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Synthesis of deuterium-labelled drugs by hydrogen–deuterium (H–D) exchange using heterogeneous catalysis

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Multi-deuterium incorporations into drugs, such as theophylline, caffeine, valpromide, phenytoin, and trimethoprim, were post-synthetically achieved by Pd/C-, Pt/C-, or/and Rh/C-catalyzed hydrogen–deuterium exchange reactions under neutral conditions using deuterium oxide as the deuterium source in the presence of hydrogen gas. The present study offers facile and convenient methods for the preparation of highly deuterated medicines, which are expected to be used as long-lasting medicines as well as internal standards for metabolic studies and for the quantitative analyses of the parent drugs.

Keywords: multiple deuteration; hydrogen–deuterium exchange; heavy-hydrogen drug; palladium on carbon; platinum on carbon; rhodium on carbon; deuterium oxide

Introduction

The pharmaceutical industry has been investigating on deuterium substitution, since Elison *et al.* demonstrated the inhibition of the enzymatic oxidation of morphine by deuterating the N-methyl hydrogens.[1] The deuteration of drugs is expected to extend the duration of the biological activity and increase the stability based on the isotope effect; the carbon–deuterium (C–D) bonds withstand chemical or enzymatic cleavage relative to the carbon–hydrogen (C–H) bonds.[2–5] The improvement of the therapeutic activities of clinical medicines by the H–D displacements have been reported: (1) amphetamines were more readily transported into the brain and persistent in the deuterated form and the activity was maintained longer[6]; (2) halogenated anesthetics, such as selvoflane, when deuterated, were no longer oxidized to the toxic forms within the body[7]; and (3) deuterium-labelled long-chain fatty acids and fluoro-D-phenylalanine were resistant to breakdown by their target microorganisms.[8] More recently, deuterium-substituted clinical medicines, such as paroxetine (antidepressant),[9] atazanavir (HIV drug),[10] and venlafaxine (antidepressant),[11] were synthesized as long-lasting drug candidates by the pharmaceutical industry. Furthermore, much attention has been drawn to the use of deuterium-labelled drugs as tracers for the investigation of human drug metabolism[12] or as surrogates for quantitative analysis using gas or liquid chromatography combined with mass spectrometry (GC-MS or LC-MS).[13–15]

Two contrasting strategies are used for the synthesis of deuterium-labelled compounds. The first strategy is the total-synthetic approach using commercially available deuterium-labelled precursors as the starting materials, although it involves long synthetic routes and requires expensive deuterium-labelled starting materials. The second strategy is based on the post-synthetic hydrogen–deuterium (H–D) exchange reaction method, which produces deuterated compounds more rapidly and cost effectively. Although a number of post-synthetic H–D exchange reactions have been reported in the literature using metal catalysts, most of them require harsh conditions, such as strong bases or acids,[16] expensive reagents, such as D_2 gas,[17] and special pressure, base and/or acid resistant equipment.[18] Furthermore, these methods in general achieved a low degree of deuterium efficiency.[19] Hence, the development of efficient H–D exchange reactions is a challenging subject.

We have recently developed palladium on carbon (Pd/C)-hydrogen gas (H_2)-deuterium oxide (D_2O) system that led to the efficient introduction of deuterium atoms into a variety of compounds, including bioactive molecules, such as sulfamethazine (antibacterial), nalidixic acid (antibacterial), antipirine (analgesic), and allopurinol (antiurolithic).[20] Platinum on carbon (Pt/C) was found to be an effective catalyst to introduce deuterium atoms on aromatic nuclei rather than the alkyl chain compared to Pd/C.[21] Ibuprofen (analgesic) was fully deuterated using 5% Pt/C–H_2–D_2O followed by 10% Pd/C–H_2–D_2O systems.[22] Lately, the simultaneous use of Pd/C and Pt/C achieved the efficient deuteration of phentermine, which was a key intermediate for the synthesis of

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the deuterium-labelled mephentermine (antihypotensive) and oxethazaine (local anesthetic).[23]

In this paper we describe the post-synthetic H–D exchange reactions of xanthines, valpromide, phenytoin, and trimethoprim using heterogeneous Pd/C, Pt/C or/and Rh/C catalysts.

