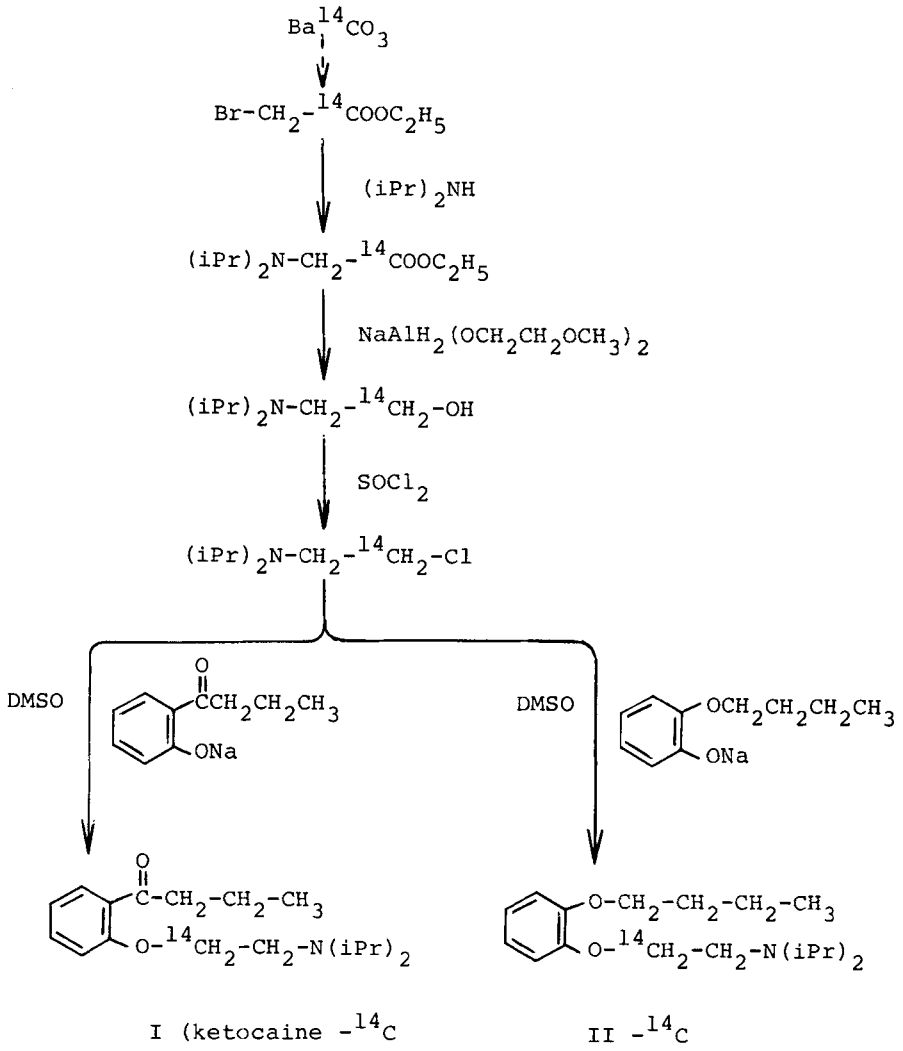
The present paper describes the preparation of the labelled compounds.



III

Ketocaine generally labelled with tritium was prepared by the Yavorsky method (5,6). The specific activity obtained (about 100 mCi/mM) using the $H_2TPO_4.BF_3$ reagent prepared from commercially available tritiated water (5Ci/ml) was high enough for most of the pharmacological investigations. However, a specific activity at least three times as high was needed for the study of organ distribution by whole body autoradiography in the mouse. This requirement could not be fulfilled by using this reagent. Platinum-catalyzed exchange with tritiated water generated in situ, a method described by Pri-Bar and Buchman (7), proved to be useful and ketocaine-^3H(G) having a specific activity of 360 mCi/mM was obtained under the conditions described in the experimental part. These conditions have been successfully used in our laboratory for labelling various compounds (8). As the specific activity of the labelled ketocaine prepared in this way was high enough for the autoradiographic studies, no attempts were made to optimize these conditions for the actual labelling.

