THE USE OF DIBORANE AND DEUTERODIBORANE IN THE SYNTHESIS OF ISOTOPICALLY LABELED AMINES

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SUMMARY

The use of diborane has facilitated the preparation of several amines labeled with carbon-14 or nitrogen-15 by reduction of the corresponding labeled nitrile or amide. Its use was particularly advantageous in the presence of aromatic halide substituents. Deuterodiborane, formed in situ from sodium borodeuteride and dimethyl sulfate, has been shown to be useful in the synthesis of 1,1-dideuteroamines.

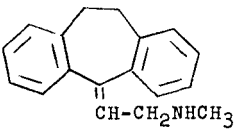
Amines prepared in this study include nortriptyline labeled with nitrogen-15, deuterium or both,; 2-(o-chlorophenoxy)ethyl-N-cyclopropylamine as the 1-^{14}C-derivative and as the 1,1-dideutero derivative; 2-(2,4-dichloro-6-phenylphenoxy)ethyl-1-^{14}C amine and its 2-chloro-6-phenyl analog; and 3-aminomethyl-^{14}C-pyridine.

INTRODUCTION

Diborane has been found (1) to have wide application as a selective reducing agent. In our work both diborane and deuterodiborane ($B_2{}^2H_6$) have been used to prepare several labeled amines, needed for drug metabolism studies, where use of lithium aluminum hydride (LAH) or lithium aluminum hydride-^{2}H either was unsuccessful or presented the problem of removal of an aromatic chloride substituent.

The availability of mass spectroscopy and, more recently,

combined gas liquid chromatography-mass spectroscopy for drug metabolism studies has increased the potential usefulness of heavy isotope labeled compounds. These compounds have application either for in vitro investigations or as an internal standard for in vivo studies (2). For this reason three derivatives of the antidepressant drug nortriptyline (I) have been prepared,



(I)

the ^{15}N analog (M+1), the dideutero (M+2), and the dideutero-^{15}N (M+3).

The method previously employed for the preparation of N-methyl-^{14}C-labeled nortriptyline (3) was not suitable for introduction of the ^{15}N label because of the impracticality of preparing the needed primary amine. A new synthesis was developed which produced the related carboxylic acid. This was converted to the N-methylamide or the N-methylamide-^{15}N which could then be reduced to the desired labeled nortriptyline. Initial attempts at reduction using LAH, however, did not yield the desired amino compound.

The known utility of diborane and the recent description (4) of its simple in situ preparation from sodium borohydride and dimethyl sulfate suggested adapting this procedure to the preparation of deuterodiborane, using NaB^2H_4. The use of diborane and deuterodiborane led to the successful preparation

of nortriptyline labeled with nitrogen-15, deuterium, or both. (Table 1). The method was extended to the preparation of a 1,1-dideuterated derivative of 2-(o-chlorophenoxy)ethyl-N-cyclopropylamine (Lilly 51641), a monoamine oxidase inhibitor which has been under study (5) in our laboratories.

The completeness of deuteration was shown by the 100 MHz NMR spectra (Varian HA 100) which were measured relative to tetramethylsilane as a standard. The NMR spectra of the deuterated and undeuterated compounds in deuterochloroform are identical except for the following two points: (1) The normal spectra give rise to a 2-proton triplet, at δ2.65 for nortriptyline and δ3.13 for the cyclopropylamino compound, corresponding to the CH_2-protons adjacent to nitrogen. This triplet is completely absent in the spectra of the deuterated materials. (2) Further evidence in regard to nortriptyline is the collapse from a triplet at δ2.34 to a doublet in the deuterated material in the signal corresponding to the allylic hydrogens. In the spectrum of the cyclopropylamino derivative the collapse is from a triplet at δ4.14 to a singlet in the signal for the methylene hydrogens adjacent to the oxygen.

Further elucidation of the purity and nature of 2-(o-chlorophenoxy)-1,1-dideuteroethyl-N-cyclopropylamine was obtained using an LKB-9000 gas-liquid chromatograph-mass spectrometer. Gas-liquid chromatography, using a 1% W98 column at 155°, indicated that the amine was pure. The molecular ion (213) was weak and the spectrum was complicated by the indication of

M+1 and M-1 ions, but strong peaks were present at 178(M-35, loss of chlorine), 127 (o-ClC_6H_4O), 86 ($CH_2C^2H_2NHC_3H_5$) and 72 ($C^2H_2NHC_3H_5$). The same mass fragmentation pattern, except for the difference in mass of the ions, was shown by authentic undeuterated material.

