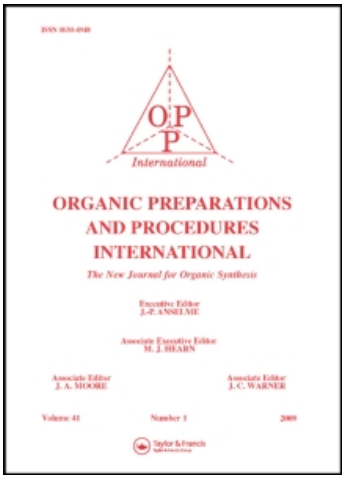

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PREPARATION OF METABOLITES OF IMIPRAMINE AND PHENYTOIN

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PREPARATION OF METABOLITES OF IMIPRAMINE AND PHENYTOIN

Submitted by (08/03/94)
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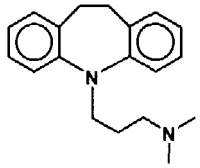
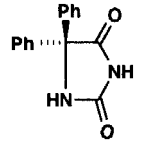
Therapeutic drug monitoring is a relatively new field directed towards individualized health care by optimizing the drug dosage to afford a safe and efficacious drug concentration in a patient's body fluids.[1] Quantification of the drug in clinical samples is most conveniently achieved by immunoassays that can be performed on various automated instruments.[2,3] There are many different types of immunoassay, such as radioimmunoassay, enzyme immunoassay and fluorescence polarization immunoasay.[4] Regardless of format, each employ a common key component which is the antibody reagent. Antibodies need to be specific in order to achieve accurate quantification of the drug in a biological sample where specificity is defined as antibody binding only to the drug of interest.[3] Development of such a reagent to small molecular weight analytes is especially challenging[5] and greatly depends on metabolite availability for specific antibody selection. A frequently encountered problem with the use of antibodies arises when the clinical sample contains molecules of similar chemical structure to the desired analyte which compete for the same antibody binding site.[6] These "similar" molecules may interfere in the immunoassay quantification of the analyte (drug) of interest.

A typical clinical sample for therapeutic drug monitoring contains a mixture of structurally related compounds (metabolites) which are produced by metabolic transformation of the drug. The most frequent metabolic pathway for the elimination of low molecular weight aromatic compounds involves hydroxylation of the aromatic ring.[7] The resulting phenols may be excreted, but are usually further transformed to their corresponding glucuronides.[8] Glucuronidation is a major pathway of drug metabolism and excretion where the anomeric carbon of the glucuronic acid moiety may be attached to a C, N or O atom.[9] The availability of drug metabolites is imperative for practical clinical research and development of immunoassays. There has been an appeal from the clinical community to make metabolites available.[1]

Imipramine (**1**) and phenytoin (**3**) are drugs which require therapeutic drug monitoring.

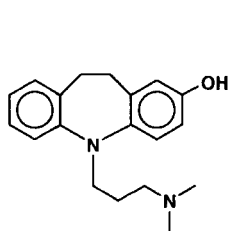


Imipramine (**1**)

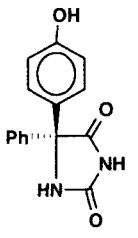


Phenytoin (**3**)

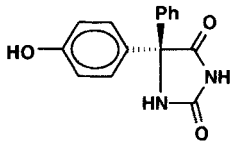
Although their corresponding metabolites (Fig. 1) have been isolated from biological fluids and identified,[10a-d] the synthesis of the glucuronides has not been described in the literature.



