SYNTHESIS OF DEUTERATED ANALYTICAL STANDARDS: KETOPROFEN-D_3 AND FLUNIXIN-D_3

T. Tobin, W. Karpiesiuk, C. Hughes, A.F. Lehner and F. Camargo

The Maxwell H. Gluck Equine Research Center, the Department of Veterinary Science and the Livestock Disease Diagnostic Center, University of Kentucky, Lexington, 40546-0099, Kentucky, USA

ABSTRACT

Synthesis of ketoprofen-d_3 and flunixin-d_3 is presented. The first step in the synthesis of flunixin-d_3 was generation of the deuterium-containing methylating agent trimethyl-d_9 sulphoxonium iodide. Iodomethane-d_3 was reacted with excess DMSO-d_6 to produce this salt which was then used to methylate m-nitrobenzotrifluoride in a nucleophilic substitution reaction in the presence of sodium hydride in DMSO-d_6 yielding 2-methyl-d_3-3-nitrobenzotrifluoride which, after catalytic hydrogenation, gave 2-methyl-d_3-3-trifluoromethylaniline. This compound was then reacted with the ethyl ester of chloronicotinic acid giving the ethyl ester of flunixin-d_3 which, after hydrolysis, provided flunixin-d_3. Crystallisation yielded fine white crystals of product.

Synthesis of ketoprofen-d_3 was accomplished using classical methods. First 3-methyl-benzophenone was brominated with N-bromosuccinimide in benzene to obtain 3-bromomethyl-benzophenone, which after reaction with tetraethylaminocyanide in methylene chloride gave the corresponding nitrile. Methylation of this material with iodomethane-d_3 produced 2-(3-benzoylphenyl)propanenitrile-d_3 which, after hydrolysis with aqueous potassium hydroxide, produced ketoprofen-d_3 as a colourless oil. Crystallisation yielded fine white crystals of product.

INTRODUCTION

Flunixin and ketoprofen (Fig 1) are non-narcotic and non-steroidal analgesic agents with anti-inflammatory and antipyretic activity (NSAID medications). Both agents may be used in the therapy of racehorses but detection of these compounds at greater than defined concentrations in post race blood or urine samples may lead to sanctions. Therefore, highly sensitive and accurate quantitative analytical tests are required. A significant need exists for deuterium labelled internal standards for use in the quantitative analysis of these compounds. Research in our laboratory is in progress toward the production of deuterated analogues of these and other compounds suitable for use as internal standards.

MATERIALS AND METHODS

Melting points were measured by using a Kofler apparatus and are uncorrected. Gas chromatography and mass spectroscopy were performed on a Hewlett-Packard 5890 gas chromatograph interfaced with an HP 5970 mass selective detector, using a DB-5 capillary column (30 meters x 0.25 mm id x 0.25 µm film thickness) (Agilent Technologies). The column temperature was programmed as follows: 2 min at 50°C, then raised to 280°C at 20°C/min. Derivatisation of both ketoprofen and flunixin was accomplished with BSTFA-1% TMCS (Pierce Chemical Company) at 70°C for 30 min before injection into the chromatograph.

Synthesis of flunixin-d_3

The first step in the synthesis of flunixin-d_3 (Fig 2) was generation of the deuterium-containing

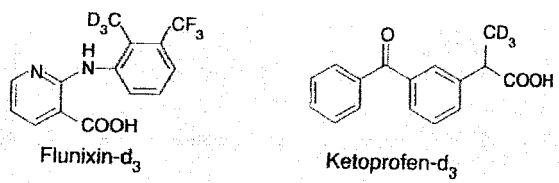

Fig 1: Structures of deuterated flunixin and deuterated ketoprofen.

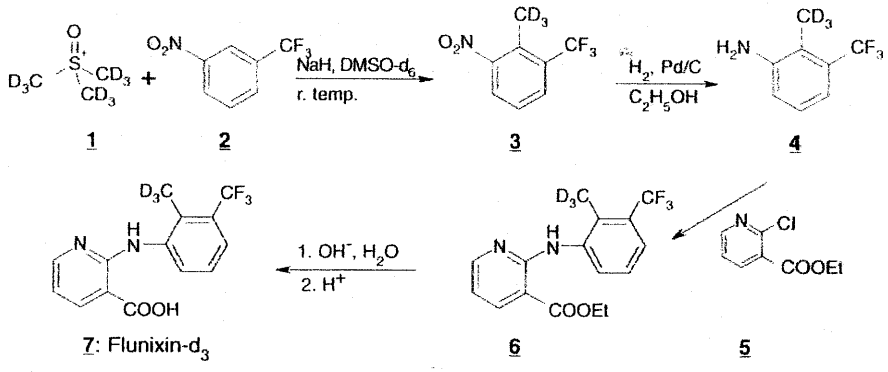

Fig 2: Synthesis of deuterated flunixin.

