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Research Article

Synthesis of [$^2H_3$]-dextromethorphan and its major urinary metabolites [$^2H_3$]-dextrorphan and [$^2H_3$]-dextrorphan-β-glucuronide

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Summary

Starting from dextromethorphan, [$^2H_3$]-dextrorphan-β-glucuronide was synthesized in four steps with [$^2H_3$]-dextromethorphan and [$^2H_3$]-dextrorphan as intermediates with an overall yield of 11%. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: dextromethorphan; dextrorphan glucuronide; deuterium label

Introduction

Dextromethorphan (6-methoxy-17-methyl-morphinan, **1**) is widely used as an anti-tussive drug without narcotic effects. Human cytochrome P450 2D6, which shows lack of function in about 8% of the Caucasian population, mediates the O-demethylation to dextrorphan (6-hydroxy-17-methyl-morphinan, **2**), which is then glucuronidated.[1] To use **1** as a probe drug for this polymorphism, a high throughput method to

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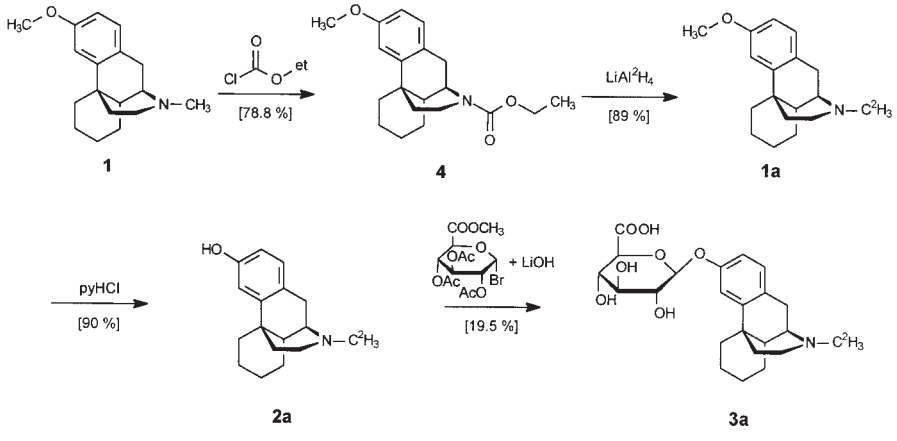
determine **1**, **2**, and dextrorphan glucuronide (6-(β-D-glucopyranuronosyl)-17-methyl-morphinan**,** **3**) in urine by LC-MS/MS is recommended. For this purpose, deuterium labelled internal standards are required.

Results and Discussion

The approach starting with **1** was used because [$^2H_3$]-dextromethorphan **1a** and [$^2H_3$]-dextrorphan **2a** are both needed as internal standards and are intermediates (Scheme 1). To introduce deuterium into **1** a modification of the method described by Elison *et al.* was used.[2] **1** was converted to 17-ethoxycarbonyl-6-methoxy-morphinan (**4**) with ethyl-chloroformate in chloroform under reflux. From traces of **1**, **4** was purified by chromatography and reduced with lithium aluminium deuteride in tetrahydrofuran to obtain **1a**, which again could be purified by chromatography. Subsequently, *O*-demethylation was achieved by the modification of the method described by Prey,[3] using a 70-fold excess of pyridine hydrochloride at 220°C in an open reaction system. Both the excess of pyridine hydrochloride and the open system were crucial in order to avoid contamination of **2a** with **2** by the exchange of the *N*-methyl-group. By crystallisation of the corresponding tartrate salt from aqueous solution, **2a** was purified. The synthesis of **2a** from **2**, via the corresponding 17-ethoxycarbonyl-6-hydroxy-morphinan and reduction with lithium aluminium deuteride gave low yields because of



Scheme 1.

incomplete formation of the carbamate, and low extraction rates of **2a** from the reaction mixture after reduction. Although no method has been described previously to synthesize **3**, several approaches to obtain aryl glucuronides using 2,3,4-tri-*O*-acetyl-1-bromo-1-desoxy-α-D-gluco-pyranosiduronic acid methyl ester (**5**)[4] and different catalysts, like cadmium carbonate[5] or sodium hydroxide[6] have been published. Carrupt and his co-workers have described a method to obtain morphine 3-glucuronide with lithium hydroxide.[7] Using the latter, both coupling with lithium hydroxide in dry methanol and the deacetylation reaction with aqueous lithium hydroxide were performed without separation of the intermediate. The use of highly pure starting materials and an accurate control of the reaction times resulted in a yield of 19.5% for **3a**. Additionally, a substantial amount of **2a** was recovered from the extraction of the alkaline reaction mixture with chloroform.

Experimental

All reactions were performed under an argon atmosphere with dried solvents. The degree of isotope labelling was determined by electrospray ionization mass spectrometry (HP 1100, Agilent Technologies, Waldbronn, Germany) and calculated by comparison to the unlabelled compound. Analytical separation of α- and β-glucuronides was achieved by HPLC-MS.

17-Ethoxycarbonyl-6-methoxy-morphinan (4)

To a mixture of 4.7 g of **1** (from 7 g of **1** hydrobromide monohydrate) and 1.6 g $NaHCO_3$ in 175 ml chloroform, 2 ml of ethyl-chloroformate was added. The reaction mixture was heated under reflux for 5 h. During this time two additional portions of 1 ml ethyl-chloroformate were added. The reaction mixture was cooled in an ice bath and the organic layer was treated twice with the same volume of 5% (w/v) $NaHCO_3$ in water. The organic solvent was evaporated under reduced pressure to obtain a slightly brown oil which was then purified by chromatography on a silica gel 60 column (Merck, Darmstadt, Germany) with ethyl acetate: hexane 1:5 (v/v) as mobile phase yielding 4.488 g of **4** (78.8% of theory) as a colourless oil.

