SYNTHESIS OF DEUTERATED ARYLALKANOIC ACIDS FOR USE AS INTERNAL STANDARDS IN GAS CHROMATOGRAPHY/MASS SPECTROMETRY ASSAYS FOR NON-STEROIDAL ANTI-INFLAMMATORY DRUGS.

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SUMMARY

A general method for the deuteration and purification of undeactivated arylalkanoic acids commonly used as non-steroidal anti-inflammatory drugs is described.

KEY WORDS

Exchange deuteration, deuterated non-steroidal anti-inflammatory drugs.

INTRODUCTION

Part of the research conducted in our laboratories requires the quantitation of small amounts (10 ng/mL or less) of many non-steroidal anti-inflammatory drugs. These drugs are usually dosed orally, and high

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performance liquid chromatography (HPLC) has the required degree of sensitivity for most clinical assays. However, there is a trend towards the topical use of these compounds and the resulting plasma levels are very low (1), therefore, a more sensitive assay technique is required to monitor the processes of absorption and excretion. Gas chromatography/mass spectrometry (GC/MS) is a technique ideally suited to the detection of low plasma concentrations of these drugs. GC/MS also has the sensitivity to detect the extremely low free levels of these drugs observed in protein binding studies (2). The most suitable internal standard for GC/MS assays are stable isotope labelled analogues of the analyte. This report describes the deuteration of several arylalkanoic acids which are suitable for use as internal standards for GC/MS assays using a modification of the approach Whitlam and Vine (2) used to synthesise deuterated ibuprofen.

EXPERIMENTAL

The following compounds were treated using the procedure detailed below: Biphenylacetic acid, naproxen (6-methoxy-∝-methyl-2-naphthaleneacetic acid), ibuprofen (∝-methyl-4-[2-methylpropyl]benzene acetic acid), indomethacin (1-[4-chloro-benzoyl]-5-methoxy-2-methyl-1H-indole-3-acetic acid), ketoprofen (3-benzoyl-methylbenzene acetic acid), diclofenac (2-[{2,2-dichlorophenyl}amino]benzeneacetic acid) diflunisal, (2',4'-difluoro-4-hydroxy[1,1'-biphenyl]-3-carboxylic acid) and salicylic acid. 50 mg of each compound was dissolved in 10 mL of dry

CH_2Cl_2. A 100 μL aliquot was evaporated to dryness and reconstituted in 2 mL of methanol. Aliquots of this solution were used for subsequent HPLC and GC/MS analysis. The remainder of the CH_2Cl_2 solution was added to 450 mg of dry $AlCl_3$ in a dry 20 mL vial, shaken and once the Friedel-Crafts complex had formed, 100 μL of D_2O (99.8 atom%) was added. The resulting mixture was stirred for 20 minutes. 1 mL of D_2O was added to decompose the reaction complex, the reaction mixture was centrifuged, 100 μL of the CH_2Cl_2 layer was withdrawn for analysis, the remainder was transfered to another vial and the reaction repeated as above. The reaction procedure was repeated one further time to give 3 deuterium exchange processes in total.

The yield of each step was calculated by HPLC analysis, and the incorporation of deuterium at each step by negative ion chemical ionisation GC/MS of the pentafluorobenzyl derivative of each compound (3). The product of the final exchange reaction was purified by preparative HPLC.

Gas Chromatography/Mass Spectrometry Analysis

GC/MS analyses were carried out on a Finnigan TSQ-46 triple stage quadrupole mass spectrometer operating in negative ion chemical ionisation mode (3). The BP 5 (SGE Australia) fused silica bonded phase capillary column (12 m x 0.2 mm I.D., 0.25 μm film thickness) was inserted directly into the ion source of the mass spectrometer. Helium (2 mL/min) was the GC carrier gas and methane, the CI reactant gas, was introduced

through the make up valve to give a final source pressure of 130 Pa. The ion source and analyser regions of the mass spectrometer were maintained at 140° C and an electron beam energy of 100 eV was used. Collision activated decomposition (CAD) mass spectra were generated using argon as the collision gas at a pressure of 0.3 Pa and the collision energy was 25 eV. The following temperature programme was used for each of the derivatives assayed. The capillary column was held at 150° C for one minute following splitless injections of 1 µL aliquots of each derivative and was then temperature programmed to 300° C at 25° C per minute. The injection port and the GC/MS interface were held at 250° C and 300° C respectively.