The dideuteronortriptyline and its dideutero-^{15}N analog were analyzed in the same manner (column temperature 180°) and were shown by gas-liquid chromatography to be pure. Due to the presence of M+1, M-1, M+2, and M-2 peaks the mass fragmentation patterns were of little use for any estimation of the extent of deuteration. That this did not indicate that the samples were impure was demonstrated by the identical behavior of nortriptyline itself. It has been found (2) that the N-trifluoroacetyl derivative is much more suitable for mass spectral analysis of nortriptyline.

2-(o-Chlorophenoxy)-ethyl-1-^{14}C-N-cyclopropylamine (Table 1) was prepared using commercial diborane for the reduction of the ^{14}C-labeled amide. This compound had previously been prepared using LAH for the reduction but the need for mild conditions to prevent removal of the o-chloro substituent led to a very low yield (16%). The use of diborane completely eliminated this problem and the amine was obtained in 61% yield.

The ability of diborane to reduce amides without removing aromatic halogens led also to the facile synthesis of two other ^{14}C-labeled compounds which were needed for metabolism studies (6). These were the 1-^{14}C-labeled demethylation inhibitors 2-(2,4-dichloro-6-phenylphenoxy)ethylamine hydrochloride (7, 8) (DPEA, Lilly 32391) and its 2-chloro-6-phenyl analog (8) (MPEA).

A further application of the diborane reduction was in the synthesis of 3-aminomethyl-^{14}C-pyridine. Reduction of nicotinamide gives a low yield of the amine but reduction of 3-cyano-^{14}C-pyridine gave the desired product in 42% yield. No product had been isolated from an attempted LAH reduction of nicotinamide. The brillant yellow color of the reaction mixture suggested the possibility that co-ordination had occurred but not the desired reduction. Co-ordination has been proposed (9), with development of an orange color, in the formation of the active agent for certain reductions with LAH in pyridine and in the production of dihydropyridine derivatives (10) in the attempted LAH reduction of a series of 3,5-dicyanopyridines.

EXPERIMENTAL

Materials and Methods.

The solution of diborane in tetrahydrofuran and the sodium borodeuteride were purchased from the Ventron Corporation. Methylamine-^{15}N hydrochloride was obtained from Merck Sharp and Dohme, Canada. Iodoacetamide-1-^{14}C, nicotinamide-7-^{14}C and chloroacetic-1-^{14}C acid were supplied by New England Nuclear Corp. All were used as obtained.

The plates used for thin-layer chromatography (TLC) were Silica gel-GF (E. Merck). Eastman Blue Brand X-ray film was used for the autoradiographs. The W98 column packing used in gas-liquid chromatography (GLC) is a methyl-vinyl silicone obtained from Applied Science Laboratories.

Reductions (1). With Generated Deuterodiborane. To 2 mmoles of sodium borodeuteride and 0.5 mmole of amide in 3 ml of dry THF,

in a flask swept with nitrogen and cooled in ice-water, was added, all at once, 2 mmoles (190 μl) of dimethylsulfate in 2 ml of dry THF. The mixture was stirred until it came to room temperature, warmed gently for 2 hours, and then refluxed for 8 hours. After cooling, it was hydrolyzed with 2 ml of 38% DCl in D_2O. It was then heated for about 2 hrs., boiling off most of the THF, made strongly basic and extracted with ether.

In the case of 2-(o-chlorophenoxy)-1,1-dideuteroethyl-N-cyclopropylamine, purification was accomplished by preparative TLC (1:1 methylcyclohexane-ethyl acetate in an ammonia atmosphere), elution with ethyl acetate, preparation of the hydrochloride, and recrystallization from isopropanol-ether.

The ether solution of the labeled nortriptyline was shaken with 10 equivalents, in small portions, of 1N HCl and then extracted with ethyl acetate to remove what appeared to be a small amount of unreacted amide. After adding about 10 drops of concd. HCl to the acidic solution and allowing it to stand overnight the product crystallized. Additional product was obtained from the ether and ethyl acetate solutions, after shaking with base, by preparative TLC (2:1 ethyl acetate-methyl cyclohexane in an ammonia atmosphere) and elution with 2:1 ethyl acetate-ethanol. The solvents were removed under reduced pressure and crystalline hydrochloride was obtained after adding 2 ml of 1N HCl.

(2) With Diborane. These reductions were carried out in the same manner except using measured amounts of the commercial 1 molar solution of diborane in tetrahydrofuran.

5-Hydroxy-5-vinyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptene.

