LABELLED COMPOUNDS OF INTEREST AS ANTITUMOUR AGENTS - V[1]. SYNTHESES OF [^{18}O]-5-METHYLISOQUINOLINONE AND 1-(FURAN-2-YL-[^{18}O]-METHOXY)-5-METHYLISOQUINOLINE

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SUMMARY

Treatment of 2-methylcinnamic acid with $H_2{}^{18}O$ at 100°C under acidic conditions leads to high incorporation of ^{18}O by exchange. Methods have been developed for chemically and isotopically efficient conversion to the corresponding [carbonyl-^{18}O] methyl ester, to [^{18}O]-5-methylisoquinolinone (an inhibitor of DNA repair) and to 1-(furan-2-yl-[^{18}O]-methoxy)-5-methylisoquinoline.

Introduction

The enzyme poly(ADP-ribose)polymerase (PARP, EC 2.4.2.30) catalyses the transfer of ADP-ribose units from NAD^+ to form a polyanionic polymer on histones and other acceptor proteins near the site of damage to DNA[2-4]. Inhibition of PARP leads to inhibition of the processes of repair of damaged sites in DNA[5-7] and thus to potentiation of the antitumour effects of many forms of radiotherapy[8-11] and chemotherapy[12-14]. Potent inhibitors of PARP include benzamides and analogues in which the conformation of the amide is constrained relative to the aromatic ring, by incorporation into a lactam[8,15,16] or by hydrogen-bonding[16]. Of these lactams, 5-substituted isoquinolinones have been shown[8,15] to be among the inhibitors with the greatest potency. During our studies on drugs which sensitise tumours to the cytotoxic effects of radiotherapy and chemotherapy[17], we required a 5-substituted isoquinolinone PARP inhibitor labelled with ^{18}O at the carbonyl oxygen. In this paper, we report our development of syntheses of methyl [carbonyl-^{18}O]-*E*-3-(2-methylphenyl)propenoate **4**, a model intermediate to check satisfactory isotopic incorporation, of [^{18}O]-5-methylisoquinolinone **7** and of a potential pro-drug derivative, 1-(furan-2-yl-[^{18}O]-methoxy)-5-methylisoquinoline **10**.

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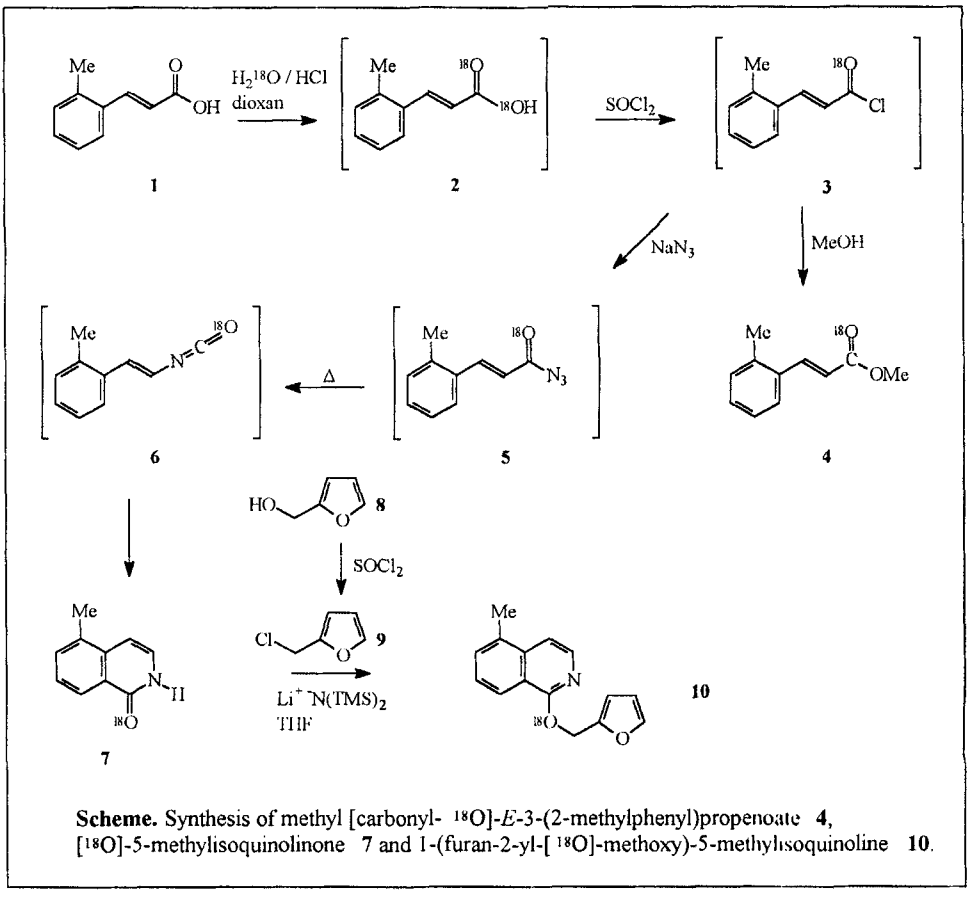
Results & Discussion

