SYNTHESIS OF DEUTERATED AND TRITIATED DERIVATIVES OF ENFLURANE

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SUMMARY

Specifically deuterated derivatives of the inhalation anesthetic enflurane [2-chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane] have been synthesized by a facile base catalyzed exchange. Tritiated enflurane has also been synthesized by this procedure.

Key Words: Enflurane, deuterium, tritium, metabolism

INTRODUCTION

Because of the occasional reports of hepatic (1) and renal (1) toxicities associated with the inhalation anesthetics halothane ($CF_3CHClBr$) and methoxyflurane ($CH_3OCF_2CHCl_2$) respectively, there has been increased interest in the development of safer alternative drugs. One compound that has become widely employed is the fluorinated ether, enflurane (1,Fig.1). Although this inhalation anesthetic appears to be safer than halothane and methoxyflurane, there have been recent reports indicating that it can produce renal changes in both man (2-4) and rat (5) similar to those seen with methoxyflurane.

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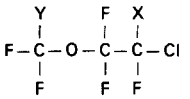

1 X = H, Y = H
2 X = D, Y = D
3 X = D, Y = H
4 X = H, Y = D
5 X = ^{3}H, Y = ^{3}H

Fig. 1. Structure of enflurane (1) and various deuterated and tritiated derivatives.

Fluoride ion appears to be responsible, at least in part, for the toxicity associated with the administration of methoxyflurane (1,5,6) and enflurane (4,5). This metabolite appears to be formed predominently in the microsomal fraction of liver by cytochrome P-450 (7). Based upon previous metabolism studies with related halogenated hydrocarbons such as chloroform (8), chloramphenicol (9,10), halothane (11) and dihalomethanes (12,13), enflurane is probably metabolized to fluoride ion by an oxidative dehalogenation mechanism (Fig.2). This reaction may occur by the initial oxidation of the C-H bond of the chlorofluoromethyl carbon (Pathway A) or by oxidation of the C-H bond of the difluoromethyl carbon (Pathway B). The alcohol intermediates in both pathways would be expected to dehalogenate spontaneously to produce acyl halides, which upon hydrolysis produce carboxylic acids and halide ion.

One way to determine experimentally the relative importance of these pathways of metabolism (Fig.2) is to compare the rates of defluorination of specifically deuterated derivatives of enflurane. Since deuterium substitution decreases the oxidative dehalogenation of chloroform (14,15), bromoform (16), and dihalomethanes (13) in rat liver microsomes, it seemed likely that the substitution of deuterium at the chlorofluoromethyl carbon should inhibit Pathway A, whereas deuterium substitution

at the difluoromethyl carbon should inhibit Pathway B (Fig.2). In order to test this idea, we have developed a procedure for the facile synthesis of specifically deuterated derivatives of enflurane. Since the only metabolite of enflurane thus far identified has been fluoride ion we have also synthesized ^{3}H-labeled enflurane, which should be useful in the isolation and identification of other metabolites of enflurane.

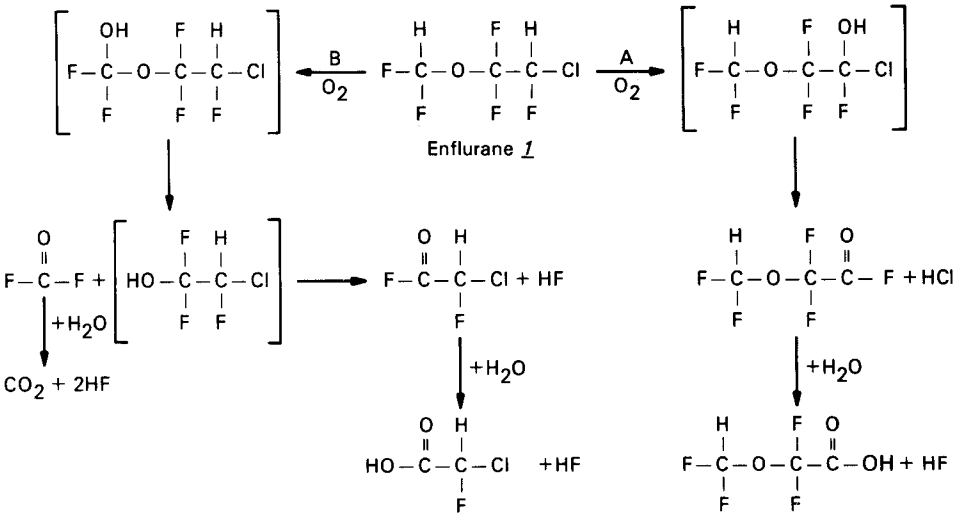

Fig. 2. Potential pathways for the oxidative metabolism of enflurane to fluoride ion.

