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A GENERAL PROCEDURE FOR ISOTOPIC (DEUTERIUM) LABELLING OF NON-STEROIDAL ANTIINFLAMMATORY 2-ARYLPROPIONIC ACIDS

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SUMMARY

Alkaline treatment of nonsteroidal antiinflammatory 2-arylpropionic acids in deuterium oxide led in all cases to isotopic exchange of the proton located at the α-position of the side chain. Monodeuteration was observed in the case of carprofen, ibuprofen, ketoprofen, fenoprofen, flurbiprofen and naproxen. Additional exchange of one or two protons of the heterocyclic ring occurred in indoprofen, suprofen and tiaprofenic acid. The isotopic labelling survived under the conditions required to perform in vitro photoallergic studies (photolysis in non-deuterated aqueous media).

Key words: 2-arylpropionic acids, deuterium labelling, photoallergy, photochemistry

INTRODUCTION

Photosensitization is the process by which drugs, in conjunction with light, may cause deleterious effects in biological systems. This process may be phototoxic or photoallergic in nature (1). Phototoxicity is more common, does not depend on an immunological mechanism and can appear whenever appropriate concentration of a photosensitizer and

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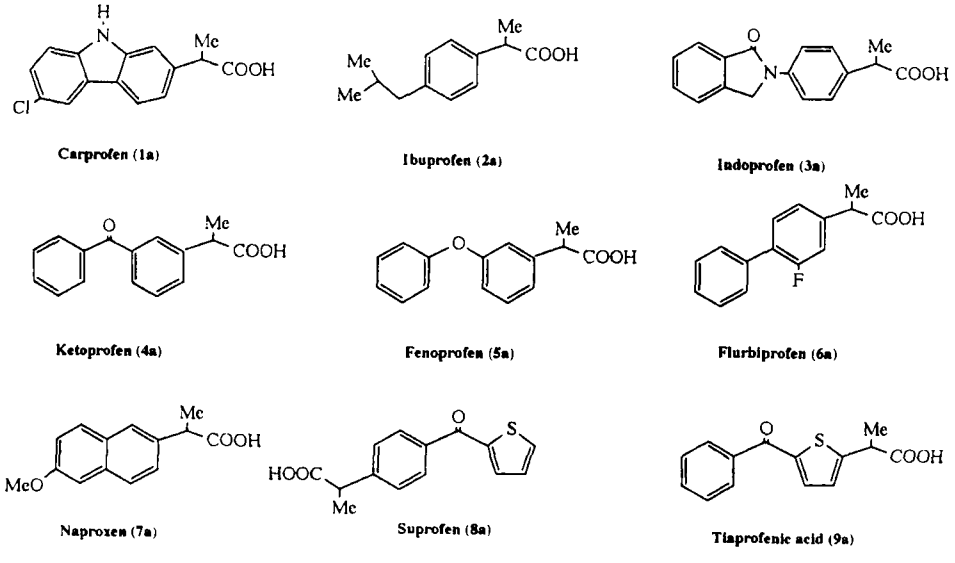
light interact at the skin surface. Photoallergy is much less frequent and develops in some patients as a hypersensitivity reaction of skin to the combined effect of light and a photosensitizer (1).

Systemically and/or topically applied drugs can induce phototoxic and/or photoallergic responses. Our interest has been focused on antiinflammatory 2-arylpropionic acids (Scheme 1), a very frequently prescribed therapeutic group with high incidence of photosensitizing reactions (2-4). Within this group, clinical reports have identified drugs causing phototoxic and photoallergic side-effects (5). A certain degree of success has been achieved in phototoxicity assessment using in vitro methods. By contrast, due to the idiosyncratic nature of photoallergy, there is no reliable and reproducible in vitro approach to anticipate its occurrence in vivo (1). A common primary event in drug allergy seems to be binding of the parent compound or its metabolites to cell macromolecules that, acting as haptens, can subsequently stimulate the immune system (6). Similarly, photobinding of a drug to cellular targets must be one of the early events in the onset of drug photoallergy.