Experimental section

General

The ^{1}H and ^{2}H NMR spectra were recorded using a JEOL AL-400 or EX-400 spectrometer (400 MHz for ^{1}H NMR; 61 MHz for ^{2}H NMR). The chemical shifts (δ) are expressed in parts per million referenced to tetramethylsilane (0.00 ppm for $CDCl_3$/^{1}H NMR), 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS) (0.00 ppm for D_2O/^{1}H NMR and for H_2O/^{2}H NMR), or solvent peaks (7.26 ppm for $CHCl_3$/^{2}H NMR; 2.49 ppm for DMSO-d_6/^{1}H NMR and for DMSO/^{2}H NMR). The deuterium content was determined using internal standards (1,4-dimethoxybenzene, *p*-anisic acid, or DSS). The EI and FAB mass spectra were taken by a JEOL JMS-SX 102A spectrometer. Deuterium isotopic distributions were determined by the comparison of peak heights of mass spectra between labelled product and unlabelled substrate according to the Bergman's methods.[24] The analytical thin-layer chromatography was carried out on pre-coated Silica Gel 60 F_{254} plates (Merck, Art 5715) and visualized with UV light or stain (bromocresol green reagent for valpromide). The 10% Pd/C and 5% Pt/C were purchased from the Aldrich Chemical Co., and the 10% Rh/C was obtained from the N.E. Chemcat Co. Deuterium oxide (99.9% isotopic purity) was purchased from Cambridge Isotope Laboratories, Inc., or Division of Spectra Gases, Inc. All other reagents were obtained from commercial sources and used without further purification. Sealed tubes (60 mL) were obtained from Taiatsu Techno Corporation.

General procedure for the H–D exchange reaction

Method A: A sealed tube was charged with a substrate (0.15–1.0 mmol), catalyst (10–30 wt% of the substrate), and D_2O (1–5 mL). The air inside the tube was exchanged with hydrogen (60 mL, approximately 2.6 mmol) by five vacuum-hydrogen gas cycles. The sealed tube was placed in an oil bath heated at 110–180°C, and stirred for 24 h. After cooling to room temperature, the reaction mixture was diluted with methanol (5 mL), and then filtered through a membrane filter (Millipore Millex-LG, 0.45 μm) to remove the catalyst. The collected catalyst was washed with methanol (2 × 5 mL), and the filtrate was concentrated in vacuo.

Method B: A 15-mL test tube was charged with a substrate (0.15–0.50 mmol), catalyst (10–20 wt% of the substrate), and D_2O (1–5 mL). After the test tube was sealed with a septum, the air inside was exchanged with hydrogen (15 mL, approximately 0.66 mmol) by five vacuum-hydrogen cycles. The mixture was stirred at 50–90°C for 24 h using a Chemistation personal organic synthesizer (EYELA, Tokyo). After cooling to room temperature, the reaction mixture was diluted with methanol (5 mL), and then filtered through a membrane filter (Millipore Millex-LG, 0.45 μm) to remove the catalyst. The collected catalyst was washed with methanol (2 × 5 mL) and the filtrate was concentrated in vacuo.

[^{2}H]Theophylline (Table 1, Entry 1)

Method A. A mixture of theophylline (90.0 mg, 0.5 mmol), 10% Pd/C (18.0 mg, 20 wt%), and D_2O (1.5 mL) was stirred at 160°C. Yield was 86.0 mg (97%). Isotopic distribution (EIMS): 2% d_2, 18% d_3, 64% d_4, 14% d_5, 2% d_6 ^{1}H NMR (DMSO-d_6, 1,4-dimethoxybenzene as an internal standard) δ 8.01 (s, 0.05H), 3.40–3.38 (s, 0.24H), 3.20–3.19 (s, 2.71H). ^{2}H NMR (DMSO) δ 8.03 (br s) 3.39 (br s).

