SYNTHESIS OF ^{14}C-LABELED AND ^{2}H-LABELED DIBUCAINE

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SUMMARY

A synthesis of dibucaine labeled with ^{14}C in the heterocyclic ring is described. The synthesis was accomplished in five steps starting with isatin and acetic anhydride-2-^{14}C as shown in Scheme I. Two deutero analogs, dibucaine-d_2 and dibucaine-d_9, were also synthesized by the reactions shown in Scheme II. Dibucaine-d_9 was synthesized by condensation of 2-chloro-N-[2-(diethylamino)ethyl]-4-quinoline carboxamide with sodium salt of n-butyl-d_9 alcohol. Dibucaine-d_2 was synthesized by condensation of 2-chloroquinoline carboxylic acid chloride with N,N-diethyl dideutero-ethylenediamine (prepared from chloroacetyl chloride) followed by reaction with sodium n-butoxide. ^{14}C-labeled dibucaine was found to undergo considerable radiolysis on storage to give 2-butoxy-quinoline-4-carboxamide as a major decomposition product.

Key Words: [^{14}C]Dibucaine, Dibucaine-d_2, Dibucaine-d_9

INTRODUCTION

Dibucaine, 2-butoxy-N-[2-(diethylamino)ethyl]-4-quinoline carboxamide is an extremely potent local anesthetic. It is used in very low concentrations as an ointment or a cream for fast relief of pain and itching due to various painful skin conditions including hemorrhoids. Dibucaine hydrochloride is also used as a spinal anesthetic. Although this anesthetic has been used since 1930, its metabolism was not studied perhaps because of the lack of sensitive methods of detection. Recently, Igarashi et al (1) reported that they detected ten metabolites in the urine of rat, rabbit and man, but total amounts of these metabolites including unchanged dibucaine accounted for only 10% of the dose after 24 hours. More than 90% of the dose which was introduced intraperitoneally

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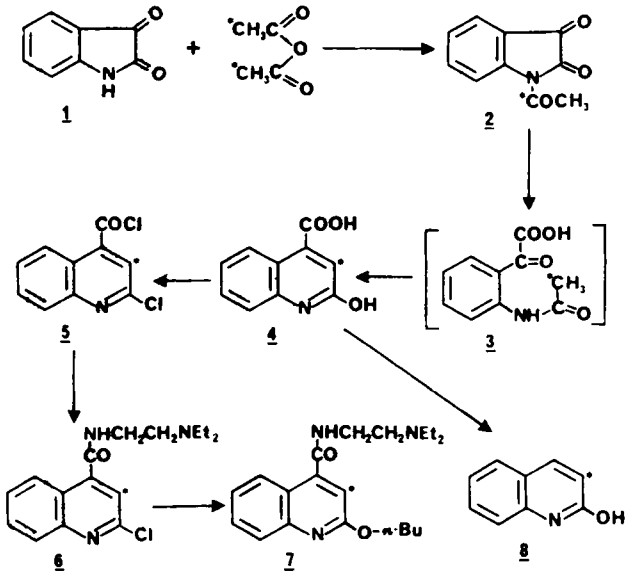
or orally, was not explained. We synthesized ^{14}C-labeled dibucaine to study its percutaneous absorption and metabolism.