SCHEME 1

In the course of a research aimed at establishing the molecular bases of 2-arylpropionic acids photoallergy, it was of interest to prepare the radioactively labelled drugs in order to study their ability to become covalently bound to proteins upon sun-light

irradiation. The desirable characteristics of a labelling procedure would be: 1) high efficiency and general applicability to all members of this therapeutic family; 2) stability of the label under the experimental irradiation conditions. Since these drugs share the propionic acid side-chain and they undergo light-induced decarboxylation (2-4), the possible locations for the isotope label would be either the carbons or the hydrogens of the α and ß positions.

In this work we have explored the possibility of exchanging the hydrogen by deuterium (7), as well as the stability of the isotopic label after UV-irradiation. Such a study appeared to be a necessary step to define conditions for optimal ^{3}H-labelling of these drugs.

MATERIALS AND METHODS

Chemicals. Carprofen (1a), indoprofen (3a), fenoprofen (5a) and suprofen (8a) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ketoprofen (4a) was a generous gift of Laboratorios Menarini (Badalona, Spain). Flurbiprofen (6a) was from Upjohn Co. (Madrid, Spain). Ibuprofen (2a) was obtained from Bayer Hispania S.A. (Madrid, Spain). Naproxen (7a) was from Laboratorios Vallés-Mestre (Barcelona, Spain), and tiaprofenic acid (9a) was from Rousell Ibérica (Madrid, Spain). Deuterium oxide (99.9% D_2O) was purchased from CEN Saclay (Gif sur Ivette, France).

Standard deuteration procedure. Fifty mg of each compound were dissolved in 30 ml of D_2O containing 5% (w/w) NaOH, and the solutions heated under reflux for 3 h. After cooling, the reaction mixture was acidified with HCl and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$ and brought to dryness to obtain the solid deuterated acids.

Irradiation of deuterated 2-arylpropionic acids. Fifty mg of each deuterated drug were dissolved with an equimolar amount of NaOH, and the volume was brought to 15 ml with PBS buffer (Phosphate buffer 50 mM, pH 7.2; 0.09% NaCl). The resulting solutions were placed in pyrex glass tubes and irradiated for 3 h in an immersion well photoreactor (Applied Photophysics, parts Ns. 3230+3307). A medium pressure mercury lamp

(OSRAM HQL, 125 W) was used as a light source. The crude photomixtures were extracted with CH_2Cl_2 and the organic phase was dried over anhydrous $MgSO_4$. The deuterated photoproducts were separated, their structures assigned on the basis of their mass spectra, and compared to the corresponding non-deuterated compounds.

Spectral properties of deuterated drugs and their major photoproducts. The most relevant features of the isolated compounds were:

[2-^{2}H]-Carprofen (D-1a): ^{1}H-NMR (CD_3COCD_3): δ 8.1-7.2 (m, 6H, aromatic), 1.5 (s, 3H, CH_3). MS: m/z (%); 276 (11), 274 (29), 231 (28), 230 (27), 229 (100), 228 (34), 195 (13), 194 (60), 193 (32), 192 (31), 191 (8).

[2-^{2}H]-*Ibuprofen* (D-2a): ^{1}H-NMR ($CDCl_3$): δ 7.4-7.0 (m, 4H, aromatic), 2.5 (d, 2H, CH_2), 2.1-1.7 (m, 1H, CH), 1.5 (s, 3H, CH_3), 0.9 (d, 6H, CH_3). MS: m/z (%); 207 (50), 164 (100), 162 (98), 120 (39), 118 (40), 107 (85), 91 (97).

[$^2H_3$]-Indoprofen (D_3-3a): ^{1}H-NMR (D_2O/NaOH): δ 7.9-7.4 (m, 8H, aromatic), 1.6 (s, 3H, CH_3). MS: m/z (%); 284 (29), 239 (100).