[^{2}H]Caffeine (Scheme 1)

First step. Method A: A mixture of caffeine (97.0 mg, 0.5 mmol), 10% Pd/C (29.1 mg, 30 wt%), and D_2O (3 mL) was stirred at 160°C for 48 h. Yield was 82.5 mg (85%).

Second step. Method A: A mixture of deuterated caffeine (49.0 mg, 0.25 mmol, obtained from the first step in Scheme 1), 10% Pd/C (9.8 mg, 20 wt%), and D_2O (1.5 mL) was stirred at 160°C. Yield was 44.0 mg (90%). Isotopic distribution (EIMS): 4% d_4, 12% d_5, 25% d_6, 38% d_7, 16% d_8, 4% d_9, and 1% d_{10}. ^{1}H NMR (D_2O, DSS as an internal standard) δ 7.88 (s, 0.02), 3.90–3.87 (s, 0.55H), 3.30–3.25 (s, 2.55H). ^{2}H NMR (H_2O) δ 7.75 (br s), 3.72 (br s), 3.27 (br s).

[^{2}H]Valpromide (Scheme 2)

First step: Method A: A mixture of valpromide (72.0 mg, 0.5 mmol), 10% Pd/C (14.4 mg, 20 wt%), and D_2O (4 mL) was stirred at 160°C. Yield was 72.0 mg (100%). Chloroform was used for workup in place of methanol.

Second step: Method A: A mixture of deuterated valpromide (50.0 mg, 0.35 mmol, obtained from first step), 10% Rh/C (21.6 mg, 30 wt%), and D_2O (4 mL) was stirred at 160°C for 48 h. Yield was 47.0 mg (94%). Chloroform was used for workup in place of methanol. Isotopic distribution (FAB$^+$ MS): 7% d_{12}, 10% d_{13}, 28% d_{14}, and 56% d_{15}. ^{1}H NMR ($CDCl_3$, 1,4-dimethoxybenzene as an internal standard) δ. 2.13–2.10 (m, 0.009H), 1.59–1.54 (m, 0.32H), 1.43–1.26 (m, 1.03H), 0.93–0.86 (m, 0.92H). ^{2}H NMR ($CHCl_3$) δ 2.09 (br s), 1.52 (br s), 1.34–1.23 (br t), 0.84 (br s).

[^{2}H]Phenytoin (Table 4, Entry 4)

Method A: A mixture of phenytoin (126 mg, 0.5 mmol), 5% Pt/C (25.2 mg, 20 wt%), 10% Pd/C (25.2 mg, 20 wt%), and D_2O (5 mL) was stirred at 180°C. Yield 99.0 mg (79%). Acetone was used for workup in place of methanol. Isotopic distribution (EIMS): 5% d_8, 23% d_9, 58% d_{10}, and 14% d_{11}. ^{1}H NMR (DMSO-d_6, 1,4-dimethoxybenzene as an internal standard) δ 7.51–7.34 (m, 0.44H). ^{2}H NMR (DMSO) δ 7.37–7.26 (br d).

[^{2}H]Trimethoprim (Table 5, Entry 4)

Method A. A mixture of trimethoprim (145 mg, 0.5 mmol), 10% Pd/C (29.0 mg, 20 wt%), 5% Pt/C (29.0 mg, 20 wt%), and D_2O (3 mL) was stirred at 180°C for 34 h. Yield 145 mg (100%). Acetone was used for workup in place of methanol. Isotopic distribution (EIMS): 3% d_3, 12% d_4, 32% d_5, 29% d_6, 15% d_7, 6% d_8, 2% d_9, and 1% d_{10}. ^{1}H NMR (DMSO-d_6, *p*-anisic acid as an internal standard) δ 7.51 (s, 0.03H), 6.61 (s, 0.17H), 3.72 (s, 3.54H), 3.61 (s, 2.04H), 3.54 (s, 0.26H). ^{2}H NMR (DMSO) δ 6.83 (br s), 3.97 (br s).