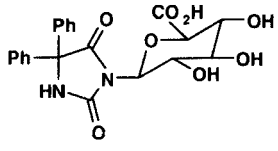
2-Hydroxyimipramine (**2**)



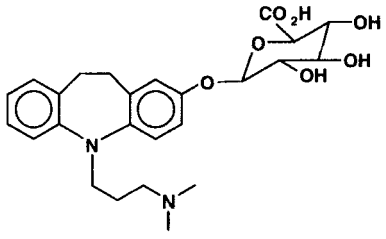
(S)-p-HPPH (**4**)



(R)-p-HPPH (**5**)



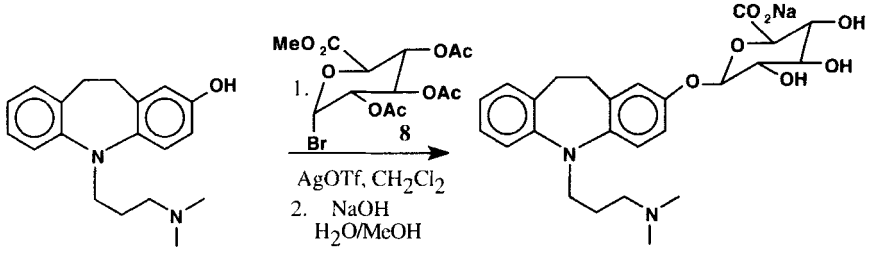
Phenytoin *N*-Glucuronide (**6**)



Imipramine 2-*O*-Glucuronide (**7**)

Fig. 1

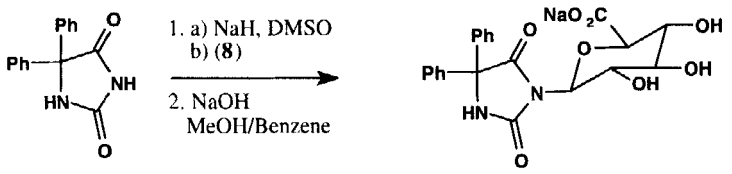
Typical methods for the synthesis of glucuronides are based on the reaction of a protected glucuronic acid moiety, such as methyl 1-bromo-2,3,4-tri-*O*-acyl glucuronate (**8**), with an aglycone. This process of glycoside formation can be accomplished under acidic or basic conditions depending upon the substrate.[11] We chose silver triflate which has been employed to couple alcohols and bromoglycosides,[12] as the reagent for the synthesis of imipramine 2-*O*-glucuronide as shown Eq. 1. Initially, 2-hydroxyimipramine (**2**)[13] was treated with methyl 1-bromo-2,3,4-tri-*O*-acyl glucuronate (**8**) to afford imipramine 2-*O*-glucuronate, which was hydrolyzed under basic conditions to give the sodium salt of imipramine *O*-glucuronide (**7**) in 16% overall yield.



(1)

2-Hydroxyimipramine (**2**) Na salt of Imipramine 2-*O*-Glucuronide (**7**)

The sodium salt of phenytoin (**3**) was coupled with methyl 1-bromo-2,3,4-tri-*O*-acyl glucuronate (**8**) as shown in Eq. 2 to afford an intermediate phenytoin glucuronate which was purified by flash chromatography. This intermediate glucuronate was hydrolyzed with sodium hydroxide in methanol/benzene to afford phenytoin *N*-glucuronide[10b] as its sodium salt in 9% overall yield from phenytoin.

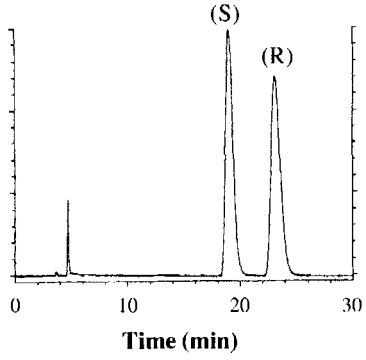


(2)

Phenytoin (**3**) Na salt of Phenytoin N-Glucuronide (**6**)

Although the overall yields of imipramine 2-O-glucuronide and phenytoin *N*-glucuronide are low, we found these overall yields to be typical of other glucuronide syntheses.