RESULTS AND DISCUSSION

Synthesis. When enflurane was mixed with two volumes of 0.4 M NaOD at room temperature for 14 hr, very little exchange occurred at either the difluoromethyl carbon or the chlorofluoromethyl carbon (Table 1). However, heating the reaction mixture to reflux for 20 hr resulted in an 87% exchange at the chlorofluoromethyl carbon as compared to a 13% exchange at the difluoromethyl carbon. Increasing the NaOD concentration to 2.0 M resulted in only moderate increases in exchange at both positions.

Table 1. Results of enflurane exchange under various conditions.[a]

NaOD (M)	Temp.	Aliquat 336[a]	Time (hr)	% Exchange[b] CF_2D	% Exchange[b] CFClD
0.4	room temp.	-	14	1	8
0.4	reflux	-	20	13	87
2.0	reflux	-	20	20	88
1.0	room temp.	+	16	85	94
1.0[c]	room temp.	+	24	98	99
c,d	reflux	+	2	10	97

[a]In a typical reaction, a mixture of 1 volume of enflurane and 2 volumes of a NaOD solution were stirred at room temperature or at reflux in the presence or absence of 0.1 equivalent of Aliquat 336 (tricaprylylmethylammonium chloride).

[b]The percent exchange at the CF_2H and CFClH positions were calculated from the ion intensities at m/e 51,52 and 67,68, respectively.

[c]Exchange was performed three times.

[d]Two volumes of Na_2CO_3 (pH 8-9) were used in place of NaOD solution.

The rate of exchange at both positions was significantly increased when a catalytic amount (0.1 equivalent) of the phase transfer catalyst Aliquat 336 (tricaprylylmethylammonium chloride) was added to the reaction mixture. For example, after 2 hr at room temperature a 28% exchange occurred at the difluoro carbon while a 94% exchange resulted at the chlorofluoro carbon. After 16 hr at room temperature the percent exchange was increased to 85% and 94%, respectively (Table 1). The incorporation of deuterium into both positions reached at least 98% when the reaction was heated to reflux and and re-exchanged two additional times with fresh NaOD solution (Table 1).

The increase in the rate of exchange produced by the phase transfer catalyst permitted the use of less alkaline conditions, thereby allowing selectivity of exchange at the more acidic chlorofluoromethyl carbon. For instance, when one volume of enflurane and 2 volumes of Na_2CO_3 solution in D_2O (pH 8-9) were mixed with 0.1 equivalents of Aliquat 336, heated at reflux for 2 hr, and the exchange repeated two additional times under identical conditions, deuterium incorporation reached 97% at the chlorofluoromethyl carbon and only 10% at the less reactive difluoromethyl carbon (Table 1).

When the 98% dideuterated enflurane (compound 2) was mixed with H_2O (pH 8-9) and 0.1 equivalents of Aliquat 336, there was a selective replacement of deuterium with hydrogen at the chlorofluoromethyl carbon. In this fashion compound 4 was prepared with deuterium incorporations of 4% and 89% at the chlorofluoromethyl and difluoromethyl carbons, respectively.

Tritium labeled enflurane (compound 5) was synthesized by reacting enflurane in the presence of ^3H_2O, 1M NaOH, and Aliquat 336 for 3 days at 67°C. In this manner, a 53% recovery of enflurane-^3H was obtained with a specific activity of 13.8 mCi/mmol. The relative incorporation of tritium at each carbon was estimated to be in a ratio of 1.00 to 1.07 at the difluoromethyl and chlorofluoromethyl positions by repeating the reaction with D_2O instead of ^3H_2O and analyzing the product by mass spectroscopy.