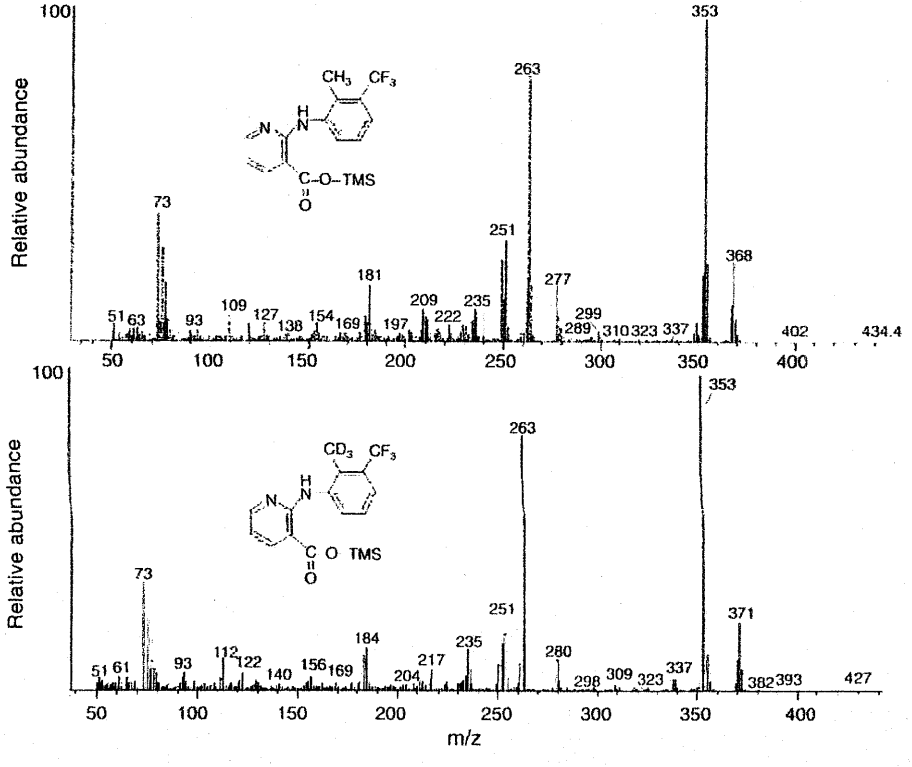

Fig 3: Mass spectra of silyl derivatives of flunixin-d_0 and flunixin-d_3 (7).

methylating agent, trimethyl-d_9 sulphoxonium iodide (1). Iodomethane-d_3 was reacted with DMSO-d_6 according to the procedure described by El-Fayoumy *et al.* (1979), giving with high yield salt 1. This salt (1) was then used to methylate 3-nitro-α,α,α-trifluorotoluene (2) in a vicarious nucleophilic substitution (VNS) reaction in the presence of sodium hydride in DMSO-d_6 at room temperature following the procedure described by Lamendola *et al.* 1989), yielding 2-methyl-d_3-3-nitro-α,α,α-trifluorotoluene (3). This reaction provided the desired product in moderate yield (50%) in mixture with un-reacted substrate (Lamendola *et al.* 1989). Due to the similar and low polarities of both substrate and product (2 and 3), these compounds are not easily separated by column chromatography and distillation is challenging due to the projected small scale reaction size (under 500 mg). Therefore, the mixture of 2 and 3 was used for the next reduction