Another unavailable set of phenytoin metabolites are its hydroxylated chiral isomers, (R) and (S)-5-*para*-hydroxyphenyl-5-phenylhydantoin.[10a] Separation of the racemic 5-*para*-hydroxyphenyl-5-phenylhydantoin (p-HPPH) by fractional recrystallization has been reported, albeit in low yield.[14] This procedure requires a large amount of the available starting racemic mixture (100 g) to afford ~9g of the each isomer. In order to establish specificity of antibodies, 50 mg of analytically pure material is sufficient. We evaluated the above procedure on a 10g scale and found that it was not dependable or even reproducible, affording material that was only ~70% optically pure. Several groups have reported analytical separation of this racemic mixture by HPLC employing various columns including: β-cyclodextrin chiral column[15a], C_{18} column with β-cyclodextrin in the mobile phase[15b], chiral α_1 acid glycoprotein (AGP)[15c] and a Chiracel OJ column.[15d] As shown below in Fig. 2, the two isomers are readily separated on an analytical ChiralPak AD column. Initially we intended to adapt this separation method on a larger scale using a semi-preparative ChiralPak AD column, however, the high cost of this column ($8500) led us to pursue a different solution. The analytical column when coupled with an autosampler, autocollector and autoinjector (100 µL injection, 4 mg p-HPPH/mL solution in ethanol) was utilized in a continuous mode of operation to afford 60 mg of the (S) isomer (**4**) and 55 mg of the (R)-isomer (**5**) in 10 days where each isomer's optical purity (ee) was >99%. Such HPLC equipment can be found in most clinical laboratories; therefore with this procedure, the desired pure isomers of hydroxy phenytoin can be conveniently and dependably prepared.



Separation of (S) and (R) isomers of p-HPPH
Fig. 2

In summary, we describe here the first synthesis of imipramine 2-*O*-glucuronide (**7**) from the known phenol (**2**) using a silver triflate coupling method and the first synthesis of phenytoin *N*-glucuronide (**6**). The convenient procedure for the preparative separation of racemic *para*-hydroxyphenylphenylhydantoin (p-HPPH) to afford pure (S) and (R) isomers, (**4**) and (**5**) is presented. These four drug metabolites will have widespread use in clinical analysis as well as in other fields of medical research.

EXPERIMENTAL SECTION

Acetobromo-α-D-glucuronic acid methyl ester (**8**) was purchased from Sigma. Amberlite XAD-2, silver triflate, racemic 5-*para*-hydroxyphenyl-5-phenylhydantoin and molecular sieves were purchased from Aldrich. 2-Hydroxyimipramine was prepared as described.[13] Silica gel 60 (230-400 mesh) was purchased from EM Science. ^{1}H, ^{13}C NMR spectra were obtained on a GE-300 NMR spectrometer and high-resolution mass spectra were determined on a Nermag 3010 instrument under FAB $(M)^+$ or $(M+H)^+$ conditions.

Imipramine-2-O-glucuronide (7).- Silver triflate (AgOTf) (141 mg, 0.55 mmol) was added to an aluminum foil covered flask containing a mixture of 2-hydroxyimipramine (**2**) (74 mg, 0.25 mmol), bromoglucuronate (**8**) (120 mg, 0.30 mmol), 4A molecular sieves (1 g) and 2.5 mL CH_2Cl_2. The reaction mixture was stirred for 18 hr in the dark, filtered off molecular sieves and stripped *in vacuo* to obtain a brown solid. Purification by flash chromatography (25 g silica gel, 10% MeOH/90% CH_2Cl_2/0.1% Et_3N; v/v/v) afforded 50 mg imipramine *O*-glucuronate (33%). ^{1}H NMR [CD_3OD:$CDCl_3$ (1:1)]: δ 7.15-6.75 (m, 7H), 5.73 (t, *J* = 8.5 Hz, 1H), 5.31 (*anomeric H*, d, *J* = 8.46 Hz, 1H), 5.23 (t, *J* = 9.56 Hz, 1H), 5.03 (t, *J* = 7.35 Hz, 1H), 4.42 (d, *J* = 10.30 Hz, 1H), 3.85-3.70 (m, 2H), 3.72 (s, 3H), 3.12 (br s, 4H), 2.53 (t, *J* = 6.25 Hz, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.80 (p, *J* = 7.53 Hz, 2H); ^{13}C NMR [CD_3OD:$CDCl_3$ (1:1)]: δ 170.3, 169.9, 169.6, 168.0, 167.2, 151.5, 148.0, 136.2, 133.6, 130.1, 126.3, 122.8, 121.1, 119.6, 117.2, 114.4, 94.7, 72.6, 70.3, 69.3, 68.7, 57.1, 53.0, 48.4, 44.4, 44.3, 32.1, 32.0, 24.6, 20.7, 20.6, 20.5; HRMS $(M+H)^+$ Calcd for $C_{32}H_{41}N_2O_{10}$ 613.2761, found 613.2747.

