Synthesis of Methyl 1-O-(4-Hydroxymethamphetamineyl)-α-D-glucopyranouronate

Rika NAKAJIMA,*a Machiko ONO,b Sadakazu AISOb and Hiroyuki AKITAb

a School of Medicine, Keio University; 33 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan; and b School of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.

Received December 24, 2004; accepted March 1, 2005

For the purpose of the direct characterization of the intact conjugated form in the urine of a methamphetamine (MA) abuser, 4-hydroxymethamphetamine (4-OHMA) glucuronate, corresponding to one of the metabolites of MA, was synthesized from the commercially available methyl 4-hydroxyphenylacetate.

Key words 4-hydroxymethamphetamine; glucuronate; synthesis

Drug abuse has become a serious problem and is increasingly widespread in the world. In Japan, methamphetamine (MA, 1) is the most frequently abused drug. Nowadays, arrests for violation of the Stimulant Control Law are on the verge of 20,000. A MA addict is identified by the detection of unchanged MA (1) and its metabolite amphetamine (AP, 2), then MA (1) analysis in urine samples is carried out routinely by a standard screening test and thin layer chromatography (TLC) method, followed by gas chromatography/mass spectrometry (GC/MS). Recently, the drug abuse situation has been internationalized. It is possible that AP (2) and/or a variety of analogues are imported from overseas. MA (1) is metabolized by way of two pathways, either by hydroxylation of the aromatic ring or demethylation of the side chain. It has been reported that the metabolites of MA (1) in urine were composed of the unchanged drug (18–27%), the free 3 and the conjugated forms (14–16%) of 4-hydroxymethamphetamine (4-OHMA, 3) and AP (2) (2–3%).1 It has been reported that analysis of a conjugated form, such as glucuronide 4 of 4-OHMA (3), in the urine samples of MA (1) abusers were carried out by comparison of the ratios of between free 4-OHMA (3) obtained by β-glucuronidase or HCl treatment, and the total 4-OHMA (3).2,3 Direct characterization of the intact conjugated form of 3 has not been reported so far.

In the present paper, we describe the synthesis of 4-OHMA glucuronide 4 for the direct characterization of an intact conjugated form in the metabolism of MA (1) abusers without hydrolysis. Moreover, 4-OHMA (3) as a metabolite of MA (1) is on the market, but it is an expensive product. Buzas et al. have synthesized 4-OHMA (3) from a controlled substance by Stimulants Raw Material in Japan,4 therefore, we now report the synthesis of glucuronate (5) of 4-OHMA (3) corresponding the above-mentioned glucuronide (4) by unregulated materials.

The synthesis of glucuronide congener (5) is shown in Charts 2–4.

Synthesis of 4-Hydroxy-N-benzylmethamphetamine (±)-13 Silylation of the commercially available methyl 4-hydroxyphenylacetate (6) gave the corresponding tert-butyl-dimethylsilyl (TBDS)-ether 7 in 95% yield, which was reduced with LiAlH4 to afford the primary alcohol 8 in 99% yield. Pyridinium chlorochromate (PCC) oxidation of 8 yielded an aldehyde 9, which was used for the next reaction without further purification. The aldehyde 9 was treated with methylthionium (MeLi) gave the secondary alcohol (±)-10 in 42% from 8. Swern’s oxidation of (±)-10 afforded a ketone 11 in 83% yield. The reaction of 11 and N-benzyl N-methylamine in the presence of 1.8m HCl/MeOH, followed by reduction with cyanoborohydride (NaBH3CN), gave the tertiary amine (±)-12 in 51% yield. Deprotection of the silyl group of (±)-12 with 1 m H2SO4 provided the desired 4-hydroxy-N-benzylmethamphetamine (±)-13 in 83% yield.

Synthesis of Glucuronide Imidate 18 Treatment of the commercially available n-(+)-glucuronolactone (14) with MeOH in the presence of triethylamine (Et3N), followed by subjectation to acetylation, gave both tetraacetyl-α-glucuronate 15 (25%) and β-glucuronate 16 (38%). By applying the reported procedure,5 α-glucuronate 15 and β-glucuronate 16 were independently treated with tributyltin methoxide (Bu3SnOMe) to provide the triacetyl-α-glucuronate 17 (60%), and (17) (99%), respectively. By applying the reported procedure,6 the reaction of 17 with K2CO3 in the presence of molecular sieves (MS, 3 Å), followed by

* To whom correspondence should be addressed. e-mail: nakaji@sc.itc.keio.ac.jp

© 2005 Pharmaceutical Society of Japan
treatment with trichloroacetonitrile (CCl₃CN), provided the desired α-imidate 18 in 75%.

