Syntheses of Deuterated Phenylpropionic Acid Derivatives

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2-(4-(2-Thienylhydroxymethyl)phenyl) propionic acid (I), 2-(4-carboxyphenyl) propionic acid (II), 2-(4-(5-hydroxy-2-thienylcarbonyl)phenyl) propionic acid (III) were labeled with multiple-deuterium for the purpose of using as internal standards in studies on the metabolism of 2-(4-(2-thienylcarbonyl)phenyl) propionic acid (suprofen, IV), anti-inflammatory agent, in man and animals by the mass fragmentography. I-d₄ was obtained in a 93% yield from IV-d₄ by reduction with sodium borohydride, and its deuterium content was 99 atom%. On the other hand, II-d₄ (99 atom% D) was obtained by four-step synthesis from 2-((4-bromophenyl-d₄))1,1-ethyleneglycol)propane (V) in a 43% yield and III-d₄ (98.4 atom% D) by five-step synthesis from V in a 12% yield.

Key Words: 2-(4-(2-thienylcarbonyl)phenyl)propionic acid metabolism, mass fragmentography, 2-(4-(2-thienylhydroxymethyl)phenyl)propionic acid, 2-(4-carboxyphenyl) propionic acid, 2-(4-(5-hydroxy-2-thienylcarbonyl) phenyl) propionic acid, multiple-deuterium labeling, mass spectrometry

1. Introduction

2-(4-(2-Thienylcarbonyl)phenyl)propionic acid (suprofen) is a nonsteroidal anti-inflammatory drug that also possesses analgesic and antipyretic activity^{1)~3)}. Among a series of phenylpropionic acid derivatives, suprofen demonstrated a very high degree of anti-inflammatory activity but a low incidence of side effects in animal models²⁾. Recently, we reported that 2-(4-(2-thienylhydroxymethyl)phenyl)propionic acid (I), 2-(4-carboxyphenyl)propionic acid (II), and 2-(4-(5-hydroxy-2-thienylcarbonyl)phenyl) propionic acid (III) were detected in urine of rats given suprofen⁴⁾. In order to select an animal model (most similar to man) to carry out studies that cannot be done in man, we have attempted to clarify the metabolism of suprofen in man and various animal species by the mass fragmentography. Therefore, it has become desirable for the metabolic studies to prepare these metabolites labeled with stable isotope as internal standards

for this technique. Multiple deuterated compounds are generally useful in the study of drug metabolism by the mass spectrometry, partly because highly enriched materials are commonly available. We previously reported that highly enriched suprofen-d₄ (IV) and 2-((4-bromophenyl-d₄))1,1-ethyleneglycol)propane (V) were synthesized from bromotoluene-d₃ in a good yield⁵⁾. Thus, this paper deals with the syntheses of I-d₄, II-d₄, and III-d₄ from IV or V.

2. Results and Discussion

The synthetic scheme of three deuterated phenylpropionic acid derivatives from IV or V is shown in Fig. 1. The reduction of IV with sodium borohydride in methanol gave the alcohol (I-d₄) in a 93% yield after purification by recrystallization from ethanol. Syntheses of II-d₄ and III-d₄ were carried out using V as the starting material. The acetal (V) was reacted with magnesium turnings in absolute tetrahydrofuran to yield the Grignard's reagent, which

Fig. 1 Syntheses of 2-(4-(2-thienylhydroxymethyl)phenyl- d_4)propionic acid (I- d_4), 2-(4-carboxyphenyl- d_4)propionic acid (II- d_4) and 2-(4-(5-hydroxy-2-thienylcarbonyl)phenyl- d_4)propionic acid (III- d_4).

was then treated with solid carbon dioxide or the imidazolidine to obtain the corresponding adduct (VI or VII). Finally, II- d_4 was obtained by deacetalization, and then oxidation with Jones' reagent. On the other hand, III- d_4 was synthesized by deacetalization, followed by oxidation and then debutylation in the presence of catalytic amount of *p*-toluenesulfonic acid. Consequently, the overall yield of the four-step synthesis of II- d_4 from V amounted 43% after purification by recrystallization from ethyl acetate, while that of the five-step synthesis of III- d_4 from V amounted 12% after purification by thin-layer chromatography (TLC). The melting points, elemental analyses, and infrared (IR), mass (MS), and nuclear magnetic resonance (NMR) spectra of the products similarly synthesized from unlabeled suprofen and 2-((4-bro-

mophenyl)1,1-ethyleneglycol)propane as shown in Fig. 1 were consistent with those of authentic samples^{4),9}.

