SYNTHESIS OF [^{3}H] QUINOCETONE

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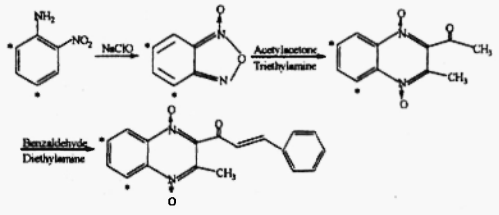
Abatract : [^{3}H]Quinocetone was prepared from [^{3}H]o-nitroaniline with a radiochemical yield of 59.2%. [^{3}H]o-nitroaniline, as starting material, was oxidized with NaClO to [^{3}H]benzofurazan oxide. [^{3}H]Benzofurazan oxide was recombined with acetylacetone to 3-methyl-2-acetyl-quinoxaline-1,4-dioxide with [^{3}H] in parent structure. In the last reaction, 3-methyl-2-acetyl-quinoxaline- 1,4-dioxide with [^{3}H] was condensed with benzaldehyde to the title compound-[^{3}H]Quinocetone. The specific radioactivity of the labeled product was 12.14mCi/mmol and its radiochemical purity was >98%.

Introduction

Quinocetone is called 3-methyl-2-cinnamicacyl-quinoxaline-1,4-dioxide. It has been used as a novel animal drug and as a feed additive for antibacterial growth promoters. Its pharmacology, toxicology, clinic characters were investigated previously[1-5]. Quinocetone may replace olaquindox and could be applied extensively in poultry and livestock farming. Moreover, Quinocetone (including its parent structure) meets the requirements of modern growth promoters. In order to investigate its absorption, distribution and excretion in animals in the clinical practice, quinocetone labeled with ^{3}H in parent structure was synthesized as a radioactive tracer. The synthetic route was chosen in the following way: Firstly, [^{3}H]o-nitroaniline, as starting material, was oxidized with NaClO to [^{3}H]Benzofurazan oxide. [^{3}H]Benzofurazan oxide was recombined with acetylacetone to 3-methyl-2-acetyl-quinoxaline-1,4-dioxide with [^{3}H] in parent structure. In the last reaction, 3-methyl-2-acetyl-quinoxaline-1,4-dioxide with [^{3}H] was condensed with benzaldehyde to



the title compound - [^{3}H]Quinocetone. The scheme of synthesis is presented in Figure-1.

Remark : * represents possible site of tritium

Fig-1 : Scheme of the synthesis of [^{3}H]Quinocetone

Experimental

All chemicals were obtained commercially at the highest available purity. Chemical analysis was carried out using a Waters 510 HPLC system. TLC analysis was carried out using Merck silica gel coated glass plates. Radiochemical purity was recorded on a thin layer radioscanner Model RTLS-A. Radioactive samples were counted with a liquid scintillation counter, Model LKB-12D. IR spectra were obtained in Thermo Nicolet Nexus FTIR-470 Spectrometer for KBr pellets.

Radiosynthesis of [^{3}H] Benzofurazan oxide (BFO) [6]

A solution of sodium hypochlorite was prepared immediately before it was to be used. A mixture of 50g(1.25 mol) of sodium hydroxide and 200ml of water was swirled until the solid dissolved. The solution was cooled to 0°C, and 100g of crushed ice was added. The flask was then placed in an ice bath, and chlorine gas from a tank was bubbled through the solution until 41g(0.58mol) was absorbed. An excess of chlorine should be avoided. The solution of sodium hypochlorite was kept in the dark at 0°C until needed.

A mixture of potassium hydroxide and 95% ethanol in a flask was heated on a steam bath until the solid dissolved. [^{3}H]O-nitroaniline was dissolved in the warm alkalic solution. The resulting deep red solution was then cooled to 0°C, and the sodium hypochlorite solution was added slowly with good stirring over the course of 10 minutes. After half an hour, the flocculent yellow precipitate was collected on a Buchner funnel, washed with 20mL of water, and air-dried for 3 hours at 40°C. The crude product was purified by recrystallization from a solution made up from 95% ethanol and water (3:1, V/V). Material insoluble in the hot solvent was removed by filtration, and the hot filtrate was allowed to cool to room temperature. The yield of yellow [^{3}H]benzofurazan oxide was above 70%, m.p. 72~73°C, as shown in Table-1.

Table 1 : The results of synthesizing benzofurazan oxide (abbr. BFO) at different conditions

o-nitroaniline mmol	mci	NaClO mmol	Chemical yield mmol	%	Radiochemical yield %
12	0.50	24	9.6	80.0	81.8
9.2	59.3	19.0	6.9	75.4	----

Radiosynthesis of [^{3}H]3-methyl-2-acetyl-quinoxaline-1,4-dioxide(abbr. MAQO) [7-8]

[4,5]benzofurazan oxide was measured accurately and dissolved in certain quantity of acetylacetone and anhydrous ethanol under heating condition. After triethylamine was added, the solution continued to be swirled for 30 minutes at 40~50℃. When the solution was cooled to about 35℃, the precipitate appeared. After 30mL of methanol was added and the solution was placed for 7 hours, the precipitate was filtrated and washed with 30mL of methanol. The yellow [^{3}H]MAQO was air-dried for 1 hours at 40~60℃, m.p.155~156℃. The obtained results were shown as table 2.