A common synthetic route was used for the ^{14}C labelling of ketocaine and compound II. These labelled compounds were prepared by the condensation of 2-diisopropylaminoethyl-1-^{14}C chloride with the sodium salts of o-hydroxybutyrophenone and o-butoxyphenol respectively. The labelled 2-diisopropylaminoethyl chloride in turn was synthesized from $Ba^{14}CO_3$ in a four step synthesis. At first ethyl bromoacetate-1-^{14}C was prepared and reacted with diisopropylamine to yield ethyl diisopropylaminoacetate-1-^{14}C. This ester on reduction with sodium bis(2-methoxyethoxy) aluminium hydride gave 2-diisopropylaminoethanol-1-^{14}C which was chlorinated with thionyl chloride to yield 2-diisopropylaminoethyl-1-^{14}C chloride. The latter was isolated and purified as the hydrochloride and condensed with the sodium salts of the corresponding phenols.



I (ketocaine -^{14}C II -^{14}C

Scheme I

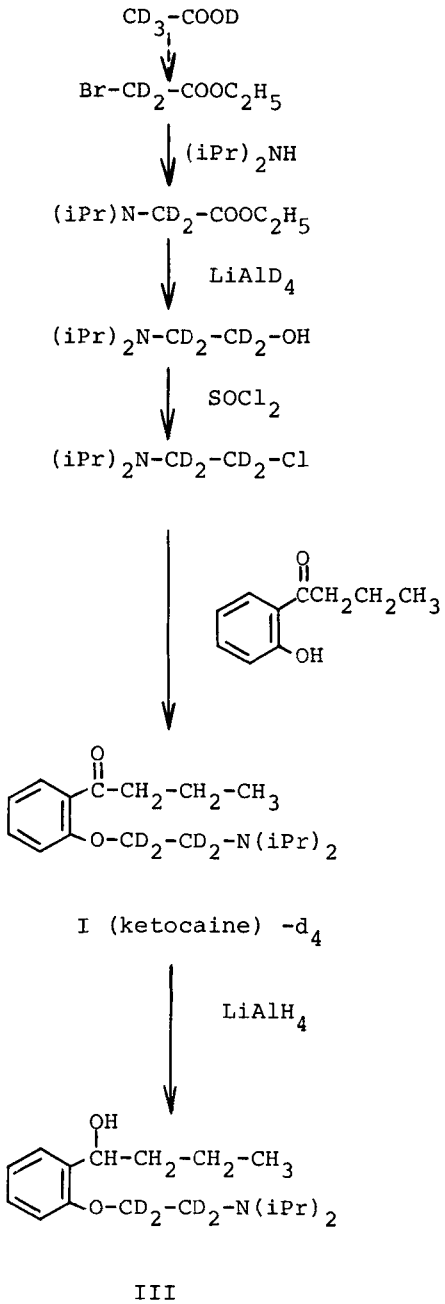
The synthesis was started with 6 mM of $Ba^{14}CO_3$ having a specific activity of 20.8 mCi/mM. The yield of pure distilled ethyl bromoacetate-1-^{14}C was 60 % based on the carbonate. Inactive ethyl bromoacetate was added at this stage and the molar specific activity was adjusted to 7.5 mCi/mM which was then maintained throughout the further reaction steps. The overall yield of ketocaine-^{14}C based on $Ba^{14}CO_3$ was 12 % and that of compound II-^{14}C 15 %. The radiochemical purity of these labelled compounds was verified by thin layer chromatography.

The principles of the syntheses described above are shown in Scheme I.

Ketocaine labelled with deuterium was prepared by a similar manner. Ethyl bromoacetate-d_2 was prepared from the commercially available acetic acid-d_4 in a yield of 64 %. The reaction of ethyl bromoacetate-d_2 with diisopropylamine gave ethyl diisopropylaminoacetate-d_2 which was reduced with lithium aluminium deuteride to 2-diisopropylaminoethanol-d_4. The alcohol was then reacted with thionyl chloride to yield 2-diisopropylaminoethyl-d_4 chloride which was isolated, purified and analyzed as the hydrochloride. It was then coupled with o-hydroxybutyrophenone by using the phase transfer alkylation technique. Ketocaine-d_4 was formed in an overall yield of 21 % based on acetic acid-d_4.

The reduction of ketocaine-d_4 with lithium aluminium hydride in ether gave compound III in a yield of 46 %.

The principles of the synthesis of the deuterated compounds are shown in Scheme II.