The properties of the synthesized compounds are summarized in Table 1. The IR spectra (KBr) of three deuterated products were greatly changed in the regions between 640 and 865 cm^{-1} when compared with their nonlabeled compounds; the spectrum of I- d_4 is characteristic in the regions at 640, 805, 820 and 845 cm^{-1} , that of II- d_4 in the regions at 675, 815 and 830 cm^{-1} , and that of III- d_4 in the region at 855 cm^{-1} . MS fragmentation of I- d_4 , II- d_4 , and III- d_4 was similar to that of their nonlabeled compounds; the prominent ions in the MS spectra of I- d_4 were present at m/z 181 ($\text{OCC}_6\text{D}_4\text{CH}(\text{CH}_3)\text{COOH}^+$) (base peak), 266 (M^+), and 111 ($\text{C}_4\text{H}_8\text{SCO}^+$), those of II- d_4 at m/z 153 ($\text{M}-\text{COOH}^+$) (base

Table 1 Physical properties of phenylpropionic acids and (phenyl- d_4) propionic acids

Compound	IR $\nu_{\text{max}}^{\text{KBr}}$ (cm ⁻¹)	NMR (DMSO- d_6)*	MS m/z (M ⁺)	Elemental analysis (calculated)		mp(°C)
				C	H	
I	3380(OH) 1690(CO)	1.40(3H, d, $J=7.5\text{Hz}$, CH_3), 3.77(1H, q, $J=7.5\text{Hz}$, CH), 6.02(1H, s, ph-CH), 6.8~ 7.8(7H, m, th and ph)	262	64.22 (64.11)	5.54 (5.38)	111
I- d_4	3380(OH) 1695(CO)	1.44(3H, d, $J=7.5\text{Hz}$, CH_3), 3.75(1H, q, $J=7.5\text{Hz}$, CH), 6.00(1H, s, ph-CH), 6.21 (1H, br, s, OH), 6.8~7.4 (3H, m, th)	266	63.43 (63.15)	6.67 (6.79)	110~112
II	1690(CO)	1.30(3H, d, $J=7.5\text{Hz}$, CH_3), 3.85(1H, q, $J=7.5\text{Hz}$, CH), 7.1~7.9(4H, m, ph), 9.5 (1H, br, OH)	194	61.72 (61.85)	5.30 (5.19)	221~222
II- d_4	1695(CO)	1.26(3H, d, $J=7.5\text{Hz}$, CH_3), 3.88(1H, q, $J=7.5\text{Hz}$, CH)	198	60.45 (60.59)	7.40 (7.10)	221~222
III	3350(OH) 1725(CO)	1.48(3H, d, $J=7.5\text{Hz}$, CH_3), 3.84(1H, q, $J=7.5\text{Hz}$, CH), 6.02(1H, d, $J=4.1\text{Hz}$, th), 7.07(1H, d, $J=4.1\text{Hz}$, th), 7.10~7.65(4H, m, ph)	276	61.15 (60.87)	4.30 (4.38)	85
III- d_4	3350(OH) 1720(CO)	1.44(3H, d, $J=7.5\text{Hz}$, CH_3), 3.85(1H, q, $J=7.5\text{Hz}$, CH), 6.03(1H, d, $J=4.1\text{Hz}$, th), 7.09(1H, d, $J=4.1\text{Hz}$, th)	280	60.35 (60.00)	6.19 (5.73)	85

I : 2-(4-(2-Thienylhydroxymethyl)phenyl)propionic acid

II : 2-(4-Carboxylphenyl)propionic acid

III : 2-(4-(5-Hydroxy-2-thienylcarbonyl)phenyl)propionic acid

* s: singlet, d: doublet, q: quartet, br: broad, m: multiplet, th: thienyl, ph: phenyl

peak) and 198 (M⁺), and those of III- d_4 at m/z 181 ($\text{OCC}_6\text{D}_4\text{CH}(\text{CH}_3)\text{COOH}^+$) (base peak) and 280 (M⁺).

Figures 2~4 show the NMR spectra of I, II, III, and their deuterated compounds in dimethylsulfoxide- d_6 (DMSO- d_6) with their partial MS spectra. The assignment of the signals in their NMR spectra is summarized in Table 1. As the signals due to aromatic protons of the benzene ring completely disappeared in the NMR spectra of I- d_4 , II- d_4 , and III- d_4 , it was clarified that deuterium atom was not eliminated during synthetic steps of the phenyl- d_4 compounds which involved the reduction with NaBH₄ or the oxidation reaction with Jones' reagent and deacetalization with hydrochloric acid. Thus, the deute-

rium content of I- d_4 and II- d_4 calculated from the molecular ions of MS spectra (Figs. 2 and 3) was 99 atom% D, which was equal to that of the starting materials. On the other hand, the deuterium content of III- d_4 was calculated from the fragment ion at m/z 181 (base peak). As shown in Fig. 4, the relative intensity of the ions m/z 180 to m/z 181 was 6.8%, indicating 98.4% D; which was almost equal to that of starting material. Since deuterium atom was not eliminated during synthetic steps of III and II- d_4 (99 atom% D each) at all, the slight elimination of deuterium in III- d_4 was due to the debutylation of III by heating in the presence of *p*-toluenesulfonic acid.

