LABELLED COMPOUNDS OF POTENTIAL BIOLOGICAL INTEREST
IV. Tritium labelling of some cyclizing compounds.[+]

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SUMMARY

Quaternary ammonium compounds formed at the site of action by spontaneous cyclization of tertiary ω-haloalkylamine precursors may have interesting pharmacological properties. In order to study the pharmacokinetics of the cyclization principle, a series of new tertiary ω-haloalkylamines derived from p-chloroamphetamine has been synthesized both unlabelled and labelled with tritium at high specific activity.

Key Words: Cyclizing compounds, Tritium, Reductive alkylation, p-Chloroamphetamine derivatives.

The preparation and pharmacological investigation of cyclizing ω-haloalkylamines has been reported recently (1-4). Results of these studies showed that interesting pharmacological properties, such as a local anaesthetic effect of long duration, can be achieved by somequaternary ammonium compounds which are formed at the sites of action by spontaneous cyclization of the corresponding tertiary ω-haloalkylamine precursors. The quaternary compounds themselves pass the cell membranes very slowly, which results in poor oral absorption and inability to pass the blood-brain barrier. However, the tertiary amine precursors in the basic form are able to pass the membranes and thereby serve as a good transport form for the quaternary compounds. The application of this cyclization

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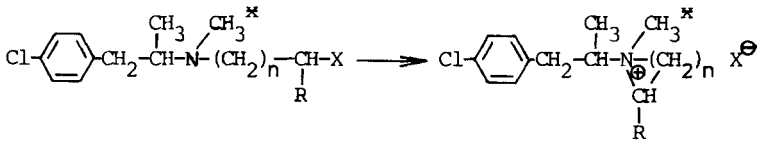
principle seemed to be promising enough to justify more detailed pharmacokinetic studies on suitable model substances. For this purpose a series of ω-haloalkylamines having different rates of cyclization were prepared. It was known from previous studies (loc.cit.) that the rate of cyclization was influenced by the length of the alkyl chain, the nature of halogen atom and the position of the latter, i.e. whether it was attached to secondary or tertiary carbon. In order to facilitate the analysis of the haloalkylamines and the quaternary compounds formed from them, the substances were also prepared labelled with a radioactive isotope. As the metabolism of the tertiary amines is a complicating factor in these investigations making the kinetic analysis more difficult, the biotransformation had to be suppressed. The tertiary haloalkylamine derivatives of p-chloroamphetamine (A) labelled in the N-methyl group seemed to be most suitable in fulfilling this requirement. These compounds are stable to monoamine oxidase as well as to metabolic hydroxylation in the aromatic ring. However the oxidative N-demethylation is very difficult to hinder regardless of other structural elements, but by having the radioactive label in the N-methyl group, the metabolic demethylation results in non-radioactive metabolites and thereby causes no disturbing factor in the analysis. In order to achieve high specific activity, tritium labelling of the N-methyl group was chosen.

In the present paper we wish to report the chemical synthetic part of the work. The results of the pharmacokinetic investigation will be published elsewhere. (5).

Five new ω-haloalkylamines (A) were prepared. The tertiary compounds are stable in the form of their salts but undergo spontaneous cyclization as bases according to equation 1 (cf. fig. 2. in ref. 2.):

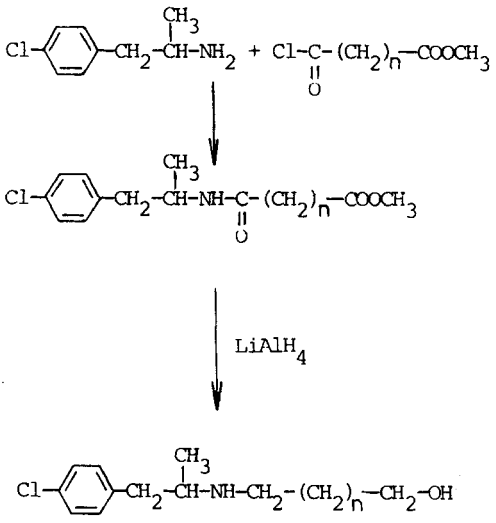


A * = tritium labelled B (equation 1)

The substances synthesized are listed in the table below. Compounds I-III were also prepared tritium-labelled in the N-methyl group.

