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Hydrogen-deuterium exchange of indole-3-propionic acid with deuterated trifluoromethanesulfonic acid

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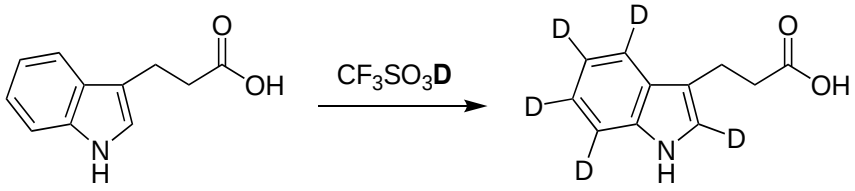
Abstract

Indole-3-propionic acid (IPA), a member of the plant auxin family, plays an important role in biological activity. There are few reports on the preparation of deuterated IPA. Deuterated trifluoromethanesulfonic acid (TfOD) is a hydrogen-deuterium exchange reagent used for aromatic compounds. The hydrogen-deuterium exchange of IPA and indole-3-alaknoic acid derivatives with TfOD was examined in detail.



Keywords: Hydrogen-deuterium exchange, indole-3-propionic acid, indole-3-acetic acid, indole-3-butanoic acid

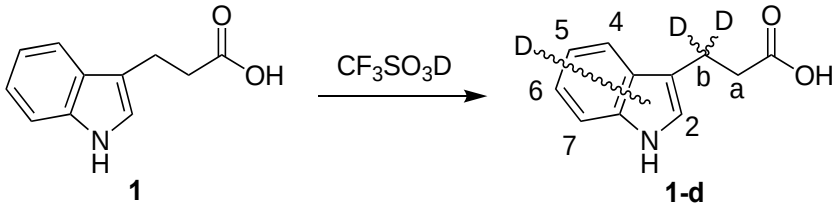
Introduction

With the progress of techniques for mass analysis, stable isotope-labeled bioactive ligands have become attractive tools in the field.[1] Deuterium is one of the simplest stable isotopes and can be utilized for post-introductions to bioactive compounds. Hydrogen-deuterium exchange (H/D exchange)[2-4] at a specific position and universal hydrogen-deuterium exchange in acidic conditions[5] and/or metal catalysis[6] in aromatics have been reported in the literature. Indole-3-propionic acid (IPA) is well known as not only a plant auxin but also a potent neuroprotective antioxidant and drug for Alzheimer's disease. Partial deuteration on the indole moiety of IPA was reported using photolysis in D_2O, with deuteration degrees up to 40 %.[7] There are few reports on a high H/D exchange for the indole moiety on IPA. Recently, we developed a method for effective H/D exchange for natural aromatic α-amino acids and their corresponding peptides with deuterated trifluoromethanesulfonic acid (TfOD) at a temperature lower than room temperature without a side-effect on stereochemistry.[8-11] We report in this paper the details of the H/D exchange of IPA and indole-3-alaknoic acid derivatives with TfOD under mild conditions.

Results and Discussion

Indole-3-propionic acid (IPA, **1**) was dissolved in deuterated trifluoromethanesulfonic acid (TfOD, 40 eq) at rt or 30 °C. The progress of H/D exchange was monitored using ^{1}H-NMR. The results are summarized in Table 1. The priority of H/D exchange for the indole ring was observed as H-2 > H-5 > H-4, 6 and 7. A partial H/D exchange was observed at β-methylene. Complete H/D exchange could not be achieved when the reaction was conducted at rt within 5 days, but no deuterated starting material 1 was observed from ^{1}H-NMR analysis. The reaction conducted at 30 °C for 4 days afforded over 80% H/D exchange at all protons expect the α-position (Table 1). The reaction at higher temperature promoted side reactions for **1**.