Results and discussion

Deuteration of xanthines (theophylline and caffeine)

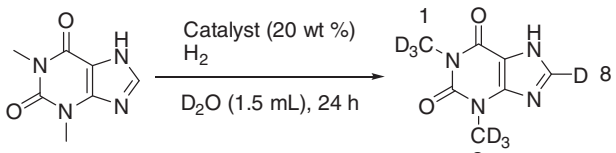
The structurally related xanthine alkaloids, such as caffeine and theophylline, are widely used in medical care.[25] Caffeine is used as a psychoactive drug, while theophylline is used as a bronchodilator.[25] The International Sports Authorities regulate the presence of the xanthines in biological samples,

e.g., caffeine and theophylline are prohibited in racing horses.[26] Labelled xanthines are used as internal standards to analyze the quantity of these xanthines in the body fluids of the horses using isotope dilution-mass spectrometry coupled with gas or liquid chromatography in anti-doping and forensic laboratories.[26] The deuterium-labelled theophylline ([$^2H_6$]theophylline) was conventionally synthesized via methylation of either 7-benzylguanine with CD_3I or 6-aminouracil with $(CD_3O)_2SO_2$, while the methylation of theophiline using CD_3I afforded the corresponding N_7-deuterium-methylated [$^2H_3$]caffeine.[26] Although the synthetic methods were reliable, expensive deuterium-labelled methylating reagents were necessary.

When theophylline (0.5 mmol) was stirred with 10% Pd/C (20 wt%: 20% of theophylline weight) in D_2O (1.5 mL) under an H_2 atmosphere at 160°C, satisfactory and selective deuterium incorporation was achieved on the methyl group at the N_3-position and the 8-position of the purine nucleus (Table 1, Entry 1).[27] The low deuterium incorporation into the N_1-methyl group would be attributed to the steric hindrance caused by the neighboring two keto groups at the 2 and 6-positions. The lowering of the temperature to 140°C maintained the high deuterium contents (Entry 3), although they dropped at the N_3-methyl group by either increasing or further decreasing the temperature (180 or 90°C; Entries 2 and 4). Other activated carbon-supported transition metal catalysts, such as 10% Rh/C, 10% Ru/C, 10% Au/C, and 5% Pt/C, were found to be far less active for the H–D exchange reaction on the N_3-methyl group (Entries 5–8). However, the use of Pt/C led to the completely selective deuterium incorporation at the 8-position (Entry 8).[28]

The H–D exchange reaction of caffeine (0.5 mmol) was investigated using the 10% Pd/C (20 wt%)–D_2O (1.5 mL)–H_2 system that afforded the best deuterium efficiency in the case of theophylline (Table 2). When the reaction was carried out at

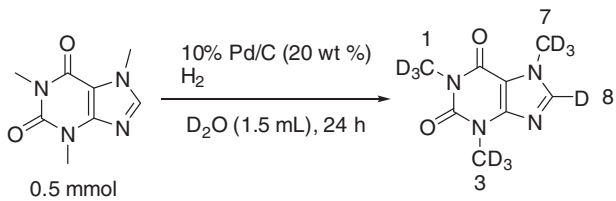
Table 1. H–D exchange reaction of theophylline



Entry	Catalyst	Temp (°C)	D content (%)[a]			Yield (%)
			N_1 (CD_3)	N_3 (CD_3)	8 (D)	
1	10% Pd/C	160	10	92	95	94
2	10% Pd/C	180	0	63	96	89
3	10% Pd/C	140	4	96	98	94
4	10% Pd/C	90	4	21	98	88
5	10% Rh/C	160	1	54	98	97
6	10% Ru/C	160	13	15	96	92
7	10% Au/C	160	8	11	98	92
8	5% Pt/C	160	0	0	96	88

[a]Determined by ^1H NMR.