Table 2 The results of synthesizing 3-methyl-2-acetyl-quinoxaline-1,4-dioxide (abbr. MAQO) at different conditions

BFO		Acetylacetone	Triethylamine	Chemical Yield		Radiochemical yield	
mmol	mci	ml	ml	mmol	%	mci	%
9.6	0.409	1.92	2.60	6.4	66.7	0.26	63.5
6.9		1.43	1.89	3.3	47.9	----	

Radiosynthesis of [^{3}H]quinocetone

[^{3}H] MAQO was added to a solution of benzaldehyde (molar ratio of MAQO and benzaldehyde was 1:2) in 10ml anhydrous ethanol under electromagnetic stirring and heating. After the solid dissolved, the solution was cooled to 30~50℃ and then diethylamine(molar ratio of diethylamine and benzaldehyde was 1:2) was added. The solution reacted at approximately 40.0°C for 3 hours and placed at room temperature for 24 hours. The precipitate was filtrated and wash with 95% ethanol until no red color material existed.

The product obtained was characterized by chromatography with its authentic sample and its radiochemical purity was checked by sillica gel thin layer chromatography followed by autoradiography and TLC scanning. The following solvent system was used for chromatography: $CH_3CO_2C_2H_5$: $CHCl_3$ (1:1v/v).

A single compact spot on the autoradiography and a single peak in the TLC radiochromatogram scan were obtained which corresponded to the spot of the authentic sample, as revealed by I_2 spot detection test, showing that the product was radiochemically pure (R_f=0.58). The radiochemical purity and chemical purity of [^{3}H] quinocetone were

estimated to be higher than 98% and 99%, respectively. The results obtained from two similar experiments are shown in table 3. [^{3}H]Quinocetone(2.9mmol, 35.2mCi, 12.14mCi/mmol) was obtained from [^{3}H] o-nitroaniline with a radiochemical and chemical yield of about 59% and 31%, respectively.

Table 3 : The results of synthesizing quinocetone at different conditions

MAQO mmol mci	Chemical yield mmol %	Chemical yield (from o-nitroaniline) %	Radiochemical yield mci %	Radiochemical yield (from o-nitroaniline) %
6.4 0.26	5.6 87.5	46.7	0.22 84.6	44.4
3.3	2.9 87.4	31.6	35.2	59.2

Results and Discussion

The key step in this experiment was the synthesis of [^{3}H] MAQO. MAQO was categorized to quinoxalines. There were several methods for the synthesis of quinoxalines, two of which were important[9-11]. Firstly, o-nitroaniline, as starting material, was oxidized to benzofurazan oxide and then condensed with β-diketone compound orβ-ketone acid compound directly to quinoxaline-N,N-dioxide. The quinoxaline-N,N-dioxide was reduced to corresponding quinoxaline. Secondly, o-phenylenediamine, as starting material, was reacted with α -bromine or hydroxyimino substituted β-diketone compound orβ-ketone acid compound to quinoxaline compound. The quinoxaline compound was oxidized to corresponding quinoxaline-N,N-dioxide. Based on available materials, feasible work and higher yield, the synthesis route from o-nitroaniline was selected.

Radiosynthesis of [^{3}H] benzofurazan oxide (BFO)

The synthesis of [^{3}H] benzofurazan oxide from o-nitroaniline has been investigated. Many oxidants may be selected and haloid was one of them. Hypochlorite and bromate were used in general. For cheap and available materials, sodium hypochlorite was adapted. However, the temperature of reactive mixture should be kept close to 0°C to avoid decomposition of the sodium hypochlorite and prevent formation of tarry materials that occurs at 10~12°C.

Radiosynthesis of [^{3}H] MAQO

Standardization of the synthesis of [^{3}H] MAQO was carried out on a ten millimolar scale based on BFO. Separation was achieved on a Nova-pak C_{18} (5μm; 4.6mm×250mmi.d.)

ıatographical column (Waters), operated at 30.0±0.5°C temperature. The mobile phase consisted of methanol-water (20:80, v/v). The UV detector wavelength was 229nm and the flow rate was 1.0 ml·min^{-1}.

The different molar ratios between different reagents or solvents were tested, shown as table 4. The results showed that the molar optimized ratio between BFO and acetylacetone was 1:2 in complete reaction of BFO, while that between triethylamine and acetylacetone was 1:1. Although the quantity of acetylacetone may be so more to act as solvent of BFO, the yield of MAQO will reduce. When the reactive solid didn't dissolve, appropriate anhydrous ethanol may be added to accomplish solution.

Table 4 : The results of synthesizing 3-methyl-2-acetyl-quinoxaline-1,4-dioxide (abbr. MAQO) at different reagents or solvents

BFO mmol	Acetylacetone mmol	Triethylamine mmol	Anhydrous ethanol ml	MAQO mmol	Chemical yield %
10	15	15	2	5.81	58.1
10	15	20	2	5.75	57.5
10	20	15	2	5.23	52.3
10	20	20	2	6.01	60.1
10	30	30	2	4.60	46.0
10	30	30	0	4.90	49.0

Radiosynthesis of [^{3}H] quinocetone

The results from the two experiments are shown in Table3. The product obtained from each experiment was characterized and its chemical purity was checked by silica gel thin layer chromatography using the solvent systems indicted above. The IR spectrum of [^{3}H] quinocetone was also the same as the IR spectrum of the authentic standard. Ethanol as reaction solvent was important for high yield of [^{3}H] quinocetone[12]. To make MAQO react completely, many preparation tests were made to set up the molar ratio of materials. The optimized molar ratio between MAQO and benzaldehyde was 1:2, and that between diethylamine and benzaldehyde as well. When the weight of MAQO was no more than 1.0g, 10 ml anhydrous ethanol as solvent was better.

Based on the yield of quinocetone from o-nitroaniline, it was concluded that o-nitroaniline, BFO and MAQO with tritium might be more reactive than the same compounds without tritium.

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