SYNTHESIS OF TRITIUM-LABELLED IOHEXOL

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SUMMARY

In an efficient three-step synthesis [^{3}H] glycerol was converted into α-tosyl- [^{3}H] glycerol (yield: 77%), from which [^{3}H] iohexol was prepared in an overall radiochemical yield of 25% and a specific activity of 400 μCi/mmol. Performing the synthesis on a microscale, the overall yield decreased to only 4%, but a 99% pure [^{3}H] iohexol with a specific activity of 1 Ci/mmol was obtained.

Key words: Iohexol, Contrast agent, [^{3}H]-α-Glyceryl-p-toluenesulfonate.

INTRODUCTION

The use of non-ionic water soluble contrast media for myelography is universally accepted in neuroradiology, because of their low neurotoxicity in comparison to ionized contrast media (1).
Still some side-effects have been reported such as headache and nausea (25%) and less common psychological symptoms (2-5%) as delusion, sensory disturbance, derealization and depersonalization (2).

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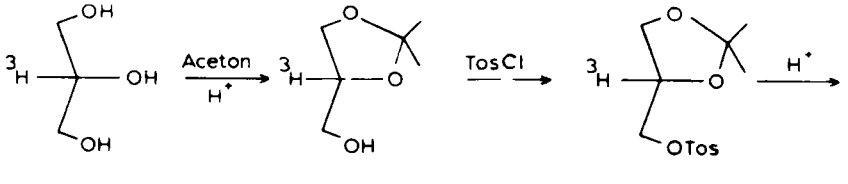
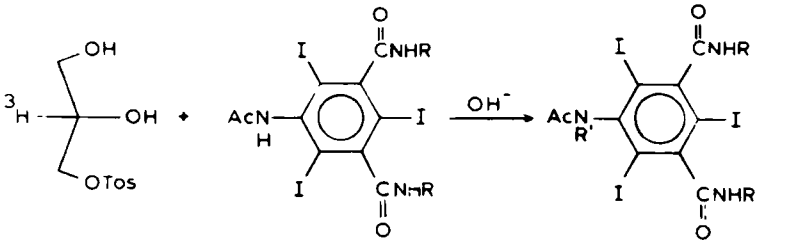
In studies using computerized tomography (CT) it has been noticed that there is a relatively large uptake of non-ionic contrast agents in the brain with a constant distribution and wash-out pattern.
To obtain more detailed information about the uptake and distribution of non-ionized contrast media in the brain, iohexol was labelled with tritium, so that autoradiography could be performed on rabbit brain tissue.

RESULTS AND DISCUSSION

The synthesis of α-tosyl-[^{3}H]glycerol was performed by a modified procedure of Van Lohuizen *et al.*(3). One gram of 2-[^{3}H]glycerol (1) was converted with acetone in the presence of p-toluenesulfonic acid and molecular sieves into the acetonide 2, which was then allowed to react with p-toluenesulfonylchloride to give the tosylate 3.



1 2 3



4 5 6

R = $CH_2CHOHCH_2OH$ R' = $CH_2C^3HOHCH_2OH$

Synthesis of [^{3}H] iohexol

Treatment of 3 in methanol with trifluoroacetic acid afforded α-tosyl-[2-^{3}H]glycerol (4) in an overall yield of 77%.

Performing the same sequence of reactions on a microscale (0.05 mmol [2-^{3}H]glycerol, specific activity 1 Ci/mmol) gave 4 in an overall radiochemical yield of 30%.

Subsequently, α-tosyl-[^{3}H]glycerol was reacted with the sodium salt of 5 in cellosolve to give [^{3}H]iohexol (6) in 33% yield.

