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Tsuzuki, Hirohisa
Center of Advanced Instrumental Analysis, Kyushu University

Mukumoto, Mamoru
Department of Molecular Science and Technology Graduate School of Engineering Science Kyushu University

Udagawa, Jun
Department of Anatomy Shimane Medical University

Mataka, Shuntaro
Institute of Advanced Material Study Kyushu University

他

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Deuterium Labelling of L-Tyrosine with Raney Alloys in Alkaline Deuterium Oxide Solutions

Hirohisa TSUZUKI,*[1] Mamoru MUKUMOTO,*[2]
Jun UDAGAWA,*[3] Shuntaro MATAKA and Masashi TASHIRO¶

The synthesis of deuteriated L-tyrosines with Raney alloys in alkaline deuterium oxide solutions, involving reductive debromination of brominated L-tyrosines and hydrogen-deuterium (H-D) exchange of L-tyrosines, without causing racemization, is presented.

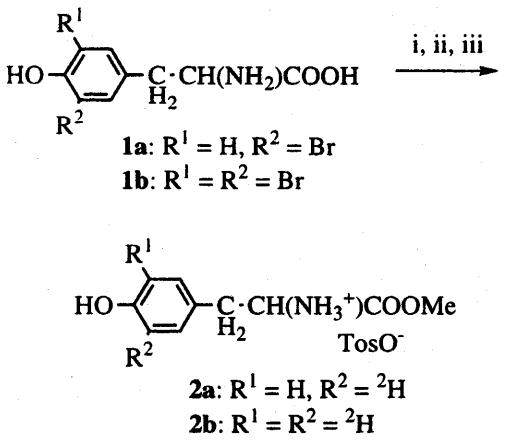
Introduction

We have previously reported that a heterogeneous catalytic system utilizing a Raney alloy in alkaline deuterium oxide solution provides a versatile procedure for synthesizing compounds labelled with deuterium(s).[1a)-e)] L-Tyrosine is widely known as an essential amino acid and is found as an important metabolic intermediate in many kinds of phenyl propanoids. Deuteriated L-tyrosines have attracted much attention in biological studies, and several syntheses have already been published.[2)] In the present study, we have applied the methodology mentioned above towards deuterium labelling of L-tyrosines focusing mainly on the hydrogen-deuterium (H-D) exchange as a key reaction, and the results are herein described.

Results and Discussions

At the outset, the direct purification of deuteriated products from an aqueous reaction mixture which was obtained by the treatment of brominated L-tyrosines with a cupper-aluminum (Cu-Al) alloy was found to be extremely difficult, due to the slightly soluble nature of L-tyrosine in both water and any organic solvents. Furthermore, $Al(OH)_3$, which was generated on neutralization of the reaction mixtures, hampered the purification. We attempted to isolate deuteriated L-tyrosines as their derivatives which are soluble in an organic solvent. Finally, we arrived at the somewhat tedious procedure whereby the deuteriated L-tyrosines were isolated as their tosylate in a two-step sequence by *in situ* *N*-Boc-protected-deprotected reaction and subsequent esterification with methyl *p*-toluenesulfonate in neutral media.[3)]

In preference to deuteriation, the deuterium replacement of the mobile hydrogens in L-tyrosine by employment of a small amount of D_2O under sonication was effective to



Scheme 1 *Reagents and conditions*: i, Cu-Al alloy, 10% NaOD-D_2O, 40 °C, 1 h; ii, *t*-Boc, *n*-BuOH, r.t., 12 h; iii, TosOMe, MeOH, reflux, 10 h

Table 1 Reductive debromination of brominated L-tyrosines (**1a-b**) using Cu-Al alloy in 10% NaOD-D_2O at 40 °C for 1 h

Run	Substrate	Y. (%)[a]	D-content (%)[b]	e.e. (%)[c]
1	**1a**	36	94	95
2	**1b**	34	93	97

[a]Isolated yields shown; [b]Deuterium content determined by ^1H NMR; [c]e.e. determined by HPLC.