Table 1. H/D exchange of indole-3-propionic acid (**1**) with TfOD



1 **1-d**

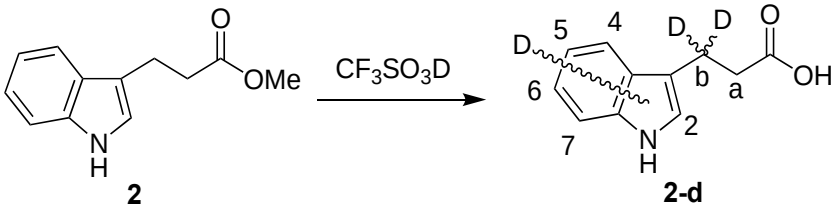
Temperature	Time (h)	2	4	5	6	7	α	β
rt	12	92	58	88	50	50	0	26
	36	93	69	90	51	47	0	38
	120	93	86	90	76	71	0	64
30 °C	6	94	46	75	35	32	0	14
	12	94	57	87	44	47	0	27
	36	94	85	89	68	61	0	49
	48	96	91	91	79	71	0	58
	60	96	92	91	80	76	0	60

	72	96	94	92	80	85	0	79
	96	96	94	92	82	90	0	86

IPA methyl ester (IPA-OMe, **2**) was subjected to H/D exchange with TfOD in a shortened reaction time (Table 2). H/D exchange on the indole moiety of IPA-OMe (**2**) was faster than that of IPA (**1**). The deuterium incorporation at the β-position of the propionic acid moiety proceeded in a time-dependent manner. On the other hand, the deuterium incorporations on the indole moiety proceeded in a temperature-dependent manner until 60 °C. Methyl ester was hydrolyzed in alkaline conditions, and deuterium enrichment on the indole ring of IPA was obtained.

The established methods for IPA (**1**) and IPA-OMe (**2**) were applied to other indole alkanoic acid derivatives. Indole-3-acetic acid (IAA, **3**), which is the shortest alkanoic acid moiety, was treated with TfOD (Table 3). Deuterium incorporations were observed at 4 °C. The α-methylene of IAA did not participate in the H/D exchange. H/D exchange on the indole ring was achieved within an hour at 40 °C.

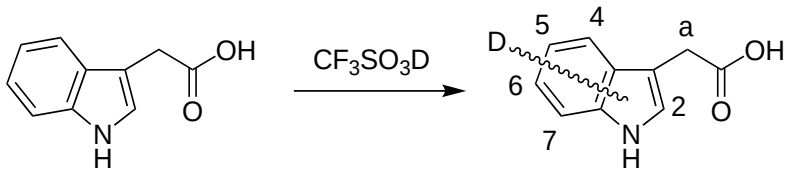
Table 2. H/D exchange of indole-3-propionic acid methyl ester (**2**) with TfOD



2 **2-d**

Temperature	Time (h)	2	4	5	6	7	α	β
rt	1	93	19	37	20	16	0	0
	2	94	27	63	26	32	0	0
	6	95	44	65	53	36	0	6
	12	97	67	89	64	32	0	26
40 °C	1	95	57	87	62	47	0	0
	2	95	65	90	62	51	0	5
	3	97	77	91	62	60	0	7
	4	97	81	92	62	60	0	10
	12	97	84	93	83	78	0	48
	72	97	90	93	88	89	0	84
60 °C	1	92	86	89	85	84	0	0
	2	92	89	91	85	85	0	10
	3	92	90	91	87	86	0	18
	4	92	92	92	90	88	0	25

Table 3. H/D exchange of indole-3-acetic acid (**3**) with TfOD

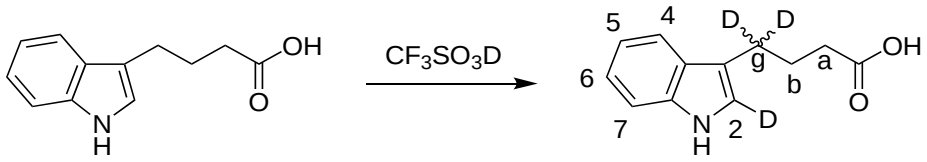


3 **3-d**

Temperature	Time (min)	2	4	5	6	7	α
4 °C	60	0	2	0	0	3	0
	480	83	83	80	58	78	0
40 °C	15	82	80	88	76	69	0
	30	88	87	93	86	81	0
	45	91	90	93	90	87	0
	60	90	90	93	90	86	0

Indole-3-butyric acid (IBA, **4**) was treated with TfOD (Table 4). The deuterium incorporations were specifically observed at the 2-position of the indole and γ-position of the alkanoic moiety at rt. The reaction conducted at 40 °C improved this incorporation into the indole rings.

