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Synthesis of a Water-Soluble Prodrug of Entacapone

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Abstract—Entacapone was reacted with phosphorous oxychloride in dry pyridine to yield a phosphate ester. The phosphate promoiety increased aqueous solubility of the parent drug by more than 1700- and 20-fold at pH 1.2 and 7.4, respectively. The phosphate ester provides adequate stability ($t_{1/2}$ = 2227 h; pH 7.4) towards chemical hydrolysis, and allowed for release of the parent drug via enzymatic hydrolysis in liver homogenate. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Entacapone[1] (*E*)-2-cyano-*N,N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide (**1**) is a new 3,4-dihydroxy-5-nitrobenzylidine derivative and a potent inhibitor of COMT[2] (catechol-*O*-methyltransferase). COMT is the main enzyme responsible for the metabolism of L-dopa (3,4-dihydroxyphenyl-L-alanine), together with aromatic amino acid decarboxylase (AADC).[2] L-Dopa is a precursor to dopamine, which is deficient in the brains of patients suffering from Parkinson's disease (PD).

Traditional PD treatment consists of co-administration of oral L-dopa with an AADC inhibitor. During such treatment, COMT becomes the main enzyme to metabolize L-dopa. Despite this coupled therapeutic approach, no more than 5–10% of orally administered L-dopa enters the brain. Administration of entacapone, together with L-dopa and an AADC inhibitor, leads to increased L-dopa bioavailability and to prolonged elimination of L-dopa.[3]

Although entacapone is in clinical use as an adjunct to L-dopa therapy in PD, its bioavailability is low after oral administration and characterized by large interindividual variation.[4] The reason for its poor bioavailability is unknown, but it may be, at least partly, due to low aqueous solubility and slow dissolution at the pH of the stomach and the small intestine.[5]

The aim of the present study was to synthesize a phosphate ester of entacapone in order to increase its aqueous solubility and dissolution rate. The phosphate ester **2** (Scheme 1) was expected to be enzymatically hydrolyzed by phosphate esterases[6–9] and thus serve as a prodrug of entacapone.

Chemistry

The activity of a COMT-inhibitor is markedly increased when the nucleophilicity of the catecholic hydroxyls decreases.[10] This is due to the strong electron withdrawing effect of the nitro-group and the conjucated structure of the molecule which together allow for the dispersal of a negative charge. Thus the nitrocatechol hydroxyls of entacapone are more acidic than in a catechol, which affects their reactivity.

A common method for the phosphorylation of phenols with concentrated phosphoric acid and phosphorous pentoxide[11] proved to be unsuccessful for the phosphorylation of the nitrocatechol structure. Also, the treatment with another common phosphorylating agent, di-*t*-butyl-*N,N*-diisopropylphosphoramidite in presence of 1*H*-tetrazole, failed to yield **2**.[12,13]

Synthesis via phosphorus oxychloride in dry pyridine gave **2** in 45% yield. The steric repulsion of the nitro-group most probably favors phosphate to attach hydroxyl at C-3.[14]

The drawback of the present synthetic route was the formation of the cyclic phosphate[15] **3**, which formed at

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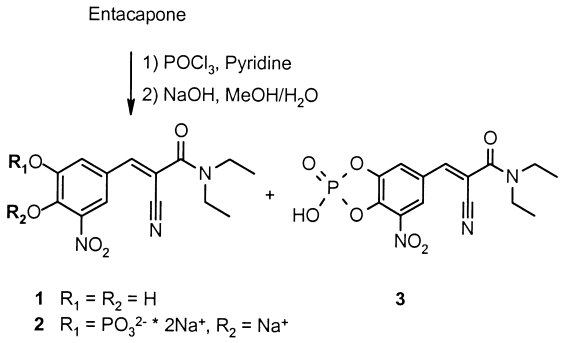

Scheme 1. Synthesis of the entacapone monophosphate.