High Performance Liquid Chromatography Analysis.

HPLC analyses were performed using a model 600 multi-solvent delivery system equipped with a model 490 programmable multiwavelength detector which was operated at 254 nm, a model 712 automatic injection device and a model 740 data module (Millipore-Waters, Australia). Quantitative analyses were carried out on a 250 mm x 4.6 mm I.D. Ultrasil 10 µm C18 reversed phase column (Beckman Instruments, Sydney, Australia), the mobile phase consisted of 40% ethanol : 60% water : 0.02% acetic acid adjusted to pH 5.6 with ammonia solution and the flow rate was 2 mL/min which produced a column pressure of 90-95 bar. Semi-preparative analyses were performed on a 250 mm x 10 mm I.D., Techsil 10µm C18 reversed phase column (Activon, Sydney, Australia) using the same mobile phase at a flow rate of 4.5 mL/min.

RESULTS AND DISCUSSION

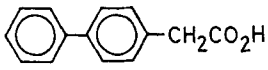
The percentage yield and percentage incorporation into the aromatic rings for each exchange reaction for naproxen, indomethacin, ibuprofen and biphenylacetic acid are shown in Table 1. No exchange, or complete degradation, occurred for ketoprofen, diclofenac, diflunisal and salicylic acid. Of these eight compounds all are arylacetic acid derivatives except diflunisal and salicylic acid which are benzoic acid derivatives. Significant incorporation, i.e. where it was possible to reduce the amount of d_0 to less than 1% after three exchange reactions, only occurred in those compounds in which the aryl ring was activated with respect to electrophilic aromatic substitution.

The effectiveness of the exchange reaction is related closely to the theoretical reactivity of each of the molecules with respect to Friedel-Crafts alkylation. A comparison of the CAD mass spectrum of the indomethacin anion containg the ^{37}Cl isotope (m/z 358) with the product of the third exchange reaction for indomethacin containg the ^{35}Cl isotope (m/z 358) provides evidence for this statement.

Figure 1 is the CAD mass spectrum of the indomethacin anion containing the ^{37}Cl isotope, m/z 358 corresponds to the indomethacin anion, m/z 314 to loss of CO_2, m/z 158 to loss of $C_7H_4O^{37}Cl$ and CH_3 from the decarboxylated indomethacin anion and m/z 113 corresponds to $C_6H_4{}^{37}Cl$.

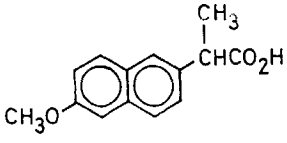
Figure 2 is the CAD mass spectrum of the indomethacin anion of the deuterated product which contains the ^{35}Cl isotope. The mass

Biphenylacetic acid



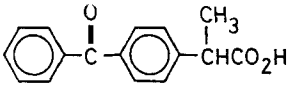
exchange reaction number	Incorporation (%) d_0	d_1	d_2	d_3	d_4	d_5	d_6	d_7	d_8	d_9	Yield (%)
1	2	1	2	3	7	16	28	28	14	1	60
2	1	1	1	-	-	1	3	11	32	50	49
3	-	-	-	-	-	-	-	-	33	66	27

Naproxen



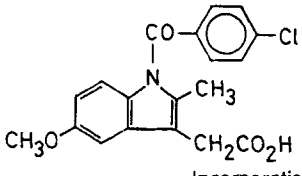
exchange reaction number	Incorporation (%) d_0	d_1	d_2	d_3	d_4	d_5	d_6	Yield (%)
1	21	44	10	11	11	2	0	88
2	4	52	12	13	14	4	0	ND
3	-	51	11	14	16	5	1	34

Ibuprofen



exchange reaction number	Incorporation (%) d_0	d_1	d_2	d_3	d_4	Yield (%)
1	4	4	10	46	35	45
2	-	-	5	15	81	37
3	-	-	-	-	100	26

Indomethacin



exchange reaction number	Incorporation (%) d_0	d_1	d_2	d_3	d_4	d_5	d_6	d_7	Yield(%)
1	10	39	50	-	-	-	-	-	78
2	5	10	85	-	-	-	-	-	ND
3	3	6	92	-		-	-	-	30

ND = Not Determined, - = <1.0%

Table 1: The percentage incorporation of deuterium into each compound studied and the percentage yield of the reaction (numbers may not total 100 due to rounding).

