SYNTHESIS OF ^{14}C AND ^{3}H LABELLED 6-NITRO-7-SULFAMOYLBENZO[F]QUINOXALIN-2,3-DIONE

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SUMMARY

A ^{14}C labelled form of 6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1] was synthesized in two steps from 1,2-diamino-5-sulfamoylnaphthalene [2] and 30 mCi ^{14}C-oxalic acid. A total of 5.5 mCi was isolated as a yellow solid with a radiochemical purity > 99%. The specific activity was 99 mCi/mmol.
A ^{3}H labelled form of [1] was synthesized in two steps from 10-bromo-7-sulfamoylbenzo-[f]quinoxalin-2,3-dione [4] and tritium gas. The product was isolated with a radiochemical purity of >98% and a specific activity of 10 Ci/mmol.

Key words: Radio-labelled 6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione, NBQX, glutamate antagonist, AMPA.

INTRODUCTION

6-Nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1] is a potent glutamate antagonist with high affinity for alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) sites[1]. The compound is effective as a neuroprotectant for cerebral ischemia[2,3,4,5,6,7], and it shows potent antiparkinsonian effects in primates and rats[8].

To be able to do studies on the distribution and metabolic fate of [1], a radioisotopic labelled form of the compound was needed. The preferred isotope for such experiments is ^{14}C, located in a stable place in the molecule.

The synthetic pathway of [1] allows ^{14}C to be incorporated in the heterocyclic ring via ^{14}C labelled oxalic acid which is commercially available.

0362–4803/92/050365–10$05.00
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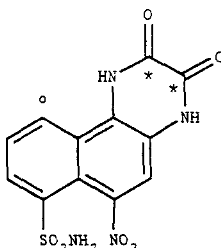
Received 19 November, 1991
Revised 14 February, 1992

The tritiated form of [1] with high specific activity was also needed for in vivo and in vitro binding studies. Labelling was done by catalytic reduction of a bromo precursor using $^3H_2$ as tritium source, which can be supplied with high specific activity.

The labelled positions:

• = ^{14}C position

o = ^3H position



[1]

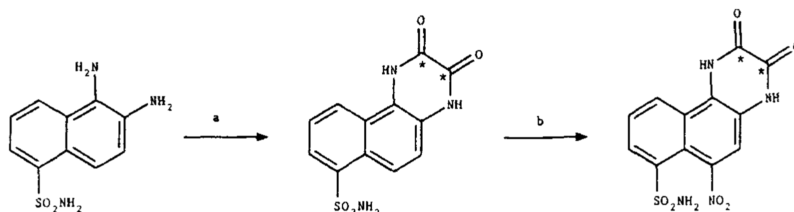
6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione (NBQX, FG 9202, NNC 07-9202).

RESULTS AND DISCUSSION

It was important that the method chosen for the ^{14}C-labelling gave a product with high radiochemical purity and specific activity, and essential, that the position of the radionuclide made it possible to follow the metabolic fate of the molecule. It was also preferable that the synthetic way included only a few steps with radioactive labelled material.

In view of these facts, incorporation of ^{14}C in the heterocyclic ring was chosen. (2,3-^{14}C)-6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1a] was synthesized in two steps from [2] and ^{14}C-oxalic acid, as shown in the following reaction scheme:

SCHEME 1 :



2 3 1a

a : ($^*COOH)_2$, HCl (aq), 100 °C

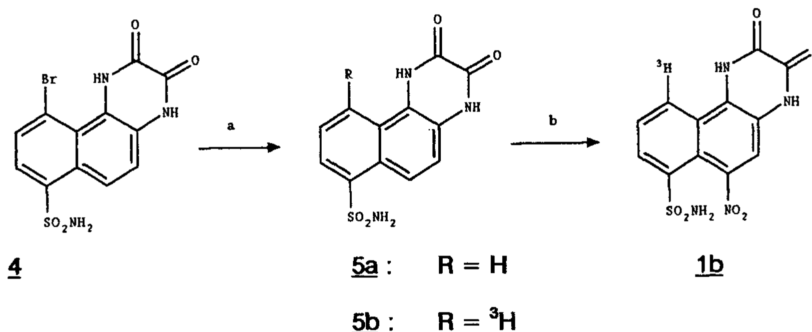
b : HNO_3/H_2SO_4, 0-5 °C

A mixture of trifluoroacetic acid (0.1%) and tetrahydrofuran was chosen as eluent for the HPLC purification step which allowed all the eluent ingredients to be removed by evaporation.