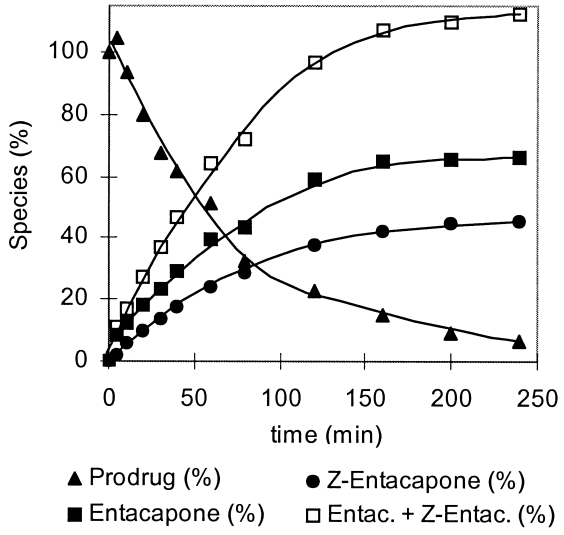

Figure 1. Hydrolysis of **2** and formation of entacapone and *Z*-entacapone in a rabbit liver homogenate (pH 7.4) at 37 °C.

the expense of **2**. An another method employed to avoid the formation of **3** involved the treatment of entacapone with a dimethyl protected phosphorous oxychloride,[16] followed by the cleavage of methyl-groups[17] to yield **2**. Unfortunately, dimethyl chlorophosphate also proved to be unreactive with entacapone.[18] To our knowledge **2** is the first monophosphate derivative of the 3,4-dihydroxy-5-nitrobenzylidine structure.

Results and Discussion

The aqueous solubility of **2** was over 30 mg/mL at both pH 1.2 and 7.4, which is significantly higher than that of entacapone (0.017 mg/ml at pH 1.2 and 1.75 mg/mL at pH 7.4). The phosphate ester also shows high stability ($t_{1/2}$ = 2227 h at pH 7.4 and 37 °C) towards chemical hydrolysis[19,20] in a buffered solution. In addition, no degradation was observed in 80% human serum[21] (pH 7.4)[20] over 21 h, which indicates that enzymes present in plasma are not able to hydrolyse **2**. In contrast to human serum, phosphate ester **2** was hydrolysed to entacapone and *Z*-entacapone (*E*/*Z* ratio 1.6) in a rabbit liver homogenate[22] with a half-life of 58 min (Fig. 1). *Z*-Entacapone is the main metabolite found in human plasma. It has been demonstrated in vitro that in plasma entacapone is slowly converted to its *Z*-isomer reaching (*E*/*Z*) ratio 2.1.[23] The reason for the slow enzymatic hydrolysis of **2** is most probably the electron withdrawing effect of the nitro-group or steric hindrance between the phosphate- and the nitro-groups for phosphatase enzymes.

Conclusion

The present synthetic route is a suitable method for the phosphorylation of nitrocatechol structures with reasonable yield. Phosphate as a promoiety group markedly increased the aqueous solubility of the parent drug.

Phosphate ester **2** showed high stability in both phosphate buffer and human serum, and released entacapone quantitatively in liver homogenate, which fulfills prodrug criteria.

Experimental

Entacapone (OR-611) was obtained from Orion Pharma (Espoo, Finland).