Coupling Reaction between 4-Hydroxy-N-benzylmethamphetamine (±)-13 and α-Imidate 18 The reaction of phenol 13 and α-imidate 18 in the presence of BF₃·Et₂O afforded the coupled product 19 as a diastereomeric mixture in 97% yield. Deprotection of the acetyl group of 19 with 1 M NaOH gave the deacetylated compound along with the partial hydrolysis of a methyl ester group, which was used for the next reaction without further purification. This mixture was subjected hydrogenolysis in the presence of 10% Pd(OH)₂–C to provide a debenzylated mixture which was treated with MeOH in the presence of 1.8 M HCl/MeOH for the purpose of the esterification of the partially generated carboxylic acid to give the desired compound 5 in 57% yield.

In conclusion, for the purpose of the direct characterization of the intact conjugated form (glucuronide congener) in the urine samples of a methamphetamine (MA) abuser, the diastereomeric mixture of glucuronates (5) under an alkaline condition should afford the corresponding glucuronide (4), which should be identical with the intact conjugated form. Methyl 4-hydroxyphenylacetate (6) was converted to 4-hydroxy-N-benzylmethamphetamine ((±)-13) in 7 steps. Coupling reaction of (±)-13 and methyl 2,3,4-triacetyl-1-O-(trichloroacetimidoyl)-α-D-glucopyranouronate (18) derived from D(+)glucurono-6,3-lactone (14) in the presence of BF₃·Et₂O afforded the glucuronide congener 19, which was subjected to deprotection to give the methamphetamine glucuronate (5).

Experimental

1H-NMR spectra were recorded by a JEOL AL 400 spectrometer (Tokyo, Japan). Spectra were taken with 5—10% (w/v) solution in CDCl₃ with Me₄Si as an internal reference. The fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS-600H (matrix; dithiothreitol : α-thioglycerol 1: 1 mixture) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrophotometer. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

Methyl 4-tert-Butyldimethylsiloxyphenylacetone (7) A mixture of methyl 4-hydroxyphenylacetate (6; 10.00 g, 60 mmol), imidazole (12.25 g, 180 mmol) and tert-butyldimethylsilyl chloride (TBDMSCl; 13.57 g, 90 mmol) in N,N-dimethylformamide (DMF; 60 ml) was stirred for 30 min at 0 °C. The reaction mixture was diluted with H₂O and extracted with Et₂O. The organic layer was washed with brine and dried over MgSO₄. Evaporation of the organic solvent gave a crude product, which was chromatographed on silica gel (400 g, hexane/AcOEt=20:1) to afford 7 (15.99 g, 95%) as a colorless oil.

IR (neat) cm⁻¹: 2950, 1741, 1511. δ¹H-NMR δ: 0.17 (6H, s), 0.96 (9H, s), 3.53 (2H, s), 3.67 (3H, s), 6.76 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz). Anal. Calc'd for C₁₅H₂₄SiO₃: C, 64.24; H, 8.63. Found: C, 63.96; H, 8.58.

2-(4-tert-Butyldimethylsiloxyphenyl) Ethanol (8) To a stirred suspension of lithium aluminium hydride (LiAlH₄; 1.40 g, 37 mmol) in Et₂O (60 ml) at 0 °C was added dropwise a solution of 7 (10.38 g, 37 mmol) in

Chart 2

Chart 3

Chart 4
Et2O (100 ml), and the reaction mixture was stirred for 1.75 h at room temperature. The reaction mixture was treated with acetonitrile (6 ml) and H2O, and extracted with EtO. The organic layer was washed with brine and dried over MgSO4. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (195 g, hexane/AcOEt = 5:1) to give 8 (9.26 g, 99%) as a colorless oil.

IR (neat) cm⁻¹: 3393, 2938, 1509. ¹H-NMR δ: 0.18 (6H, s), 0.97 (9H, s), 1.54 (1H, s), 2.74 (2H, t, J = 6.0 Hz), 3.79 (2H, t, J = 6.0 Hz), 6.77 (2H, d, J = 8.0 Hz), 7.06 (2H, d, J = 8.0 Hz). Anal. Caled for C11H15NO: C, 78.54; H, 8.17. Found: C, 78.56; H, 8.14.

1-(4-Hydroxyphenyl)-2-n-benzyl-3-n-methylaminophenol ((±)-13) A solution of 12 (0.60 g, 1.6 mmol) and 1 ml of Ac2O (solution 4 ml) in MeOH (8 ml) was stirred for 3.5 h at room temperature, then the reaction mixture was treated with NaHCO3 (0.21 g). The reaction mixture was diluted with H2O and extracted with EtO. The organic layer was washed over MgSO4 and evaporated in vacuo to provide a residue, which was chromatographed on silica gel (30 g, benzene/AcOEt = 20:1) to give 13 (0.34 g, 83%) as a colorless oil.