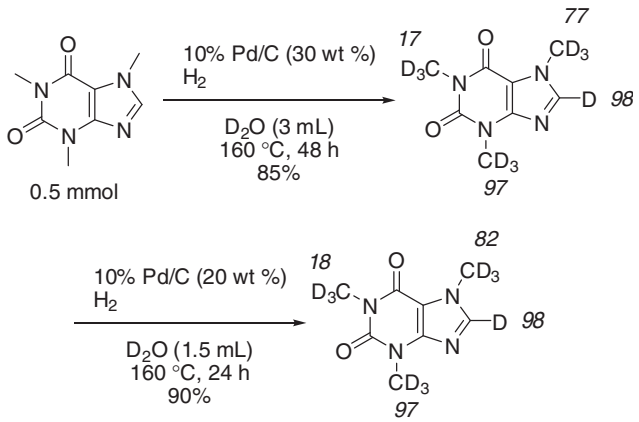
Table 2. Pd/C-catalyzed H–D exchange reaction of caffeine



Entry	Temp (°C)	D content (%)[a]				Yield (%)
		N_1 (CD_3)	N_3 (CD_3)	N_7 (CD_3)	8 (D)	
1	90	0	23	0	96	98
2	160	19	90	57	96	98
3	180	22	82	51	96	98

[a]Determined by ^1H NMR.

90°C, the H–D exchange reaction took place with a 96% deuterium efficiency at the 8-position, but the methyl groups at the N_1-, N_3-, and N_7-positions were hardly deuterated (Entry 1). As the temperature was raised, the deuterium efficiency of the N_3-methyl group significantly increased (Entries 1–3), and the quantitative deuterium incorporation at the N_3-methyl was achieved at 180°C with a medium deuterium efficiency at the N_7-methyl group (Entry 3). For the reaction using increased amounts of 10% Pd/C (30 wt%) and D_2O (3 mL) at 160°C for 48 h, a 77% deuterium efficiency was obtained even at the N_7-methyl group with high D contents kept at the N_3- and 8-positions (Scheme 1). The resulting deuterated caffeine was again stirred in D_2O (1.5 mL) under an H_2 atmosphere at 160°C for 24 h in the presence of 10% Pd/C (20 wt%) (Scheme 1). Although the deuterium atoms were not sufficiently introduced into the N_1-methyl group (15%), reuse of the substrate after the first deuteration improved the deuterium efficiency at the N_7-position to 82% (Scheme 1).



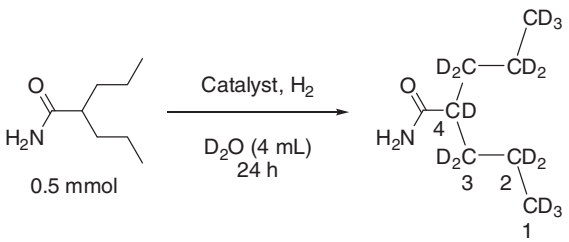
Scheme 1. Stepwise deuteration of caffeine. Deuterium efficiencies are indicated in italics.

Deuteration of anticonvulsants (valpromide and phenytoin)

Anticonvulsants, such as valpromide and phenytoin, are used to treat epilepsy and to provide relief from non-seizure neurological and psychiatric disorders, such as migraines, neuropathy pain, and mood disorders.[29, 30] Deuterium-labelled phenytoin has been used as a tracer in a bioavailability study using GC-MS to evaluate the clearance values by analyzing its concentration in plasma.[31] Pharmacokinetic studies of phenytoin are important because of its narrow therapeutic concentration range, hence small changes in the bioavailability can cause medical accidents. Valpromide has a very short half-life (less than 1 h), and a high clearance value.[32] The exchange of the valpromide hydrogens with deuterium could therefore be expected to increase the half-life and lower the clearance value due to the isotope effect. Fully deuterated phenytoin, [$^2H_{10}$]phenytoin, was synthesized by the modified Bucherer–Bergs reaction of [$^2H_{10}$]benzophenone,[33] which was prepared from [$^2H_6$]benzene and [$^2H_5$]benzoyl chloride,[34] while no synthesis of the deuterated valpromide has been reported in the literature.