The imipramine *O*-glucuronate (25 mg, 0.041 mmol) was dissolved in 10 mL of 0.1N methanolic NaOH, stirred for 4 hr and stripped *in vacuo* to afford a solid. Purification was achieved by column chromatography (8 g Amberlite XAD-2[prewashed with 100 mL EtOH then 100 mL H_2O], eluted with 100 mL H_2O followed by 200 mL EtOH) to give an off-white solid followed by preparative tlc (1000 μ C_{18}, 20% H_2O/80% MeOH/0.5% NH_4OH; v/v) to yield 9 mg (**7**) (47%) as its sodium salt. ^{1}H NMR (CD_3OD): δ 7.10-6.80 (m, 7H), 5.41 (d, *J* = 7.60 Hz, 1H), 3.97 (d, *J* = 10.07 Hz, 1H), 3.85 (t, *J* = 7.69 Hz, 1H), 3.71 (t, *J* = 6.41 Hz, 2H), 3.57 (dd, *J* = 9.59, 7.22 Hz, 1H), 3.48 (t, *J* = 9.88 Hz, 1H), 3.11 (br s, 4H), 2.35 (t, *J* = 5.86 Hz, 2H), 2.12 (s, 6H), 1.71 (p, *J* = 6.22 Hz, 2H); ^{13}C NMR (CD_3OD): δ 177.5, 154.1, 149.7, 145.0, 137.6, 134.6, 131.1, 127.4, 123.3, 122.1, 120.4, 119.2, 116.3, 99.5, 74.7, 74.0, 73.1, 72.6, 58.8, 48.6, 45.4(2C), 33.4, 32.9, 26.6; HRMS $(M+H)^+$ Calcd for $C_{25}H_{33}N_2O_7$ 473.2288, found 473.2288.[16]

Phenytoin N-Glucuronide (6).- Diphenylhydantoin (**3**) (300 mg, 1.2 mmol) was added to a suspension of sodium hydride (50 mg, 1.2 mmol) in DMSO and stirred for 1 hour under nitrogen. Acetobromo-α-D-glucuronic acid methyl ester (570 mg, 1.5 mmol) was added to the reaction mixture, stirred for 3 days and carefully quenched with brine (30 mL). After extraction of the mixture with ethyl acetate (2 x 50 mL) and drying the extracts over $MgSO_4$, the solvents were removed *in vacuo* to afford a solid which was purified by flash chromatography (30% EtOAc/70% hexanes; v/v) to afford methyl diphenylhydantoin *N*-glucuronate (210 mg, 31%). ^{1}H NMR: δ 7.40-7.28 (m, 10H), 6.00 (t, *J* =

10.91 Hz, 1H), 5.55-5.35 (m, 2H), 5.20 (t, J = 10.95 Hz, 1H), 4.42 (d, J = 12.27 Hz, 1H), 3.70 (s, 3H), 2.05-1.95 (m, 9H); ^{13}C NMR: δ 171.6, 171.3, 171.1, 170.5, 168.6, 140.1 (2C), 129.8 (4C), 129.7 (4C), 128.1 (2C), 91.3, 79.3, 75.3, 71.3, 70.7, 70.4, 53.4, 20.5, 20.4, 20.2; HRMS $(M+H)^+$ Calcd for $C_{28}H_{28}N_2O_{11}$ 569.1771, found 569.1756.

