SYNTHESIS OF ^{14}C LABELLED HEPTACAINE AND CARBISOCAINE, NEW LOCAL ANAESTHETICS.

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ABSTRACT

The published four step synthesis of N-{2-(2-heptyloxyphenylcarbamoyloxy)-ethyl}-piperidinium chloride (5) and N-{2-(2-heptyloxyphenylcarbamoyloxy)-propyl}-diethylammonium chloride (6), which starts from o-acetamidophenol and 1-bromoheptane was scaled down and modified for radioisotope work. After chromatography 1064 MBq (28,7 mCi) of [heptyl-1-^{14}C]heptacaine 5 and 840 MBq (22,6 mCi) of [heptyl-1-^{14}C]carbisocaine 6 were isolated. Radiochemical yields of 5 and 6 on starting 1-bromo[1-^{14}C]heptane were 30% and 25%, respectively.

KEY WORDS : local anaesthetics, [^{14}C]heptacaine, [^{14}C]carbisocaine, 1-bromo[1-^{14}C]heptane.

INTRODUCTION

The use of hydrochlorides of phenylcarbamoyloxyalkyl amines as local anaesthetics was for the first time suggested by Fromherz as early as in 1914 (1, 2). Since that time a lot of derivatives differently substituted at phenylamino or alkoxy portion of molecule have been prepared and tested for their biological activities(3). Two compounds of this type are most promising (4, 5); N-{2-(2-heptyloxyphenylcarbamoyloxy)-ethyl}-piperidinium chloride (heptacaine)(5) and N-{2-(2-heptyloxyphenylcarbamoyloxy)-propyl}-diethylammonium chloride (carbisocaine) (6) . Their

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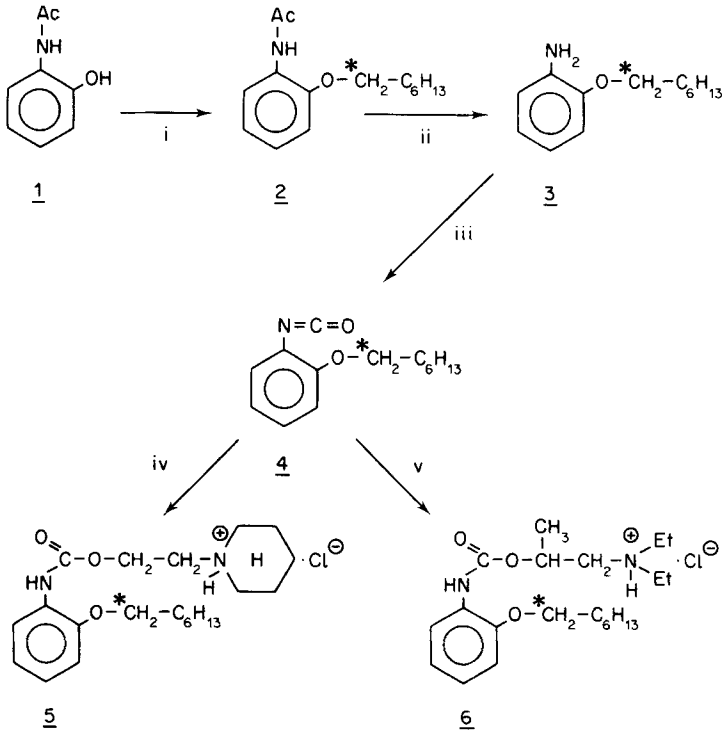
local anaesthetic activity is several hundred times higher than that of standarts (procaine, cocaine) and heptacaine (5) is several times more potent antiarythmic than lidocaine without increased toxicity. For further studies ^{14}C labelled materials were needed.

Published synthesis of heptacaine (5) and carbisocaine (6) (4, 5) are based on addition of appropriate amino alcohol to 2-heptyloxyphenylisocyanate (4). The synthesis of 4 starts from o-acetamidophenol (1), which is alkylated by Williamson method (6) with 1-bromoheptane, the amino group is deblocked by acid hydrolysis and eventually converted to isocyanato group by reaction with phosgene (7). We decided to follow the aformentioned scheme and use the 1-bromo[1-^{14}C]heptane for introducing ^{14}C label into the aromatic part of the molecule.

