Microwave-Assisted Direct H/D Exchange Reactions of Dimetridazole and Metronidazole in Alkaline D_2O

Akira Miyazawa,[*1] **Haruki Shimodaira,**[1,2] **and Yuji Kawanishi**[1]

[1]National Institute of Advanced Industrial Science and Technology (AIST), Central 5, Tsukuba, Ibaraki 305-8565

[2]S. I. Technology Group Tsukuba Laboratories, Taiyo-Nippon Sanso Corporation, 10 Ohkuba, Tsukuba, Ibaraki 300-2611

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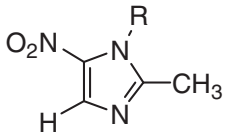
E-mail: a.miyazawa@aist.go.jp

Upon microwave irradiation, a ring proton at the 4-position and protons at the 2-methyl positions of dimetridazole, 1,2-dimethyl-5-nitroimidazole, and metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, were selectively and rapidly exchanged with deuterium at an exchange efficiency in excess of 98 atom % within 30 min.

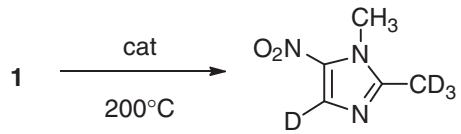
Series of compounds labeled with deuterium(s) are important tools for mechanistic investigations of chemical reactions, metabolic pathways, and as internal standards for the quantitative measurement of contaminating toxic chemicals in foods and the environment. Quantitative analysis methods that use deuterated internal standard compounds, in general, require that the mass difference be sufficiently large to distinguish between the non-deuterated target compounds and the deuterated internal standard in GC- and LC-MS analyses using isotope dilution techniques.[1] Although deuterated compounds are required for precise quantitative determination, commercially available deuterated compounds are generally expensive and in limited supply. Thus, development of simple and cost-effective deuteration protocols is an important contribution to health and the environment. Dimetridazole (**1**) (1,2-dimethyl-5-nitroimidazole) and metronidazole (**2**) (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole), are well-known and widely used agricultural and food-producing animal drugs for the treatment of trichomoniasis, histomoniasis, and coccidiosis.[2] The molecular structures of **1** and **2** are depicted in Figure 1. Cohen and coworkers[3] reported kinetic studies of the H/D exchange reaction of **1** in a MeOD–D_2O–NaOD solution. The deuterated product was **1**-d_3, a deuterated *C*-methyl derivative, and the introduction of deuterium on the imidazole ring was not observed. The yield of **1**-d_3 was low due to a competing decomposition reaction that proceeded under the base-catalyzed reaction conditions. Miyano[4] reported that the methyl protons at the 2-position of metronidazole (**2**) could be exchanged with deuterium to give **2**-d_3 with high deuterium content. However, their protocol required large quantities of the reactants, and the yield of **2**-d_3 was around 30%. Direct H/D exchange is the most cost-effective method for obtaining deuterated **1** and **2**. However, to the best of our knowledge, both ways are problematic in the yields and long reaction time.



1 dimetridazole (R=CH_3)
2 metronidazole (R=CH_2CH_2OH)

Figure 1.

Table 1. Direct H/D Exchange Reaction of **1**



Entry	Method[a)]	Cat[b)]	Yield[c)] /%	D-content/atom %	
				2-CD_3	4-D
1[d)]	CH	none	quant	1	38
2	CH	none	quant	6	92
3[d)]	MW	none	quant	2	66
4	MW	none	quant	19	99
5	CH	Na_2CO_3	72	87	81
6	MW	Na_2CO_3	59	99	99
7	MW	Pd/C	quant	21	99
8	MW	Pt/C	quant	20	99

a) CH and MW means conventional heating and microwave heating, respectively. b) 10 mol % of Na_2CO_3 or metal catalysts were used. c) Isolated yields are shown. d) Reactions carried out at 160 °C.

This decade has seen a tremendous increase in the number of microwave-assisted organic syntheses, which can accelerate reactions and occasionally enhance selectivity.[5] Several examples of microwave-assisted direct H/D exchange reactions in D_2O have been reported.[1a] Our previous studies of microwave-assisted reactions in water (including D_2O) showed remarkable acceleration and occasionally enhanced selectivity for deuteration.[6] Here, we report the rapid and cost-effective direct H/D exchange reaction of **1** and **2** in D_2O under microwave irradiation to provide deuterated **1** and **2** for use as internal standards for the quantitative analysis of the agrochemicals **1** and **2**.

