SYNTHESIS OF TWO ^{14}C-LABELLED CATECHOL-O-METHYLTRANSFERASE INHIBITORS

Carita Karlsson and Erkki Honkanen

Orion Corporation Ltd., Orion Pharmaceutica, Research Center, SF-02101 Espoo, Finland.

SUMMARY

^{14}C-labelled 3-(3,4-dihydroxy-5-nitrophenylmethylidene)-2,4-pentanedione (*4*) and ^{14}C-labelled E-N,N-diethyl-2-cyano-3-(3,4-dihydroxy- 5-nitrophenyl)acrylamide (*5*) have been synthesized from [carbonyl-^{14}C]vanillin.

Key words: carbon-14 labelling, synthesis, COMT-inhibitor.

INTRODUCTION

Catechol-O-methyltransferase (COMT) enzyme is present in most tissues, both in the periphery and in the central nervous system. It has an important role in the extraneuronal inactivation by O-methylation of the phenolic hydroxyl group of catecholamine neurotransmitters and exogenous catechol-structured compounds and drugs e.g. L-DOPA. In the treatment of Parkinson's disease the drug of choice is still L-DOPA in combination with decarboxylase inhibitors like carbidopa or benserazide, which reduce peripheral dopamine formation and allow more L-DOPA to reach the brain. However, the 3-O-methylation route then becomes dominant which leads to poor clinical potency or side effects. At present there is no effective and selective COMT inhibitor that could be used therapeutically for the treatment of Parkinson's disease by inhibiting selectively the metabolism of L-DOPA.

Recently two research groups independently reported the development of potent and selective COMT inhibitors [1,2] which seems to be very promising and may open new possibilities for the treatment of Parkinson's disease. Both of these inventions are based on 3-nitrocatechol structures. Two COMT inhibitors namely 3-(3,4-dihydroxy-5-nitrophenylmethylene)-2,4-pentanedione (OR-462, NITECAPONE) and E-N,N-diethyl-2-cyano-3- (3,4-dihydroxy-5-nitrophenyl)acrylamide (OR-611) are now in clinical trials. The aim of this study was to synthesize these compounds labelled with carbon-14 which would be necessary in studying the metabolism and pharmacokinetic properties of these drugs.

MATERIALS AND METHODS

Radioactivity was measured on an LKB Wallac 1219 rackbeta liquid scintillation counter using National Diagnostics, Ecoscint scintillant. Radiochemical purity of the products was determined by high performance liquid chromatography on a liquid chromatograph with Radiomatic Instruments' FLO-ONE radioactivity detector. Radiochemical purity of intermediates was determined by thin layer chromatography on Merck silica gel 60 F_{254}. [carbonyl-^{14}C]Vanillin was obtained from Amersham International plc. All solvents

0362–4803/91/020237–03$05.00

Received September 17, 1990
Revised October 5, 1990

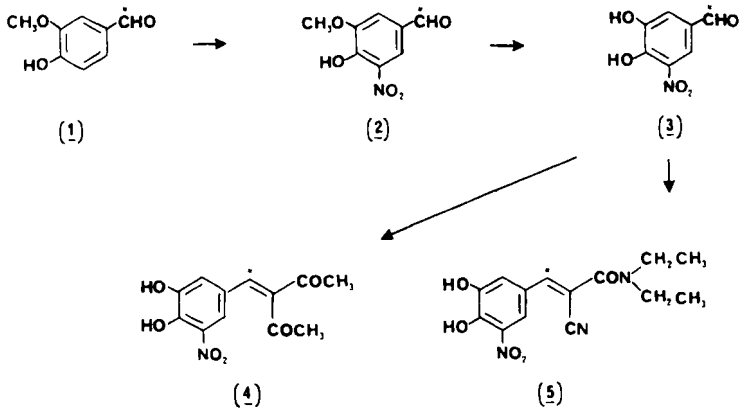
and other reagents were reagent grade and were obtained from commercial sources. All synthetic and analytical operations were initially performed with unlabelled compounds, and the structures of unlabelled intermediates and products were confirmed spectroscopically.

SYNTHESIS

The syntheses of the unlabelled 3-(3,4-dihydroxy-5-nitrophenylmethylidene)-2,4-pentanedione (*4*) and E-N,N-diethyl-2-cyano- 3-(3,4-dihydroxy-5-nitrophenyl)acrylamide (*5*) have been reported previously [1,3]. The starting material for the syntheses of both labelled compounds was 3,4-dihydroxy-5-nitro[carbonyl-^{14}C]benzaldehyde (*3*), which was obtained by nitration of [carbonyl-^{14}C]vanillin followed by demethylation with concentrated hydrobromic acid. Compound (*3*) was condensed with 2,4-pentanedione by using hydrogen chloride as catalyst to give compound (*4*) in 49 % yield. For the synthesis of E-N,N-diethyl-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)acrylamide the labelled aldehyde (*3*) was condensed with N,N-diethyl-cyanoacetamide in basic conditions (piperidine acetate) to give compound (*5*) in 14 % yield.