Compound	n	R	X
I	3	H	Cl
II	4	H	Cl
III	4	H	Br
IV	3	CH_3	Cl
V	3	CH_3	Br

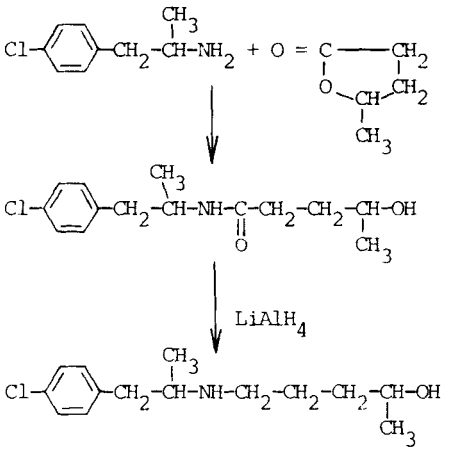
A common synthetic route of preparation for all the five compounds was used. At first the secondary ω-hydroxyalkylamines were prepared. Acylation of p-chloroamphetamine with the acid chloride of succinic acid mono methylester or of glutaric acid mono methylester respectively gave an amidoester which was reduced with $LiAlH_4$ to the secondary hydroxyalkylamines corresponding to compounds 1-3 as shown in equation 2:



n = 2, 3

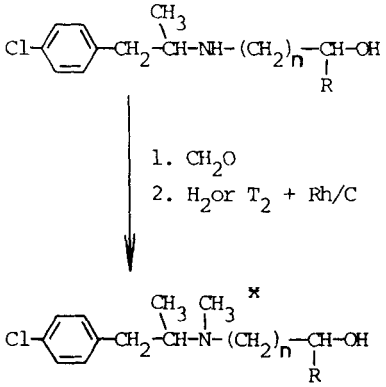
(equation 2)

The secondary hydroxyalkylamine corresponding to compounds 4 and 5 was prepared by acylation of p-chloroamphetamine with γ-valerolactone and subsequent $LiAlH_4$ reduction of the hydroxyamide formed (equation 3):



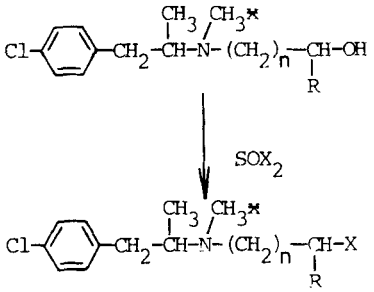
(equation 3).

The secondary ω-hydroxyalkylamines, obtained in yields of 50-70 %, were then N-methylated by reductive alkylation. In the case of the preparation of the tritiated compounds the label was introduced in this reaction step. In order to achieve high specific activity the secondary amines were reacted with formaldehyde followed by catalytic tritiation using carrier-free tritium gas as shown in equation 4. Care was taken in the choice of a suitable catalyst in order to avoid simultaneous hydrogenolytic loss of the aromatic chlorine. Freifelder (6) recommends the use of rhodium on carbon at 2-3atm. pressure in similar cases. In our experiments, this catalyst proved to be very useful even at atmospheric pressure in the case of catalytic hydrogenation, or at reduced pressure in the case of tritiation. The methylated products could be obtained in nearly quantitative yields. The specific activities of the tritiated products were of the order of 20 Ci/mM.



