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Synthesis of ^{14}C-labelled phenethyl isothiocyanate and the cysteine conjugate S-(N-phenethylthiocarbamoyl)cysteine and use under physiological conditions

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SUMMARY

2-Phenyl-[1-^{14}C]-ethyl isothiocyanate and S-(N-phenyl-[1-^{14}C]-ethylthiocarbamoyl)cysteine were synthesised from 2-phenyl-[1-^{14}C]-ethylamine. Phenylethylamine was reacted with thiophosgene to make phenethyl isothiocyanate (yield: 35%, based on phenylethylamine). Phenethyl isothiocyanate was converted to the cysteine conjugate S-(N-phenethylthiocarbamoyl)cysteine by reaction with cysteine and crystallisation (yield: 86%, based on phenethyl isothiocyanate). The compounds have been used to study the cellular and subcellular labelling of tumour cells during the induction of apoptosis in human tumour cells by phenethyl isothiocyanate and S-(N-phenethylthiocarbamoyl)cysteine *in vitro*.

KEY WORDS: ^{14}C-phenethyl isothiocyanate, S-(N-phenethylthiocarbamoyl)cysteine, apoptosis.

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INTRODUCTION

Phenethyl isothiocyanate (PEITC) is formed from dietary glucosinolates. Glucosinolates are naturally occurring thioglucosides present in cruciferous vegetables, broccoli, cabbage, cauliflower, turnip, radish and watercress (1). They are degraded non-enzymatically and enzymatically by myrosinases during food preparation, cooking and chewing to isothiocyanates, thiocyanates, nitriles and epithiocyanoalkanes. One of the most prevalent glucosinolates is gluconasturtin that degrades to form PEITC. Dietary isothiocyanates such as PEITC and synthetic analogues have recently been of intense interest for their anti-carcinogenic activities and potential use in the chemoprevention of cancer (2). Chemopreventive activity is thought to be associated with inhibition of the metabolic activation of carcinogens by cytochrome P450 isozymes (3) and increased excretion of carcinogens by inducing increased activities of glutathione S-transferases involved in elimination of carcinogens by the mercapturic acid pathway (4,5). Cysteine adducts of dietary isothiocyanates, S-(N-thiocarbamoyl)cysteine derivatives have similar cancer chemopreventive activities (5,6). A further feature of the pharmacological activity of dietary isothiocyanates and related S-(N-thiocarbamoyl)cysteine derivatives was their anticancer activity *in vitro*.

PEITC inhibited the growth of human leukaemia 60 (HL60) cells *in vitro* and induced apoptosis. The median growth inhibitory concentration GC_{50} value was 1.49 μM. Closely related mercapturic acid metabolites of PEITC had similar antitumour activities. There was decreased toxicity to corresponding differentiated cells (7). These studies suggest that dietary isothiocyanates may have selective toxicity to the malignant phenotype (8). PEITC-induced apoptosis was characterised by activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1 (MEKK1), a sustained activation of stress-activated protein kinase c-Jun N-terminal kinase 1 (JNK1) (9), activation of caspases (10,11) and induction of oxidative stress (10).

In this note, we describe the synthesis of ^{14}C-labelled PEITC and the cysteine conjugate S-(N-phenethylthiocarbamoyl)cysteine ([^{14}C]PETC-Cys) used in a study of the cellular and sub-cellular binding of PEITC to tumour cells during the commitment to apoptosis.

MATERIALS AND METHODS

Materials

2-Phenyl-[1-^{14}C]-ethylamine hydrochloride (50-60 mCi/mmol; 250 μCi) in ethanol was purchased from Biotrend, Koln, Germany. The ethanol was removed in vacuo. Thiophosgene was purchased from Aldrich Chem. Co. Ltd (Poole, Dorset, U.K.). L-Cysteine, Trypan blue and dimethyl sulphoxide and were purchased from Sigma Chem. Co. Ltd. (Poole, Dorset, U.K.). Tissue culture medium RPMI 1640 and foetal calf serum were purchased from Gibco Europe Ltd (Paisley, Scotland).

Synthesis of 2-phenyl-[1-^{14}C]-ethyl isothiocyanate ([^{14}C]PEITC)

2-Phenyl-1-[^{14}C]ethylamine hydrochloride (0.79 mg, 5 μmol 250 μCi) and phenethylamine (0.245 mmol, 30.7 μl) was dissolved in 350 μl of water in a small glass vial and sodium hydroxide solution (10 M, 20 μl) added. The solution was cooled to 0°C, and 250 μl of dichloroethane, 29 mg of calcium carbonate, and 20 μl of thiophosgene was added. The mixture was stirred for 18 h at room temperature and then warmed to 37°C and diluted with 0.5 ml of 5 M hydrochloric acid. The solution was extracted with 5 x 2 ml of dichloroethane. The combined extracts were washed with water (5 ml), dried over anhydrous sodium sulphate, filtered and the dichloromethane evaporated with a stream of dry nitrogen to leave the product, [^{14}C]PEITC. The latter was analysed by ^1H NMR and thin layer chromatography (TLC) on silica gel 60 F_{254} with mobile phase of hexane.

