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First synthesis and full characterization of mexiletine *N*-carbonyloxy β-D-glucuronide

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ABSTRACT

A simple, convergent synthesis of the *N*-carbonyloxy β-D-glucuronide of mexiletine (sodium salt) in moderate yield is described. The compound is now available as an authentic reference standard for analytical studies, enabling more detailed investigation on the metabolism of mexiletine.

1. Introduction

Mexiletine, 1-(2,6-dimethylphenoxy)propan-2-amine (Fig. 1), is an orally effective class IB antiarrhythmic agent.[1,2] It has also been shown to be effective in the treatment of myotonic syndromes[3–6] and for neuropathic pain resulting from both cancer[7] and diabetes mellitus.[8,9] Its analgesic efficacy for neuropathic pain has been recently confirmed in a meta-analysis.[10] In human, mexiletine undergoes extensive liver metabolism (<10% of an administered oral dose is recovered unchanged in urine) by numerous oxidative pathways (phase I metabolism) involving aliphatic and aromatic hydroxylation, dealkylation, deamination, and N-oxidation.[11–13] The major oxidation metabolites, namely *p*-hydroxymexiletine (PHM), hydroxymethylmexiletine (HMM), *m*-hydroxymexiletine (MHM), and *N*-hydroxymexiletine (NHM) are shown in Figure 1.

Phase I metabolites, as well as mexiletine, undergo a further glucuronidation reaction (phase II metabolism) which plays a pivotal role in the elimination of the drug from the body. In fact, approximatively 30% of the dose is excreted as glucuronide metabolites.[14,15] However, there is some confusion about the chemical structure of the main glucuronic acid conjugate. For a long time, *N*-hydroxymexiletine glucuronide has been indicated as the major conjugated metabolite of mexiletine, representing one of the major metabolic pathway in the disposition of the drug.[16–18]

Recently, Senda et al. reported a novel chemical structure of the major mexiletine glucuronide in human urine, sowing a seed of doubt on the expected in vivo formation of *N*-hydroxymexiletine conjugate.[19] By using LC/MS/MS and HPLC analyses, they established that the glucuronide contained a carbonyloxy moiety in its structure, besides mexiletine and glucuronic acid moieties. Their results indicated that the structure of the isolated metabolite was the β-D-glucuronic acid conjugate of *N*-carbonyloxymexiletine (**1**, Scheme 1) which appears to be one of the major metabolites of mexiletine in human urine. In fact, its estimated urinary recovery corresponded to about 80% of total mexiletine glucuronide and over 25% of the dose of the drug.[19] On the other hand, the formation of such carbamyl *O*-β-D-glucuronides from drugs containing an amino group has already been described before.[20–23] Since authentic reference standard for mexiletine carbonyloxy β-D-glucuronide was unavailable,[19] a chemical synthesis was indeed required to provide a reliable analytical standard useful for unambiguous identification of the main metabolic product of mexiletine conjugation. In particular, being mexiletine glucuronidation enantioselective, favoring the *R* isomer,[14,17,18,24,25] and since the *R* isomer of mexiletine performs as the eutomer,[26–30] we focused our efforts on the synthesis of carbonyloxy β-D-glucuronide of (*R*)-mexiletine (**1**). The voltage-gated sodium channels' blocking activity of the newly synthesized compound (sodium salt, **1**·**Na**, Scheme 1) has already been evaluated[31] as a part of a program aimed at the study of mexiletine metabolite effects on skeletal muscle.[32,33] **1**·**Na** does not retain the parent drug's activity.[31] The synthesis and full characterization of **1**·**Na** are reported herein.