CD3-COOD
Br-CD2-COOC2H5
(iPr)2NH
(iPr)N-CD2-COOC2H5
LiAlD4
(iPr)2N-CD2-CD2-OH
SOCl2
(iPr)2N-CD2-CD2-Cl
O
CCH2CH2CH3
OH
O
C-CH2-CH2-CH3
O-CD2-CD2-N(iPr)2
I (ketocaine) -d4
LiAlH4
OH
CH-CH2-CH2-CH3
O-CD2-CD2-N(iPr)2
III

Scheme II

EXPERIMENTAL

Melting and boiling points reported are uncorrected.

Specific activity of the labelled compounds was measured in a Packard TriCarb liquid scintillation spectrometer (Model 3320) using internal standardization (Hexadecane-1,2-^{3}H and hexadecane-^{14}C resp. from the Radiochemical Centre, Amersham).

Thin layer chromatography. - Precoated silica gel plates (F_{254}, 0.25 mm layer thickness, E. Merck, Darmstadt) were used. Spots were detected by visual examination under UV-light. The distribution of radioactivity along the chromatogram was determined by the use of a Berthold "Dünnschicht Scanner II".

Analysis of the deuterium-labelled isolated intermediates and final products was carried out by GC-MS using an LKB 2091 instrument.

Preparation of ketocaine-^{3}H(G)

a) Yavorsky method

Yavorsky reagent was prepared as described previously (6). Ketocaine hydrochloride (1 g) was dissolved in 6 g of the reagent (710 mCi/g) and heated at 70°C for 24 hours. Water (10 ml) and 30 % aqueous NaOH (10 ml) were then added to the chilled solution. The ketocaine base was extracted with ether. The hydrochloride was prepared by adding HCl in ether and recrystallized twice from ethanol-ether. Yield: 630 mg (63 %). M.p.: 127.5-129.5°C (an authentic sample melts at 129-130°C). Specific activity: 292 μCi/mg= 95.8 mCi/mM. TLC in both acetone-25 % NH_4OH (15:1) and cyclohexane-isopropanol-acetone-25 % NH_4OH (90:6:3:0.1) showed a single radioactive peak at R_F values of 0.83 and 0.22 respectively, corresponding to the R_F value of an authentic sample.

b) Platinum-catalyzed exchange with tritiated water

Ketocaine hydrochloride (105 mg) was dissolved in 2 ml of dry dioxan and 50 mg of Adam's platinum oxide was added. The reaction flask was then attached to a vacuum line, cooled in liquid nitrogen and evacuated. Tritium gas (25Ci = 0.47 mM, i.e. 10 % excess over the calculated amount required for the reduction of $PtO_2.H_2O$) was then introduced from a UT_3 reservoir and the mixture was stirred at room temperature for 20 hours. (The reduction of $PtO_2.H_2O$ was complete after 1 hour). The mixture was then refrozen, evacuated and the flask was disconnected from the line. After dilution with methanol, charcoal was added which facilitated the removal of the reduced, partly colloidal platinum by subsequent filtration through a short hyflo column. The solvents were evaporated under reduced pressure and the labile tritium was removed by addition of several portions of methanol and repeatedly removing the solvent by evaporation. The crystalline residue was recrystallized from ethanol-ether. Yield: 55 mg. (52 %). Specific activity: 1.1 mCi/mg = 360 mCi/mM. TLC showed a single radioactive peak in both of the solvent systems described under a).

Preparation of the ^{14}C-labelled compounds

Ethyl bromoacetate-1-^{14}C was prepared from $Ba^{14}CO_3$ (1.2 g = 6 mM; 20.8 mCi/mM) by methods previously described in the literature (9). The product was distilled in a microdistillation apparatus at atmospheric pressure. B.p.: 159-160°C. Yield: 600 mg (60 % based on $Ba^{14}CO_3$). The product was diluted with inactive ethyl bromoacetate to a specific activity of 7.5 mCi/mM.

Ethyl diisopropylaminoacetate-1-^{14}C. - Diisopropylamine (910 mg = 9 mM) was dissolved in 7 ml of toluene and a solution of ethyl bromoacetate-1-^{14}C (630 mg = 3.8 mM) in 1.5 ml of toluene was added dropwise with cooling in an ice bath. The mixture was then stirred at room temperature for 17 hours whereupon it was heated under reflux for 7 hours. After cooling 5 ml of 2M HCl were added, the salt

formed in the reaction was removed by filtration and the phases were separated. The acidic aqueous solution was washed with ether and then made alkaline by adding 10 ml of 30 % aqueous NaOH. The product was extracted with ether. The ethereal solution was dried with K_2CO_3 and the ether was removed by evaporation under reduced pressure. A colourless oil was obtained which was used in the next reaction step without further purification. Yield: 447 mg (64 % based on ethyl bromoacetate-1-^{14}C).