spectrum is very similar to that in Figure 1 except that the ion at m/z 160 replaces that at m/z 158 indicating that the parent ion of deuterated

indomethacin contains deuterium in the indole portion of the molecule. The ion at m/z 111 corresponds to $C_6H_4{}^{35}Cl$, no ion is present in this mass spectrum which indicates incorporation of deuterium into the

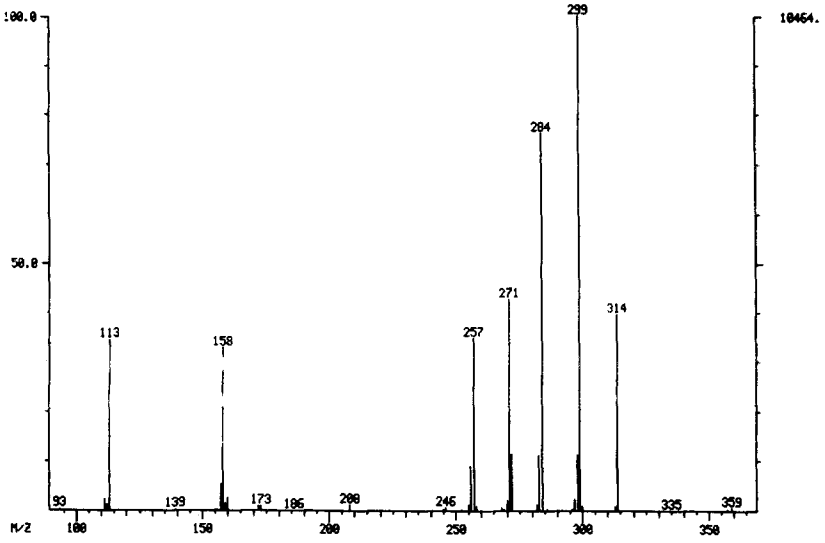


Figure 1. The CAD mass spectrum of the indomethacin anion (m/z 358) containing the ^{37}Cl isotope

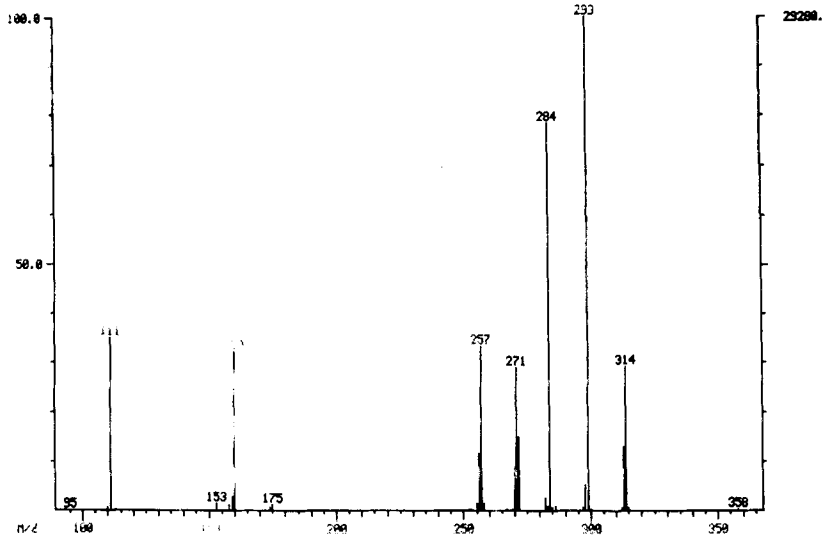


Figure 2: The CAD mass spectrum of the deuterated indomethacin anion (m/z 358) containing the ^{35}Cl isotope

chlorobenzene portion of the deuterated indomethacin molecule.

In summary therefore analysis of the fragmentation patterns shown in Figures 1 and 2 shows that incorporation has only occurred in the indole portion of the molecule and that no exchange into the chlorobenzoyl ring has taken place. The deuterium in the aromatic rings did not back-exchange on treatment with dilute acid.

The synthetic sequence described here is a rapid and inexpensive procedure for the production of deuterium labelled analogues for a wide variety of non-steroidal anti-inflammatory drugs. We have used this approach to synthesise deuterium labelled internal standards for use in the development of GC/MS assays for ibuprofen, biphenylacetic acid and indomethacin and are extending this work to develop a GC/MS method to screen urine samples for the presence of a range of non-steroidal anti-inflammatory drugs.

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