A convenient synthesis of isoquinolinones by Curtius rearrangement of cinnamyl azides and thermal cyclisation, in a one-pot process, has been described by Eloy and Deryckere[18]. The Curtius reaction involves a true rearrangement of the intermediate acyl nitrene to form the isocyanate. Thus ^{18}O present in the carbonyl group should be retained in the isocyanate. The subsequent mechanistic steps, thermal isomerisation of the C=C and cyclisation, also take place under conditions where the oxygen atom should be retained in the isoquinolinone. The synthetic route, therefore, requires the ^{18}O-labelled 2-methylcinnamyl azide **5** as a starting material for the synthesis of the ^{18}O-labelled 5-methylisoquinolinone **7**.

We considered that **5**, in turn could be derived from the corresponding ^{18}O-labelled acid chloride **3** and the ^{18}O-labelled carboxylic acid **2**. An initial series of model experiments was devised to develop a procedure for incorporation of ^{18}O into 2-methylcinnamic acid **1**, forming **2**, and for conversion to the acid chloride **3**, under conditions which would not permit loss of ^{18}O by exchange. ^{18}O was incorporated by heating the carboxylic acid **1** in 1,4-dioxan with twenty equivalents of $H_2{}^{18}O$ under acidic conditions to promote exchange. The solvent and excess water were removed by distillation in a closed system to give the isotopomer **2**. Since this acid is capable of losing ^{18}O by exchange with atmospheric water, it was rapidly converted to the corresponding acid chloride **3** with thionyl chloride To check the level of incorporation of ^{18}O, the acid chloride was quenched with methanol. Mass spectrometry showed the ester **4** to contain 8.0 ± 0.7% ^{18}O in the carbonyl group, confirming that the exchange process had proceeded satisfactorily and that little ^{18}O had been lost during the subsequent reactions.

Now that the initial exchange reaction had been developed and conversion to the acid chloride **3** had been established, attention was focussed on the main isoquinolinone-forming process. The ^{18}O-labelled acid chloride **3** was prepared as before but on a multimillimole scale The acid chloride **3** should not be subject to exchange of ^{18}O under aqueous conditions without hydrolysis, so conversion to the acyl azide **4** was effected by treatment with sodium azide in aqueous acetone. Any material which may have been hydrolysed to the acid would not be converted to the acyl azide by this process. The Curtius reaction was carried out in boiling diphenyl ether at *ca.* 260°C, forming the isocyanate **6**, which was isomerised $E \rightarrow Z$ and cyclised under the same conditions, giving the ^{18}O-labelled 5-methylisoquinolinone **7** in good yield. Again, the isotopic composition was determined by mass spectrometry; **7** was shown to contain 8.0% ^{18}O at the carbonyl oxygen. This represents the same incorporation as was seen for the methyl 2-methylcinnamate **4**, indicating no loss of ^{18}O in steps after the acid chloride.



Scheme. Synthesis of methyl [carbonyl- ^{18}O]-*E*-3-(2-methylphenyl)propenoate **4**, [^{18}O]-5-methylisoquinolinone **7** and 1-(furan-2-yl-[^{18}O]-methoxy)-5-methylisoquinoline **10**.