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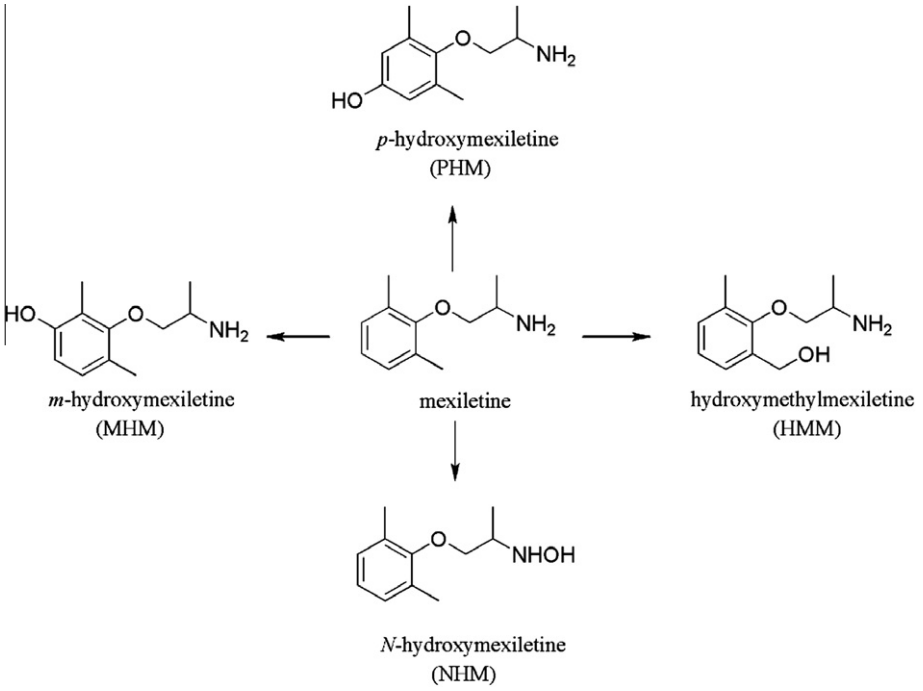
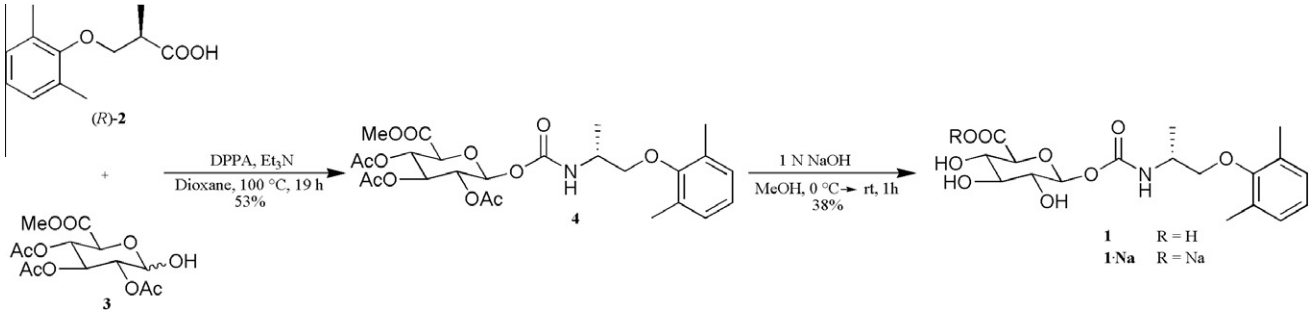


Figure 1. Phase I metabolic pathways of mexiletine in human.



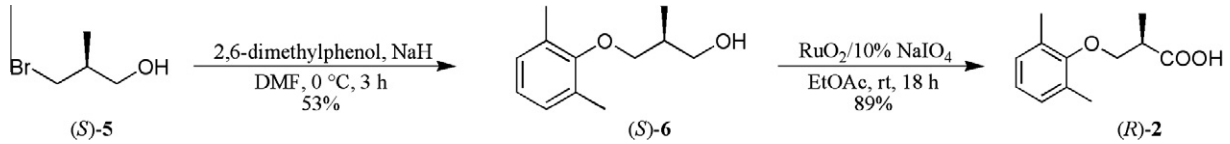
Scheme 1. Convergent synthesis of mexiletine *N*-carbonyloxy β-D-glucuronide as its sodium salt (**1**·**Na**).

2. Results and discussion

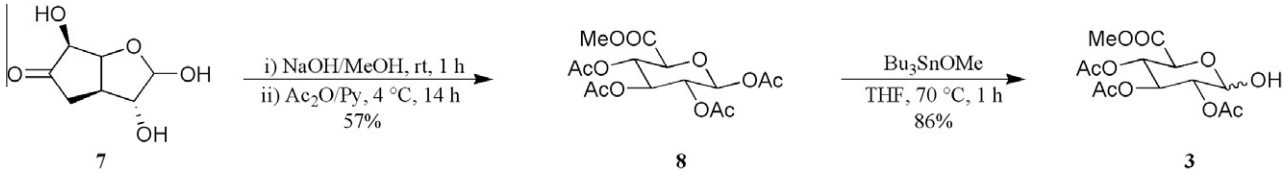
The sodium salt of (*R*)-mexiletine carbonyloxy β-D-glucuronide (**1**·**Na**, Scheme 1) was prepared following a convergent synthetic route based on the coupling between two building blocks, the carboxylic acid (*R*)-**2** and the hemiacetal **3**, as the key step.