RESULTS AND DISCUSSION

In the preliminary non radioactive experiments we verified the modifications required by the work with high both total and molar activities - scaling down and simplifying of isolation operations.

After first step - heptylation of phenol 1 with sodium ethanolate and 1-bromoheptane - the unreacted phenol 1 was removed from ethereal solution of 2-heptyloxyacetanilide (2) by extraction with 10% sodium hydroxide solution. After evaporation of diethyl ether the crude 2 was directly hydrolyzed with 18% hydrochloric acid. 2-Heptyloxyaniline (3) was extracted from the reaction mixture (after being made alkaline by conc. ammonia solution) with diethyl ether. We found, that distillation of 3 is not necessary and that for successfull transformation of 3 to the 2-heptyloxyphenylisocyanate (4) its through drying by repeated evaporations with absolute ethanol is sufficient measure. Reaction of 3 with phosgene in benzene solution gave smoothly isocynate 4 ; excess phosgene and solvent were removed by evaporation. For the good yields of 2-heptyloxycarbanilates 5 and 6 it was essential to add the toluene solution of 4 dropwise to the boiling solution of appropriate amino alcohol. When the reactants were first mixed together and then refluxed or the addition was reversed the yields were substantialy lower. The chromatographic purification on silica gel column gave free bases of 5 and 6 which were transformed to the hydrochlorides 5 and 6 by ethereal hydrogen chloride. Yields of 5 and 6 based on 1-bromoheptane



i EtONa/EtOH, $Br-{}^{*}CH_2-C_6H_{13}$

ii HCl, H_2O

iii $COCl_2$, benzene

iv $HO-CH_2-CH_2-N$ (H) , toluene

v $HO-CH(CH_3)-CH_2-N(Et)_2$, toluene

SCHEME I

were 35% and 21%, respectively. The identity of the products 2, 3 and 4 was confirmed by the comparison of their m.p. or b.p. with published data. They were m.p. 45 °C for 2 b.p. 165 °C/13 Pa for 3 and m.p. 145 °C for 4 (8). The confirmation of identity of prepared 5 and 6 was done by the comparison of their analytical profile (see Table I) either with published data (ref. 9 for 5) or that of authentic specimen (for 6).

TABLE I

Analytical profiles of heptacaine (5) and carbisocaine (6)

		heptacaine		carbisocaine
m.p.		117-119 °C		94-97 °C
UV [a]	$\lambda_{max.}$ [nm]	206 (ε = 29 110)		207 (ε = 29 110)
		236 (ε = 10 280)		236 (ε = 10 280)
		280 (ε = 3 120)		280 (ε = 3 120)
IR [b]	ν [cm^{-1}]	3400	(N-H)	3440
		2935, 2864		2975, 2870
		1737	(C=O)	1735
		1540	amide II band	1540
		1497, 1402		1495, 1402
		1238	$(C\text{-}O\text{-}C)_{as.}$	1230
		1214	$(C\text{-}O\text{-}C)_{sym.}$	1215
^1H NMR [c]	[δ]	1,12-1,56 m 19 H		1,34-1,51 m 22 H
		3,37 m 6 H	N^+-CH	3,19
		3,97 t 2 H	Ar-O-CH	4,01
		4,51 t 2 H	CO-O-CH	5,31 m 1 H
		6,93 s 1 H	N^+-H	6,95
		7,72 m 4 H	H_{Ar}	7,61
		8,16 s 1 H	CO-NH-Ar	8,01
MS [d]	[m/e]	362	$M^{\cdot +}$	364
		for $C_{21}H_{34}O_3N_2$		for $C_{21}H_{36}O_3N_2$
		calc. 362,5		calc. 364,5