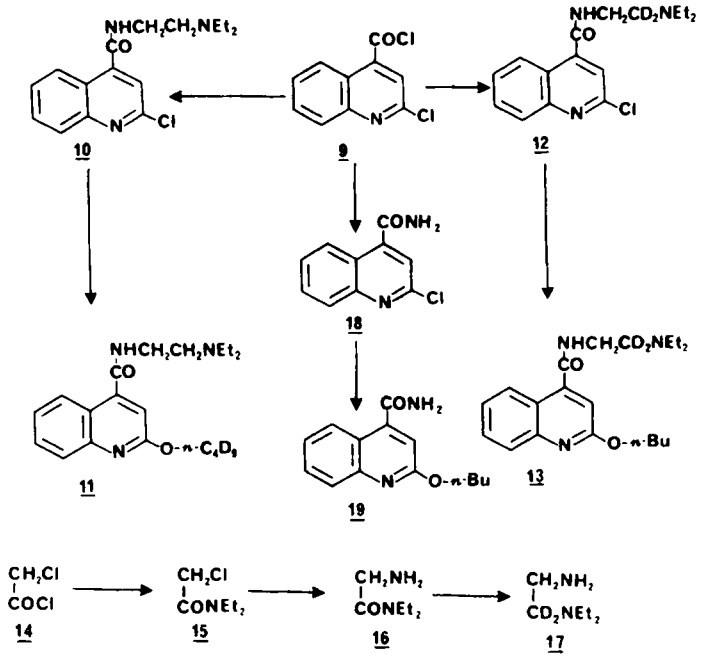
METHODS AND RESULTS

The synthesis of [^{14}C]dibucaine (7) was achieved in five steps starting with isatin (1) and acetic anhydride-2-^{14}C by the reaction sequence shown in Scheme I. N-Acetylisatin (2), obtained by acetylation of isatin, was heated with sodium hydroxide solution according to a published method (2) to give 2-hydroxyquinoline-4-carboxylic acid (4) which must have been formed by hydrolytic ring opening of 2 to 3 followed by ring closure of 3. Thermal decarboxylation of 4 at 350 - 360° gave 2-hydroxyquinoline (8) with retention of all the radioactivity showing thereby that the label was in the heterocyclic ring. The hydroxy acid 4 was heated with phosphorous pentachloride to obtain the acid chloride 5 which was then converted to [^{14}C]dibucaine by adapting a method described in a patent (3). The acid chloride 5 was condensed with N,N-diethylethylene-

Scheme I



Scheme II



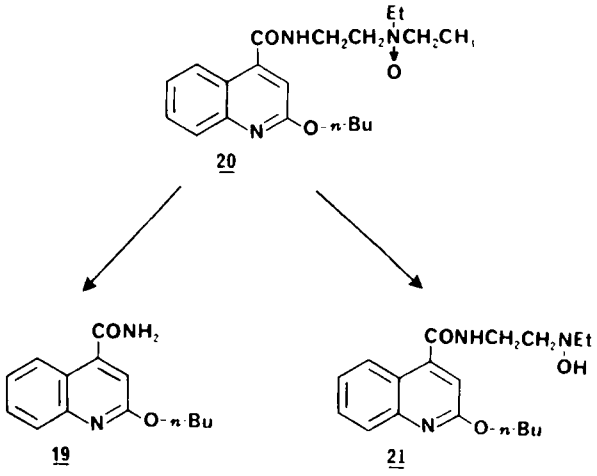
diamine and the resulting amide 6 was subsequently reacted with sodium n-butoxide to give 7.

Two deutero analogs, dibucaine-d_9 (11) and dibucaine-d_2 (13), were also synthesized as shown in Scheme II for the development of a sensitive GC-MS method for the analysis of dibucaine in biological fluids. The synthesis of these two deuterated compounds started from 2-hydroxyquinoline-4-carboxylic acid which was prepared by the published method (2) from N-acetylisatin. 2-Chloroquinoline-4-carboxylic acid chloride (9), prepared from the above hydroxy acid by heating with phosphorous pentachloride was reacted with N,N-diethylethylenediamine and the resulting amide 10 was then condensed with sodium salt of n-butyl-d_9 alcohol to give 11. For the synthesis of dibucaine-d_2 (13), the acid chloride 9 was reacted with N,N-diethyldideuteroethylenediamine (17) to give the labeled amide 12 which was subsequently reacted with sodium n-butoxide to give 13. Compound 17 required for the above synthesis was prepared in three steps from chloroacetyl

chloride (14). Reaction of 14 with diethylamine gave the amide 15 which was then treated with ammonia. The resulting amide 16 on reduction with lithium aluminum deuteride gave 17.