* = position of ^{3}H label
R = H, CH_3
n = 3, 4
(equation 4)

The last step in the syntheses was the preparation of the corresponding chloro- and bromo-derivatives. This was performed by using thionyl chloride and thionyl bromide respectively according to equation 5. The chloro-compounds were obtained in 50 - 90% yield, while the bromoderivatives were formed in yields of 30 - 40%. The products were isolated as oxalates.



* = position of ^{3}H label
R = H, CH_3
n = 3, 4
X = Cl, Br
(equation 5)

The radiochemical purity of the labelled forms of compounds I-III was determined by radio - thin layer chromatography. Compounds I and II could be obtained with radiochemical purities of > 97% after recrystallization, while in the case of compound III the radiochemical purity was not better than 85%, so that this product had to be further purified by thin layer chromatography.

All three labelled compounds were unstable on storage. Compound I was prepared from the tertiary hydroxy-derivative at a specific activity level of about 20 Ci/mM, but this high specific activity proved to be impractical from the point of view of storage. The final product was therefore diluted with pure inactive carrier to a specific activity of 1.3 Ci/mM. The preparation of compounds II and III was performed by using diluted tertiary hydroxyderivatives and the final products obtained had specific activities of 2.9 and 3.6 Ci/mM respectively. However, the stability on storage, even of these diluted products, was limited. The labelled compounds were stored in a freezer at -20°C as solids.

The radiochemical purity of compound I decreased after two months of storage to a value of about 70%. The main product of radiolysis proved

to be the corresponding cyclized quaternary derivative (about 27%) and two further unidentified radioactive peaks representing about 3% of the total activity could be detected. The impurities could easily be removed by recrystallization.

Compound II showed somewhat better storage properties. In spite of its higher specific activity, the radiochemical purity of this product decreased only to 83% after six months of storage at -20°C, the main impurity (14%) being again the cyclized quaternary derivative. A single recrystallization increased the radiochemical purity to 97%. The better stability of compound II can be expected as the rate of its cyclization in the pharmacokinetic studies was also much lower (5).

The radiochemical purity of compound III decreased to 38% after four months of storage. The rest of the radioactivity could be detected partly as the cyclized quaternary derivative (37%), and partly as unidentified decomposition products (25%). Repurification of this product could not be effected without further dilution with pure inactive carrier. By doing so, the recrystallized product had a specific activity of 2.4 Ci/mM and a radiochemical purity of 85%. Aliquot samples of this product were purified by thin layer chromatography immediately before use (5).

EXPERIMENTAL

Melting- and boiling-points reported are uncorrected.

Analyses. All unlabelled intermediates and final products were analyzed for C, H, N and halogen. In cases were the intermediates were further reacted without purification, a sample for analysis was prepared separately. The microanalyses were carried out at the laboratories of Dr A Bernhardt, Mühlheim, West Germany. The analytical results were within $\pm$0.4% of the theoretical values.

IR and NMR spectra of the isolated intermediates and final products were recorded by using a Perkin Elmer 720 IR spectrophotometer and a Varian T60 NMR spectrometer respectively.

Thin layer chromatography. Precoated silica gel plates (F_{254}, 0.25 mm layer-thickness, E. Merck, Darmstadt) were used. Acetone- 25% NH_4OH (15:1) was employed as the developing solvent system except for the bromo derivatives (compounds III and V) which seemed to undergo cycli-

zation during chromatography in this system. For the latter the solvent system acetone- acetic acid (15:2) proved to be applicable. The Rf values for the different intermediates and final products are given below:

Acylated p-chloroamphetamines	0.75 - 0.80
Secondary ω-hydroxyalkyl derivatives	0.55 - 0.60
Tertiary ω-hydroxyalkyl derivatives	0.65 - 0.70
Tertiary ω-chloroalkyl derivatives	0.75 - 0.80
Tertiary ω-bromoalkyl derivatives in acetone - acetic acid (15:2)	0.45 - 0.50
Cyclized quaternary derivatives in both solvent systems	0.00 - 0.05

Specific radioactivities were measured in a Packard TriCarb liquid scintillation spectrometer (Model 3320) using internal standardization (Hexadecane - 1,2 - ^{3}H from the Radiochemical Centre, Amersham).