(*R*)-**2** was prepared by modifying a procedure used in the literature (Scheme 2).[34] The preparation of **3** started from commercially available D-glucuronolactone (**7**) which was submitted to a ring-opening reaction with NaOH in methanol followed by acylation with acetic anhydride in pyridine at 4 °C to give methyl tetra-*O*-acetyl-D-glucopyranuronate (**8**) (Scheme 3).[35]

Low temperature was necessary to obtain the β isomer of **8** in high percentage. After recrystallization from EtOAc/petroleum ether, **8** was obtained as the pure β anomer. The β-linkage of the glucuronate moiety was assessed on the basis of the trans-diaxial coupling of 8.0 Hz found for H-1 doublet. The β epimer, compared with the α-form, was desirable since it was hydrolyzed faster to hemiacetal **3** in the next step, giving higher yields. The hydrolysis was carried out by treating **8** with an equimolar amount of tributyltin methoxide in anhydrous THF affording methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (**3**) as an anomeric mixture.[36] The prepared synthons (*R*)-**2** and **3** were coupled according to the Curtius rearrangement, in the presence of diphenylphosphoryl azide and Et_3N,[37] giving the corresponding carbamate **4** exclusively as the β-anomer (Scheme 1). The stereochemistry of the glycosidic linkage of **4** was determined on the basis of the value of the coupling constant of the anomeric proton (J_{H1-H2} = 8.0 Hz). Thus,



Scheme 2. Preparation of synthon (*R*)-**2**.



Scheme 3. Preparation of synthon **3**.

β-stereospecificity of the coupling reaction might stem from the well-known higher nucleophilicity of the 1-OH group in the β- than in the α-configuration.[38] Deprotection of the acetyl groups and hydrolysis of the methyl ester of compound **4** were performed using 1 N NaOH in MeOH, followed by an acidic work-up with Amberlyst 15 ion-exchange resin.[39] At odds with what reported for other glucuronides,[39] this work-up gave the desired product **1** as its sodium salt **1**·**Na**. The reaction was stereochemically reliable, giving **1**·**Na** with exclusive β-stereochemistry at C-1, as confirmed by ^{1}H NMR analysis (J_{H1-H2} = 8.0 Hz).

3. Conclusions

In summary, a facile convergent synthesis of (*R*)-mexiletine *N*-carbonyloxy β-D-glucuronide (**1**)—the main phase II mexiletine metabolite—as its sodium salt (**1**·**Na**) has been developed.[40] Ready availability of **1**·**Na** should favor more detailed studies on the metabolism of mexiletine. Investigation on the possible extension of the route described herein to deuterated analogs of **1** has been undertaken.

Acknowledgment

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40. Procedures for key compounds.
Methyl tetra-O-acetyl-β-D-glucopyranuronate (**8**).
Prepared as reported in the literature.[35] Yield: 57%; mp 177–178 °C, lit.[35] 177–178 °C; $[\alpha]_D^{20}$ +8.6 (*c* 1, $CHCl_3$), lit.[35] $[\alpha]_D^{23}$ +7.4 (*c* 2, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 20.7 (1C), 20.8 (2C), 21.0 (1C), 53.2 (1C), 69.1 (1C), 70.3 (1C), 72.0 (1C), 73.2 (1C), 91.5 (1C), 167.0 (1C), 169.1 (1C), 169.4 (1C), 169.7 (1C), 170.1 (1C); MS (70 eV) *m*/*z* (%) 334 (M^+−43, <1), 43 (100). ^{1}H NMR spectrum was in agreement with that reported in the literature.[41]
Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranuronate (**3**).
Prepared as reported in the literature.[36] Yield: 86%; mp 103–105 °C (EtOAc/hexane), lit.[42] 98–100 °C (Et_2O); $[\alpha]_D^{20}$ +86.0 (*c* 2, $CHCl_3$), lit.[42] $[\alpha]_D^{20}$ +81.2 (*c* 0.105, $CHCl_3$); IR (KBr): 3474 (OH), 1753 (C=O) cm^{-1}; MS (70 eV) *m*/*z* (%) 275 (M^+−59, <1), 43 (100). ^{1}H NMR and ^{13}C NMR spectra were in agreement with those reported in the literature.[42]
Methyl (1′R,2S,3R,4R,5R,6R)-3,4,5-tri-O-acetyl-6-[2-(2,6-dimethylphenoxy)-1-methyl ethylaminocarbonyloxy]tetrahydro-2H-pyran-2-carboxylate (**4**).[43]
A solution of (−)-(*R*)-3-(2,6-dimethylphenoxy)-2-methylpropanoic acid [(*R*)-**2**] (0.65 g, 3.10 mmol), methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (1.55 g, 4.65 mmol) (**3**), diphenylphosphoryl azide (DPPA, 1.28 g, 4.65 mmol), and Et_3N (0.95 mL, 6.82 mmol) in 40 mL of dioxane was stirred under reflux for 19 h. After evaporation of the solvent, the residue was taken up with EtOAc, washed with 2 N HCl, and the aqueous phase was washed with EtOAc. The two organic phases were washed, separately, with 2 N NaOH and then combined and dried over anhydrous Na_2SO_4. The solvent was concentrated in vacuo to afford 1.07 g of a brown solid. The crude product was purified by flash chromatography (EtOAc/hexane 4:6) to give 0.89 g (53%) of the desired product as a yellow solid which was recrystallized from EtOAc/hexane (0.49 g, 29%): mp 156–158 °C; $[\alpha]_D^{20}$ +8.4 (*c* 1, $CHCl_3$); IR (KBr): 3343 (NH), 1748, 1726 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, *J* = 6.9 Hz, 3H), 2.02 (s, 6H), 2.04 (s, 3H), 2.23 (s, 6H), 3.65–3.85 (m overlapping s at 3.72, 2H), 3.72 (s overlapping m at 3.65–3.85, 3H), 3.95–4.10 (m, 1H), 4.16 (d, *J* = 9.9 Hz, 1H), 5.15 (t overlapping t at 5.21, *J* = 9.2 Hz, 1H), 5.21 (t overlapping t at 5.15, *J* = 9.5 Hz, 1H), 5.32 (t overlapping d at 5.36, *J* = 9.4 Hz, 1H), 5.36 (d overlapping t at 5.32, *J* = 8.2 Hz, 1H), 5.72 (d, *J* = 8.0 Hz, 1H), 6.85–7.05 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 16.5 (2C), 17.9 (1C), 20.7 (1C), 20.8 (1C), 20.9 (1C), 47.8 (1C), 53.2 (1C), 69.4 (1C), 70.2 (1C), 72.2 (1C), 73.0 (1C), 73.6 (1C), 92.7 (1C), 124.4 (1C), 129.2 (2C), 131.0 (2C), 153.3 (1C), 155.0 (1C), 167.0 (1C), 169.7 (2C), 170.2 (1C); MS (70 eV) *m*/*z* (%) 205 (M^+−333, 56), 122 (100). Anal. Calcd for $(C_{25}H_{33}NO_{12}\cdot 0.5\ H_2O)$: C, 54.74; H, 6.25; N, 2.55. Found: C, 54.45; H, 6.03; N, 2.76.