2-Diisopropylaminoethanol-1-^{14}C. - The crude product of the previous reaction (447 mg = 2.4 mM) was dissolved in 4 ml of dry benzene and a solution of sodium bis-(2-methoxyethoxy)-aluminium hydride (900 mg = 3.1 mM) in 2.5 ml of dry benzene was added dropwise with cooling in an ice bath. The mixture was then heated at 80°C for 1 hour. After cooling, 6 ml of saturated aqueous Na_2SO_4 were added, the mixture was filtered and the phases were separated. The aqueous phase was extracted with ether and the ethereal extract together with the benzene phase was dried and finally evaporated under reduced pressure to give a yellow oil which was used in the next reaction step without further purification. Yield: 338 mg (97 % based on ethyl diisopropylaminoacetate-1-^{14}C).

2-Diisopropylaminoethyl-1-^{14}C chloride hydrochloride. - The product of the previous reaction (338 mg = 2.3 mM) was dissolved in 5 ml of dry chloroform, HCl in ether was added and evaporated under reduced pressure. The residue was redissolved in 5 ml of dry chloroform and a solution of thionyl chloride (560 mg = 4.7 mM) in 5 ml of dry chloroform was added dropwise with cooling in an ice bath. The mixture was then stirred at room temperature for 20 hours. The solvent and excess thionyl chloride was removed under reduced pressure and the residue was recrystallized from ethanol-ether. Yield: 275 mg (59 % based on the starting alcohol). M.p.: 129-132°C. Specific activity: 36.9 μCi/mg = 7.4 mCi/mM.

2'-(2-Diisopropylaminoethoxy-1-^{14}C)-butyrophenone hydrochloride (Ketocaine-^{14}C hydrochloride). - o-Hydroxybutyrophenone (113 mg = 0.68 mM) and pulverized NaOH (56 mg = 1.4 mM) were mixed with 4 ml of benzene and 0.4 ml of dimethyl sulphoxide. An almost clear solution was obtained. The benzene was distilled off under reduced pressure in order to remove the water formed and a new portion of 4 ml of benzene was added. 2-Diisopropylaminoethyl-1-^{14}C chloride hydrochloride (137 mg = 0.68 mM) was then added in one portion to the solution which was heated under reflux for 3 hours. After cooling 4 ml of benzene was added and the solution was washed with 3x2 ml of water. The solvent was then removed under reduced pressure, the residue was dissolved in ether and HCl in ether was added. After evaporation to dryness under reduced pressure the crude product was recrystallized from 2-pentanone. Yield: 126 mg (57 % based on 2-diisopropylaminoethyl-1-^{14}C chloride hydrochloride; 12 % overall yield based on $Ba^{14}CO_3$). M.p.: 128.5-131°C. Specific activity: 23 μCi/mg = 7.5 mCi/mM. TLC in acetone-25 % NH_4OH (15:1) showed a single radioactive peak at R_F = 0.83.

1-Butoxy-2-(2-diisopropylaminoethoxy-1-^{14}C)-benzene hydrochloride (Compound II-^{14}C hydrochloride). - This compound was prepared from 0.68 mM of o-butoxyphenol by essentially the same procedure as described above. The crude product was recrystallized from ethyl acetate. Yield: 156 mg (69 % based on 2-diisopropylaminoethyl-1-^{14}C chloride hydrochloride; 15 % overall yield based on $Ba^{14}CO_3$). M.p.: 101.5-104.5°C. Specific activity: 22.7 μCi/mg = 7.5 mCi/mM. TLC in acetone-25 % NH_4OH (15:1) showed a single radioactive peak at R_F = 0.84 which corresponds to the R_F value of an authentic sample.

Preparation of the deuterium-labelled compounds.

Ethyl bromoacetate-d_2. - Bromine (20 g = 125 mM) was added dropwise to a mixture of acetic acid-d_4 (Fluka; 3.2 g = 49 mM) and red phosphorus (0.6 g = 18 mM) with cooling in a cold water bath. The reaction mixture was then heated under reflux for 3 hours. After cooling, the excess bromine was removed by flushing with dry argon. Dry ethanol (7.2 g = 156 mM) was then added dropwise with cooling in a cold water bath. The mixture was heated at 100°C for 5 minutes. After cooling, 10 ml of water and 10 ml of ether were added and the phases were separated. The aqueous phase was extracted with ether, the combined ethereal solutions were washed with saturated aqueous $NaHCO_3$ and dried. The ether was removed under reduced pressure and the residue (6.9 g) was distilled at atmospheric pressure. Yield: 5.2 g (64 %). B.p.: 159-160°C.