Scheme 1.

Synthesis of ^{14}C-labelled 3-(3,4-dihydroxy-5-nitrophenylmethylidene)- 2,4-pentanedione (*4*) and E-N,N-diethyl-2-cyano-3-(3,4-dihydroxy-5- nitrophenyl) acrylamide (*5*).



4-Hydroxy-3-methoxy-5-nitro[^{14}C]benzaldehyde (2)

[carbonyl-^{14}C]Vanillin (3.6 mCi, 53 mg, 0.35 mmol) (*1*) was dissolved in dichloromethane (1.5 ml). A solution of 1 mol HNO_3 in dichloromethane (0.525 ml) was added and the reaction mixture was stirred for half an hour at 0°C. The mixture was diluted with dichloromethane so that the precipitate was dissolved. The solution was washed with cold water (3 x 1 ml) and the organic layer dried over Na_2SO_4 and filtered. After evaporating *in vacuo* the yield was 65 mg, 0.33 mmol, 94 %. The resulting product had a radiochemical purity of approximately 97 %. TLC (silica, toluene 8: dioxane 2: acetic acid 1): Rf 0.56.

3,4-Dihydroxy-5-nitro[^{14}C]benzaldehyde (3)

The above product (2) in 48% hydrobromic acid (1.9 ml) was stirred for 4 hours under nitrogen at 110°C. A saturated solution of Na_2SO_4 (2 ml) was added and the product was extracted with ether, washed with a saturated solution of Na_2SO_4, and cold water. The organic phase was dried over Na_2SO_4, filtered and evaporated *in vacuo* to yield 57mg, 0.31 mmol. The resulting product had a radiochemical purity of approximately 93%. TLC (silica, ether): Rf 0.69.

Purification: The product was dissolved in warm toluene and treated with a small amount of charcoal, filtered and evaporated to dryness. The yield was 57 mg, 0.31 mmol, 94 %.

3-(3,4-Dihydroxy-5-nitro-phenyl[^{14}C]methylidene)-2,4-pentanedione (4)

2,4-Pentanedione (40 µl) was added to a solution of (3) (57 mg) in tetrahydrofuran (130 µl) and toluene (420 µl). HCl-gas was passed for 7 minutes into the reaction mixture at room temperature. Stirring was continued over night and the desired product was filtered and washed with toluene and petroleum ether.

Purification: The product was dissolved in warm methanol and treated with a small amount of charcoal, filtered and evaporated to dryness. The residue was dissolved in warm ether, filtered and evaporated *in vacuo* to give 46 mg, 0.17 mmol, 55 % of the desired product, having a specific activity of 31.5 µCi/mg. HPLC analysis (LiChrosorb RP-18, 25 mM sodium dihydrogen phosphate and 10 mM citric acid pH 2.2 with a gradient from 0 % methanol to 80 % methanol in 45 min, 1 ml/min) showed that the product has a purity over 99 % as determined by radioactivity detection.

E-N,N-Diethyl-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)[3-^{14}C]acrylamide (5)

3,4-Dihydroxy-5-nitro[^{14}C]benzaldehyde (3) (57 mg) was dissolved in toluene (1.6 ml) and N,N-diethyl-2-cyanoacetamide (61 mg), celite (38 mg), piperidine (6 µl), acetic acid (9 µl) and molecular sieves (3Å) were added to the reaction mixture. The resulting mixture was stirred for 5 hours at 115°C under nitrogen atmosphere, then a solution consisting of 10 µl of H_2SO_4 and of 200 µl acetic acid was added. After about 5 minutes the warm reaction mixture was filtered, concentrated and stirred for 5 hours at 0 °C. The precipitate was filtered off and washed with cold toluene and dried thoroughly *in vacuo*. The resulting product was recrystallized from a solution containing toluene, hydrogen bromide and acetic acid to give 15 mg, 0.05 mmol, 16 % of the desired product, having a specific activity of 30.1 µCi/mg. HPLC analysis (Spherisorb ODS2, 25 mM sodium dihydrogen phosphate and 10 mM citric acid pH 2.2 with a gradient from 0 % methanol to 90 % methanol in 30 min, 1 ml/min) showed the product to be 99 % pure as determined by radioactivity detection.

ACKNOWLEDGEMENT

The authors wish to thank M.Sc. (pharm.) Tom Wikberg for HPLC-analysis.

REFERENCES

1. Bäckström R., Honkanen E., Pippuri A., Kairisalo P., Pystynen J., Heinola K., Nissinen E., Linden I-B., Kaakkola S., and Pohto P. - J. Med. Chem. *32*: 841 (1989).

2. Borgulya J., Bruderer H., Bernaur K., Zürcher G., and Da Prada M. - Helv. Chim. Acta *72*: 952 (1989).

3. Pippuri A., Honkanen E., and Haarala J., - British pat. appl. 8924838.9 (1989).