Studies of the direct H/D exchange reactions of **1** and **2** commenced by optimizing the reaction temperature and heating method. The results of direct H/D exchange of **1** are summarized in Table 1. Solutions of **1** in D_2O were heated at 160 and 200 °C for 1 h using conventional or microwave heating methods. In all cases, increasing the reaction temperature accelerate the H/D exchange of the imidazole ring proton up to

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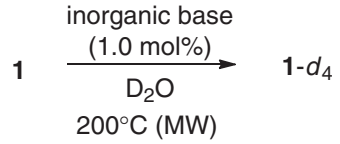
Table 2. Optimization of the Direct H/D Exchange of **1**



Entry	Base	Time /min	Yield[a] /%	D-content/atom %[b]	
				2-CD_3	4-D
1	Na_2CO_3	15	80	98	98
2	Na_2CO_3	30	77	99	99
3	Na_2CO_3	60	59	99	99
4	Ka_2CO_3	30	61	98	99
5	Ka_2CO_3	60	53	98	98
6	Cs_2CO_3	60	37	98	99

a) Isolated yields are shown. b) Based on the peak integrals obtained by ^{1}H NMR.

99 atom % at 200 °C with microwave heating. However, the rate of the H/D exchange at the 2-methyl bound to a ring carbon were slow compared to ring proton, and the deuterium content estimated was less than 20 atom % (Entries 1–4). No H/D exchange reactions were observed at the methyl protons bound to nitrogen. The deuterium content could, therefore, be estimated based on the integral of the unchangeable methyl peak as an internal reference in the ^{1}H NMR spectrum. Albright[7] reported that **1** readily reacted with organic or inorganic bases to generate reactive anions. Their results suggested to us the addition of base to our H/D exchange reaction. Although the addition of Na_2CO_3 (10 mol %) decreased the yield of desired deuterated product due to a decomposition reaction,[3] both protons at the 2-methyl and ring positions were successfully exchanged with deuterium to yield an extremely high deuterium content (up to 99 atom %) under microwave irradiation (Entry 6). Unfortunately, all attempts to isolate and identify the decomposition products were unsuccessful. Pd/C and Pt/C, well-known H/D exchange reaction catalysts[8] for imidazoles, did not yield significant advantages over the reaction conducted in D_2O only (Entries 7 and 8).

Several common inorganic bases, Na_2CO_3, K_2CO_3, and Cs_2CO_3, were evaluated under these reaction conditions (Table 2). Under the reaction conditions listed in Table 1, all inorganic bases yielded high deuterium exchange efficiencies at both the 2-methyl and 4-ring positions of up to 99 atom %, whereas H/D exchange at the 1-methyl position did not occur. The yield of the desired **1**-d_4 depended upon the inorganic base used. A high deuterium content and yield were obtained using Na_2CO_3 as the catalyst. The use of K_2CO_3 and Cs_2CO_3, which are stronger bases than Na_2CO_3, decreased the yield due to a competing decomposition reaction (Entries 3, 5, and 6). These results indicated that Na_2CO_3 was the best catalyst for the direct H/D exchange reaction of **1**. To avoid decomposition, we studied the time dependence of the reaction. The microwave-assisted reactions conducted at 200 °C for 30 and 15 min yielded **1**-d_4 in 77% and 80% yields, respectively, and the deuterium content of **1**-d_4 remained unchanged (Entries 1–3). Thus H/D exchange at the 2-methyl and imidazole ring positions was very rapid under the conditions tested, and prolonging the reaction

