JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 457–461.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.941

Research Article

Synthesis of [^{2}H]-labelled phase-I-metabolites using 1-[^{2}H]-pyridinium hydrochloride

Georg Heinkele and Thomas E. Mürdter*
Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Auerbachstrasse 112, D-70376 Stuttgart, Germany

Summary

Simultaneous *O*-demethylation and hydrogen/deuterium exchange of aryl-methyl-ethers can be obtained using 1-[^{2}H]-pyridinium hydrochloride as reagent at 220°C for 6 h. This reaction was applied to dextromethorphan and various non-steroidal anti-inflammatory drugs. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: *O*-demethylation; pyridinium deuterochloride; deuterium label; [$^2H_3$]-3-hydroxymorphinan

Introduction

Often, the first step of metabolism of drugs containing aryl-methyl-ether moieties is oxidative *O*-demethylation by a member of the cytochrome P450 enzyme family to the corresponding phenols. For the determination of drug and metabolite concentrations in pharmacokinetic and metabolism studies LC-MS technique is state of the art. Accurate quantification with minimal sample preparation efforts recommends the use of stable isotope-labelled internal standards. One approach to deuterium-labelled phenols is hydrogen/deuterium exchange using strong acids in deuterium oxide. However, this often needs prolonged reaction times and a high reaction temperature with the need of elevated pressure.[1] On the other hand, *O*-demethylation of parent drugs can be obtained in a melt of pyridinium hydrochloride as described by Prey.[2] Therefore, we decided to use 1-[^{2}H]-pyridinium hydrochloride (**1**) as reagent for simultaneous *O*-methyl cleavage and labelling of the aromatic system.

*Correspondence to: Thomas E. Mürdter, Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Auerbachstrasse 112, D-70376 Stuttgart, Germany. E-mail: thomas.muerdter@ikp-stuttgart.de

Contract/grant sponsor: Robert Bosch Foundation

Received 17 January 2005
Revised 9 February 2005
Accepted 11 February 2005

Results and discussion

The reagent was prepared by adding 1.2 equivalents of deuterium chloride in deuterium oxide to one equivalent of either dry pyridine or dry [$^2H_5$]-pyridine and evaporating deuterium oxide and the excess of deuterium chloride under reduced pressure. The remaining colourless highly hygroscopic solid must be kept under a moisture-free argon atmosphere to avoid any contamination with water which leads to a decrease in labelling efficiency.

Reaction conditions were evaluated using dextromethorphan (3-methoxy-*N*-methyl-morphinan, **2**) as starting material. By the reaction of **2** with a 50–100-fold excess of **1** at 220°C we obtained [$^2H_3$]-dextrorphan (5,7,8-[$^2H_3$]-3-hydroxy-17-methyl-morphinan, **3**) in one step. Labelling efficiencies under different conditions are given in Table 1. In addition, we examined whether the labelling efficiency could be improved by the use of [$^2H_6$]-pyridinium hydrochloride. Surprisingly, neither a prolonged reaction time nor the use of perdeuterated pyridinium hydrochloride significantly increased the labelling efficiency.

The same reaction was applied to other aryl-*O*-methyl compounds as well as some non-steroidal anti-inflammatory drugs (Table 2). Except for indomethacin which showed degradation to an unidentified product deuterium labelling and yields were acceptable. However, strongly activating substituents are pivotal to obtain a reasonable labelling efficiency. This was clearly seen when ibuprofen and ketoprofen were used as the starting material. Under the same reaction conditions ibuprofen was predominantly di-substituted with almost complete exchange at the 2-position of the propionic acid moiety and some exchange of all arylic hydrogens whereas ketoprofen only showed mono-substitution in the side chain (Figure 1).

To compare the labelling efficiency of the present method with the acid-catalyzed hydrogen/deuterium exchange, dextrorphan was labelled by both methods. As shown in Table 2 in contrast to the use of **1** exchange in acidified deuterium oxide resulted in the formation of di-substituted dextrorphan rather

Table 1. Influence of reaction conditions on the formation of 3 as determined by MS

			Isotope distribution					
Reagent	Molar ratio	Time (h)	$^2H_0$	$^2H_1$	$^2H_2$	$^2H_3$	$^2H_4$	Yield (%)
py^{2}HCl	1:50	6	0.08	2.8	25.5	67.2	4.4	93
py^{2}HCl	1:100	3	0.9	13.8	56.3	27.9	1.0	n.d.[a]
py^{2}HCl	1:100	6	0.04	2.3	24.3	71.6	1.8	85[b]
py^{2}HCl	1:100	28	0.2	4.8	26.2	51.8	13.6	64
[$^2H_5$]py^{2}HCl	1:50	6	0.04	2.1	24.9	69.5	3.5	86

[a] Not determined.
[b] After column chromatography.