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*[1]Center of Advanced Instrumental Analysis, Kyushu University
*[2]Department of Molecular Science and Technology, Graduate School of Engineering Science, Kyushu University
*[3]Department of Anatomy, Shimane Medical University
¶ Present address: Tohwa Institute for Science, Tohwa University

prevent the lowering of the deuterium content in the products. The conventional dehalogenation method was extended to the selective deuteriation of L-tyrosines and proved to be straightforward; 3-bromo- and 3,5-dibromo-L-tyrosines (**1a-b**) were reductively debrominated with a Cu-Al alloy in 10% NaOD-D_2O to the corresponding L-[3-$^2H_1$]- and [3,5-$^2H_2$]-tyrosines (**2a-b**) as their tosylate in 94 and 93% deuterium content, respectively (Scheme 1 and Table 1).

The deuterium content of the products was determined by using ^1H NMR spectroscopy. It should be noted that pronounced racemization did not occur and no additional deuterium incorporation was observed under the above reaction conditions.

It has recently been demonstrated that benzylic hydrogens are susceptible to H-D exchange when using a cobalt-aluminum (Co-Al) alloy in 20% Na_2CO_3-D_2O, affording the [α,α'-$^2H_2$] compounds in high deuterium contents and in favorable yields.[1e] In addition, it was found that some H-D exchange of optically active benzylic hydrogens proceeds with complete retention of stereochemistry.[1c] We subsequently turned our attention to deuterium labelling of L-tyrosine (**3**) by means of the H-D exchange technique in order to explore the feasibility of this approach toward amino acids bearing an aromatic ring.

The H-D exchange reactions of L-tyrosine (**3**) were performed with Raney alloys, such as nickel-aluminum (Ni-Al) and Co-Al alloys, in alkaline deuterium oxide and the results are summarized (Fig. 1 and Table 2).

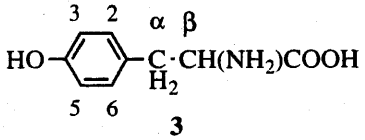

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Fig. 1

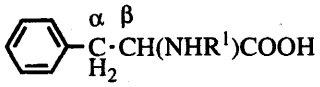
Table 2 H-D exchange reaction of L-tyrosine (**3**) in alkaline D_2O at 90 °C for 2 h

Run[a]	Product	Y.(%)[b]	D-content (%)[c]
1	**2c**	65	α:79; β:13; 2,6-Ph: 6; 3,5-Ph:41
2	**2d**	45	α:97; β:57; 2,6-Ph: 2; 3,5-Ph:94
3	**2e**	26	α:93; β:91; 2,6-Ph:13; 3,5-Ph:92

[a]Run 1: Co-Al alloy, 20% Na_2CO_3-D_2O, d.e.> 99%; run 2: Ni-Al alloy, 20% Na_2CO_3-D_2O, d.e.> 99%; run 3: Ni-Al alloy, 10% NaOD-D_2O, d.e.> 85%; [b]Isolated yields shown; [c]Deuterium content determined by ^1H NMR.

The optical purities of **2c-e**, which were determined with HPLC, were expressed in d.e. (%), since deuteriums were incorporated into also the α-positions. The deuterium content of deuteriated L-tyrosines (**2c-e**) was highly dependent upon the kind of Raney alloy and alkaline solution used. When **3** was treated with the Co-Al alloy in 20% Na_2CO_3-D_2O at 90 °C for 2 h, 41% deuterium was surprisingly incorporated at the *o*-position to the hydroxyl group in addition to the benzylic position (run 1). The use of a Ni-Al alloy brought about high deuterium incorporation into both the 3,5- and benzylic positions. It is noteworthy that the racemization at the β-carbon did not occur even though 57% of the methine proton in **3** was replaced with deuterium (run 2). The best results were obtained under the more demanding conditions when L-tyrosine (**3**) was treated with the Ni-Al alloy in the presence of 10% NaOD-D_2O; the deuterium content at the β-position amounted to 91% and that at the *o*-position of the hydroxyl group and at the benzyl position 92 and 93%, respectively, in 85% optical purity (run 3). The lowering of the optical purity as compared to runs 1-2 was foreseen to some extent since the use of a strong base such as NaOD-D_2O solution is expected to cause racemization as a result of enolization.