Vinylmagnesium bromide was prepared on a 0.87 molar scale according to the procedure of Seyferth (11). Then a solution of 150g (0.72 mole) of 10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one in 150 ml of dry THF was added at a rate which maintained the temperature at 40-50°. After completion of the addition, the mixture was heated at 60° for one hour and was allowed to stand overnight. The mixture was then chilled in an ice-bath and was hydrolyzed with 600 ml of satd. NH_4Cl solution. It was stirred for an hour, extracted with ether, washed with water, and dried over $MgSO_4$. Removal of the ether and distillation gave 150g (82%) of product which boiled at 139-141° at 0.3 mm.

Anal. Calcd for $C_{17}H_{16}O$: C, 86.40; H, 6.83; O, 6.77. Found: C, 86.15; H, 6.91; O, 7.03.

10,11-Dihydro-5H-dibenzo[a,d]cycloheptene-$\Delta^{5,\beta}$-ethyl Chloride.

Hydrogen chloride gas was passed into a solution of 50g (0.21 mole) of the above vinyl carbinol in 500 ml of dry chloroform, at a rate to promote reflux, until saturated and for thirty minutes thereafter. The solvent was removed and the product was distilled at 160-165° at 0.8 mm to give 39.1g (79%).

Anal. Calcd for $C_{17}H_{15}Cl$: C, 80.15; H, 5.94; Cl, 13.92. Found: C,80.43; H, 6.18; Cl, 14.18.

10,11-Dihydro-5H-dibenzo[a,d]cycloheptene-$\Delta^{5,\beta}$-propionitrile.

A mixture of 25.5g (0.1 mole) of the chloro compound and 4.9g (0.1 mole) of NaCN in 250 ml of ethanol was heated at reflux overnight. The solid present was isolated by filtration, triturated with water, and recrystallized from ethanol to give

product melting at 142-145°.

Anal. Calcd for $C_{18}H_{15}N$: C, 88.13; H, 6.16; N, 5.71. Found: C, 87.90; H, 6.34; N, 5.64.

Additional product was obtained by taking the reaction mixture to dryness. After crystallization from ethanol it did not depress the m.p. of the first material isolated. The combined yield was 10g (41%).

10,11-Dihydro-5H-dibenzo[a,d]cycloheptene-$\Delta^{5,\beta}$-propionic acid. The nitrile (10g, 0.041 mole) was hydrolyzed with KOH (9.1g, 0.13 mole) in ethylene glycol (33 ml) following a procedure described by Prout and co-workers (12). The acid isolated was crystallized from ethanol-water to give 5.9g (55%) melting at 115-117°.

Anal. Calcd for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10; O, 12.11. Found: C, 81.84; H, 6.26; O, 12.28.

N-Methyl-10,11-dihydro-5H-dibenzo [a,d]cycloheptene-$\Delta^{5,\beta}$-propionamide-^{15}N. The acid chloride was prepared from 660 mg (2.5 mmoles) of the acid and 0.5 ml (2.9 mmoles) of oxalyl chloride in 24 ml of dry benzene. Reaction with 240 mg (1.5 mmoles) of methylamine-^{15}N hydrochloride, 1 ml of water and 3 ml of 5N NaOH gave 435 mg of a cream colored product which melted at 125-130°. Preparative TLC (EtOAc-methylcyclohexane, 2:1) and EtOAc elution gave 330 mg (48%) of product melting at 132-134°. Analysis by gas-liquid chromatography (1% W98 column, 220°)-mass spectrometry showed the product to be pure with a molecular ion of 278. Fragments corresponded to $CH\text{-}CH_2CO^{15}NHCH_3$ (86), $CH_2CO^{15}NHCH_3$ (73) and $CO^{15}NHCH_3$ (59).

N-Methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptene-$\Delta^{5,\beta}$-propionamide was prepared according to the same general procedure. The crude product melted at 133-135°, and a sample was purified by recrystallization from dilute EtOH and then from EtOH to give product which melted at 137-139°.

Anal. Calcd. for $C_{19}H_{19}NO$; C, 82.28; H, 6.90; N, 5.05. Found: C, 82.50; H, 7.08; N, 5.24.

o-Chlorophenoxyacetic-1-^{14}C Acid. To 256 mg (2 mmoles) of o-chlorophenol in 0.6 ml of 5N NaOH solution was added 94.5 mg (1 millicurie, 1 mmole) of chloroacetic-1-^{14}C acid and 1 ml of water for a rinse. The mixture was heated at 60-70° for 7 hours. It was then acidified with 6N HCl, extracted with ether, and the extracts were washed with water. The product was extracted with $NaHCO_3$ solution and was isolated by acidification with 6N HCl and filtration to give 61 mg melting at 147-149° (13). Ether extraction of the acidified solution gave an additional 10 mg of product for a total yield of 38%. This was used without further purification.

o-Chlorophenoxy-N-cyclopropylacetamide-1-^{14}C. To the acid chloride prepared from 71mg (0.38 mmole) of the acid, using oxalyl chloride, was added a solution of 82mg (1.44 mmoles) of cyclopropylamine in 3 ml of benzene. The yield of crude amide was 63mg (73%) melting at 92-94° (authentic sample, 92-94). It was not purified prior to reduction.