Table 2. Application of the method to different compounds

Starting material	Demethylation	Isotope distribution[a]								Yield (%)[b]	Entry in Figure 1
		$^2H_0$	$^2H_1$	$^2H_2$	$^2H_3$	$^2H_4$	$^2H_5$	$^2H_6$	$^2H_7$		
Dextrorphan	No	0.24	4.9	31.3	60.5	3.2	–	–	–	95	A
Dextrorphan[c]	No	0.05	3.5	92.8	3.6	0.0	–	–	–	68	B
5	*O* and *N*[d]	0.07	3.6	35.9	46.9	11.6	1.9	–	–	37[e]	C
Methoxy-phenamine	Yes	0.28	8.2	54.1	32.4	4.9	0.3	–	–	96	D
Naproxene	Yes	0.00	0.0	0.2	2.1	13.2	34.6	37.0	13.1	98	E
Ibuprofen	No	1.83	42.6	46.6	8.2	0.7	–	–	–	98	F
Ketoprofen	No	5.87	87.8	5.3	1.2	–	–	–	–	102	G
Indomethacin	Yes	–	–	–	–	–	–	–	–	0	–

All reactions were performed with a 1:100 excess of **1** for 6 h at 220°C.
[a] As determined by MS.
[b] Yields of the raw product without further purification. Purity was greater than 95% as confirmed by HPLC-UV.
[c] For comparison exchange was performed with $^2H_2SO_4$ in ^2H_2O at 125°C for 48 h.
[d] *N*-demethylation via ethoxy-carbamate.
[e] After re-crystallization from ethyl acetate.

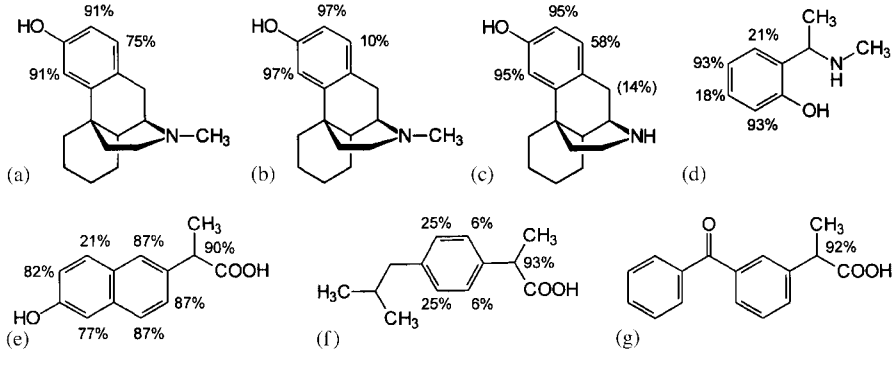

Figure 1. Localization and efficiency of labelling as determined by ^{1}H-NMR spectroscopy; values in parenthesis could not be assigned to this position by NMR but are likely to be at this position

than **3**. The efficiency of exchange at different positions as identified by ^{1}H-NMR is shown in Figure 1.

Furthermore, a two-step reaction sequence was used to synthesize [$^2H_3$]-3-hydroxymorphinan (**4**) from **2**: Firstly, **2** was converted to *N*-ethoxycarbonyl-3-methoxy-morphinan (**5**) as described previously.[3] Subsequently, cleavage of the carbamate and the *O*-methyl-group as well as hydrogen/deuterium exchange was obtained with **1** at 220°C for 6 h.

Experimental

Used glass ware was dried by heating under a stream of dry argon. All reactions were performed under an argon atmosphere. The degree of isotope

labelling was determined by electrospray ionization-mass spectrometry (HP 1100, Agilent Technologies, Waldbronn, Germany) and calculated by comparison to the unlabelled compound. Identification of the localization of deuterium labels was done by comparison of ^{1}H-NMR spectra of labelled and unlabelled compounds at 250 MHz (Bruker, Karlsruhe, Germany).