Decarboxy-[$^2H_1$]-indoprofen (D_1-3b): MS: m/z (%); 240 (57), 225 (100).

[2-^{2}H]-Ketoprofen (D-4a): ^{1}H-NMR ($CDCl_3$): δ 7.9-7.3 (m, 9H, aromatic), 1.4 (s, 3H, CH_3). MS: m/z (%); 255 (30), 210 (38), 181 (16), 178 (59), 105 (100), 77 (76), 51 (18).

Decarboxy-[2-^{2}H]-ketoprofen (D-4b): MS: m/z (%); 211 (30), 181 (20), 134 (100), 105 (72), 77 (46), 51 (6).

[2-^{2}H]-Fenoprofen (D-5a): ^{1}H-NMR ($CDCl_3$): δ 7.6-6.9 (m, 9H, aromatic), 1.5 (s, 3H, CH_3). MS: m/z (%); 243 (71), 198 (100), 119 (17), 105 (40), 104 (37), 91 (52), 77 (44).

[2-^{2}H]-Flurbiprofen (D-6a): ^{1}H-NMR ($CDCl_3$): δ 7.7-7.2 (m, 8H, aromatic), 1.6 (s, 3H, CH_3). MS: m/z (%); 245 (43), 200 (100), 185 (13), 184 (20), 180 (19), 179 (24).

[2-^{2}H]-Naproxen (D-7a): ^{1}H-NMR ($CDCl_3$): δ 7.8-7.0 (m, 6H, aromatic), 3.9 (s, 3H, OCH_3); 1.5 (s, 3H, CH_3). MS: m/z (%); 231 (50), 186 (100), 171 (76), 142 (23), 116 (15).

Decarboxy-[2-^{2}H]-naproxen (D-7b): MS: m/z (%); 187 (39), 172 (100), 129 (19).

[$^2H_2$]-Suprofen (D_2-8a): ^{1}H-NMR ($CDCl_3$): δ 7.9-7.2 (m, 6H, aromatic), 1.5 (s, 3H, CH_3). MS: m/z (%); 262 (34), 217 (43), 188 (12), 134 (8), 112 (100).

[$^2H_3$]-Suprofen (D_3-8a): ^{1}H-NMR ($CDCl_3$): δ 7.9-7.2 (m, 5H, aromatic), 1.5 (s, 3H, CH_3). MS: m/z (%); 263 (27), 218 (40), 189 (18), 134 (16), 113 (100).

Decarboxy-[$^2H_2$]-suprofen (D_2-8b): MS: m/z (%) 218 (47), 188 (43), 175 (11), 134 (100), 112 (90).

Decarboxy-[$^2H_3$]-suprofen (D$_3$-8b): MS: m/z (%); 219 (41), 189 (32), 176 (9), 134 (100), 113 (52).

[2-^{2}H]-Tiaprofenic acid (D-9a): ^{1}H-NMR ($CDCl_3$): δ 8.2-7.1 (m, 7H, aromatic), 1.6 (s, 3H, CH_3). MS: m/z (%); 261 (33), 216 (100), 105 (75), 77 (75).

[$^2H_2$]-Tiaprofenic acid (D$_2$-9a): ^{1}H-NMR ($CDCl_3$): δ 8.2-7.1 (m, 6H, aromatic), 1.6 (s, 3H, CH_3). MS: m/z (%); 262 (56), 217 (100), 105 (30), 77 (47).

Decarboxy-[2-^{2}H]-tiaprofenic acid (D-9b): MS: m/z (%); 217 (42), 140 (100), 105 (41), 77 (40).