Phosphorus oxychloride (183 mg, 1.2 mmol) was added at 0 °C to entacapone (300 mg, 0.98 mmol) in dry pyridine (10 mL) and the mixture was allowed to stir for 2 h without cooling. Water (10 mL) was added and the mixture was stirred for 30 min. Pyridine was evaporated and the residue was made acidic (pH 1) with concd phosphoric acid. Unreacted entacapone and cyclic phosphate **3** were extracted with EtOAc. The aqueous phase was saturated with NaCl and extracted with $CHCl_3$. Evaporation of the solvent gave a yellow powder (170 mg, 45%), which was dissolved into a water: methanol (1:1) solution and treated with 3.3 eqiuv of 1N NaOH. The product was crystallised from the water: methanol mixture to yield **2** as red needles. The NMR spectra were measured on a Bruker 400 WB spectrometer (400.1 MHz, D_2O) ^{1}H NMR δ: 1.30 (6H, br, NCH_2–CH_3), 3.60 (4H, br, NCH_2–CH_3), 7.50 (1H, s, C*H*=C), 7.83 (1H, d, $^3J_{HH}$ = 2.5 Hz, $^4J_{PH}$ = −1.0 Hz, Ar-*H*2), 8.40 (1H, d, $^3J_{HH}$ = 2.5 Hz, Ar-*H*6); ^{13}C NMR (100.6 MHz) δ: 14.5, 15.6, 43.8, 47.3, 100.5, 117.5, 120.5, 125.0 (d, $^3J_{CP}$ = 5.7 Hz, Ar2), 126.6, 140.0, 151.7 (d, $^3J_{CP}$ = 5.7 Hz, Ar4), 154.3, 160.1 (d, $^2J_{CP}$ = 6.4 Hz, Ar3), 169.3; ^{31}P NMR (162.0 MHz) δ: −2.45; ESI-MS: 430.3 (M + 1 + 2*Na). Anal. calcd for $C_{14}H_{13}N_3O_8PNa_3$*$2H_2O$: C, 34.51; H, 3.52; N, 8.62. Found: C, 34.72; H, 3.08; N, 8.57.

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14. The ^{1}H NMR spectrum shows a quartet for the ArH[2] and couplings 2.5 Hz and 1.0 Hz. The former is coupling to the ArH6 aromatic proton and the latter indicates $^{4}J_{PH}$ coupling. If phosphate is attached to hydroxyl at C-4, then very small or no $^{5}J_{PH}$ coupling would be observed in the aromatic protons. ^{13}C NMR supports this observation since the C-2, C-3 and C-4 peaks are shifted downfield relative to the native entacapone spectrum, and $^{2}J_{CP}$ and $^{3}J_{CP}$ couplings are observed.
15. 367.2 (M^{+}, 100%) by ESI-MS.
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18. In dry pyridine or with a catalytic amount of DMAP (dimethylaminopyridine). Stirred at ambient temperature for 3 h.
19. The hydrolysis rate of **2** was determined in phosphate buffer solution (0.16 M) at pH 7.4 and at 37 °C. An appropriate amount of **2** was dissolved in pre-heated (37 °C) phosphate buffer, the solution was placed in a thermostated waterbath (37 °C) and sampled at suitable intervals. The samples were analyzed by HPLC.[20]
20. The HPLC used for the determination of in vitro samples consisted of a UV detector (set at 254–304 nm, depending on compound). Separations were performed with a Purospher RP-18 reverse-phase column, (12.5 cm×4.0 mm i.d., 5 μm), (Merck, Darmstadt, Germany). The conditions: injection volume, 50 μL; column temperature, 40 °C; flow rate, gradient/isocratic at 1.0 mL/min. The mobile phase consisted of various proportions of methanol and citrate/phosphate buffer pH 2.2, depending on the compound.
21. The hydrolysis rate of **2** was determined in 80% human serum. An appropriate amount of **2** was dissolved in 1.0 mL of preheated deionized water and 4.0 mL preheated serum was added. Solutions were kept in a water-bath at 37 °C and sampled (500 μl) at appropriate time intervals. Ethanol (1.0 mL) was added to terminate enzymatic activity in the serum after which the sample was centrifuged. Supernatant was withdrawn after centrifugation and analyzed with HPLC.[20]
22. Hydrolysis rate of **2** in 10% (w/v) rabbit liver homogenate, diluted with borate buffer (isotonic pH 7.4), was determined at 37 °C. Rabbit liver was homogenized with four volumes of isotonic borate buffer (pH 7.4). The homogenate was centrifuged and stored at −80 °C until analysis. Prior to the hydrolysis study the 20% liver homogenate was diluted to 10% with isotonic borate buffer (pH 7.4). An appropriate amount of **2** was dissolved in pre-heated 10% liver homogenate and the solution was incubated at 37 °C. Samples (200 μL) were withdrawn at appropriate time intervals and cold methanol (400 μL) was added to samples to terminate enzymatic activity, after which the samples were centrifuged. The supernatant was withdrawn and analyzed by HPLC.[20]
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