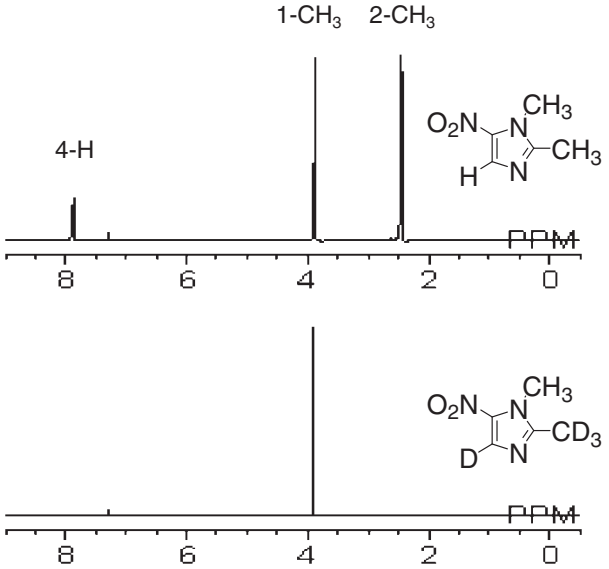
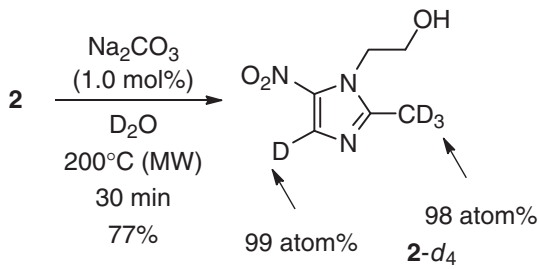


Figure 2. ^{1}H NMR of **1** and **1**-d_4 (Entry 2 in Table 2).



Scheme 1. H/D exchange reaction of **2**.

caused decomposition of the product. Finally, the optimal H/D reaction conditions of **1** were found to include heating at 200 °C by microwave for 30 min in D_2O.

The ^{1}H NMR spectrum of **1**-d_4 is depicted along with that of **1** for comparison (Figure 2). The two singlet signals of the 2-methyl- and imidazole ring protons of **1** nearly disappeared upon enrichment with deuterium. In the expanded spectra, the three 2-methyl signals observed for deuterated CH_2D, CHD_2, and the unchanged CH_3 appeared as a triplet, a quintet, and a singlet, respectively. The sum of the integrals of these peaks was estimated to be 0.05H relative to that of the 1-methyl group.

The direct H/D exchange reaction of **2** under the same conditions (Entry 2 in Table 2) gave **2**-d_4 in 77% yield, with a deuterium content of 98 atom % for the 2-methyl position and 99 atom % for the ring position (Scheme 1). The protons attached to the ethylene group adjacent to the nitrogen were unchanged.

To test the availability of **1**-d_4 as a standard sample labeled with deuteriums, a mixed solution of **1** and **1**-d_4 in equal molar quantities (10 ppm each) was analyzed by GC-MS. The total ion chromatogram and mass number distribution are shown in Figure 3. A clear single peak observed in the total ion chromatogram indicated that the retention times of **1** and **1**-d_4 were identical (Figure 3a). MS analysis of the mixed solution showed five mass numbers at 141, 142, 144, 145, and 146. Among them, 141 and 142 originated from **1**, and the others

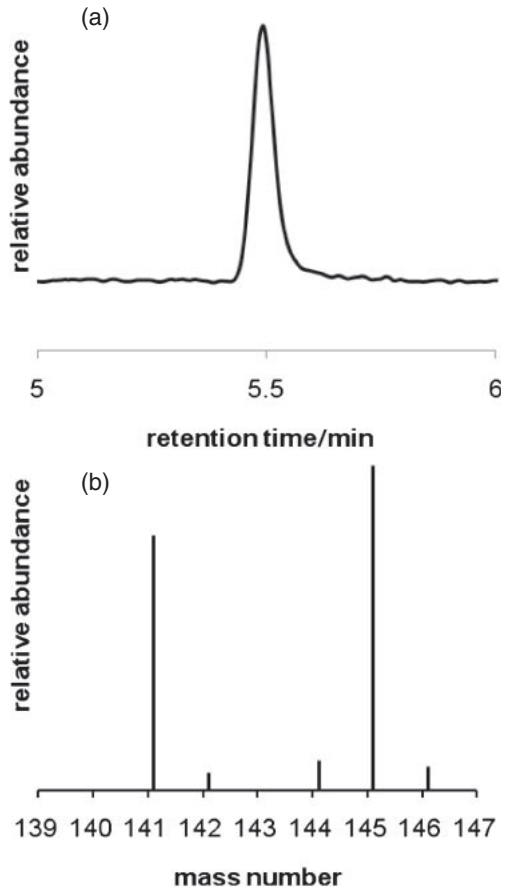


Figure 3. Total ion chromatogram (top) and mass distribution (bottom) of a mixed solution containing **1** and **1**-d_4 during GC-MS analysis.

originated from **1**-d_4. Thus mass distributions of each compound were completely separated (Figure 3b). The results clearly showed that the compound **1**-d_4 obtained in this study met the requirements for a standard sample labeled with deuteriums.