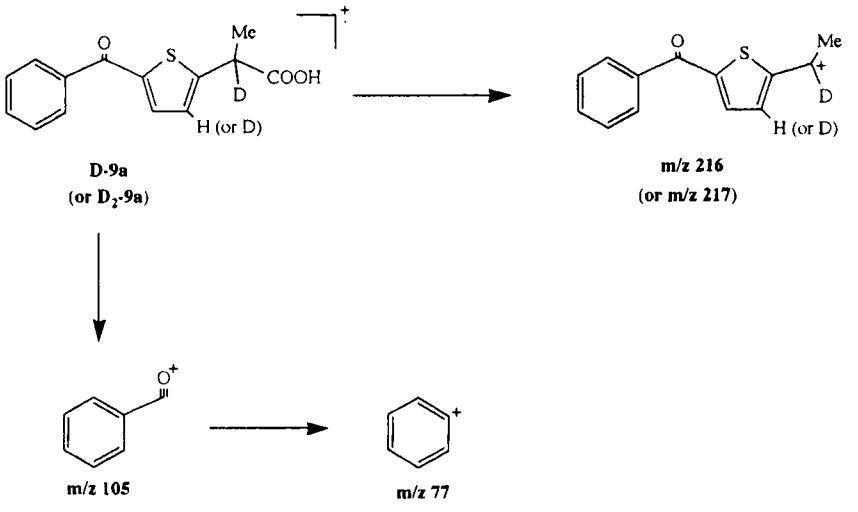
Decarboxy-[$^2H_2$]-tiaprofenic acid (D$_2$-9b): MS: m/z (%); 218 (47), 141 (100), 105 (41), 77 (41).

RESULTS AND DISCUSSION

Heating a solution of selected 2-arylpropionic acids in D_2O containing NaOH (5% w/w) at reflux temperature during 3 h, resulted in a considerable exchange of hydrogen by deuterium. The percentage of non-labelled drug, as indicated by the relative abundance of the M^+ peak, varied from 50% (ibuprofen, fenoprofen and carprofen), to 15% (tiaprofenic acid) or even less than 5% (flurbiprofen, indoprofen, ketoprofen, naproxen and suprofen).

As a general rule, one deuterium was found at the α-position. This was assessed by comparative analysis of the ^{1}H-NMR spectra, which displayed a typical singlet at δ ca. 1.5 ppm, corresponding to the methyl protons, instead of the characteristic doublets observed for non-deuterated precursors at the same chemical shift. A parallel decrease of the quartet signal at δ 3.8, assignable to the α-carboxyl protons was observed upon deuteration.

In the case of tiaprofenic acid, a careful analysis of the fragmentation pattern (Scheme 2), evidenced that a second deuterium atom was located at the heterocyclic ring. The ^{1}H-NMR spectrum showed a marked decrease of the signal corresponding to the H-3 proton (doublet at δ 7.1 ppm), indicating that this was the deuteration position. Partial irreversible degradation occurred under the general labelling conditions and hence a less basic medium (1% NaOH), shorter reaction time (1 h) and lower temperature (75 °C) were employed. This resulted in a clean, complete and selective exchange of the hydrogen at the α-position. The fragmentation pattern of this compound is also indicated in Scheme 2.