IR (KBr) cm⁻¹: 3404, 1608, 1509, 1243. ¹H-NMR δ: 0.98 (3H, s), 2.23 (3H, s), 2.40 (1H, dd, J = 15.0, 10.0 Hz), 2.91 (1H, qdd, J = 6.0, 10.0, 6.0 Hz), 2.94 (1H, dd, J = 15.0, 6.0 Hz), 3.59 (1H, d, J = 14.0 Hz), 3.62 (1H, d, J = 14.0 Hz), 6.71 (2H, d, J = 8.0 Hz), 6.98 (2H, d, J = 8.0 Hz), 7.18—7.31 (5H, m). Anal. Caled for C13H12NO: C, 79.76; H, 8.29; N, 5.49. Found: C, 79.56; H, 8.38; N, 5.46. FAB-MS (m/z): [M+H⁺]⁺ 256.

Methyl 1,3,4-Tetraacetyl-α-D-glucopyranuronate (15, 16) A solution of glucuronolactone (14. 5.0 g, 45 mmol) and MeOH (60 ml) was stirred for 2 h at room temperature, and then MeOH was removed in vacuo. The resulting syrup was dissolved in pyridine (20 ml) and acetic anhydride (30 ml). The mixture was allowed to stand in the refrigerator for 5 d, then concentrated in vacuo. EtOAc was added to the resulting residue, and the less soluble product was filtered to give a solid (β-isomer 16). The mother liquor was evaporated to give a crude oil (13.82 g), which was chromatographed in silicagel (50 g, hexane/AcOEt = 3:1) to yield 16 (6.43 g, 38%). The NMR data of 16 were identical with those of the reported 15.8 The crude oil from the mother liquor was again chromatographed on silicagel (160 g, hexane/AcOEt = 4:1) to provide 15 (4.30 g, 25% as α-isomer).

15: ¹H-NMR δ: 1.68 (3H, s), 2.00 (3H, s), 2.00 (3H, s), 2.14 (3H, s), 3.30 (3H, s), 4.57 (1H, d, J = 10.0 Hz), 5.07 (1H, dd, J = 10.0, 4.0 Hz), 5.18 (1H, dd, J = 10.0, 10.0 Hz), 5.47 (1H, dd, J = 10.0, 10.0 Hz), 6.35 (1H, d, J = 10.0 Hz), 7.17—7.30 (5H, m). The NMR data of 16 were identical with those of the reported 15.8

Methyl 2,3,4-Triacetyl-α-D-glucopyranuronate (17) A mixture of 15 (2.0 g, 5.3 mmol) and tributyltin methoxide (Bu₃SnOMe; 1.57 ml, 5.3 mmol) in CH₂Cl₂/CH₃CH₂CH₃ (30 ml) was stirred for 5 h at 90 °C, and the whole mixture was evaporated to give a crude residue. It was chromatographed on silicagel (70 g, hexane/AcOEt = 3:1) to give 17 (1.06 g, 60%) as a colorless oil. The NMR data of 17 were identical with those of the reported 17.8

A mixture of 16 (1.0 g, 2.7 mmol) and Bu₃SnOMe (0.8 ml, 2.7 mmol) in CH₂Cl₂/CH₃CH₂CH₃ (15 ml) was stirred for 2 h at 90 °C. The reaction mixture was worked up in the same way as for 15 to give 17 (0.90 g, 99%) as a colorless oil. The NMR data of 17 were identical with those of the reported 17.8

Methyl 2,3,4-Triacetyl-α-D-glucopyranuronate (17) A mixture of 17 (1.50 g, 4 mmol), K₂CO₃ (0.94 g, 6.8 mmol) and molecular sieves (MS 3 Å, 0.5 g) in CH₂Cl₂ (5 ml) under an argon atmosphere was stirred for 25 h at 0 °C. A solution of trichloroacetone (Cl₃CCN; 1.73 g, 12 mmol) in CH₂Cl₂ (5 ml) was added to the above mentioned reaction mixture, and the whole mixture was stirred for 2 h at the same temperature. The reaction mixture, including a solid, was filtered and the filtrate was treated with 7% aqueous NaHCO₃, then concentrated in vacuo. The resulting residue was chromatosgraphed on silicagel (50 g, hexane/AcOEt = 5:1) to give 18 (1.44 g, 75%) as a solid. The NMR data of 18 were identical with those of the reported 18.