Table 4. H/D exchange of indole-3-butyric acid (**4**) with TfOD



4 **4-d**

Temperature	Time (h)	2	4	5	6	7	α	β	γ
rt	0.5	61	0	0	0	0	0	0	14
	1	74	0	0	0	0	0	0	36
	2	75	0	0	0	0	0	0	57
	3	79	0	0	0	0	0	0	61
	4	85	0	0	0	0	0	0	79
	6	86	0	0	0	0	0	0	89
	8	88	0	0	0	0	0	0	92
40 °C	1	95	26	46	17	10	0	5	82
	1.5	96	35	51	23	12	0	5	83
	2	98	44	66	37	16	0	6	88
	3	98	50	72	42	20	0	5	88
	4	98	55	79	51	22	0	5	90

Conclusions

Deuterium incorporation via H/D exchange is one of the simplest ways to prepare deuterated compounds, which are utilized to elucidate not only of biological activities but also reaction mechanisms. It has been reported that many H/D exchange reactions under acidic conditions proceed at higher temperature and with a longer reaction time. Indole-3-alkanoic acids are well known as plant growth regulators (auxins), but there are few reports for the preparation of their deuterated derivatives for IAA[12,13] and IBA.[14] The many reported reactions were conducted at high temperature. H/D exchange with deuterated trifluoromethanesulfonic acid (TfOD), which was recently reported by us, can overcome these problems. The reactions can be conducted in a temperature-dependent manner according to the chemical stability of the ligands without the use of special reagents (e.g., complex metal). The detailed analysis of H/D exchange of indole-3-alkanoic acids revealed that the reaction proceeded swiftly at low temperature. The effective H/D exchange of indole-3-alkanoic acids will be helpful not only in plant regulation studies but also in drug development.

Experimental Section

General. All reagents used were analytical grade. Deuterated trifluoromethanesulfonic acid was purchased from Aldrich. NMR spectra were measured by using an EX 270 spectrometer (JEOL, Tokyo, Japan). HRMS-ESI spectra were obtained with a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA). H/D exchange of Indole-3-alakanoic acid derivatives were conducted up to 150 mg scale for each compound.

3-(1*H*-Indol-3-yl-2,4,5,6,7-d$_5$)propanoic-2,2-d$_2$ acid (1-d). 3-(1*H*-indol-3-yl)propanoic acid **1** (26.5 mg, 0.14 mmol) was dissolved in TfOD (0.5 ml, 5.65 mmol) at 0 °C. The reaction mixture was stirred at 30 °C for 96 h and slowly poured into 3.5 ml D_2O on ice. The aqueous solution was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate: *n*-hexane, 1:1) to afford colorless solid (19.8 mg, 72 %). ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.47 (0.06H, s, H-4), 7.27 (0.10H, s, H-7), 7.03 (0.18H, s, H-6), 6.96 (0.08H, s, H-5), 6.95 (0.04H, s, H-2), 3.00 (0.28H, t, *J* 7.6 Hz, H-b), 2.62 (2H, t, *J* 7.6 Hz). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 177.5, 138.0, 128.4, 122.8 (t, *J* 24.0 Hz), 121.8 (t, *J* 25.1 Hz), 118.7 (t, *J* 22.8 Hz), 114.8 (t, *J* 23.1 Hz), 111.8 (t, *J* 25.1 Hz), 36.0, 21.8 (m). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{11}H_5D_7NO_2^+$ 197.1302, found 197.1284.