The H–D exchange reaction of valpromide using the Pd/C–H_2–D_2O combination did not proceed well at room temperature (approximately 25°C) and 110°C (Table 3, Entries 1 and 2), while an increase in the temperature to 160°C remarkably enhanced the deuterium efficiency to ca. 50% on all carbon atoms (Entry 3). The use of 10% Rh/C (30% of the substrate weight)[35] in place of 10% Pd/C lowered the deuterium incorporation (Entry 4), but it was improved to more than 55% on all carbon atoms by the reuse of the moderately deuterated valpromide obtained in Entry 4 for the H–D exchange reaction (Entry 5) or the extension of the reaction time from 24 to 48 h (Entry 6).

Table 3. The H–D exchange reaction of valpromide

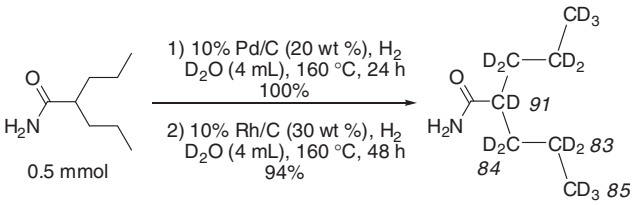


Entry	Catalyst	Temp (°C)	D content (%)[a]				Yield (%)
			1 (CD_3)	2 (CD_2)	3 (CD_2)	4 (CD)	
1	10% Pd/C (20 wt%)	rt	3	4	6	9	97
2	10% Pd/C (20 wt%)	110	6	3	8	10	92
3	10% Pd/C (20 wt%)	160	52	48	48	52	100
4	10% Rh/C (30 wt%)	160	24	23	12	33	97
5[b]	10% Rh/C (30 wt%)	160	55	55	61	72	96
6[c]	10% Rh/C (30 wt%)	160	67	66	71	82	80

[a]Determined by ^1H NMR.
[b]The product of Entry 4 was used as the starting material.
[c]48 h.

Based on these results, over an 80% deuterium efficiency of valpromide was finally achieved by the successive H–D exchange reactions at 160°C using 10% Pd/C (20 wt%) for 24 h and then using 10% Rh/C (30 wt%) for 48 h (Scheme 2).

We have already reported that 5% Pt/C more effectively catalyzed the H–D exchange on the aromatic nuclei rather than 10% Pd/C in D_2O under an H_2 atmosphere.[36] When Pt/C was used as the catalyst for the H–D exchange reaction of phenytoin, which bears two benzene rings, low-to-moderate deuterium efficiencies were obtained depending on the temperature (Table 4, Entries 1–3). We also reported the synergistic effect of Pd/C and Pt/C on the simultaneous use for the H–D exchange reaction of various arenes to increase the deuterium incorporation even at the sterically hindered positions.[23, 36] The combined use of 5% Pt/C and 10% Pd/C at 180°C led to the expected quantitative deuteration of phenytoin.

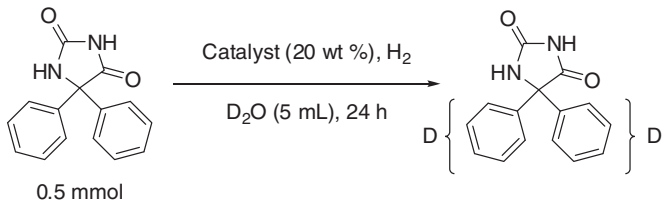


Scheme 2. Stepwise deuteration of valpromide. Deuterium efficiencies are indicated in italics.