The ready deuterium incorporation into the 3,5-positions in **3** might be explained by the fact that the introduction of a polar hydroxyl group causes much stronger adsorption of the aromatic ring on the active catalyst surface, thereby favoring H-D exchange. Indeed, as shown in Fig. 2 and Table 3, treatment of L-phenylalanine (**4a**) with a Co-Al alloy in 20% Na_2CO_3-D_2O at 90 °C leads to exchange only at the benzylic and β-positions (83 and 32% respectively); no deuteriums were incorporated in the aromatic ring. These results are consistent with previous findings on 4-methoxybenzyl alcohol.[1e]



4a: R^1 = H
4b: R^1 = CHO
4c: R^1 = Boc

Fig. 2

Table 3 H-D exchange reaction of L-phenylalanines (**4a-c**) in 20% Na_2CO_3-D_2O for 2 h

Run[a]	Substrate	Y.(%)[b]	D-content (%)[c]
1	**4a**	65	α: 83 β: 32 Ph: 0
2[d]	**4b**	67	α: 60 β: 0 Ph: 7
3[d]	**4c**	91	α: 0 β: 0 Ph: 0

[a]Run 1: Co-Al alloy, 90 °C; run 2: Ni-Al alloy, 60 °C; run 3: Ni-Al alloy, 60 °C; [b]Isolated yields shown; [c]Deuterium content determined by ^1H NMR; [d]Under ultrasound irradiation.

In the studies of the H-D exchange of *N*-formyl- and -Boc-protected phenylalanines (**4b-c**), which was designed

to assess the steric factor, no deuterium exchange occurred at the both β-positions. Hence, it is strongly suggested that both a hydroxyl and amino group are essential if extensive H-D exchange is to occur.

The site(s) of deuterium incorporation can be determined directly by using ^{2}H NMR spectroscopy as well as indirectly by ^{1}H NMR spectroscopy. For example, in the ^{2}H NMR spectrum of **2b** the deuterium signals were observed as a singlet at 6.89 ppm and in that of **2e** as singlets at 6.87, 4.28, and 3.08 ppm in an approximately 2:1:2 intensity ratios, respectively. Furthermore, in the ^{13}C NMR spectrum of **2b** the deuterium-bound C(3) was observed as a triplet of *J* 22.9 Hz at 115.15 ppm. The ^{13}C NMR spectrum of **2e** showed a triplet of *J* 21.8 Hz at 115.16 ppm, a triplet of *J* 20.0 Hz at 53.26 ppm, and a quintet of *J* 20.0 Hz at 34.43 ppm, which are assigned as the C(3), C(β), and C(α), respectively. These results clearly show that deuterium atoms were situated in the appropriate positions of these compounds.