Scanning of the chromatograms of the labelled products was carried out in a Berthold "Dünnschicht Scanner II".

The description of the chemical syntheses which follows here has been divided into four groups:

1. Acylations of p-chloroamphetamine
2. $LiAlH_4$ reduction of the amides
3. Reductive methylation of the hydroxyalkyl amines
4. Preparation of the chloro- and bromo-derivatives

1. ACYLATIONS OF p-CHLOROAMPHETAMINE

N-p-Chlorophenylisopropyl-3-carbomethoxypropionamide (1a). - To a cold (-10°C) solution of 22g (107mM) p-chloroamphetamine hydrochloride and 41.5g (320mM) ethyldiisopropylamine in 300 ml of dichloroethane was added during 20 minutes 16.6g (110mM) of the acid chloride of succinic acid monomethylester in 75 ml of dichloroethane. The mixture was heated at 70°C for 15 minutes. The precipitated salt was removed by filtration and the filtrate was washed with 30 ml of 1M HCl and 4x25 ml of water. After drying over $MgSO_4$ the solvent was removed at reduced pressure and the residue (29.5g) was recrystallized from 300 ml of diisopropyl ether yielding 26.8g (89%) of a white crystalline product. M.p.: 96-98°C.

N-p-Chlorophenylisopropyl-4-carbomethoxy butyramide (16) was prepared by essentially the same procedure as described above by using the acid chloride of glutaric acid monomethylester as the acylating agent. The crystalline product was formed in a yield of 82%. M.p.: 83-85°C.

N-p-Chlorophenylisopropyl-4-hydroxyvaleramide (1c). - A mixture of p-chloroamphetamine (17.4g., 103 mM), γ-valerolactone (10.4g., 100mM) and water (20ml) was heated under reflux for 95 hours under a gentle stream of argon. The mixture was cooled and extracted three times with chloroform. The collected extracts were washed with 2M HCl, saturated $NaHCO_3$ and saturated Na_2SO_4 solutions. The organic layer was then evaporated to dryness. The residual oil (16g) solidified on adding a mixture of ether-petroleum ether (1:1). The solvent mixture was decanted and the residue washed with cold diisopropyl ether yielding 7.7g (28%) of a white solid which was reacted in the next reaction step without further purification. A sample prepared for analysis by recrystallization from diisopropyl ether had a melting point of 65-68.5°C.

2. $LiAlH_4$ REDUCTION OF THE AMIDES

N-(4-Hydroxybutyl)-p-chloroamphetamine (2a). - A solution of 12g (40mM) 1a in 500 ml of dry ether was slowly added to a chilled (0°C) suspension of 8.6g (170mM) $LiAlH_4$ om 250 ml of ether during 1.5 hours. After heating under reflux for 24 hours the excess of hydride was decomposed by the subsequent addition of ethyl acetate (13.8ml), ethanol (20.8ml) and saturated Na_2SO_4 (55.5ml). The saltmixture was removed by filtration and washed with ether. The combined ethereal solutions were extracted with 50 ml of 1M HCl. The hydrochloric acid extract was washed with 5x25 ml of ether, made alkaline with 10M NaOH and the amine was extracted with 4x50ml of ether. After drying over $MgSO_4$ the ether was removed in vacuo. The residual oil (9.5g., 92%) was distilled at reduced pressure using a short column filled with glass helices. The fraction boiling at 140-143°C/0.4 mm Hg was collected. Yield: 5g (54%).

N-(5-Hydroxypentyl)-p-chloroamphetamine (2b) was prepared from 1b by the same procedure as described above giving an oil (8.4g., 98%) which soon solidified after standing at room temperature. Recrystallization from cyclohexane gave white crystals (6.5g., 75%). M.p.: 49-50°C.