Stability of [^{14}C]dibucaine. In contrast to dibucaine and its deuterated analogs, [^{14}C]dibucaine (7) was found to undergo considerable decomposition by radiolysis. A sample of 7 stored at -5° for two years contained only 30% unchanged dibucaine. Thin layer chromatography showed one major decomposition product and four minor products. The major product was isolated by chromatography and identified as 2-butoxyquinoline-4-carboxamide (19) by comparison with a synthetic sample prepared from 2-chloroquinoline-4-carboxylic acid chloride (9). Compound 9, on treatment with ammonia, gave the amide 18 which was then reacted with sodium n-butoxide to obtain 19. One of the minor radiolysis products was identified as the N-oxide (20) by comparison of its TLC behavior with that of authentic N-oxide of dibucaine prepared by oxidation of dibucaine with 3-chloroperoxybenzoic acid. The synthetic N-oxide decomposed at 160 - 165° with evolution of a gas to give a mixture of compounds 19 and 21 (Scheme III). The radiolysis of [^{14}C]dibucaine may thus have occurred by the

Scheme III



formation and decomposition of its N-oxide. Compound 21 which was formed by elimination of ethylene (Cope reaction) from the N-oxide was not, however, detected as a radiolysis product. It is interesting to note here that neither 19 nor 21 was detected as a metabolite by Igarashi et al (2).

EXPERIMENTAL

Melting points are uncorrected. Thin layer chromatography (TLC) was performed on silica gel 60 F-254 plates (E. Merck) of 0.5 mm thickness and column chromatography with 70 - 230 mesh silica gel 60 (E. Merck). Acetic anhydride-2-^{14}C was purchased from New England Nuclear Corporation of Boston, Massachusetts, U.S.A., n-butyl-d_9 alcohol from MSD Isotopes of Montreal, Canada, and lithium aluminum deuteride from Aldrich Chemical Co. of Milwaukee, Wisconsin, U.S.A. Nuclear magnetic resonance spectra were obtained in $CDCl_3$ solution with a 90 MHz Varian EM 390 Spectrometer and EI mass spectra with a Finnigan 3300 mass spectrometer and an AEl MS-902 instrument.

N-Acetylisatin-^{14}C (2). To a solution of 1.28 g of isatin in 2 ml of pyridine was added 890 mg of acetic anhydride-2-^{14}C(50 mCi) and the mixture was stirred at room temperature for 22 hr. It was then cooled in an ice bath and decomposed by the addition of ice water. The precipitated solid was filtered and washed with water followed by ether. It was then air-dried to yield 1.30 g (80%); m.p. 140-142°; reported (2) m.p. 141-142°.

2-Hydroxyquinoline-4-carboxylic acid-^{14}C (4). The above solid was dissolved in 34 ml of 0.4N NaOH solution and then heated under reflux for 2 hr. After cooling in ice bath, the solution was neutralized to Congo red by adding 6N HCl solution. The precipitated solid was filtered immediately and washed with water. The solid was then dried at 80° under vacuum for 24 hr; yield 0.87 g (67%); m.p. 340-344°; reported (2) m.p. 346-347°.

2-Chloroquinoline-4-carboxylic acid chloride-^{14}C (5). The above acid was mixed with 2.1 g of PCl_5 and the mixture was heated for 4 hr at 110°. To the mixture was then added 10 ml of toluene and it was evaporated under re-

duced pressure to remove $POCl_3$ and toluene. After drying in high vacuum a solid was obtained; m.p. 88-89°; yield 780 mg (75%).

2-Chloro-N-[2-(diethylamino)ethyl]-4-quinoline carboxamide-^{14}C (6). The above solid was dissolved in 5 ml of toluene and cooled to 0°. To the cooled solution was then added 1.5 g of N,N-diethylethylenediamine in 3 ml of toluene. The mixture was stirred at room temperature for 2 hr and then 10 ml of 2N sodium carbonate solution was added to it. The toluene layer was separated, dried ($MgSO_4$) and distilled under reduced pressure. The residue was crystallized from petroleum ether to yield 845 mg of a solid (80%); m.p. 72-73°; reported (3) m.p. 74°.