Methyl 3-(1*H*-indol-3-yl-2,4,5,6,7-d$_5$)propanoate (2-d). 3-(1*H*-indol-3-yl)propanoic acid (**1**, 2.0 g, 10.6 mmol) was dissolved in 53 ml methanol. Concentrated H_2SO_4 (1.7 ml) was added at 0 °C. The reaction mixture was stirred at room temperature overnight, poured into 88 ml of H_2O, extracted with CH_2Cl_2 (100 ml × 2), dried with $MgSO_4$, filtered, and concentrated to afford methyl 3-(1*H*-indol-3-yl)propanoate (**2**) as a pale brown solid (1.80 g, 84%). Compound **2** (143.0 mg, 0.70 mmol) was dissolved in TfOD (2.5 ml, 28.3 mmol) at 0 °C. The reaction mixture was stirred at 60 °C for an hour and slowly poured into 20 ml of D_2O on ice, and then extracted with $CHCl_3$ (10 ml × 3). The organic layer was washed with brine (5 ml × 3), dried over $MgSO_4$, filtered, and concentrated to afford pale yellow solid (103.0 mg, 70%)

^{1}H-NMR (270 MHz, CD_3OD) δ: 7.47 (0.11H, s, H-4), 7.27 (0.15H, s, H-7), 7.03 (0.10H, s, H-6), 6.96 (0.08H, s, H-5), 6.95 (0.08H, s, H-2), 3.72 (s, 3H), 3.00 (2H, t, *J* 7.6 Hz), 2.62 (2H, t, *J* 7.6 Hz). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 177.5, 138.0, 128.4, 122.8 (t, *J* 23.8 Hz), 121.8 (t, *J* 25.0 Hz), 118.7 (t, *J* 22.0 Hz), 114.8 (t, *J* 22.2 Hz), 111.8 (t, *J* 25.1 Hz), 51.5, 34.7, 20.6. HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{12}H_9D_5NO_2^+$ 209.1333, found 209.1350.

Hydrolysis of 2-d. Compound **2-d** (63.0 mg, 0.30 mmol) was dissolved in methanol (3.5 mL), and 1 M NaOH (1.2 mL) was added slowly. The reaction mixture was stirred at room temperature overnight and washed with

CH_2Cl_2. The aqueous layer was acidified with 2 M HCl and extracted with ethyl acetate (20 ml × 2). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (ethyl acetate: n-hexane, 1:1) to give 3-(1*H*-indol-3-yl-2,4,5,6,7-d_5)propanoic acid as a colorless solid (56.2 mg, 96 %).

2-(1*H*-Indol-3-yl-2,4,5,6,7-d_5)acetic acid (3-d). 2-(1*H*-indol-3-yl)acetic acid **3** (24.6 mg, 0.14 mmol) was dissolved in TfOD (0.5 ml, 5.65 mmol) at 0 °C. The reaction mixture was stirred at 40 °C for 45 min and slowly poured into 3.5 ml of D_2O on ice, and then extracted with ethyl acetate. The organic layer was washed with brine, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate: n-hexane, 1:1) to afford a colorless solid (24.8 mg, 98%). ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.52 (0.10H, s, H-4), 7.33 (0.13H, s, H-7), 7.14 (0.09H, s, H-6), 7.08 (0.10H, s, H-5), 7.00 (0.07H, s, H-2), 3.72 (2H, s). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 176.5, 137.9, 128.6, 124.4 (t, *J* 26.8 Hz), 121.9 (t, *J* 24.6 Hz), 119.0 (t, *J* 24.6 Hz), 118.4 (t, *J* 24.6 Hz), 108.7 (t, *J* 25.0 Hz), 31.9. HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{10}H_5D_5NO_2^+$ 181.1020, found 181.1045.

4-(1*H*-Indol-3-yl-2-d)butanoic-4,4-d_2 acid (4-d). 4-(1*H*-indol-3-yl)butanoic acid **4** (34.4 mg. 0.17 mmol) was dissolved in TfOD (0.6 ml, 6.78 mmol) at 0 °C. The mixture was stirred at rt for 8 h and slowly poured into 3.5 ml D_2O on ice. The aqueous solution was extracted with ethyl acetate. The organic layer was washed with brine, dried with $MgSO_4$, and filtered. The residue was purified by column chromatography (ethyl acetate: n-hexane, 1:1) to afford a colorless solid (31.7 mg, 91%). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.60 (1H, t, *J* 3.8 Hz, H-4), 7.35 (1H, d, *J* 7.9 Hz, H-7), 7.19 (0.12H, t, *J* 7.3 Hz, H-6), 7.11 (1H, t, *J* 7.3 Hz, H-5), 6.99 (0.07H, s, H-2), 2.83 (0.16H, dd, *J* 12.5, 6.6 Hz, H-g), 2.43 (2H, t, *J* 7.4 Hz), 2.05 (2H, q, *J* 6.9 Hz) ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ: 179.2, 136.3, 127.4, 122.0, 119.3, 118.9, 115.1 (t, *J* 24.6 Hz), 111.1, 33.4, 24.9, 24.2 (m). HRMS-ESI (*m/z*) $[M + H]^+$ calcd for $C_{12}H_{11}D_3NO_2^+$ 207.1207, found 207.1188.