N-(4-Hydroxypentyl)-p-chloroamphetamine (2c) was prepared from 1c by essentially the same procedure as described under 2a giving 5.1g of an oily product which crystallized after standing at room temperature for

several months. The oil was used in the next reaction step without further purification.

3. REDUCTIVE METHYLATION OF THE HYDROXYALKYLAMINES

N-Methyl-N-(4-hydroxybutyl)-p-chloroamphetamine (3a). - To a solution of 2.4g (10mM) 2a in 25 ml of abs. ethanol, 0.32g (10.5mM) paraformaldehyde was added. The suspension was kept at room temperature for 48 hours with occasional shaking. A clear solution was obtained. After adding 0.4g of 5% rhodium on carbon catalyst, the mixture was hydrogenated at atmospheric pressure. The calculated amount of hydrogen was taken up within 2 hours. The catalyst was removed by filtration and the solvent was evaporated at reduced pressure giving an oil (2.5g., 96%) which was halogenated without further purification.

N-Methyl-N-(5-hydroxypentyl)-p-chloroamphetamine (3b) and

N-Methyl-N-(4-hydroxypentyl)-p-chloroamphetamine (3c) were prepared by the same procedure as described above. The oily products formed in yields of 96% and 98% respectively were halogenated without further purification.

N-Methyl-^{3}H-N-(4-hydroxybutyl)-p-chloroamphetamine (3a-^{3}H) and

N-Methyl-^{3}H-N-(5-hydroxypentyl)-p-chloroamphetamine (3b-^{3}H) were prepared similarly. These preparations were carried out on a 0.5 mM scale on a vacuum manifold using carrier-free tritium gas. The products, formed in yields of 97%, showed a single radioactive peak upon thin layer chromatography at Rf values corresponding to those of the authentic unlabelled samples.

4. PREPARATION OF THE CHLORO- AND BROMO-DERIVATIVES

N-Methyl-N-(4-chlorobutyl)-p-chloroamphetamine oxalate (compound I).-

To a solution of 2.4g (9.6mM) 3a in 60ml of dry chloroform, 2.3g (19mM) freshly distilled thionyl chloride was added. The solution was allowed to stand at room temperature for 1 hour and then heated under reflux for 2 hours. Evaporation to dryness under reduced pressure gave 3.1g of a clear yellow oil. This was dissolved in 25 ml of water, washed with 2x15 ml of ether, made alkaline by adding solid Na_2CO_3 and the base was extracted with ether. The ethereal extract was dried and added to a

solution of anhydrous oxalic acid in ether. The white precipitate (2.9g) was recrystallized from 90% ethanol saturated with ether giving 2.1g (61%) of a white crystalline product, M.p.: 108-113°C. Recrystallization from acetonitrile increased the melting point to 116-118°C.

N-Methyl-N-(5-chloropentyl)-p-chloroamphetamine oxalate (compound II) was prepared by essentially the same procedure as described above. The yield of white crystalline oxalate was 87%. M.p.: 124-126°C.

N-Methyl-N-(5-bromopentyl)-p-chloroamphetamine oxalate (compound III) was prepared similarly by using thionyl bromide instead of thionyl chloride. The yield of the pure bromo compound was 38%. M.p.: 109-111°C.

N-Methyl-N-(4-chloropentyl)-p-chloroamphetamine oxalate (compound IV) was formed under the same conditions as described for compound I in a yield of 51%. The melting point of the pure product was 113-115°C.

N-Methyl-N-(4-bromopentyl)-p-chloroamphetamine oxalate (compound V) was prepared similarly. The yield of the pure bromo compound was 31%. M.p.: 96-100°C.

The labelled forms of compounds I-III were prepared by essentially the same procedure as described for the unlabelled derivatives. These preparations were carried out on the 0.5-1 mM scale. The questions connected with the specific activities and radiochemical purities, as well as storage properties of the labelled products were discussed in the general part.

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