[^{14}C]Dibucaine (7). To a solution of 130 mg of sodium in 15 ml of n-butanol was added 845 mg of the solid obtained in the last step. The mixture was heated under reflux for 2 hr and then evaporated under reduced pressure. The residue was chromatographed on a column of silica gel. Elution with 3% methanol in chloroform gave 760 mg of a solid after removal of the solvent. The solid was crystallized from petroleum ether to yield 735 mg of [^{14}C]dibucaine (78%); m.p. 63-64°; reported (3) m.p. 64°. The NMR spectrum showed peaks at 8.7-9.1, 7.2-8.3, 4.3-4.7, 3.6-4.3, 2.8-3.6 and 0.8-2.3 ppm, and it was identical to that of authentic dibucaine. TLC in methanol/ethyl acetate (1:1), and in ethyl acetate/acetic acid/water (75/15/10) showed only one UV visible spot, and only one radioactive peak after scanning with a Berthold radioscanner. The overall chemical yield from isatin was 25% and the radiochemical yield based on acetic anhydride-2-^{14}C was 12% to give 6.05 mCi of 7.

2-chloroquinoline-4-carboxylic acid chloride (9). This was prepared by heating a mixture of PCl_5 and 2-hydroxy-quinoline-4-carboxylic acid which was prepared from N-acetylisatin by the published method (2).

2-chloroquinoline-4-carboxamide (18). A slow stream of ammonia was passed through a solution of 3 g of 9 in 15 ml of toluene for 2 hr. Water was then added to the mixture and toluene layer separated. The toluene solution was dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow

solid which was crystallized from ethanol; m.p. 258-260°; yield 2.5 g. Anal. Calcd. for $C_{10}H_7N_2OCl$: C, 58.11; H, 3.39; N, 13.56. Found: C, 58.03; H, 3.57; N, 13.56.

2-Butoxyquinoline-4-carboxamide (19). To a solution of 400 mg of sodium in 20 ml of n-butanol was added 2 g of 18 and the mixture refluxed for 4 hr. After work-up, the product was crystallized from ethyl acetate; yield 1.85 g (75%); m.p. 162-164°. Anal. Calcd. for $C_{14}H_{16}N_2O_2$: C, 68.85; H, 6.56; N, 11.48. Found: C, 69.06; H, 6.79; N, 11.39.

N-Oxide of dibucaine (20). To a solution of 2 g of dibucaine in 100 ml of chloroform was added 2 g of 3-chloroperoxybenzoic acid. The solution was allowed to stand at room temperature. TLC in methanol showed that the oxidation was complete in 2 hr. The chloroform solution was extracted with 1N H_2SO_4 solution and the acid extract was made basic by adding 6N NaOH solution. The mixture was extracted with chloroform and the chloroform solution washed with brine. The residue obtained after evaporation of chloroform was crystallized from chloroform/ether to give 1.6 g of the N-oxide; m.p. 122-123°; reported (4) m.p. 122-123°. The NMR spectrum showed peaks at 9.7-9.9, 7.0-8.2, 4.3-4.6, 3.6-4.0, 2.8-3.3, 0.8-1.9 ppm.

Pyrolysis of dibucaine-N-oxide. The N-oxide (1 g) was taken in a test tube and heated in an oil bath. When the bath temperature reached 130°, the N-oxide melted and decomposed with evolution of gas. Heating was continued further until the temperature reached 160-165°. The melt was cooled and extracted with toluene. The toluene extract was chromatographed on a column of silica gel and eluted with ethyl acetate/toluene (1/1). Two major fractions were obtained. The first fraction with R_f = 0.53 on TLC in the above solvent system gave 275 mg of a white solid; m.p. 162-164° which was not depressed on mixing with 19. The second fraction with R_f = 0.35 gave 380 mg of a white solid; m.p. 121-122°. Anal. Calcd. for $C_{18}H_{25}N_3O_3$ (21): C, 65.26; H,7.55; N, 12.69. Found: C, 65.28; H, 7.49; N, 12.44. The NMR spectrum showed peaks at 6.8-8.1, 6.2-4.6, 3.3-3.7, 2.3-2.8 and 0.8-1.9 ppm.