Ethyl diisopropylacetate-d_2 was prepared by essentially the same method as that described under the preparation of the ^{14}C-labelled analogue and was used in the next reaction step without purification.

2-Diisopropylamino ethanol-d_4. - Lithium aluminium deuteride (Fluka; 228 mg = 5.4 mM) was suspended in 30 ml of ether and the crude ethyl diisopropylaminoacetate-d_2 (1.2 g = 6.2 mM) in 10 ml of ether was added dropwise with cooling in an ice bath. The mixture was then stirred at room temperature for 3 hours. The excess of $LiAlD_4$ was destroyed by the subsequent addition of ethyl acetate (0.33 ml), ethanol (0.5 ml) and saturated aqueous Na_2SO_4 (1.33 ml) with cooling in an ice bath. The precipitated salts were removed by filtration and the filtrate was evaporated under reduced pressure. The residue (787 mg = 80 %) was transformed to the hydrochloride by dissolving it in ether and then addding HCl in ether. The ether was then removed under reduced pressure. The crystalline residue was used in the next reaction step without re-crystallization.

2-Diisopropylaminoethyl-d_4 chloride hydrochloride. - The crude hydrochloride from the previous reaction (5 mM) was dissolved in 15 ml of dry chloroform and thionyl chloride (1.2 g = 10 mM) in 10 ml of dry chloroform was added dropwise with cooling in an ice bath. The mixture was stirred at room temperature for 20 hours. After removal of the solvent under reduced pressure the residue was recrystallized from ethanol-ether. Yield: 1 g (98 %). M.p.: 130-132°C. GC-MS showed that the incorporation of deuterium into the given positions was > 99 %.

2'-(2-Diisopropylaminoethoxy-d_4)-butyrophenone hydrochloride (Ketocaine-d_4 hydrochloride). - A mixture of 2-diisopropylaminoethyl-d_4 chloride hydrochloride (500 mg = 2.45 mM) and o-hydroxybutyrophenone (406 mg = 2.45 mM) was dissolved in 3 ml of methylene chloride. To this solution was added a solution of tetrabutylammonium hydrogen sulphate (832 mg = 2.45 mM) and NaOH (300 mg = 7.5 mM) in 4 ml of water. The mixture was vigorously stirred at room temperature for 7 hours. The methylene chloride phase was then separated and the solvent was removed under reduced pressure. Water (5 ml) and ether (5 ml) were then added to the residue and the phases were separated. The aqueous phase was extracted with ether. The solvent was removed under reduced pressure from the combined ethereal solutions. The residue (535 mg = 74 %) was redissolved in ether and HCl in ether was added. The ether was evaporated under reduced pressure and the residue was recrystallized first from 2-pentanone and finally from ethanol-ether. Yield: 497 mg (61 %; overall yield from acetic acid-d_4: 21 %). M.p.: 127-129°C. GC-MS showed that the incorporation of deuterium into the given positions was > 99 %.

1-(1-Hydroxybutyl)-2-(2-diisopropylamonoethoxy-d_4) - benzene hydrochloride (Compound III hydrochloride). - Ketocaine-d_4 base (152 mg = 0.52 mM; prepared from the hydrochloride in the usual way) dissolved in 5 ml of ether was added dropwise to a suspension of $LiAlH_4$ (11.4 mg = 0.3 mM) in 5 ml of ether with cooling in an ice bath. The mixture was heated

under reflux for 3 hours. The excess of $LiAlH_4$ was destroyed by the subsequent addition of ethyl acetate (18 µl), ethanol (28 µl) and aqueous Na_2SO_4 (75 µl). The precipitated salts were removed by filtration and the filtrate was evaporated under reduced pressure. The hydrochloride was prepared by dissolving the residue (130 mg) in ether and adding HCl in ether to the solution. The crude hydrochloride was recrystallized from ethanol-ether. Yield: 76 mg (46 %). M.p.: 135-136.5°C. GC-MS showed that the incorporation of deuterium into the given positions was > 99 %.

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