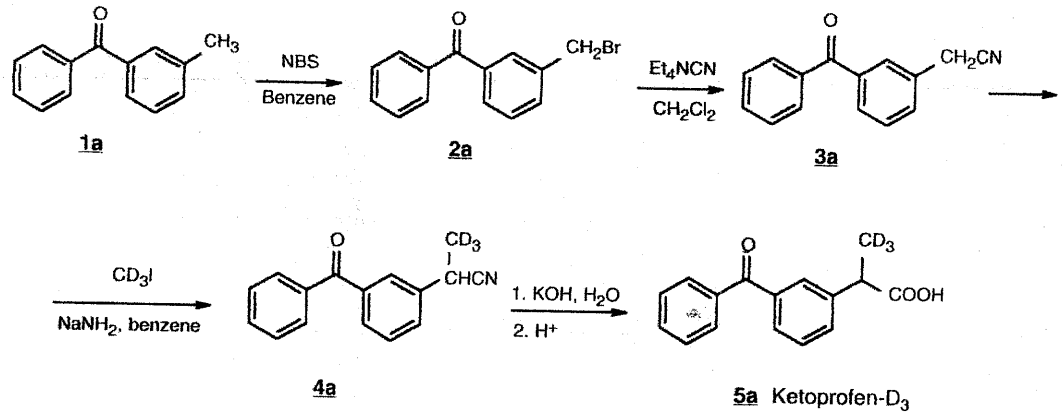

Fig 4: Synthesis of deuterated ketoprofen.

step. In this step the nitro group was reduced to amino group by classic hydrogenation using 10% palladium on charcoal as the catalyst in ethyl alcohol. After a 5 h reaction at 60°C, both nitro compounds 2 and 3 were converted to their corresponding aniline derivatives (**4** and 3-triflurometylaniline). This mixture was separated by column chromatography using an eluent system of n-hexane-*tert*-butylmethylether 9:1, to yield methyl-d_3-trifluoromethylaniline (**4**) with a 5% admixture of 3-triflurometylaniline. Therefore compound **4** was additionally purified upon crystallisation from n-hexane at -10°C, and next reacted for 12 h with the equimolar amount of ethyl ester of chloronicotinic acid (**5**) (Matrix Scientific Inc.) in ethylene glicol at 160°C, according to the procedure described by Jaouhari and Quinn (1994). The ethyl ester of flunixin-d_3 **6** was purified upon column chromatography (n-hexane – ethyl acetate, 3:1) giving a high yield (80%) of the condensation product. Finally, hydrolysis for 4 h at 70°C with potassium hydroxide in methanol/water (4:1) followed by acidification yielded flunixin-d_3. Crystallisation from acetone – n-hexane gave 50 mg of fine crystals, mp 216–219°C.

Synthesis of ketoprofen-d_3

Synthesis of deuterated ketoprofen (Fig 4) was accomplished using classical methods, first 3-methylbenzophenone (**1a**) was brominated with N-bromosuccinimide in benzene to obtain 3-bromomethylbenzophenone (**2a**), which after reaction with tetraethylaminocyanide in methylene chloride gave the corresponding nitrile (**3a**). After silica gel chromatography (petroleum ether/ethyl acetate 4:1), methylation of this material was achieved with methyl-d_3 iodide in the presence of sodium amide in toluene-benzene. The obtained 2-(3-benzoylphenyl)propanenitrile-d_3 (**4a**) was purified from the dimethylated product upon column chromatography (petroleum ether/ethyl acetate, 5:1). Next, compound (**4a**) dissolved in small amount of ethyl alcohol was added to an aqueous potassium hydroxide solution (2%) and heated under reflux for 24 h. After diluting with water, washing the aqueous ketoprofen potassium salt solution with ethyl ether, acidification, extraction with ethyl acetate, and evaporation of the solvent, crude ketoprofen-d_3 (**5a**), as colorless oil, was obtained. Crystallisation from hexane-ethyl ether gave fine white crystals of **5a**, mp 95–96°C.

Results and Discussion

Our synthetic pathway (Fig 2) to flunixin-d_3 has yielded the desired deuterated product (Fig 3) with an isotopic purity of greater than 98%, and no detection of the d_0 analogue, as established by GC/MS of the silyl derivative. Work is in progress to further purify this product.

Ketoprofen-d_3 has been produced with satisfactory yield and high chemical and isotopic purity as fine white crystals. Isotopic purity as determined by GC/MS of the silyl derivative is greater than 99% ketoprofen-d_3, with 0.72% ketoprofen-d_0 calculated from the ratio of the 311/314 m/z spectral peaks (Fig 5).