Mass Spectral Evaluation. The fragmentation pattern of enflurane (1) permitted the facile assignment of both position and extent of deuteration (Fig.3). For instance, the intense ion at m/e 51 corresponds to the difluoromethyl group. The mass of this ion is unchanged in compound 3, but moves up 1 amu to m/e 52 in compounds 2 and 4. The ion doublet, in a ratio of approximately 3 to 1 at m/e 67, 69 represents the chlorofluoromethyl group. This ion pair is at m/e 67,69 in compound 4 and at m/e 68, 70 in compounds 2 and 3. The mass spectra of the deuterated derivatives clearly demonstrate that the ion at m/e 117 of enflurane is composed of a difluoromethoxydifluoromethyl and a 1,1-chlorofluoro-2,2-difluoroethyl fragment in a ratio of approximately 1 to 1.

EXPERIMENTAL

2-Chloro-1-(difluoromethoxy-d)-1,1,2-trifluoroethane-2-d (2).

A mixture of 5.0 ml (41 mmol) of 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane (Enflurane, Ohio Medical Products) (1), 200 mg (0.41 mmol) of tricaprylylmethylammonium chloride (Aliquat 336, General Mills Chemicals, Inc.) and 10 ml of 1.0 M NaOD (1 ml of 40% NaOD, Aldrich, 99% D; and 9 ml of D_2O, Merck, 99% D) were stirred at reflux. After 24 hr, the mixture was cooled to room temperature, the aqueous layer was replaced by an additional