1-[^{2}H]-pyridinium hydrochloride (**1**)

To 1 equivalent of dried pyridine (stored over molecular sieve 0.3 nm) slowly 1.2 equivalent of 35 wt% deuterium chloride in deuterium oxide was added. From the acid solution excess of deuterium chloride and deuterium oxide was removed under reduced pressure at 80°C. The remaining white solid was stored desiccated over phosphorous pentoxide until use.

[$^{2}H_{6}$]-pyridinium hydrochloride

[$^{2}H_{6}$]-pyridinium hydrochloride was prepared by the same method: To 2.24 ml of [$^{2}H_{5}$]-pyridine (28 mmol) 3 ml of 35 wt% deuterium chloride in deuterium oxide was added and dried under reduced pressure. The obtained white solid (3.3 g) was used without further purification.

General procedure for hydrogen/deuterium exchange

A mixture of 0.35 mmol of dried aryl-methyl-ether and 4 g of **1** was slowly heated under an argon atmosphere to 220°C in an oil bath. The depth of immersion of the flask into the oil was adjusted to minimize sublimation of **1** to the upper part of the flask. After 6 h at 220°C the reaction mixture was cooled to room temperature under a stream of argon. The resulting yellow-to-brown solid was dissolved in 10 ml of water and 10 ml of 5 M NaOH was added prior to extraction with ethyl acetate. The organic layer was dried over sodium sulphate and evaporated to dryness under reduced pressure.

For carboxylic acids the aqueous layer was directly extracted with ethyl acetate. The free acids were obtained by evaporation of the organic layer to dryness.

[$^{2}H_{3}$]-dextrorphan (**3**)

According to the general procedure described above, 0.88 mmol of **2** were melted with 10.2 g of **1** for 6 h at 220°C. The brown oily residue of the ethyl acetate extract (222 mg, 97% yield) was further purified by column chromatography on a silica gel 60 column (Merck, Darmstadt, Germany) with ethyl acetate:methanol:triethylamine 90:10:1 (v/v/v) as mobile phase yielding 194 mg (85%) of a colourless oil which crystallized during storage at −20°C. For isotope distribution see Table 1.

[$^2H_3$]-3-hydroxymorphinan (**4**)

As described previously, **5** was prepared from **2** with ethyl-chloroformate and $NaHCO_3$ in chloroform.[3] In contrast to the general procedure described above, 1 mmol of **5** (330 mg) was melted with 6.1 g (50 mmol) of **1** at 220°C for 6 h. The brown solid was dissolved in 25 ml of water and made alkaline by adding 20 ml of 5 M NaOH. This solution was extracted 4 times with 50 ml of chloroform. The organic layer was dried and evaporated to dryness to give 144 mg (58%) of a yellow solid. Further purification by treatment with charcoal in methanol followed by crystallization from methanol resulted in 90 mg (37%) colourless crystals. For MS and NMR data see Table 2 and Figure 1.

[$^2H_2$]-dextrorphan by exchange with $^2H_2SO_4$

In a PTFE-reactor 100 mg of **2** were heated in 10 ml ^2H_2O and 2 ml $^2H_2SO_4$ (98%) at 125°C for 48 h. The reaction mixture was made alkaline by adding 10 M NaOH and the product extracted with ethyl acetate. The organic layer was dried over sodium sulphate and evaporated to dryness to give a brown solid (68 mg). For MS and NMR data see Table 2 and Figure 1.

Conclusion

Simultaneous *O*-demethylation and deuterium labelling of aryl-methyl-ethers can be achieved by the method described utilizing **1**, which is easily prepared from pyridine and deuterochloric acid. This reaction can be used for a variety of different compounds, however, it has its limitations due to the need for activating substituents in the aromatic ring system and the high temperatures required.

Acknowledgements

We thank Mrs A. Pikulski at the University of Stuttgart, Institute of Organic Chemistry, for the acquisition of NMR spectra. This work was supported by the Robert Bosch Foundation, Stuttgart, Germany.

References

1. Werstiuk NH. Hydrogen isotope labelling of aromatic compounds in aqueous medium at elevated temperature. In *Synthesis and Application of Isotopically Labelled Compounds 1991*, Buncel E, Kabalka GW (eds). Elsevier Science Publishers BV: Amsterdam, 1992; 303–308.
2. Prey V. *Berichte* 1941; **74**: 1219–1225.
3. Heinkele G, Schänzle G, Mürdter TE. *J Label Compd Radiopharm* 2002; **45**: 1153–1158.