After HPLC purification, the radiochemical purity was >99% by radio-HPLC and the total radiochemical yield was 18%. The high specific activity of ^{14}C-oxalic acid was preserved in the product [1a], and determined to be 99 mCi/mmol by MS.

(10-^{3}H)-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1b] was prepared following a two step procedure. The synthetic route is shown below:

SCHEME 2 :



a : $^{3}H_2$, PdO, DMF, TEA, 50 °C

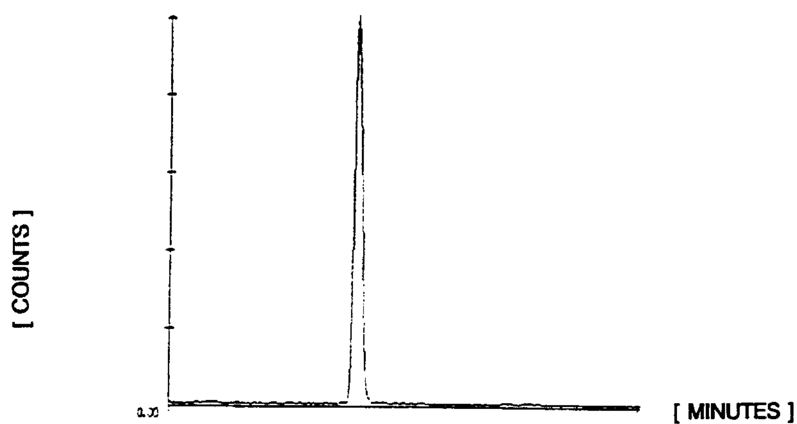
b : HNO_3/H_2SO_4, heat

The bromo precursor [4] was synthesized by a 7-step procedure starting from 1-amino-5-nitronaphthalene[9].

Tritiation of [4] with tritium gas went smoothly. The radiochemical impurities was less than 20% after evaporation of solvent, and reverse phase radio-HPLC showed only very polar impurities.

The nitration step b was difficult to get started when using crude [5b], but addition of a small amount of a similar "cold" reaction mixture starting with pure [5a], started the reaction.

Figure 1 : HPLC Radiochromatogram and UV-chromatogram of ^{14}C-NNC 07-9202 [1a]



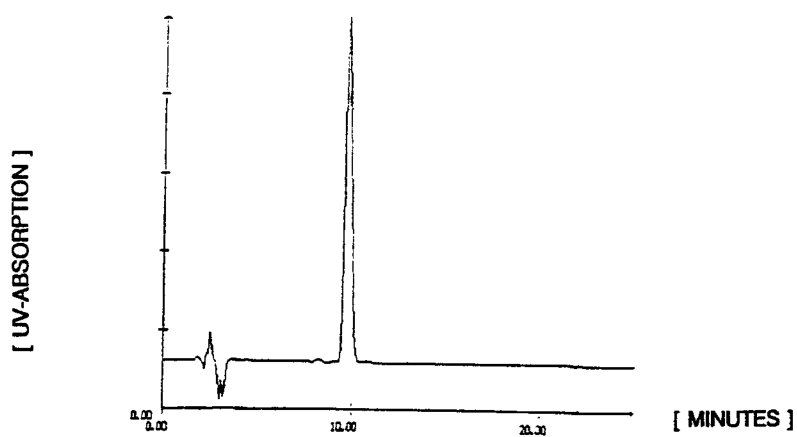
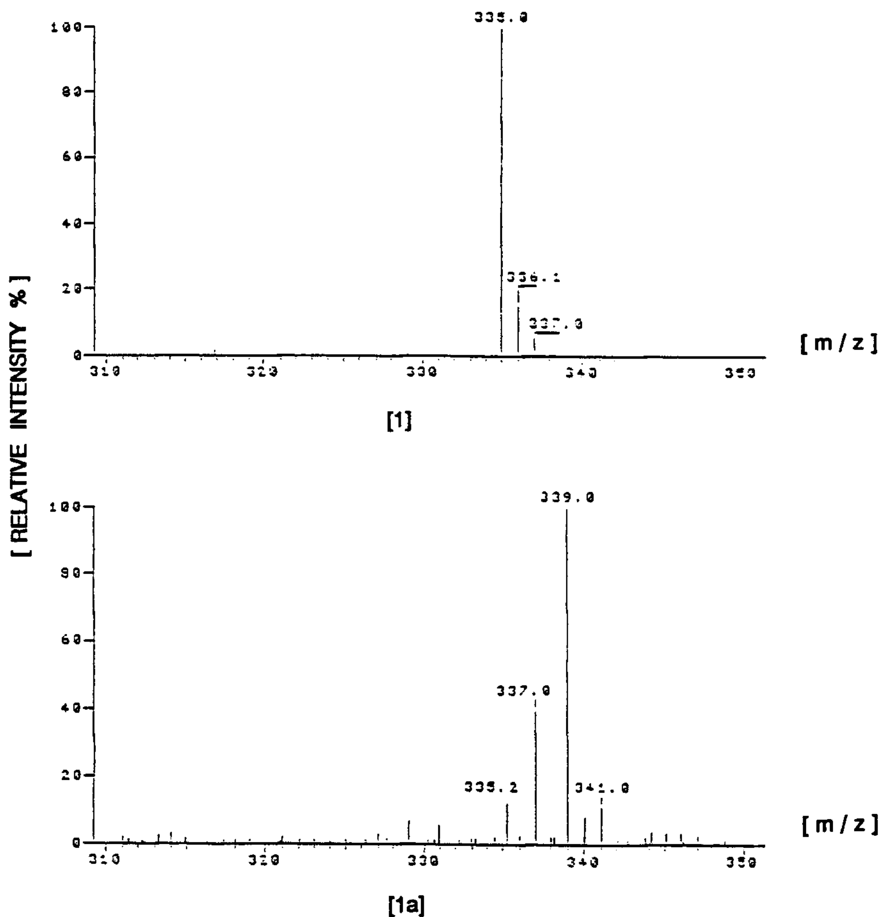