[18,18,18-$^2H_3$]-6-methoxy-17-methyl-morphinan (1a)

To a solution of 1 g of lithium aluminium deuteride in 20 ml THF cooled in an ice bath 2.03 g of **4** in 20 ml THF was added dropwise. After completing addition, the reaction mixture was subsequently stirred at room temperature for 30 min and under reflux for 2 h. The reaction mixture was then cooled in an ice bath and the excess of lithium aluminium deuteride was removed by slowly adding 20 ml of THF: water 9:1 (v/v), followed by 2 ml of water. The reaction mixture was further diluted with 75 ml of water and made alkaline by adding 50 ml of 2 M NaOH. THF was removed under reduced pressure and the remaining aqueous mixture was extracted three times with chloroform. The combined organic layers were re-extracted with water, dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure to give 1.506 g of **1a** as a colourless solid (89% of theory). Anal.: Calculated for $C_{18}H_{22}{}^2H_3NO$: C, 78.78; H, 8.08; ^2H, 2.20; N, 5.10; O, 5.83. Found: C, 78.73; H, 8.12; ^2H, 2.21; N, 5.09. Isotope distribution: $^2H_0$, 0.0; $^2H_1$, 0.0; $^2H_2$, 2.3; $^2H_3$, 97.8.

[18,18,18-$^2H_3$]-6-hydroxy-17-methyl-morphinan (2a)

A mixture of 36 g of pyridinium chloride and 1.2 g of **1a** was heated at 220°C for 4.5 h, with a slight stream of argon led through the reaction flask and a glass tube as reflux condenser. After cooling the reaction mixture in an ice bath, the solid was dissolved in 120 ml of water, made alkaline by the addition of 150 ml of 3 M NaOH and was extracted 3 times with 100 ml of chloroform. The combined organic layers were re-extracted with water, dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure. Further purification on a silica gel 60 column with ethyl acetate : triethylamine 100:1 (v/v) as mobile phase gave 1.022 g of **2a** (90% of theory) as an almost colourless solid. For the preparation of **2a** tartrate monohydrate 826 mg of **2a**, which was recovered as a yellow solid from the synthesis of **3a**, was dissolved in a solution of 476 mg of *d*-tartratic acid in 11 ml of water and treated with charcoal. After filtration and reduction of the solvent volume colourless crystals of **2a** tartrate precipitated. Anal.: Calculated for $C_{17}H_{20}{}^2H_3NO{*}C_4H_6O_6$: C, 61.45; H, 6.38; ^2H, 1.47; N, 3.41. Found: C, 61.49; H, 6.44; ^2H, 1.48; N, 3.38. Isotope distribution: $^2H_0$, 0.0; $^2H_1$, 0.1; $^2H_2$, 2.5; $^2H_3$, 97.1.

[18,18,18-$^2H_3$]-6-(β-D-glucopyranuronosyl)-17-methyl-morphinan (3a)

To a solution of 1.022 g of **2a** in 8.5 ml methanol, 90 mg of LiOH and 1.402 g of **5** were successively added and stirred at room temperature for 30 min. Following the addition of 259 mg of LiOH in water, the reaction mixture was stirred for another 30 min. Unchanged **2a** was recovered by extraction with chloroform and the evaporation of the organic solvent. The aqueous layer was acidified with acetic acid and extracted again with chloroform. This second organic extract was discarded. The water was evaporated under reduced pressure and the remaining white solid was purified by chromatography on a Lobar B column packed with Lichroprep RP 18 (40–64 μm) (Merck, Darmstadt, Germany). Hydrophilic by-products were washed from the column with water : methanol 85:15 (v/v) then **3a** was eluted with water : methanol 1:1 (v/v). This latter chromatographic system was also suitable to separate **3a** from minor amounts of its α-isomer. Evaporation of the solvent yielded 300 mg (19.5% of theory) of **3a** as a slightly yellow solid which was recrystallised from water: methanol 1:1 (v/v) to obtain colourless needles. Anal.: Calculated for $C_{23}H_{28}{}^2H_3NO_7{*}1\frac{1}{4}H_2O$: C, 60.18; H + ^{2}H, 7.36, N, 3.05. Found: C, 60.05; H + ^{2}H, 7.31, N, 3.03. ^{13}C-NMR: 24.16, 25.67, 28.18, 37.53, 38.35, 41.78, 45.38, 50.46, 62.94, 74.70, 75.70, 78.37, 79.47, 103.08, 116.96, 117.73, 131.54, 132.36, 142.40, 158.93, 178.14. Isotope distribution: $^2H_0$, 0.0; $^2H_1$, 0.0; $^2H_2$, 2.5; $^2H_3$, 97.4.

6-(β-D-glucopyranuronosyl)-17-methyl-morphinan (3)

Unlabelled reference compound **3** was synthesized from **2** in the same way as **3a** and was recrystallized from water: methanol 1:1 (v/v) to obtain colourless crystals. Anal.: Calculated for $C_{23}H_{31}NO_7{*}H_2O$: C, 61.18; H, 7.37; N, 3.10. Found: C, 61.46; H, 7.24; N, 3.15. ^{13}C-NMR: 24.16, 25.67, 28.18, 37.53, 38.35, 41.79, 43.27, 45.40, 50.58, 63.04, 74.70, 75.70, 78.37, 79.46, 103.09, 116.97, 117.77, 131.56, 132.36, 142.40, 158.34, 178.15.

Conclusion

In summary, we obtained deuterium labelled dextromethorphan and its metabolites, dextrorphan and dextrorphan β-glucuronide in good yield

and purity within one reaction sequence. In addition, this work is the first to describe the synthesis of dextrorphan glucuronide.

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