Experimental

All melting points were determined by a Yanagimoto micro-apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Jasco IR-700 spectrophotometer. ^{1}H NMR spectra were measured on a JEOL EX-270 and JEOL GSX-500 NMR spectrometers at 270 MHz and 500 MHz using Me_4Si as an internal standard. Chemical shift values are expressed in δ (ppm) downfield from Me_4Si and coupling constants are in Hz. ^{13}C{^{1}H} NMR spectra were recorded on a JEOL GSX-500 NMR spectrometer at 125.65 MHz by setting dimethyl sulfoxide-d_6 signal to 39.5 ppm as a standard. ^{2}H NMR spectra were recorded with a JEOL EX-270 NMR spectrometer at 41.34 MHz by setting dimethyl sulfoxide-d_6 signal to 2.30 ppm as a reference. Mass spectra were taken on a JEOL JMS OISG-2 mass spectrometer at 75 eV in a direct inlet system. A Transonic T460 ultrasonic laboratory cleaner (35 KHz, 68 W, Elma Inc.) was used in sonication procedures. The optical purities of **2c-e** were determined with a Shimadzu HPLC LC-6A using a SUMICHIRAL-OA-5000 column (4.6 mm I.D. x 15 cm) in a mobile phase, 1 mM $CuSO_4$ in a solution of water and methanol (85:15 / v:v).

Materials.—Deuterium oxide (D_2O, 99.9 atom%) and 40% NaOD-D_2O (99.5 atom%) were purchased from ISOTEC Inc. Ni-Al (Ni:Al = 50:50 *w*%), Co-Al (Co:Al = 50:50 *w*%) and Cu-Al (Cu:Al = 50:50 *w*%) alloys were available from Kishida Chemical Co., Ltd. 3-Bromo-L-tyrosine (**1a**) was prepared in the same way as 3-bromo-D,L-tyrosine.[4] 3,5-Dibromo-L-tyrosine (**1b**) was purchased from Sigma Chemical Company and was used without further purification.

3-Bromo-L-tyrosine (**1a**): 30%, colourless needles (H_2O), mp 242-244 ℃; ν_{max}/cm^{-1} (KBr) 3242, 1595, 1436, 1327, 1232, 1038, 835, 748; δ_H (D_2O, 270 MHz) 7.30 (d, 1H, *J* 2.3 Hz), 6.94 (dd, 1H, *J* 8.3, 2.3 Hz), 6.64 (d, 1H, *J* 8.3 Hz), 3.41-3.31 (m, 1H), 2.94-2.62 (m, 2H); *m/z* 261 (M^+, 16), 259 (M^+, 25), 246 (37), 244 (57), 215 (90), 213 (100); Found: C, 41.38; H, 4.11; N, 5.15. $C_9H_{10}O_3NBr$ requires C, 41.56; H, 3.89; N 5.39%.

General procedure for the H-D exchange method.—A mixture of L-tyrosine (**3**) (544 mg, 3.0 mmol) and D_2O (1.0 ml) was stirred for 5 min under ultrasound irradiation, then was evaporated *in vacuo*. To a mixture of the deuterium-displaced **3** in 10% Na_2CO_3-D_2O (12.0 ml) was gradually added Ni-Al alloy (800 mg) over a period of 20 min at 90 ℃ and the mixture was then stirred at the same temperature for 2 h. After it was cooled to room temperature, the mixture was filtered off using a celite as aid and the inorganics were washed with a small amount of 10% aqueous Na_2CO_3. To the washings, which were combined with the filtrate, was added di-*tert*-butyl dicarbonate (756 mg, 3.3 mmol) and *tert*-butanol (10.0 ml), and the mixture was then stirred at room temperature for 12 h. The resulting suspension was carefully adjusted to pH 5 with concentrated HCl under ice-cooling and was saturated with brine. The whole mixture was extracted with *n*-butanol (30 ml x 3) and the extracts dried over $MgSO_4$ before being evaporated *in vacuo* to leave a residue, which was allowed to dry by further heating on a vacuum pump to yield L-[3,5,α,α',β-$^2H_5$]tyrosine (266 mg, 49%) as colourless needles (1% aqueous HCl), mp 277-278.5 ℃ (dec.) (lit.[5] mp 280-285 ℃ as for $^2H_0$ form); ν_{max}/cm^{-1} (KBr) 3206, 2076, 1604, 1475, 1402, 1354, 1248, 840, 767. In a separate experiment, a mixture of L-[3,5,α,α',β-$^2H_5$]tyrosine (90 mg, 0.48 mmol) and methyl *p*-toluenesulfonate (186 mg, 1.0 mmol) in dry methanol (2.0 ml) was refluxed for 10 h. After the reaction mixture had been cooled to room temperature, it was evaporated *in vacuo* to leave a residue. To it was added ether to generate a precipitate which was collected by filtration to afford L-[3,5,α,α',β-$^2H_5$]tyrosine methyl ester *p*-toluenesulfonate salt (**2d**) (167 mg, 92%, overall 45% from **3**) as colourless needles without further purification, mp 214.5-216.5 ℃ (lit.[3b] mp 210-210.5 ℃ as for $^2H_0$ form); ν_{max}/cm^{-1} (KBr) 3334, 3062, 1745, 1603, 1535, 1322, 1190, 1033, 1009, 820, 685; δ_H (dimethyl sulfoxide-d_6, 270 MHz) 9.38 (s, 1H), 8.31 (s, 3H), 7.48 (d, 2H, *J* 7.9 Hz), 7.11 (d, 2H, *J* 7.9 Hz), 6.99 (s, 2H), 6.71 (d, weak, *J* 8.9 Hz), 4.22 (s, weak), 3.69 (s, 3H), 2.95 (d, weak, *J* 11.2 Hz), 2.29 (s, 3H); δ_C (dimethyl sulfoxide-d_6) 169.40, 156.58, 145.17, 137.97, 130.22, 128.14, 125.48, 124.12, 115.16 (t, *J* 21.8 Hz), 53.26 (t, *J* 20.0 Hz), 52.54, 34.43 (quint, *J* 20.0 Hz), 20.75; δ_D 6.87 (2D, s), 4.28 (1D, s), 3.08 (2D, s).