Consequently, it was concluded that the use

Fig. 2 NMR (DMSO-*d*₆) and partial MS spectra of I (upper) and I-*d*₄ (lower).

Fig. 3 NMR (DMSO-*d*₆) and partial MS spectra of II (upper) and II-*d*₄ (lower).

of highly deuterated suprofen⁵⁾ and its metabolites (I, II, and III) as internal standards in the mass fragmentography permits detailed studies on the *in vivo* metabolism of suprofen in man and animals.

3. Experimental

All melting points are uncorrected. IR spectra were recorded with a Hitachi 285 spectrophotometer, MS spectra with a Shimadzu LKB-9000 B at 70 eV, and NMR spectra in CDCl₃ or DMSO-*d*₆ with a JEOL-MH-100 spectrometer using tetramethylsilane as an internal standard.

Fig. 4 NMR (DMSO-*d*₆) and partial MS spectra of III (upper) and III-*d*₄ (lower).

3.1 Chemicals

2-(4-(2-Thienylcarbonyl) phenyl-*d*₄) propionic acid (IV), 2-((4-bromophenyl-*d*₄)1,1-ethyleneglycol)propane (V), and 2-*tert*-butoxythiophene-5-carbonylimidazole were synthesized from bromobenzene-*d*₅ or *N,N*-carbonyldiimidazole according to the methods described in our previous reports.^{5),6)} *N,N*-Carbonyldiimidazole was purchased from Tokyo Kasei Co., Tokyo and NaBH₄, CrO₃, C₂H₅Br, magnesium turnings, and *p*-toluenesulfonic acid from Wako Pure Chemicals Ltd., Osaka. Tetrahydrofuran (THF) was dried over LiAlH₄ and distilled from LiAlH₄ before use. All other reagents and solvents used were of reagent grade.

3.2 2-(4-(2-Thienylhydroxymethyl)phenyl-*d*₄)propionic acid (I-*d*₄)

To 264 mg of IV dissolved in 20 ml of methanol and cooled to 5°C with an ice bath was slowly added 90 mg of NaBH₄ while maintaining the temperature between 0 and 5°C. After the addition was completed, stirring was continued for 1 h at room temperature. The mixture was poured into water and made acidic with 5% hydrochloric acid, which was then extracted with ethyl acetate, washed with water, and dried over Na₂SO₄. The extract was evaporated under reduced pressure to give a solid. Recrystallization from ethanol gave 247 mg of colorless needles (I-*d*₄).

3.3 2-((4-Carboxyphenyl-*d*₄)1,1-ethyleneglycol)propane (VI)

To a mixture of V (6.6 g), magnesium turnings (1.3 g), and 20 ml of THF was slowly added 0.5 ml of C₂H₅Br while maintaining the temperature at 50°C, and the mixture was refluxed for 30 min. The resulting solution was allowed to come to room temperature and then poured on a slurry of an excess of crushed solid carbon dioxide and 50 ml of ether. When no solid carbon dioxide remained, an excess of 5% hydrochloric acid was added to the mixture. The aqueous layer was extracted with ether, and the ether layer was re-extracted with 10% NaOH solution in order to remove the unreacted V; which was then made acidic and extracted with CHCl₃. The CHCl₃ extract was washed with water, dried over Na₂SO₄, and evaporated to yield a crystalline solid. Recrystallization from ethanol-H₂O (2:3, v/v) gave 3.6 g of colorless needles (VI, mp 95~96°C). IR_{max}^{KBr} cm⁻¹: 1685 (CO). NMR (CDCl₃)δ: 1.34 (3H, d, *J*=7.5 Hz, CH₃), 3.06 (1H, m, ph-CH), 3.84 (4H, s, OCH₂-CH₂O), 4.97 (1H, d, *J*=4 Hz, ph-CHCH). MS *m/z*: 226 (M⁺).

3.4 2-(4-Carboxyphenyl-*d*₄)propionic acid (II-*d*₄)

A mixture of VI (3.3 g), 2% hydrochloric acid (30 ml), and acetone (40 ml) was heated at 60~70°C for 2 h. The reaction mixture was then poured into water and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and evaporated to yield 2.6 g of an oil. To this crude oil dissolved in 20 ml of acetone and cooled to 5°C with an ice bath was slowly added 3.5 ml of Jones' reagent (950 mg of CrO₃ dissolved in 3.0 ml of 25% H₂SO₄). After the complete addition, the mixture was stirred for 1 h, then poured into water and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and evaporated to yield a crystalline solid. Recrystallization from ethyl acetate gave 2.15 g of colorless needles (II-*d*₄).