Sodium (1'R,2S,3R,4R,5R,6R)-3,4,5-trihydroxy-6-[2-(2,6-dimethylphenoxy)-1-methyl ethylaminocarbonyloxy]tetrahydro-2H-pyran-2-carboxylate (**1**·**Na**).[44]

To a cooled and stirred suspension of compound **4** (0.54 g, 1.00 mmol) in methanol (13 mL), 1 N NaOH solution (10.1 mL, 10.1 mmol) was added dropwise. The mixture was stirred in an ice bath for 30 min and then left at room temperature for 30 min. The undissolved material was removed by filtration and the filtrate was added to cold water containing Amberlyst 15 ion-exchange resin so as to reach pH 2. The mixture was filtered and the resin was washed with water/methanol (1:1). The filtrate was concentrated under vacuum and water was azeotropically removed. Crystallization of the residue from MeOH/*i*-PrOH gave 0.15 g (38%) of the title product as a yellow solid: mp 198–200 °C; $[\alpha]_D^{20}$ +0.9 (*c* 1, MeOH); IR (KBr): 3342 (NH, OH), 1720, 1614 (C=O), 1415 (C–O–C) cm^{-1}; ^1H NMR (D_2O) δ 1.11 (d, *J* = 6.9 Hz, 3H), 2.06 (s, 6H), 3.25–3.50 (m, 3H), 3.50–3.95 (m, 4H), 5.27 (d, *J* = 8.0 Hz, 1H), 6.80–7.00 (m, 3H); ^{13}C NMR (CD_3OD) δ 15.3 (2C), 16.7 (1C), 60.4 (1C), 72.2 (1C), 72.7 (1C), 74.1 (1C), 75.4 (1C), 76.7 (1C), 95.4 (1C), 123.9 (1C), 128.7 (2C), 130.7 (2C), 155.4 (1C), 155.6 (1C), 175.1 (1C); LC/MS *m*/*z* 398 [M^-]. Anal. Calcd for ($C_{18}H_{24}NNaO_9 \cdot 1.5\,H_2O$): C, 48.21; H, 6.07; N, 3.12. Found: C, 47.92; H, 5.81; N, 3.11.

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43. Compound **4** may be also referred to as methyl 5-[2-(2,6-dimethylphenoxy)-1-methylethylaminocarbonyloxy]-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate.
44. Compound **1**·**Na** may be also referred to as sodium 5-[2-(2,6-dimethylphenoxy)-1-methylethylaminocarbonyloxy]-β-D-glucopyranuronate.