Conclusions

Flunixin-d_3 has been produced with satisfactory yield and high isotopic purity. Ketoprofen-d_3 has been produced with satisfactory yield and high

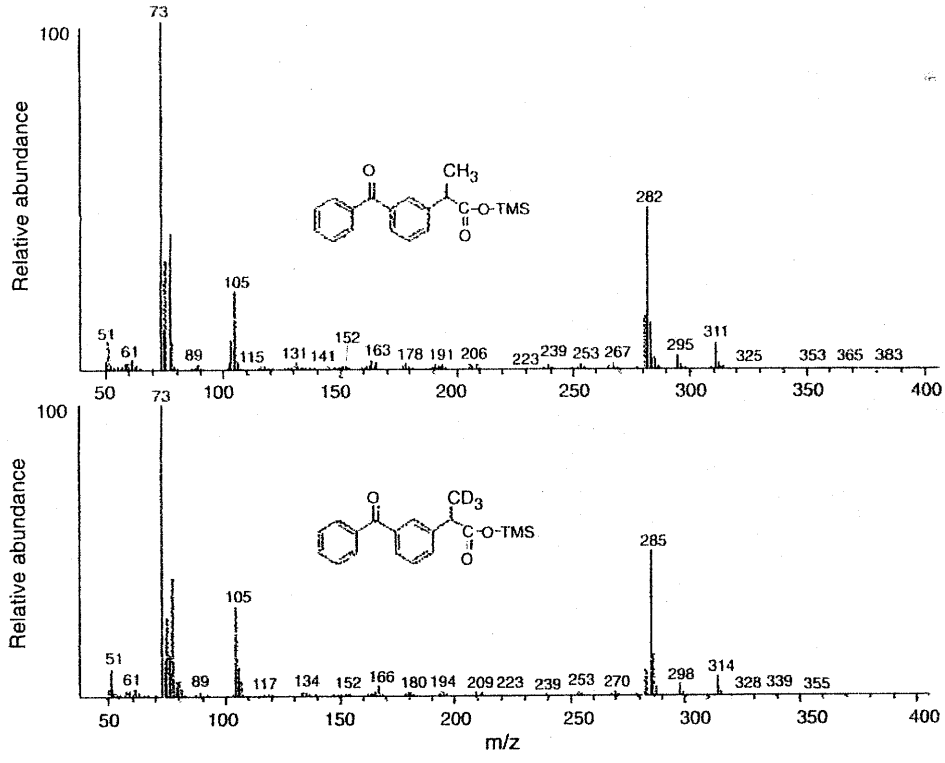

Fig 5: Mass spectra of silyl derivatives of ketoprofen-d_0 and ketoprofen-d_3 (5a).

chemical and isotopic purity. These compounds are of sufficient quality for use as internal standards in the quantitative analysis of their respective non-deuterated analogues.

ACKNOWLEDGEMENTS

Supported by Kentucky Science and Engineering Foundation Grant Agreement Number: KSEF-148-502-05-160 with the Kentucky Science and Technology Corporation. Also, research support from the National, Alabama, Arkansas, Kentucky, Pennsylvania, Ohio, Michigan, Charles Town WV, West Virginia, Florida, Tampa Bay Downs, Nebraska, Iowa, Minnesota, Louisiana, Oregon, Washington State, Arizona, Oklahoma, Texas, Ontario, and Canadian Horsemen's Benevolent and Protective Associations and a gift from General Fred Bradley.

Published as publication #363 from the Equine Pharmacology, Therapeutics and Toxicology Program at the Maxwell H. Gluck Equine Research Center and Department of Veterinary Science, University of Kentucky.

Published as Kentucky Agricultural Experiment Station Article #07-14-037 with the approval of the Dean and Director, College of Agriculture and the Kentucky Agricultural Experimental Station.

REFERENCES

El-Fayoumy, M.A.G., Bell, H.M., Ogliaruso, M.A. and Arison, B.H. (1979) Mono- and bishomo-benzotropones. 1. Synthesis and nuclear magnetic resonance spectra of 2,3-benzo-6,7-mono-homotropone and 2,3-benzo-trans-4,5:6,7-bishomotropone *J. Org. Chem.* **44**, 3057-3062.

Jaouhari, R. and Quinn, P. (1994) Improved process for the preparation of 2-methyl-3-trifluoro-methylaniline: a versatile intermediate for flunixin synthesis. *Heterocycles* **38**, 3057-3062.

Lamendola, J.F., Vashi, D. and Tyson, R.G. (1989) Synthesis of 3-Amino-2-Methylbenzotrifluoride *US Patent* **4**, 831,193.