Figure 2 : MS-Spectrum of reference sample of NNC 07-9202 [1] and ^{14}C-NNC 07-9202 [1a]



After HPLC purification, the radiochemical purity was >98%, and the specific activity 10 Ci/mmol determined by HPLC, with reference standard.

EXPERIMENTAL

(2,3-^{14}C)-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [3].

30 mCi ^{14}C-oxalic acid (0.29 mmol, specific activity given by Amersham 103 mCi/mmol) was dissolved in 1.0 ml water. To the stirred solution was added 79.5 mg (0.29 mmol) 1,2-diamino-5-sulfamoyl-naphthalene hydrochloride[10] and 1.0 ml 4.0 N HCl.

The mixture was stirred in a sealed vial for 3 hours at 100 - 105 °C. The vial was cooled to 5 °C overnight. The mixture was centrifuged, and the liquid layer removed. The dark solid was washed twice with cold water (2 x 2.0 ml), centrifuged and dried at RT at reduced pressure.

Yield: 70.1 mg dark solid. Radiochemical purity 95.5% determined by radio-HPLC analysis.

(2,3-^{14}C)-6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1a].

70.1 mg crude ^{14}C-labelled quinoxaline [3] was dissolved in 1.3 ml concentrated sulfuric acid. The stirred solution was cooled in an ice bath for 5 minutes and 11.3 μl concentrated nitric acid in 300 μl concentrated sulfuric acid was added over a period of 2 minutes.

The mixture was stirred for one hour in an ice bath and poured into 5.0 g stirred ice/water mixture. After 10 minutes the mixture was centrifuged at 2-5°C. The liquid layer was removed and the dark precipitated solid was washed twice with water (2 x 2.0 ml).

Radio-HPLC showed that the combined aqueous liquids contained considerable amounts of product. These liquids were extracted with acetonitrile (2 x 20 ml) after addition of 10 ml brine. The organic phases was concentrated to a dark solid, which was dissolved together with the precipitated solid in DMSO/water (total volume 2.6 ml).

The product was purified by reverse phase HPLC using a 250 x 16 mm C-18 column. The eluent was removed under reduced pressure and a total of 5.5 mCi of product was achieved as a yellow solid. The product was dissolved in 55% ethanol in 0.015 N NaOH. The radiochemical purity was determined to be >99% by radio-HPLC analysis. The column

retention time of the labelled product corresponded with the reference standard. The specific activity was 99 mCi/mmol determined by MS.

(10-^{3}H)-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [5b].

1.40 mg (3.77 μmol) 10-bromo-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [4] was dissolved in 1.0 ml DMF in a 5 ml vial. 0.10 ml TEA was added together with 2 mg of PdO. The vial was cooled in a dry ice/acetone bath and connected to a sealed container with 1 Ci of tritium gas.