[a] 50% methanol
[b] KBr pellets
[c] d_6-DMSO, 80 MHz
[d] E.I., 75 eV

At least 600 MBq (16,2 mCi) of each compound were required and so we had to start from 6,95 GBq (188 mCi, 4 mmol) of 1-bromo [1-^{14}C] heptane to have a sufficient margin. When the crude labelled 2 was submitted to acidic hydrolysis, two phases formed and t.l.c. revealed that no reaction occured. Even after repeated isolation-hydrolysis process there was no significant progress. Eventually we performed hydrolysis in 50% aqueous ethanol and after 5 hours at 100 °C there were still small amounts of insoluble oil * in the reaction mixture but no [^{14}C] acetanilide 2 (according to t.l.c.) . Because of prolonged reaction times and because radio-t.l.c. revealed that about 25% of activity migrates with solvent front (most probably [1-^{14}C] heptan-1-ol) we decided to purify labelled 3 by column chromatography on silica gel. It gave 2,81 GBq (76 mCi) of chromatographically pure 3 (radiochemical yield 40,5%) , which was then transformed to isocyanate 4. One half of the labelled 4 was reacted with 2-N-piperidylethanol. T.l.c. showed, that 94% of activity is in the desired base of 5. After chromatographic purification and conversion to hydrochloride 1,062 GBq (28,7 mCi, 75,6% on 4) of [heptyl-1-^{14}C] heptacaine (5) were obtained.

As expected for the secondary alcohol - 1-diethylamino-2-propanol - its reaction with isocyanate 4 was less smooth and only 70% of activity was incorporated in 6. Chromatography and conversion to hydrochloride gave eventually 0,835 GBq (22,6 mCi; 60 % on 4) of [heptyl-1-^{14}C] carbisocaine (6) . Radiochemical purity of both 5 and 6 was better than 99%. Total radiochemical yield of this four step synthesis on starting 1-bromo [1-^{14}C] heptane was 30,6% and 24% for 5 and 6, respectively.

These ^{14}C labelled local anaesthetics were used for the pharmacokinetic studies of these drugs. The results will be published elsewhere.

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*Difficulties met at "hot" experiment and never encountered before were most probably caused by silicone grease from vacuum manifold in 1-bromo [1-^{14}C] heptane, which was not purified by distillation because of its better than 98% radiochemical purity.

EXPERIMENTAL

Activities were measured on Packard 2660 apparatus in liquid scintillator SLD-31 (dioxane based; Lachema Czechoslovakia). Corrections for quenching were done by channel ratio method after preliminary gauging with standart set made in Institute for Research, Production and Application of Radioisotopes.

UV spectra were measured in 50% methanol using Specord UV-VIS spectrophotometer (Carl Zeiss, Jena). IR spectra were recorded on Pye Unicam SP 100 G apparatus using KBr technique. ^{1}H NMR spectra were obtained on 80 MHz Tesla BS 487 B Czechoslovakia apparatus in d_6-DMSO with TMS as an internal standart. Mass spectra were recorded on Jeol JMS D 100 apparatus using electron impact (75 ev) technique.

T.l.c. was performed on Silufol UV-254 plates (Kavalier Votice, Czechoslovakia) in chloroform (solvent A) or benzene - 2-propanol - conc. ammonia 200 : 40 : 1 mixture (solvent B). Modes of spot visualisation and R_f's of compounds are summarized in Table II. Radio-t.l.c. was performed on Berthold T.l.c. Scanner II combined with Berthold-Silena multichannel analyzer and HP-97 S calculator.

Evaporations were made under vacuum of oil pump (unless stated otherwise) at temperatures not exceeding 35 °C. Column chromatography was performed on Pitra silica gel 70-160 μ (Service Laboratories of Czechoslovak Academy of Sciences). As petroleum ether fraction boiling in 33-47 °C range was used.

2-[1-^{14}C] Heptoxyacetanilide (2) -- The stock ethereal solution of 6,95 GBq (188 mCi, 4 mmol) of 1-bromo[1-^{14}C]heptane (10) was concentrated approximately to 1 ml volume; 600 mg (4 mmol) of acetamidophenol (1) and 2 ml of 2 M sodium ethoxide solution were added and the mixture was left at laboratory temperature overnight. Then the mixture was heated in the 110 °C warm bath in stoppered flask for 3 h. After evaporation of ethanol in closed system the residue was treated with 20 ml of 10% NaOH solution and 2 was extracted with 20 ml of ether. Excessive emulsion formation was observed. Emulsion was partially resolved by addition of 20 ml of benzene. Organic layer was washed with 3 x 10 ml of water and evaporated to yield 910 mg (6,12 GBq, 88%) of oily 2 with R_f identical with that of standart (see Table II).