This reaction, which presumably proceeds through an epoxide intermediate (4) was very difficult to perform on a microscale (yield only 13%). However, a product with high specific activity (1 Ci/mmol), essential to measure possible receptor interactions, was obtained. Since it is known, from studies on labelled misonidazole (5), that ^{3}H-exchange does not occur under physiological conditions or at acidic or basic pHs, tritium-labelled iohexol (1 Ci/mmol) was injected intrathecally via the suboccipitally approach in Dutch rabbits. The animals were sacrificed after 3 and 6 hours, and distribution of [^{3}H]iohexol in the brain was studied by autoradiography. Preliminary results have shown that iohexol penetrates the grey matter, cerebellar cortex and the deep nuclei (6).

A study of series of tests in a concentration/volume paradigm and the extension of the distribution at the microscopic level is in progress.

EXPERIMENTAL

Radioactivity was measured by liquid scintillation counting using a Packard Model 2450 Tri-Carb Liquid Scintillation Spectrometer and appropriate standards. High performance liquid chromatography was carried out on a reversed-phase C-18 column, using a gradient of 1-13% CH_3CN/H_2O in 1 hour and a flow rate of 1 ml/min. This HPLC-system was used for the determination of the radiochemical purity of 6, and also for its preparative isolation when the reaction was performed on a microscale.

Thin layer chromatography (TLC) was carried out on silicagel F-254 plates (thickness 0.2 mm, Merck 5554).

[2-^{3}H]-α-glyceryl-p-toluenesulfonate (4)

From the commercially available solution of [^{3}H]glycerol (Amersham International plc) the ethanol was evaporated by a stream of dry air. To this was added 1 g of non- radioactive glycerol, followed by 5 ml of acetone, 10 mg of p-toluenesulfonic acid and 2 g of mol. sieves (4Å). Stirring was continued for 16 hours (according to GLC the reaction is nearly complete after 3 hours) after which MgO (excess) was added. After filtration and evaporation of the solvent, the residue was dissolved in 4 ml of pyridine, cooled to 0°C, and 2.17 g of p-toluenesulfonyl chloride was added. After 2 hours the pyridine was evaporated, the residue dissolved in 25 ml of dichloromethane and washed with 25 ml of 0.1 N HCl. Chromatography on silica with dichloromethane/hexane (3:7) as eluent, gave 2.66 g (85%) of 3 ($R_f(CH_2Cl_2)$: 0.62). Compound 3 was dissolved in 1 ml of methanol to which 1 ml of trifluoroacetic acid was added. After complete deketalization (followed by TLC, silica, CH_2Cl_2 or ethyl acetate) 25 ml of water, saturated with NaCl, was added, followed by 50 ml of ethyl acetate. The organic layer was separated, dried (Na_2SO_4) and concentrated to give 2.06 g of 4, as an oily compound which solidified on standing in a refrigerator. The overall yield from glycerol was 77%.

When the reaction was performed on a microscale, the solvent and trifluoroacetic acid were evaporated and the crude product 4 was used directly in the final step.

[^{3}H]iohexol

With vigorous stirring 4.6 g of compound 5 and 345 mg of NaOH were dissolved in 14 ml of cellosolve, keeping the temperature between 35-40°C. After complete dissolution (about 5 hours) 2.06 g of 4 in 4 ml of

cellosolve was added at once. Stirring was continued for 18 hours, after which the reaction was quenched by adding 0.3 ml of concentrated hydrochloric acid.

The reaction mixture was then concentrated *in vacuo* to a sirupy oil, and subsequently diluted with 7 ml of methanol. The mixture was stirred for 1 hour and sedimentation allowed for 4 hours which gave a solution which was then decanted onto a suction filter. The methanol solution was added over a period of 1 hour to 7 ml of vigorously stirred isopropanol, and the stirring was continued for 4 hours. Filtration and drying *in vacuo* gave the crude product. This was taken up in 30 ml of methanol/water (8:2) and treated with an excess of a desalting ion-exchange resin (Merck V). The solution was stirred for 3 hours, and then filtered. The ion-exchange resin was washed with 25 ml of water/methanol (1:1). Evaporation and drying *in vacuo* gave the salt-free product, which was recrystallized from refluxing isopropanol (reflux time 44 hours), yielding 2.2 g of 6 (33%).

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