General procedure for the reductive debromination method.—To a stirred mixture of the deuterium-displaced 3,5-dibromo-L-tyrosine (**1b**) (1.02 g, 3.0 mmol) in 10% NaOD-D_2O (12.0 ml) which was prepared from 40% NaOD-D_2O (3.0 ml) and D_2O (9.0 ml) was gradually added Cu-Al alloy (500 mg) at room temperature over a period of 15 min and the mixture was then stirred at 40 ℃ for 1 h. After cooling to room temperature, it was treated in a similar manner to that described above to afford L-[3,5-

$^2H_2$]tyrosine (188 mg, 34%), mp 275.5-276.5 ℃ (lit.[5], mp 280-285℃); ν_{max}/cm^{-1} (KBr) 3204, 2080, 1607, 1476, 1433, 1363, 1330, 1246, 1044, 912, 767, 552. In a separate experiment, L-[3,5-$^2H_2$]tyrosine (90 mg, 0.49 mmol) was converted into the corresponding *p*-toluenesulfonate salt (**2b**) (164 mg, 86%, overall 29% from **1b**) as colourless needles without further purification, mp 215.5-218.0 ℃ (lit.[3b] mp 210-210.5 ℃ as for $^2H_0$ form); ν_{max}/cm^{-1} 3336, 3044, 1746, 1586, 1537, 1481, 1436, 1257, 1185, 1126, 1034, 1009, 820, 686, 570; δ_H (dimethyl sulfoxide-d_6, 500 MHz) 9.37 (s, 1H), 8.37 (s, 3H), 7.53 (d, 2H, *J* 7.7 Hz), 7.14(d, 2H, *J* 7.7 Hz), 6.99 (s, 2H), 6.72 (d, weak, *J* 6.8 Hz), 4.19 (s, 1H), 3.66 (s, 3H), 3.03-2.93 (m, 2H), 2.29 (s, 3H); δ_C (dimethyl sulfoxide-d_6) 169.40, 156.57, 145.10, 138.01, 130.23, 128.14, 125.46, 124.20, 115.15 (t, *J* 22.9 Hz), 53.51, 52.53, 35.20, 20.74; δ_D 6.89 (2D, s).

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