Isolation of 2-butoxyquinoline-4-carboxamide-^{14}C. A sample of [^{14}C]dibucaine (300 mg) which was stored for 2 years was chromatographed on a column of silica gel and eluted successively with toluene/ethyl acetate (1/1), ethyl acetate and ethyl acetate/methanol (1/1). The toluene/ethyl acetate eluate gave 150 mg of a white solid; m.p. 162-164°; mass spectrum showed peaks at m/e 244 $(M)^+$, 201 $(M-CONH_2+1)^+$, and 188 $(M-C_4H_8)^+$. The ethyl acetate/methanol eluate gave 90 mg of [^{14}C]dibucaine.

2-Chloro-N,N-diethylacetamide (15). To a solution of 10 ml of diethylamine in 10 ml of toluene was added dropwise a solution of 4 g of chloroacetyl chloride (14) in 10 ml of toluene. The mixture was stirred for 1 hr and filtered. The filtrate was evaporated under reduced pressure and the residue distilled (b.p. 90-93°/7 mm) to give 5.4 g of the product.

2-Amino-N,N-diethylacetamide (16). The above product was stirred at room temperature with 40 ml of conc. NH_4OH for two days. Excess NH_4OH was removed by distillation and the residue extracted with ether. The ether extract was discarded and 6N NaOH solution was added to the residue until strongly basic. The mixture was extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated. The residual oil was then distilled (b.p. 68-70°/6 mm) to give 3.4 g of product.

2,2-Dideutero-(2-diethylamino)ethylamine (17). A solution of 2.5 g of the above oil in 10 ml of ether was added dropwise to a suspension of 1.1 g of lithium aluminum deuteride in 25 ml of ether with stirring. The mixture was then stirred and refluxed for 4 hr. After cooling to room temperature, the mixture was decomposed by careful addition of 3 ml of D_2O. The mixture was then filtered and the solid washed with ether. The washing and the filtrate were combined, dried ($MgSO_4$) and evaporated. The residue was distilled (b.p. 135-136°/760 mm) to give 850 mg of an oil. The mass spectrum showed peaks at m/e 118 $(M)^+$, 88 $[CD_2=N(C_2H_5)_2]^+$ and 60 $(CD_2=NHC_2H_5)^+$.

Dibucaine-d_2 (13). A toluene solution of 730 mg of the above amine 17

was condensed with 1.36 g of the acid chloride 9. The resulting amide 12 was then reacted with sodium n-butoxide in n-butanol following the procedure described for the preparation of [^{14}C]dibucaine. The product was purified by column chromatography followed by crystallization from petroleum ether to give 1.1 g of a white solid; m.p. 63-64°. The mass spectrum showed peaks at m/e 345 $(M)^+$, 273 $[M-N(C_2H_5)_2]^+$, 228 $[M-NHCH_2CD_2N(C_2H_5)_2]^+$, 200 $(228-CO)^+$, 172 $(228-C_4H_8)^+$, 144 $(172-CO)^+$ and 88 $[CD_2{=}N(C_2H_5)_2]^+$. TLC in methanol was identical to that of dibucaine.

Dibucaine-d_9 (11). This was prepared by condensing 920 mg of 10 (prepared from 9) with the sodium salt prepared from 150 mg of sodium hydride and 5 ml of n-butyl-d_9 alcohol. The product was purified by column chromatography and crystallization; yield 800 mg; m.p. 60-61° which is slightly lower than that of dibucaine. The mass spectrum showed a molecular ion peak at m/e 352 and fragment ion peaks at m/e 280, 237, 209, 172, 144 and 86. TLC in methanol was identical to that of unlabeled dibucaine.

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