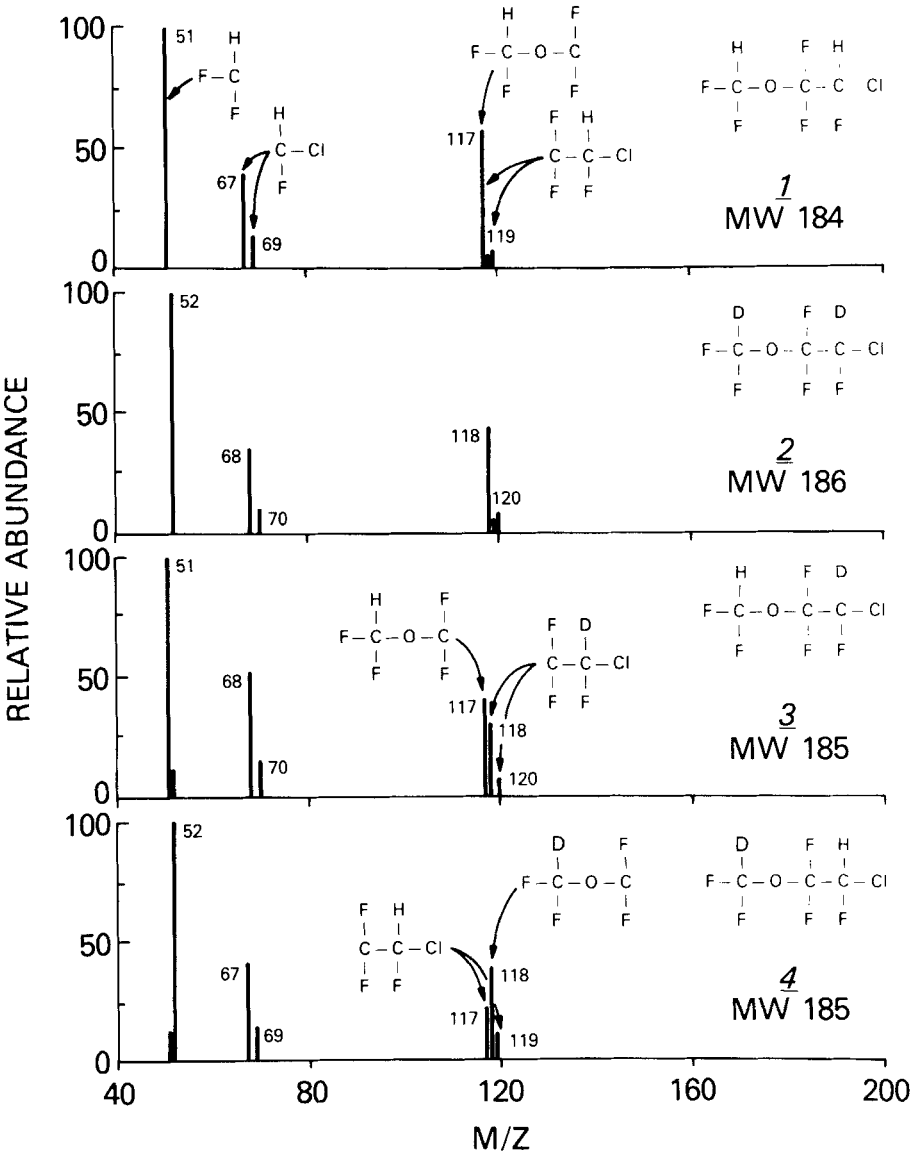

Fig. 3 Mass spectra of enflurane and deuterated derivatives.

10 ml of 1.0 M NaOD and the exchange reaction continued for another 24 hr. The exchange was repeated a third time with 10 ml of 1.0 M NaOD. After 2 hr the mixture was cooled to room temperature and the organic layer was removed and washed with 1 ml of 1N HCl and then distilled at room temperature in a micro distillation apparatus. The product was collected by immersing the collection flask in a dry ice-acetone bath. In this manner, 2.9 ml (58% yield) of enflurane-d_2 (2) was obtained. Mass spectral analysis indicated deuterium incorporation of 99% at the chlorofluoromethyl carbon and 98% at the difluoromethyl carbon.

2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane-2-d (3). A mixture of 3.5 ml (29 mmol) of enflurane (1), 177 mg (0.36 mmol) of Aliquat 336 and 7 ml of Na_2CO_3 in D_2O (pH 8-9) was stirred at reflux. After 2 hr the aqueous layer was replaced by another 7 ml of D_2O (pH 8-9) and heated at reflux for 1 hr. The exchange was repeated a third time. After 1 hr 1.15 g (33%) of enflurane-d (3) was obtained. Mass spectral analysis indicated deuterium incorporations of 97% and 10% at the chlorofluoromethyl and difluoromethyl positions, respectively.

2-Chloro-1-(difluoromethoxy-d)-1,1,2-trifluoroethane (4). A mixture of 3 ml (25 mmol) of enflurane-d_2 (2), 120 mg (0.25 mmol) of Aliquat 336 and 6 ml of aqueous Na_2CO_3 (pH 8-9) was reacted following the procedure outlined for the synthesis of compound 3. Distillation of the reaction product gave 2.0 g (66%) of enflurane-d (4) with deuterium incorporation of 89% and 4% at the difluoromethyl and chlorofluoromethyl positions, respectively.

Enflurane-^{3}H (5). A mixture of 1 ml (8.3 mmol) of enflurane (1), 120 mg (0.25 mmol) of Aliquat 336, 40 mg (1.0 mmol) of NaOH, and 1 ml of ^3H_2O (1 Ci; New England Nuclear) was placed into a 5 ml reaction vial. The reaction container was sealed with a Teflon lined septum and shaken to dissolve the NaOH. The mixture was then placed in an oil bath at 67°C and magnetically stirred. After 3 days the vial was cooled in an ice bath and the aqueous layer was removed through the septum with a syringe. The organic layer was then washed 2 times with 0.8 ml of 0.05 M HCl and 13 times with

0.8 ml H_2O to remove residual ^3H_2O. Micro distillation at 40-50°C gave 800 mg (53%) of enflurane-^3H (5) with a specific activity of 13.8 mCi/mmol.

Mass Spectra Analysis. Mass spectra were obtained on a V.G. Micromass 16F spectrometer at an accelerating voltage of 4kV, an electron energy of 70eV, and a source temperature of 220°C. The samples were introduced into the instrument both directly through a septum inlet and by injection onto an interfaced Varian 1400 gas chromatograph which was equipped with a glass column (2 mm i.d. x 1.81 m) and packed with Porapak Q, 100/120 mesh. The injector and column temperatures were 250° and 150°, respectively. Analysis of the products by gas chromatography-mass spectroscopy indicated that all of the labeled enflurane derivatives were at least 99% chemically pure.

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