TABLE II

Compound	Visualized by method	R_f	Solvent
1	I	0,1	A
2	I	0,3	A
3	II	0,6	A
		0,8	B
5	III, IV	0,5	B
6	III, IV	0,5	B
2-N-piperidyl-ethanol	IV	0,1	B
1-diethylamino-2-propanol	IV	0,1	B

Methods of spot visualizing :
I - quenching of fluorescence
II - brown spots in iodine vapor
III - red spots with Dragendorff reagent
IV - carbonization by gentle heating in flame

2-[1-^{14}C]Heptyloxyaniline (3) -- To the 910 mg of oily 2 water (3 ml) and 36% hydrochloric acid (3 ml) were added and the mixture was heated in stoppered flask in the 150 °C oil bath for 1 h. The reaction mixture has been made alkaline with conc.ammonia and has been extracted with ether. T.l.c. of organic layer showed only 2, no free aniline 3 was formed. Ether was evaporated and the residue was again treated with 6 ml of 18% hydrochloric acid for 3 h at 150 °C. After workup again major component was 2, only small amounts of 3 were formed. The solvent was evaporated and hydrolysis was eventually performed in a mixture of 1 ml of water, 3 ml of ethanol and 3 ml of 36% hydrochloric acid. The stoppered flask was heated in bath 100 °C warm. After 5 h no trace of 2 was detected by t.l.c. . The mixture was made alkaline as above, 3 was extracted with 20 ml of petroleum ether and the extract was applied on 30 g silica gel column. Column was eluted with benzene - petroleum ether 3 : 1 mixture; 10 ml fractions were collected. Fractions 12-20 were combined and evaporation of solvent gave 340 mg (2,81 GBq, 76 mCi) of chromatographically pure 3 with R_f identical with that of authentic sample (see Table II). Radiochemical yield on 2 was 46%.

2-[1-^{14}C]Heptyloxyphenylisocyanate (4) --Oily 3 (340 mg) was evaporated twice with 10 ml portions of dry benzene and 25 ml of 20% $COCl_2$ in benzene were added; the mixture was refluxed for 3 h. Excessive phosgene and solvent were first evaporated in the vacuum of water pump, then the residue was evaporated with two 10 ml portions of dry toluene. Resulting oily isocyanate 4 (383 mg) was dissolved in 10 ml of dry toluene and stored. The yield of 4, as deduced from weight increase and from its reaction with N-2-hydroxyethylpiperidine (see below) was better than 95%.

[heptyl-1-^{14}C]Heptacaine (5) -- To the refluxing solution of 303 mg (2,35 mmol) of N-2-hydroxyethylpiperidine in 3 ml of toluene, 5 ml of toluene solution of isocyanate 4 (192 mg, 1,41GBq) were dropped by syringe via reflux condenser and the mixture was refluxed for another 1 h (the reflux condenser was stoppered with calcium chloride tube). T.l.c. of the reaction mixture revealed, that 94% of activity was in the form of 5. The reaction mixture was then applied on 25 g silica gel column. The column was first rinsed with 120 ml of benzene and then eluted with solvent B; 7 ml fractions were collected. The fractions 10-20 containing free base of 5 were combined and evaporated to 235 mg of syrup. The syrup was treated with 10 ml of saturated diethyl ether solution of hydrogen chloride; the excessive reagent was evaporated in the vacuum of water pump. The residue of 258 mg (1,063 GBq, 28,7 mCi) of [heptyl-1-^{14}C]heptacaine (radiochemical yield 76% on 3) crystallized spontaneously. Its radiochemical purity was better than 99%, according to t.l.c., and its m.p., UV and IR agreed with those published (see Table I). Molar activity was 1,74 Gbq.mmol^{-1}. For storing the crystalline 5 was dissolved in 20 ml of water containing 2% of ethanol.

[heptyl-1-^{14}C]Carbisocaine (6) -- To the refluxing solution of 300 mg of 1-diethylamino-2-propanole in 3 ml of toluene, 5 ml of toluene solution of labelled isocyanate 4 were dropped as described for 5. The mixture was refluxed 2,5 h. After the same workup as described for 5 205 mg (835 MBq, 22,6 mCi) of syrupy 6 were obtained (60% on 3). Radiochemical purity was better than 99%, according to t.l.c., and its UV and IR spectra were identical with those of authentic specimen (see Table I). Molar activity was 1,74 GBq.mmol^{-1}. The stock solution was prepared by dissolving syrupy 6 in 20 ml of water containing 2% of ethanol.

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