The system was evacuated and the seal broken with a magnetic hammer. The mixture was stirred in the presence of tritium gas for 2 hours at 50°C. DMF was evaporated at reduced pressure and the remaining tritiated material dissolved in 2.0 ml absolute ethanol.

Yield: 30 mCi of crude [5b]. Radiochemical purity 80 % by radio-HPLC analysis and the specific activity determined to be 11 Ci/mmol by HPLC with reference standard.

(10-^{3}H)-6-nitro-7-sulfamoylbenzo[f]quinoxalin-2,3-dione [1b].

7.5 mCi crude [5b] was dissolved in 0.20 ml concentrated sulfuric acid. 40 μl of a 0.1 vol % nitric acid in concentrated sulfuric acid was added to the stirred mixture. Heating and addition of 20 μl of a similar nitration mixture starting with pure [5a] was needed to start the reaction.

The mixture was stirred 2 hours at RT. 8.0 ml of water was added and pH adjusted to 4 by NaOH (aq). The aqueous phase was saturated with NaCl, and extracted several times with acetonitrile.

The combined organic layers were stored at -22 °C for some days. The precipitated salts were removed and the organic phase was evaporated at reduced pressure.

The remaining tritiated material was dissolved in 0.40 ml DMSO/water mixture and purified by reverse phase HPLC using a 250 x 4.6 mm C-18 column.

2.1 mCi of [1b] was achieved. The radiochemical purity was determined to be >98% by radio-HPLC analysis. The specific activity was determined to be 10 Ci/mmol by HPLC with reference standard.

High Performance Liquid Chromatography

HPLC analyses were performed using a Merck HPLC pump L-6200 with a Rheodyne Injector (20 μl loop) and a Merck UV-detector L-4000 (operated at 220 nm).
Separations were accomplished with a Novo C-18 column (250 x 4.6 mm) at RT, using an eluent of 92% trifluoroacetic acid (0.1%, pH 2.0) and 8% tetrahydrofuran. The flow rate was 1.0 ml/min. Radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One Beta detector A-200, using a 500 μl liquid flow cell. The ratio of column effluent to liquid scintillator (Pico-aqua^{TM}, Packard) was 1:3. Data collection was done by FLO-ONE/ Data- software on a PC-XT computer.

Radioactivity counting

Determination of total radioactivity was done on a Packard 2000 CA tri-carb liquid scintillation analyzer, using 20 ml counting vials and Pico-aqua^{TM}, Packard liquid scintillator.

MS

The mass spectrometer was a Finnigan-MAT TSQ 70B triple-quadropole instrument equipped with a Finnigan-MAT thermospray ion source, interface and controller.
TSMS operating conditions were as follows: Vaporizer, 110°C, block, 240°C, manifold pressure $3x10^{-5}$ torr, scan range, m/z 310 to m/z 350, scan time 0.50 s., electron multiplier set to 1800 V. The TS source was operated in the negative ion, "filament off" and "discharge off" mode.
The quadropole filters were optimised on the system ions m/z 113, 227, 300, 385 and 401. The mobile phase was 0.04% trifluoroacetic acid/tetrahydrofuran, 88/12 delivered at 1 ml/min. The make-up flow was 0.5 ml/min. 1% Diethylamin.
Samples were injected as loop-injection.

Chemicals, Reagents and Columns

[U-^{14}C] oxalic acid: spec. act. 103 mCi/mmol Amersham CFQ 6281.

$^{3}H_2$-gas spec.act. 58 Ci/mmol, NEN, NET-092.

1,2-diamino-5-sulfamoylnaphthalene, hydrochloride: Novo Nordisk, CNS Division.

10-bromo-7-sulfamoylbenzo[f]-quinoxalin-2,3-dione: Novo Nordisk, CNS Division.

Nitric acid: Merck p.a. (100%) Art. 455.

Sulfuric acid: Merck p.a. (95-97%) Art. 731.

4.0 N hydrogenchloride: Prepared from Merck titrisol® (9970).

Trifluoroacetic acid: Janssen (99%) Art. 13.972.04.

Tetrahydrofuran: Merck, Lichrosolv®, Art. 8101.

Dimethylsulfoxide: Merck Art. 802912.

5.0 N Sodiumhydroxide: Prepared from Merck titrisol® (9956).

Ethanol: DDSF, 99.9%.

Water: All water used was Millipore filtered by Milli Q water system.

HPLC column 250 x 4.6 mm: Novo C-18, 120 Å, 5 μm.

HPLC column 250 x 16 mm: Novo C-18, 100 Å, 7 μm.

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