Sodium hydroxide (67 mg, 1.7 mmol) was added to a solution of diphenylhydantoin *N*-glucuronate (190 mg, 0.334 mmol) in a methanol (12 mL)/benzene (10 mL) mixture under nitrogen and stirred for 40 min. The reaction mixture was concentrated *in vacuo* and purified by preparative tlc (C_{18} reverse phase plates, 75% MeOH/25% H_2O; v/v) to give phenytoin *N*-glucuronide as its sodium salt (**6**) (41 mg, 31%). ^{1}H NMR: δ 7.45-7.25 (m, 10H), 5.06 (d, J = 9.46 Hz, 1H), 4.45 (t, J = 9.34 Hz, 1H), 3.82 (d, J = 9.23 Hz, 1H), 3.60 (t, J = 9.16 Hz, 1H), 3.44 (t, J = 9.00 Hz, 1H); ^{13}C NMR: δ 174.9, 174.0, 156.7, 141.0 (2C), 129.6 (4C), 129.4 (2C), 128.4 (4C), 82.4, 79.2, 79.0, 73.1, 70.7, 69.3; HRMS $(M)^+$ Calcd for $C_{21}H_{19}N_2NaO_8$ 451.1117, found 451.1140.[16]

Separation of (R) and (S) para-hydroxyphenylphenylhydantoin (p-HPPH).- The HPLC System consisted of a Beckman System Gold [Programmable Solvent Module 126, Diode Array Detector 168, Autosampler 507], Foxy 200 fraction collector, ChiralPak AD analytical column (0.46 cm x 25 cm) with a ChiralPak AD analytical guard column (Chiral Technologies, Inc., Exton, PA).

Analytical HPLC Procedure.- Using the above described HPLC system a 1 mg/mL solution of either racemic p-HPPH or a purified isomer in ethanol was injected (5 µL) onto the column (80% hexane:20% isopropanol, 1 mL/min flow rate, λ = 200-800 nm using photodiode array detector) which afforded baseline separation of the two isomers with retention times of 18.8 and 23.9 minutes as shown in Figure 5.

Semi-preparative HPLC Procedure.- A 4 mg/mL solution of racemic p-HPPH in ethanol was prepared. Injection and collection of the separated enantiomers was automated using the auto-sampler and fraction collector. Injections (100 µL) were made every 30 minutes onto the column (80% hexane:20% isopropanol, 1 mL/min flow rate, λ = 270 nm). Fractions for the two enantiomers were collected using the slope detection (peak detection) feature of the Foxy 200. Each sample injection would reset the fraction collector thus simplifying collection to two 500 mL flasks After 240 hours of continuous operation, each fraction [Peak #1 (S-isomer, 60 mg) and Peak #2 (R-isomer, 55 mg)] was concentrated and dried *in vacuo* to afford white solids.

S-(-)-5-*para*-Hydroxyphenyl-5-phenylhydantoin (4).- ^{1}H NMR [CD_3OD]: δ 7.40- 7.30 (m, 5H); 7.15 (d, J = 8.74 Hz, 2H); 6.75 (d, J = 8.79 Hz, 2H); ^{13}C NMR [CD_3OD]: δ 177.54, 158.71, 158.61, 141.34, 131.79, 129.53, 129.38, 129.26, 128.08, 116.24, 72.47; MS $(M+NH_4)^+$ 286; optical rotation $[\alpha]_D^{23}$= -16°(c=0.44, MeOH, lit.[15], c=0.43, $[\alpha]_D^{23}$= -25.7°); analytical HPLC retention time 18.8 min (>99%).

R-(+)-5-*para*-Hydroxyphenyl-5-phenylhydantoin (5).- ^{1}H NMR [CD_3OD] δ 7.40-7.30 (m, 5H); 7.15 (d, J = 8.74 Hz, 2H); 6.75 (d, J = 8.79 Hz, 2H); ^{13}C NMR [CD_3OD] δ 177.54, 158.71, 158.61, 141.34, 131.79, 129.53, 129.38, 129.26, 128.08, 116.24, 72.48; MS $(M+NH_4)^+$ 286; optical rotation $[\alpha]_D^{23}$= +18°(c= 0.44, MeOH; analytical HPLC retention time 23.9 min (>99%).

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16. The glucuronide metabolites **6** or **7**, or their sodium salts, were not stable to heat and slowly decomposed during drying to afford compounds which did not give satisfactory elemental analysis.