2-(2,4-Dichloro-6-phenylphenoxy)acetamide-1-^{14}C. To the NaOEt from 25 mg (1.1 mmoles) of sodium in 1 ml of absolute ethanol was added 240 mg (1 mmole) of 2,4-dichloro-6-phenylphenol and

10 ml of dry toluene. The solution was reduced to half volume by boiling and there was then added 1 mCi (185 mg, 1 mmole) of iodoacetamide-1-^{14}C. The mixture was stirred and refluxed gently for 5 hours. After cooling and adding 10 ml of $CHCl_3$, the solution was washed twice with 1N NaOH solution and then three times with water. The washes were drawn off with a dropper. The dried ($MgSO_4$) solution was taken to dryness under reduced pressure to give 240 mg of crude amide. This was purified on two preparative TLC plates (2 mm. thickness) using benzene-ethyl acetate (1:1) and eluting with ethyl acetate. The colorless product (175 mg, 59%), melting at 142-143° after softening, was used without further purification. The reported (14) melting point for the unlabeled amide is 143°.

2-Chloro-6-phenylphenoxyacetamide-1-^{14}C was prepared in 65% yield by the same procedure on a 0.5 mmole scale using 92.5 mg (1 millicurie) of iodoacetamide-1-^{14}C. The crude amide melted at 157-158° and was reduced directly without the TLC purification employed above. The literature (14) melting point for unlabeled amide is 159°.

3-Cyano-^{14}C-pyridine was prepared in 70% yield by distillation from a mixture of nicotinamide-7-^{14}C and P_2O_5 according to the method of LaForge (15). It was used without further purification.

3-Aminomethyl-^{14}C-pyridine dihydrochloride. To 3.5 ml (3.5 mmoles) of 1M B_2H_6 in THF was added 146mg (1.4 mmoles) of the above nitrile in 8 ml of dry THF. An initial orange color faded

Table I

LABELED AMINES FROM DIBORANE OR DEUTERODIBORANE REDUCTIONS OF AMIDES

PRODUCT	mmoles amide	mmoles B_2H_6	Yield %	mp °C[1]	Thin-layer System	Specific Activity μCi/mg, mCi/mmole
^{14}C-Labeled:						
DPEA-1-^{14}C	0.53	1.5	57	177-179°	C_6H_6-EtOAc 1:1	2.35, 0.75
MPEA-1-^{14}C	0.32	1.5	26	163-165°	EtOH-EtOAc 1:1	7.65, 2.18
o-$ClC_6H_6OCH_2{}^{14}CH_2NHC_3H_5$	0.28	2.0	61	120-121°	Methylcyclohexane-EtOAc 1:1 in ammonia atmosphere	6.16, 1.53
Deuterium and ^{15}N-Labeled:						
Nortriptyline-^{15}N	0.50	2.00	9	213-215°		
Nortriptyline-$^{2}H_2$	0.62	1.24($B_2{}^{2}H_6$)	17	212-214°		
Nortriptyline-^{15}N-$^{2}H_2$	0.50	2.00($B_2{}^{2}H_6$)	20	211-213°		
o-$ClC_6H_4OCH_2$-$C^{2}H_2NHC_3H_5$	1.00	2.00	37	120-122°		

[1]In addition to the melting points which agreed with those of authentic samples, purity and identity were further confirmed for the ^{14}C-compounds by thin-layer chromatography, co-chromatography with authentic material, and autoradiography. In all cases the products gave a single radioactive spot. The deuterium and nitrogen-15 labeled compounds all gave a single peak on gas-liquid chromatography.

to yellow during one hour stirring and became colorless during 3 hours refluxing. Hydrolysis was accomplished with 1 ml of ethanol and 2 ml of 6N HCl. The mixture was made basic with saturated KOH and was extracted with ether.

The solution was dried over $MgSO_4$. The dihydrochloride (105mg, 42%) melted at 222-227° after shrinking. The specific activity was 2.73 μCi/mg. Thin-layer chromatography (EtOH-EtOAC 1:1 in an NH_3 atmosphere) co-chromatography with authentic material, autoradiography, and scintillation counting of the radioactive sections showed the product to have a radiochemical purity of 95%.

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