6-[^{125}I]-Iodo-2-methyl-1,4-naphthoquinol Bis(diammonium phosphate): a New Potential Anti-tumour Drug

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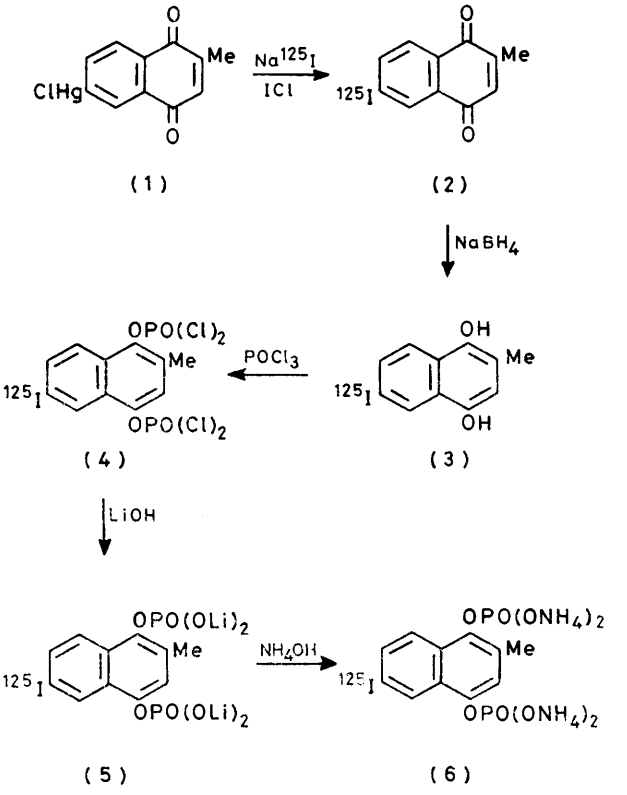
Summary 6-[^{125}I]-Iodo-2-methyl-1,4-naphthoquinol bis-(diammonium phosphate) has been prepared as a potential radiochemotherapeutic drug with a specific activity of 1·82 mCi/mg; laboratory studies have shown comparable uptake and cytotoxicity for tumour cells cultured *in vitro* under anoxic and euoxic conditions.

IODINE-125, from the point of view of both its half-life (60 days) and manner of decay, appears eminently suitable as a radionuclide for incorporation into organic compounds for therapeutic purposes. It decays by electron capture, followed by the emission of low-energy Auger electrons with subcellular ranges.[1] Microdosimetric calculations have indicated that, in small target spheres of diameter <50 nm, more energy is, on average, deposited by a decaying iodine-125 atom than by a high LET α-particle traversing an identical sphere.[2] This may well explain its extreme biological toxicity to DNA in microbial[3] and tumour cells.[4]

Substituted naphthoquinol diphosphates are known to be selectively taken up by some malignant tumour cells;[5] in particular, Synkavit[6] can enhance the effect of *X*-irradiation both *in vitro*[7] and *in vivo*.[8,9] Tritiated and iodinated (^{131}I) derivatives[10] have been used to some effect in palliative therapy and scanning for certain advanced human tumours.[11,12] Here, we have incorporated iodine-125 into the unsubstituted aromatic ring of 2-methyl-1,4-naphthoquinol bis(diammonium phosphate), with the aim of producing an effective radiochemotherapeutic drug for occult metastases.

The synthesis of 6-[^{125}I]-iodo-2-methyl-1,4-naphthoquinol bis(diammonium phosphate) (**6**) is outlined in the Scheme. An ethanolic suspension of 6-chloromercuri-2-methyl-1,4-naphthoquinone (**1**)[13] (20 mg) was refluxed with iodine-125 (70 mCi) and ICl (9 mg) for 1½ h. After filtration, (**2**) was precipitated by the addition of excess of



SCHEME

KI solution (7% w/v), then washed. The radiochemical yield was 37—48%. The product (**2**) was dissolved in MeOH (5 ml) and reduced by $NaBH_4$ (15 mg) in the presence of Dowex 50-W-X8 (H^+) resin. The filtrate was

evaporated to dryness;† toluene (1 ml) was added, and the mixture then re-evaporated. Compound (**3**) was dissolved in dry pyridine (2 ml), and phosphorylated by the dropwise addition of $POCl_3$ (0·1 ml) at 4 °C. The solution was evaporated to a semi-solid residue (**4**). A mixture (12 ml) of CH_2Cl_2–H_2O–sat. LiOH solution (11 : 11 : 2) was added and the mixture vigorously stirred; the pH was maintained at 8—11. The separated aqueous phase contained 60—80% remaining activity; this was passed down a column of Dowex 50-W-X8 (H^+) resin and collected, finally under mild suction. The column was then washed with EtOH (10 ml). T.l.c. [cellulose; Bu^nOH–H_2O–$MeCO_2H$ (5 : 3 : 2)] was performed on samples from both solutions; the R_F value for the bis(dihydrogen phosphate) of (**6**) is 0·45—0·50. Aqueous and ethanolic solutions were evaporated to dryness; a few drops of distilled water were added to each. If the t.l.c. was satisfactory, both solutions were mixed and NH_4OH (*d* 0·880) was added until the pH was 8—9. The final solution contained 6-[^{125}I]-iodo-2-methyl-1,4-naphthoquinol, polyphosphate complexes, and (**6**).

Separation and purification of (**6**) was carried out by paper chromatography (Whatman 3MM); downward elution with Bu^nOH–H_2O–$MeCO_2H$ for 16 h. Autoradiography enabled isolation of (**6**) (R_F *ca.* 0·5); this was eluted with distilled water. The total activity was 4·5—6·3 mCi (radiochemical yield 6·4—9·0%); u.v.: λ_{max} (0·01 M HCl) 230 (ϵ 35,800), 247 (39,900), and 290 nm (5,250). The specific activity of (**6**) was 1·32—1·82 mCi/mg from 70 mCi of iodine-125.

Safety: syntheses were performed in a grade B laboratory[14] The Radiation Centre, Birmingham University, over periods of 4—5 days. All stages were carried out in a ventilated enclosure; apparatus was shielded by 5 cm thick lead bricks. Baseline thyroid uptake measurements were carried out daily. Prophylactic KI (120 mg/day) was taken orally. There was no measurable uptake of iodine-125 into the thyroid gland. Total exposure to the torso ($\leqslant$160 mrem) and finger tips ($\leqslant$120 mrem) was minimal. Total body counting is advisable if repetitive syntheses are to be undertaken.

Preliminary experiments[15] on tumour cells (HEp2) cultured *in vitro* with (**6**) under anoxic and euoxic conditions showed a comparable cellular uptake with the behaviour of high LET radiation.

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† All evaporations carried out *in vacuo* (10 mTorr).

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