For the synthesis of the *O*-furanylmethyl derivative **10**, two synthetic approaches were possible; (*i*) reaction of furan-2-[^{18}O]-methanol, as its alkoxide, with 1-chloro-5-methylisoquinoline or (*ii*) alkylation of the anion of the isoquinolinone **7** with an electrophilic furanylmethyl compound. The former was discounted, as this would require development of a synthesis of a new ^{18}O-labelled intermediate, the furanmethanol. Nevertheless, the reaction of unlabelled sodium furanmethoxide with 1-chloroisoquinoline was investigated but was found to lead only to destruction of the furan at the high temperature of the reaction. The second approach also presented some problems, as furanylmethyl electrophiles are notoriously unstable.

2-Chloromethylfuran **9** was prepared by treatment of the corresponding alcohol **8** with thionyl chloride by the general method of Tarrago *et al*[19]. This unstable material was allowed to react with the lithium anion of the ^{18}O-labelled 5-methylisoquinolinone **7** at reflux in tetrahydrofuran. To counteract the loss of **8** by decomposition during this alkylation, it was used in large excess. The ^{18}O-labelled furanylmethoxyisoquinoline **10** was obtained in good chemical yield and with excellent

isotopic enrichment (8.0% ^{18}O). The equivalence of the isotopic enrichment of **7** and **10** demonstrates that no loss of ^{18}O has occurred during the alkylation.

Conclusion

Efficient techniques have been developed for the incorporation of ^{18}O into a *Ar*-substituted cinnamic acid by exchange with $H_2{}^{18}O$ and for subsequent conversion to the carbonyl-^{18}O-labelled isoquinolinone **7** and the furanylmethyl ether **10**. These methods should be applicable to syntheses of other ^{18}O-labelled cinnamate esters and to other isoquinolinones which do not bear strong electron-withdrawing groups. The results of biological and biomimetic studies with **7** and **10** will be reported elsewhere.

Experimental

Jeol GX270 and EX400 instruments furnished the NMR spectra of solutions in $CDCl_3$; the internal standard was tetramethylsilane. Melting points are uncorrected. Solvents were evaporated under reduced pressure. The chromatographic stationary phase was silica gel. Brine refers to a saturated solution of sodium chloride in water. [^{18}O]-Water (10 atom %) was obtained from the Aldrich Chemical Company.

Methyl [carbonyl-^{18}O]-*E*-3-(2-methyl)phenylpropenoate (4). *E*-3-(2-methyl)phenylpropenoic acid **1** (53 mg, 330 μmol) was boiled under reflux with [^{18}O]-water (120 μl, 10 atom %) and hydrogen chloride (1.0 M in diethyl ether, 150 μl) in dry 1,4-dioxan (1.5 ml) for 24 h. The solvents and excess reagent were distilled off under nitrogen and the residue was stirred with thionyl chloride (3.0 ml) and dimethylformamide (10 μl) for 16 h. The excess reagent was evaporated. Methanol (2.0 ml) was added and the mixture was stirred for 1 h. Chromatography (ethyl acetate) yielded **4** (46 mg, 79%) as a pale yellow oil (lit.[20] unlabelled compound is an oil): δ_H 2.43 (3 H, s, Ar-Me), 3.81 (3 H, s, OMe), 6.36 (1 H, d, J = 15.9 Hz, HC=C), 7.18-7.28 (3 H, m, Ar 3,4,5-H_3), 7.54 (1 H, d, J = 7.0 Hz, Ar 6-H), 8.00 (1 H, d, J = 15.9 Hz, C=CH); δ_C 19.74, 51.62, 118.8, 126.29, 126.35, 129.97, 130.73, 133.32, 137.59, 142.49, 167.40; *m/z* (EI) 178 (2.7%) (M), 176 (33%) (M), 163 (2.0%) (M - Me), 161 (23%) (M - Me), 147 (9%) (M - MeO), 145 (90%) (M - MeO).