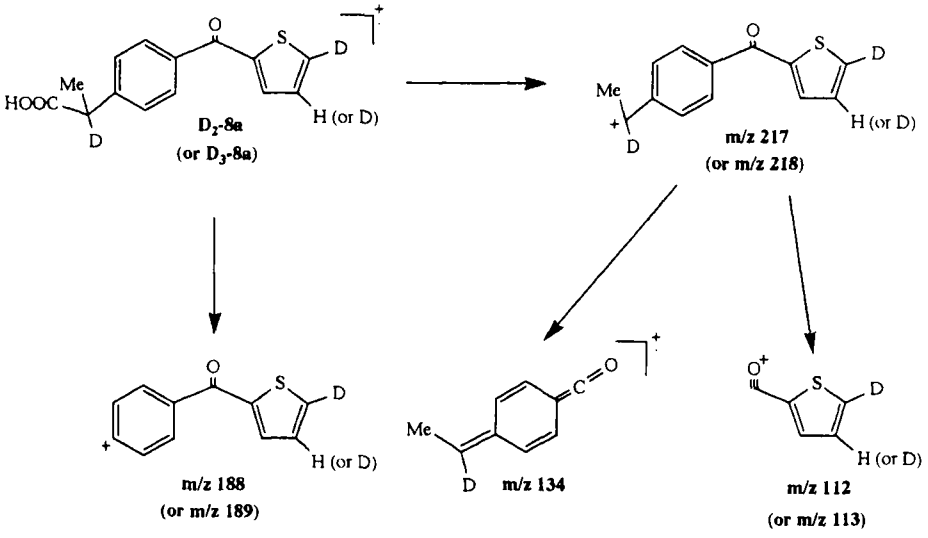


SCHEME 2

The same trend was observed for suprofen. The mass spectrum, with a characteristic M^+ +3 ion was interpreted in terms of double deuteration of the tiophene ring. This conclusion was drawn from the fragmentation which is shown in Scheme 3. A further piece of evidence was provided by the ^{1}H-NMR spectrum, which showed the disappearance of the H-2 (δ 7.1) and H-3 (δ 7.2) protons. As in the case of tiaprofenic acid, partial degradation occurred and hence, deuteration of suprofen was attempted at lower temperature (75 °C). After 24 h, complete exchange of two protons (at the α-carboxyl and thiophene H-2 positions) was observed. The MS assignment is also given in Scheme 3. The lack of deuteration at the H-3 position of the heterocyclic ring under these milder reaction conditions is in agreement with the expected reactivity of thiophenes towards bases, characterised by preferential deprotonation at H-2 (8).

Finally, multiple H exchange was also observed for indoprofen. The MS spectrum showed an intense peak corresponding to the M^+ +3 ion. The ^{1}H-NMR-spectrum showed, besides α-deuteration (detected in the normal way by changes in the signals corresponding to the propionic acid side chain), a substantial decrease of the singlet assignable to the methylene protons of the lactam ring. The efficient exchange at this position can be explained by the well-established acidity of benzylic protons.

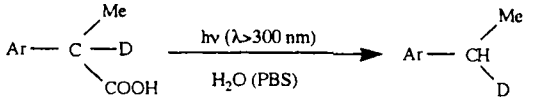
After achieving a successful deuteration of the different 2-arylpropionic acids, it was of interest to investigate whether the isotopic label could survive the usual irradiation conditions employed for in vitro photoallergy experiments (1). It has been established that



SCHEME 3

these drugs, when irradiated with light of appropriated wavelength, can undergo photochemical decarboxylation, giving rise to benzylic radicals which finally collapse to stable photoproducts bearing an ethyl side-chain (2-4). Thus, it appeared relevant for further studies, to determine whether the above mentioned reactive radicals (species presumably involved in photobinding to biomolecules) and hence their final photoproducts, still carried the deuterium label.

As anticipated, this was found to be the case. In spite of the fact that the irradiations were performed in non-deuterated aqueous media, no appreciable D/H exchange was observed in the course of the reaction (Scheme 4). Some of the drugs (1a, 2a, 5a and 6a)



D-4a, D-7a, D-9a
D_2-8a, D_2-9a
D_3-3a, D_3-8a

D-4b, D-7b, D-9b
D_2-8b, D_2-9b
D_3-3b, D_3-8b

SCHEME 4

did not produce the corresponding decarboxylated analogue when exposed to pyrex-filtered light ($\lambda > 300$ nm), which mimics the spectral output of sunlight. The other drugs (3a, 4a, 7a, 8a and 9a) were markedly photolabile. A careful MS analysis evidenced that the isolated photoproducts (if any), as well as the recovered starting acids conserved the same number of deuterium atoms, at identical positions (see data in experimental section).

In conclusion, alkaline treatment of 2-arylpropionic acids in D_2O provides a general and efficient methodology for the isotopic labelling of these drugs, which survives under the conditions required to perform in vitro photoallergic studies. The simplicity of this experimental procedure is especially suitable for its future extension to the corresponding radioactive tritium analogues.

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