Synthesis of S-(N-phenyl-[1-^{14}C]-ethylthiocarbamoyl)cysteine ([^{14}C]PETC-Cys)

This was synthesised by the method for PETC-Cys described (7). [^{14}C]PEITC (0.04 mmol, 6.5 mg) and L-cysteine (0.1 mmol, 12 mg) were dissolved in 200 μl of 50% (v/v) ethanol containing 5 mM sodium phosphate buffer at pH 6.6 and reacted at room temperature for 1 h. The precipitate formed was sedimented by centrifugation (6000 g, 10 min), washed with 80 μl of 70% ethanol and dried in vacuo. The product was analysed by ^1H NMR and thin layer chromatography (TLC) on silica gel 60 F_{254} with mobile phase of propanol: acetic acid: water, 10:5:5 (v/v/v).

RESULTS

Synthesis of 2-phenyl-[1-^{14}C]-ethyl isothiocyanate ([^{14}C]PEITC)

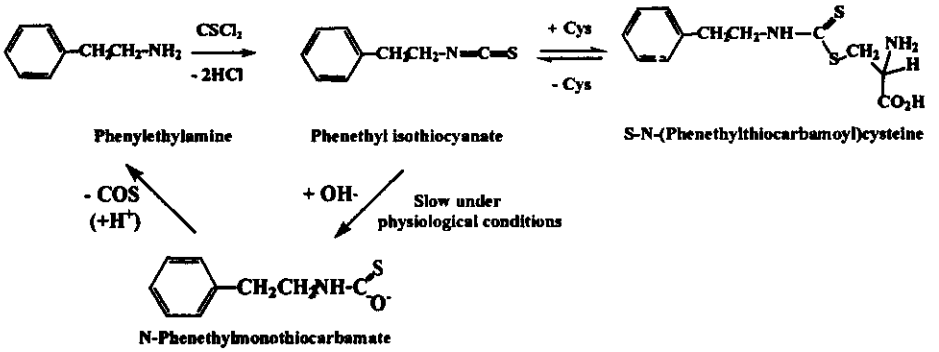
[^{14}C]PEITC was synthesised from [^{14}C]phenylethylamine as described above. The ^{1}H NMR spectrum of PEITC was characterised by (δH ppm (J Hz)): 2.96 ($J_{1,2}$ 6.7) 1-H(2H), 3.91 ($J_{1,2}$ 6.7) 2-H(2H), 7.30 (5H) phenyl. The R_f value was 0.30 and the yield was 36% (based on [^{14}C]phenylethylamine).

Synthesis of and S-(N-phenyl-[1-^{14}C]-ethylthiocarbamoyl)cysteine ([^{14}C]PETC-Cys)

[^{14}C]PETC-Cys was synthesised from [^{14}C]PEITC by reaction with cysteine. PETC-Cys gave a ^{1}H NMR spectrum identical to that previously described (7). The R_f value was 0.85 and the yield was 86% (based on [^{14}C]PEITC), which was the usual high yield for preparation of PETC-Cys by this method.

DISCUSSION

[^{14}C]PEITC was prepared by the reaction of phenylethylamine with thiophosgene, and [^{14}C]PETC-Cys by the reaction of PEITC with cysteine – Scheme 1. Similar derivatives of



Scheme 1. The formation of phenethyl isothiocyanate and S-(N-phenethylthiocarbamoyl)-cysteine from phenylethylamine (synthesis), and spontaneous hydrolysis of phenethyl isothiocyanate (slow under physiological conditions).

benzyl isothiocyanate have been described (12). The formation of the cysteine adduct PETC-Cys is reversible but under the conditions described, the conjugate can be isolated and is stable when

anhydrous. Our recent studies have shown that PEITC hydrolyses in aqueous solution under physiological conditions, pH 7.4 and 37°C, to phenylethylamine. The half-life of PEITC was 141 ± 7 min. The approximate chemical relaxation time τ for an initial concentration of 50 μM PETC-Cys under physiological conditions was *ca.* 22 min (assuming negligible PEITC hydrolysis during the relaxation to equilibrium) -- unpublished observations. These instabilities of PEITC and PETC-Cys should be considered in the use of these compounds in biological experiments.

Dietary isothiocyanates have been of interest recently for their ability to inhibit the phase I metabolic activation of pro-carcinogens and enhance the phase II conjugation and elimination of carcinogens (13). They are prospective chemopreventive agents for cancer (14), although some concern as to their ability to promote carcinogenesis has recently arisen (15). The anti-proliferative, antitumour activity of dietary isothiocyanates and their cysteine conjugates is a further development. This antitumour effects may suppress the growth of pre-clinical tumours and thereby make additional contributions to the well-established decreased cancer incidence associated with a vegetable-rich diet (16). [^{14}C]-Labelled derivatives will assist in investigating further the mechanism of induction of apoptosis by these compounds.

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