[^{18}O]-5-Methylisoquinolinone (7). *E*-3-(2-methyl)phenylpropenoic acid **1** (450 mg, 2.8 mmol) was boiled under reflux with [^{18}O]-water (1.00 ml, 10 atom %) and hydrogen chloride (1.0 M in diethyl ether, 1.5 ml) in dry 1,4-dioxan (15 ml) for 12 h. The solvents and excess reagent were distilled off under nitrogen and the residue was stirred with thionyl chloride (5 ml) and dimethylformamide

(10 μl) for 1.5 h. The excess reagent was evaporated. The acid chloride **3**, in acetone (5 ml), was added to sodium azide (630 mg, 9.7 mmol) in water (2 ml) and acetone (1 ml) at 0°C. The mixture was stirred for 30 min at this temperature. The acyl azide **5** was extracted with dichloromethane and was washed with brine and was dried ($MgSO_4$ / $CaCl_2$). Diphenyl ether (3 ml) was added and the dichloromethane was evaporated at ambient temperature. The residue was added to boiling diphenyl ether (10 ml) during 10 min and the solution was boiled under reflux for 2 h. Evaporation and chromatography (ethyl acetate / hexane 1:1) gave **7** (270 mg, 61%) as a pale yellow solid: mp 178-180°C (lit.[18] mp 184-185°C for the unlabelled compound); δ_H 2.55 (3 H, s, Me), 6.71 (1 H, d, J = 7.3 Hz, 4-H), 7.25 (d, J = 6.4 Hz, 3-H), 7.40 (1 H, dd, J = 7.9, 7.3 Hz, 7-H), 7.52 (1 H, d, J = 7.0 Hz, 6-H), 8.30 (1 H, d, J = 7.9 Hz, 8-H); δ_C 19.17, 103.47, 125.18, 126.10, 126.39, 127.47, 133.45, 137.18, 164.90 (one C_q was not observed); *m/z* (EI) 161.0726 ($C_{10}H_9N^{18}O$ requires 161.0727) (8.7%) (M), 159.0682 ($C_{10}H_9N^{16}O$ requires 159.0684) (100%) (M)

1-(Furan-2-yl-[^{18}O]-methoxy)-5-methylisoquinoline (10). To furan-2-methanol **8** (1.00 g, 10 mmol) in chloroform (10 ml) was added pyridine (1.5 ml). The solution was cooled to -10°C and thionyl chloride (2.0 ml) in chloroform (20 ml) was added. The mixture was stirred at this temperature under nitrogen for 3 h. Hydrochloric acid (10%, 10 ml, 0°C) was added. The organic phase was washed with hydrochloric acid (10%, 0°C) and with aqueous sodium hydroxide (3%, 0°C). The solution was dried ($MgSO_4$ / K_2CO_3) and the solvent was evaporated at ambient temperature to give **9** (1.07 g, 89%) as an unstable orange liquid. The [^{18}O]-isoquinolinone **7** (250 mg, 1.6 mmol), in tetrahydrofuran (25 ml), was treated with lithium hexamethyldisilazide (1.0 M in tetrahydrofuran, 2.0 ml) and the mixture was stirred at ambient temperature for 1 h. 2-Chloromethylfuran **9** (1.00 g, 8.6 mmol), in tetrahydrofuran (25 ml) was added dropwise during 1 h at 0°C, followed by sodium iodide (20 mg). The mixture was boiled under reflux for 18 h. The evaporation residue, in ethyl acetate, was washed with water and with brine and was dried ($MgSO_4$). Evaporation and chromatography (ethyl acetate / hexane 1:5) gave **10** (224 mg, 60%) as a pale yellow oil which crystallised on standing: mp 84-86°C; δ_H 2.51 (3 H, s, Me), 5.19 (2 H, s, CH_2), 6.33 (1 H, dd, J = 3.1, 1.9 Hz, furan 4-H), 6.42 (1 H, d, J = 3.3 Hz, furan 3-H), 6.61 (1 H, d, J = 7.7 Hz, isoquinoline 4-H), 7.21 (1 H, d, J = 7.7 Hz, isoquinoline 3-H), 7.36 (2 H, m, isoquinoline 7-H + furan 5-H), 7.46 (1 H, d, J = 7.2 Hz, isoquinoline 6-H), 8.32 (1 H, d, J = 8.1 Hz, isoquinoline 8-H); δ_C 18.93, 44.33, 103.02, 109.42, 110.64, 110.83, 125.97, 126.55, 130.54, 133.08, 133.17, 135.90, 142.77, 149.78, 162.1; *m/z* (EI) 241.0990 ($C_{15}H_{13}N^{16}O^{18}O$ requires 241.0989) (3.6%) (M), 239.0943 ($C_{15}H_{13}N^{16}O_2$ requires 239.0946) (40%) (M), 81 (100%) (furan-CH_2).

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