3.5 2-((4-(5-*tert*-Butoxy-2-thienylcarbonyl)-phenyl-*d*₄)1,1-ethyleneglycol)propane (VII)

The Grignard's reagent, which was prepared

from 1.35 g of V and 300 mg of magnesium turnings, was slowly added to a stirred solution of 1.2 g of the imidazolidine[®] dissolved in 30 ml of THF[™]. The mixture was stirred at room temperature for 3 h, which was then poured into 20 ml of 2% hydrochloric acid and extracted with ether. The extract was washed successively with water, NaHCO₃ solution, and water, dried over Na₂SO₄, and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel (Wako gel C-200) column with the elution medium of benzene-ethyl acetate=10:1 (v/v). Evaporation of the eluate gave 903 mg of VII (50%) as a solid (mp 73~75°C). IR_{max}^{KBr} cm⁻¹: 1635 (CO). NMR-(CDCl₃)δ: 1.37 (9H, s, OC(CH₃)₃), 1.38 (3H, d, *J*=8 Hz, CH₃), 3.06 (1H, m, ph-CH), 3.80 (4H, s, OCH₂CH₂O), 4.96 (1H, d, *J*=5 Hz, ph-CHCH), 6.34 (1H, d, *J*=3.5 Hz, th), 7.33 (1H, d, *J*=3.5 Hz, th). MS *m/z*: 364 (M⁺).

3.6 2-(4-(5-*tert*-Butoxy-2-thienylcarbonyl)-phenyl-*d*₄)propionic acid (VIII)

A mixture of VII (850 mg), 2% hydrochloric acid (10 ml) and acetone (15 ml) was heated at 60°C for 1.5 h, which was then poured into water and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and evaporated to give 685 mg of an oil. To this crude oil dissolved in 15 ml of acetone and cooled to 5°C with an ice bath was slowly added 1.5 ml of Jones' reagent (405 mg of CrO₃ dissolved in 1.5 ml of 25% H₂SO₄). After the complete addition, stirring was continued for 1 h at below 5°C. The solution was then extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and evaporated to yield a crude oil, which was then chromatographed over a silica gel column with the elution medium of CHCl₃-acetone=6:1 (v/v). Evaporation of the eluate gave 354 mg of VIII (45%) as a colorless oil. IR_{max}^{Nujol} cm⁻¹: 1715 (CO). NMR (CDCl₃)δ: 1.38 (9H, s, OC(CH₃)₃), 1.45 (3H, d, *J*=7.5 Hz, CH₃), 3.77 (1H, q, *J*=7.5 Hz, CH), 6.34 (1H, d, *J*=4 Hz, th), 7.33 (1H, d, *J*=4 Hz, th). MS *m/z*: 336 (M⁺), 99 atom % D.

3·7 2-(4-(5-Hydroxy-2-thienylcarbonyl)-phenyl- d_4)propionic acid (III- d_4)

The oil (310 mg of VII) was mixed with a crystalline *p*-toluenesulfonic acid (5 mg), and the mixture was heated at 120°C to evolve isobutylene at once. After gas evolution was ceased (5 min), the mixture was diluted with water and then extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 , and evaporated to give a crude oil (242 mg). The crude oil was chromatographed on a Silica gel 60 F₂₅₄ (2 mm thickness) pre-coated plate (E. Merck, Darmstadt, Germany) with CHCl_3 -acetone-acetic acid=20:3:1 (v/v/v) to give 138 mg of III- d_4 (54%) as a pale yellow solid (amorphous).

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要 旨

フェニルプロピオン酸誘導体の重水素標識化合物の合成

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抗炎症剤2-(4-(2-チエニルカルボニル)フェニル)プロピオン酸(スプロフェン, IV)のヒトおよび動物における代謝をマスフラグメントグラフ法で検討するときの内部標準物質として, 2-(4-(2-チエニルヒドロキシメチル)フェニル)プロピオン酸(I), 2-(4-カルボキシフェニル)プロピオン酸(II)および2-(4-(5-ヒドロキシ-2-チエニルカルボニル)フェニル)プロピオン酸(III)の重水素多重標識体を合成した。I- d_4 は, IV- d_4 を水素化ホウ素ナトリウムで還元することにより収率93%で得られ, その重水素含量は99 atom%であった。一方, II- d_4 (99 atom% D)は2-((4-ブロムフェニル- d_4)1,1-エチレンジグリコール)プロパン(V)から4ステップの反応を経て総収率43%で, またIII- d_4 (98.4 atom% D)はVから5ステップの反応を経て総収率12%で得られた。