In summary, we have developed a simple and cost-effective method for the synthesis of highly deuterated dimetridazole and metronidazole in microwave-assisted direct H/D exchange reaction. The application of our protocol to other nitroimidazoles is being investigated in our laboratory.

Experimental

All chemicals used in this study were used as received without further treatment. Column chromatography was performed on Wakogel C-300 silica gel. The deuterium contents of deuterium oxide was 99.9 atom %. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz. All ^{1}H NMR spectra were referenced internally using residual chloroform signals in $CDCl_3$ (7.26 ppm).

Direct H/D exchange reaction of **1** was conducted as follows (Entry 2 in Table 2); 1,2-Dimethyl-5-nitroimidazole, dimetridazole, (141 mg, 1.0 mmol), Na_2CO_3 (10 mg, 0.1 mmol), and 3 mL of D_2O (99.9 atom %, purchased from ISOTEC) were placed in a 10 mL pressure-resistant glass ampoule. The ampoule was sealed with a Teflon® covered cap and placed into a cavity of microwave irradiation apparatus (CEM Discover, NC, USA) using continuous microwave irradiation applied with stirring to maintain a 200 °C temperature, without cooling, for 30 min. After the reaction, the reaction mixture was allowed to cool at room temperature, followed by extraction with ether. The organic layer was separated, dried over Mg_2SO_4 and evaporated. The resulting slightly yellow solid was purified by silica gel column chromatography with a mixed solution of hexane and ethyl acetate (1:1 in volume). The main fraction collected was evaporated and recrystallized from ethyl acetate to afford the desired **1**-d_4 in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm} 7.92 (0.01H, s, ring-*H*), 3.91 (3H, s, C*H*$_3$), 2.48–2.44 (0.05H, quintet, $J_{H-D} = 2.3$ Hz, C*H*D$_2$).

Metronidazole-d_4 (**2**-d_4): ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm} 7.96 (0.01H, s, ring-*H*), 4.42 (2H, t, $J = 5.3$ Hz, C*H*$_2$), 3.82 (2H, t, $J = 5.3$ Hz, C*H*$_2$), 2.38 (0.07H, quintet, $J_{H-D} = 2.3$ Hz, C*H*D$_2$).

References

1 a) J. Atzrodt, V. Derdau, T. Fey, J. Zimmermann, *Angew. Chem., Int. Ed.* **2007**, *46*, 7744. b) P. Mottier, I. Huré, E. Gremaud, P. A. Guy, *J. Agric. Food Chem.* **2006**, *54*, 2018.

2 a) S. Fraselle, V. Derop, J.-M. Degroodt, J. V. Loco, *Anal. Chim. Acta* **2007**, *586*, 383. b) M. Cronly, P. Behan, B. Foley, E. Malone, L. Regan, *J. Chromatogr., B* **2009**, *877*, 1494.

3 C. Rav-Acha, L. A. Cohen, *J. Org. Chem.* **1981**, *46*, 4717.

4 M. Miyano, J. N. Smith, *J. Heterocycl. Chem.* **1982**, *19*, 659.

5 C. O. Kappe, D. Dallinger, *Mol. Diversity* **2009**, *13*, 71.

6 a) A. Miyazawa, K. Saitou, K. Tanaka, T. M. Gädda, M. Tashiro, G. K. S. Prakash, G. A. Olah, *Tetrahedron Lett.* **2006**, *47*, 1437. b) A. Miyazawa, M. Tashiro, G. K. S. Prakash, G. A. Olah, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 791. c) T. M. Gädda, X.-Y. Yu, A. Miyazawa, *Tetrahedron* **2010**, *66*, 1249.

7 J. D. Albright, R. G. Shepherd, *J. Heterocycl. Chem.* **1973**, *10*, 899.

8 C. Hardacre, J. D. Holbery, S. E. J. McMath, *Chem. Commun.* **2001**, 367.