Deuteration of trimethoprim

Trimethoprim is a diaminopyrimidine derivative with a high potency and specificity as a bacterial dihydrofolate reductase (DHFR) inhibitor.[37] Trimethoprim is effective for the treatment of acute infections of the urinary and respiratory tracts.[38–41] The deuterated analogs of trimethoprim ([$^2H_2$]trimethoprim, [$^2H_3$]terimethoprim, and [$^2H_6$]trimethoprim) have been used to study the binding of trimethoprim with DHFR by high resolution X-ray and NMR spectroscopy.[42] Trimethoprim-d_2 was prepared by the introduction of deuterium atoms on the benzene ring

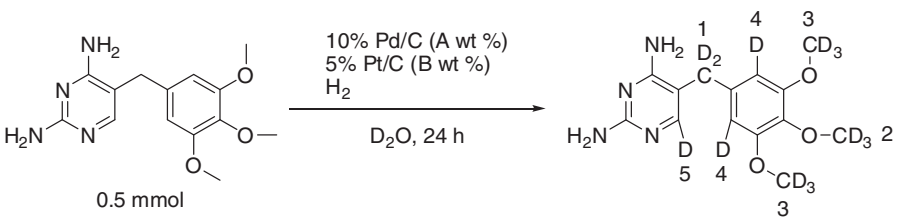
Table 4. The H–D exchange reaction of phenytoin



Entry	Catalyst	Temp (°C)	D content (%)[a]	Yield (%)
1	10% Pt/C	rt	11	100
2	5% Pt/C	160	50	100
3	5% Pt/C	180	70	100
4	5% Pt/C+10% Pd/C	180	96	79

[a]Determined by ^1H NMR.

Table 5. The H–D exchange reaction of trimethoprim



Entry	Catalyst loading		D_2O (mL)	Temp (°C)	D content (%)[a]					Yield (%)
	A	B			1	2	3	4	5	
1	10	10	1	rt	33	35	15	39	49	100
2	10	20	2	160	41	7	13	28	94	100
3	20	20	3	180	71	11	19	38	95	86
4[b]	20	20	3	180	87	32	41	92	97	100

[a]Determined by ^1H NMR.
[b]Reaction time was 34 h.

of trimethoprim by the H–D exchange reaction in heated 1 M DCl,[39] while multi-step syntheses through deuterated 3,4,5-trimethoxybenzaldehyde were adopted for the preparation of [$^2H_3$]trimethoprim and [$^2H_6$]trimethoprim.[43, 44]

When 10% Pd/C and 5% Pt/C (each 10% of substrate weight) were simultaneously used for the deuteration of trimethoprim (0.5 mmol) at room temperature with D_2O (1 mL) and hydrogen gas, the deuterium atoms were poorly incorporated (Table 5, Entry 1). The deuterium efficiency on the pyrimidine nucleus was improved to 94% by raising the temperature to 160°C and the amounts of D_2O and 5% Pt/C to 2 mL and 20 wt%, respectively (Entry 2). The ease of the H–D exchange of the pyrimidine hydrogen would be rationally explained by the directing effect of the adjacent ring nitrogen atom; the active palladium metal could be readily located near the C–H bond of the pyrimidine ring by the coordination with the lone pair of the neighboring ring nitrogen.[20] Further increase in the D_2O and 10% Pd/C quantity to 3 mL and 20 wt%, respectively, led to a significant deuterium incorporation at the benzylic position (Entry 3). Two hydrogen atoms on the benzene rings were finally replaced with deuterium atoms with a high efficiency (92%) by the extension of the reaction time to 34 h, although the extent of deuteration on the methoxy group was only slightly improved (Entry 4). The low deuterium incorporation of the methoxy group was identical to that of the alkyl groups of the alkoxyarenes previously reported by us.[45] The deuterated trimethoprim might be valuable to investigate the binding with DHFR because the location of its deuterium atoms is different from that of the previously reported deuterated trimethoprims.

Conclusion

We have successfully deuterated xanthines, valpromide, phenytoin, and trimethoprim using the heterogeneous Pd/C-, Pt/C-, or/and Rh/C-catalyzed H–D exchange reactions in the presence of D_2O and H_2. One of the most distinctive features of these methods is their simplicity; no multi-step reaction was required to access the deuterium-labelled drugs. The protocol for the H–D exchange reactions could be practical for the preparation of deuterated analogs with improved biological activities over the parent drugs, as well as providing standard materials for quantitative analyses.

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