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References

1. Arsene, C. G.; Ohlendorf, R.; Burkitt, W.; Pritchard, C.; Henrion, A.; O'Connor, G.; Bunk, D. M.; Guttler, B. *Anal. Chem.* **2008**, *80*, 4154. https://doi.org/10.1021/ac7024738
2. Dorman, G.; Olszewski, J. D.; Prestwich, G. D.; Hong, Y.; Ahern, D. G. *J. Org. Chem.* **2002**, *60*, 2292. https://doi.org/10.1021/jo00112a066
3. Ambroise, Y.; Mioskowski, C.; Djega-Mariadassou, G.; Rousseau, B. *J. Org. Chem.* **2000**, *65*, 7183. https://doi.org/10.1021/jo0012243
4. Faucher, N.; Ambroise, Y.; Cintrat, J. C.; Doris, E.; Pillon, F.; Rousseau, B. *J. Org. Chem.* **2002**, *67*, 932. https://doi.org/10.1021/jo0109669
5. Atzrodt, J.; Derdau, V.; Fey, T.; Zimmermann, J. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 7744. https://doi.org/10.1002/anie.200700039
6. Ito, N.; Watahiki, T.; Maesawa, T.; Maegawa, T.; Sajiki, H. *Adv. Synth. Catal.* **2006**, *348*, 1025.

https://doi.org/10.1002/adsc.200606053
7. Saito, I.; Muramatsu, S.; Sugiyama, H.; Yamamoto, A.; Matsuura, T. *Tetrahedron Lett.* **1985**, *26*, 5891. https://doi.org/10.1016/S0040-4039(00)98254-6
8. Murai, Y.; Wang, L.; Masuda, K.; Sakihama, Y.; Hashidoko, Y.; Hatanaka, Y.; Hashimoto, M. *Eur. J. Org. Chem.* **2013**, *2013*, 5111. https://doi.org/10.1002/ejoc.201300405
9. Wang, L.; Murai, Y.; Yoshida, T.; Okamoto, M.; Masuda, K.; Sakihama, Y.; Hashidoko, Y.; Hatanaka, Y.; Hashimoto, M. *Biosci. Biotechnol. Biochem.* **2014**, *78*, 1129 https://doi.org/10.1080/09168451.2014.917267
10. Tachrim, Z. P.; Nakagawa, S.; Nakamura, T.; Ohashi, F.; Kurokawa, N.; Wakasa, H.; Tokoro, Y.; Sakihama, Y.; Hashidoko, Y.; Suzuki, T.; Hashimoto, M. *Heterocycles* **2019**, *99*, 404. https://doi.org/10.3987/Com-18-S(F)33
11. Tachrim, Z. P.; Kurokawa, N.; Tokoro, Y.; Hashimoto, M. *Heterocycles* **2020**, *101*, 357. https://doi.org/10.3987/com-19-s(f)28
12. Magnus, V.; Bandurski, R. S.; Schulze, A. *Plant Physiol.* **1980**, *66*, 775. https://doi.org/10.1104/pp.66.4.775
13. Brown, B. H.; Neill, S. J.; Horgan, R. *Planta* **1986**, *167*, 421. https://doi.org/10.1007/bf00391349
14. Yamada, T.; Arai, K.; Kikuchi, R.; Okamoto, S. *ACS Omega* **2021**, *6*, 19956. https://doi.org/10.1021/acsomega.1c02940