Horninhydroxamic acid (13) and 18 A mixture of 13 (0.1214 g, 0.048 mmol), 18 (0.45 g, 0.094 mmol) and MS 3 Å (0.2 g) was stirred under reduced pressure, and CH₂Cl₂ (10 ml) was added to the above mentioned mixture. The whole mixture was stirred for 10 min at room temperature under an argon atmosphere. Boron trifluoride ether complex (BF₃·Et₂O; 0.06 ml, 0.42 mmol) was added to the above mentioned whole mixture at 0 °C and the mixture reaction was stirred for 3 h at room temperature. The reaction mixture was filtered and the filtrate was washed with 7% aqueous NaHCO₃. The organic layer was washed over MgSO4 and evaporated to give a crude residue, which was chromatographed on silicagel (20 g, benzene/AcOEt = 5:1) to provide 19 (0.225 g, 97%) as a colorless oil.

IR (KBr) cm⁻¹: 1755, 1375. ¹H-NMR δ: 0.96 (3H, s, J = 6.0 Hz), 2.22 (3H, s), 2.43 (1H, dd, J = 16.0, 12.0 Hz), 2.92 (1H, dd, J = 16.0, 6.0 Hz), 2.94 (1H, qdd, J = 12.0, 6.0, 6.0 Hz), 3.56 (1H, d, J = 14.0 Hz), 3.71 (3H, s), 4.15 (1H, d, J = 10.0 Hz), 5.09 (1H, d, J = 8.0 Hz), 5.22—5.28 (1H, m), 5.29—5.35 (2H, m), 6.88 (2H, d, J = 8.0 Hz), 7.05 (2H, d, J = 8.0 Hz), 7.17—7.30 (5H, m). Anal. Caled for C₂₂H₁₉NO₂: C, 63.04; H, 5.62; N, 2.45. Found: C, 63.26; H, 6.71; N, 2.19. FAB-MS (m/z): [M+H⁺]⁺ 572.

Methyl 1-O-(4-Hydroxyphenethylamino)-α-D-glucopyranuronate (5) A solution of 19 (0.1875 g, 0.3 mmol) and 1 ml NaOH solution (2.4 ml) in MeOH (3 ml) was stirred for 1.5 h at 0 °C, and the reaction mixture was acidified with 1 ml HCl (3 ml). The whole mixture was stirred for 20 min at 0 °C and evaporated in vacuo. The resulting residue was dissolved in MeOH and the precipitate was filtered. The filtrate was concentrated in vacuo to provide a residue which was dissolved in MeOH (2 ml). The MeOH solution was subjected to hydrogenolysis in the presence of 10% palladium hydroxide on carbon (Pd(OH)₂·C; 0.1 g) for 12 h at ambient temperature. The reac-
tion mixture was filtered and the filtrate was evaporated to provide a residue. The residue was dissolved in MeOH (1 ml) in the presence of MS 3 Å (0.15 g). 1.8 M HCl (gas)/MeOH solution (2 ml) and the whole mixture was stirred for 1 h at room temperature. The reaction mixture was filtered and the filtrate was treated with 7% aqueous NaHCO₃ (1 ml) and evaporated in vacuo. The resulting residue was chromatographed on silica gel (10 g, CHCl₃/MeOH/H₂O 5: 5: 1) to give 5 (0.061 g, 57%) as a colorless oil.

IR (KBr) cm⁻¹: 3395, 1735, 1629, 1516, 1233. ¹H-NMR (CD₃OD, 60 °C, 400 MHz) δ: 1.24 (3H, d, J=7.0 Hz), 2.70 (3H, s), 2.75 (1H, dd, J=9.0, 14.0 Hz), 3.09 (1H, dd, J=6.0, 14.0 Hz), 3.42 (1H, qdd, J=7.0, 9.0, 6.0 Hz), 3.47—3.52 (2H, m), 3.64 (1H, dd, J=10.0, 10.0 Hz), 3.75 (3H, s), 4.00 (1H, d, J=10.0 Hz), 4.96 (1H, d, J=8.0 Hz), 7.05 (2H, d, J=9.0 Hz), 7.20 (2H, d, J=9.0 Hz). ¹³C-NMR (CD₃OD, 45 °C, 125 MHz) δ: 15.85 (q), 31.05 (q), 39.53 (t), 52.93 (q), 57.99 (d), 73.01 (d), 74.68 (d), 76.83 (d), 77.31 (d), 102.56 (d), 118.50 (d), 131.29 (s), 131.57 (d), 132.05 (s), 170.95 (s). FAB-HR-MS (m/z) C₁7H₂₆NO₇: Calcd 356.1709. Found. 356.1706.

Acknowledgments The authors thank Dr. Tatsuya Murai for his kind help in the performance of this work.

References