THE PREPARATION OF CARBON-14 LABELLED 1-[4-(2-DIETHYLAMINO-ETHOXY) PHENYL]-1, 2-DIPHENYL-2-CHLOROETHENE (CLOMIPHENE) AND 1-[4-(2-(N-PYRROLIDINYL) ETHOXY) PHENYL]-2-(4-METHOX-YPHENYL)-2-NITRO-2-PHENYLETHENE (CI-628, NITROMIPHENE)

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SUMMARY

The preparation of [^{14}C] clomiphene and [^{14}C] nitromiphene from [methylene-^{14}C] benzyl chloride is described. The overall radio-chemical yield and specific activity of the former are 53% and 0.53 mCi/mmol and those of the latter are 26% and 0.23 mCi/mmol. The method described is of micromolar scale designed for maintaining cost economy while yielding final products of sufficient radioactivity for *in vitro* and *in vivo* metabolism studies.

Key Words: Clomiphene, nitromiphene, carbon-14, Reacti-Vial System.†

INTRODUCTION

The introduction of tamoxifen (1) as a therapeutically advantageous drug in the treatment of breast cancer has generated considerable interest in the triarylethylene antiestrogens.[1] In addition to their antitumor properties, they can invoke estrogenic and antiestrogenic responses by target tissues.[2] Although their mechanism of action remains unknown, there is speculation that their biological activity is in part mediated by metabolites.[3,4] Of the triarylethylene anties-

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†Reacti-Vial™, trade mark the property of Pierce Chemical Company.

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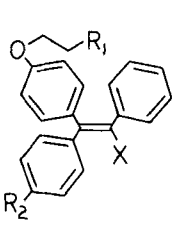


	R_1	R_2	X
1	NMe_2	H	Et
2	NEt_2	H	Cl
3	N⌒	OMe	NO_2

trogens which are used clinically or pharmacologically, only tamoxifen has received a detailed examination of its metabolism.[5] In this laboratory, we have studied the microsomal metabolism of clomiphene (2)[6,7] and nitromiphene (3)[8] using non-labelled suspected metabolites as comparative standards in thin-layer chromatographic analysis of incubation extracts. We have now embarked on a more analytically sensitive approach to supplement our previous studies by using radiolabelled cogeners of these compounds. Herein we report the syntheses of [^{14}C] clomiphene and [^{14}C] nitromiphene from [methylene-^{14}C] benzyl chloride. The procedure which we have developed is of micromolar proportions and ideally suited for maintaining cost economy in purchasing expensive radiolabelled intermediates. The specific activity generated, while low compared to that required for radioligand - receptor binding studies, has been, as we expected, quite sufficient for monitoring the biotransformation of these compounds.

MATERIALS

[Methylene-^{14}C] benzyl chloride was purchased from Pathfinder Laboratories Incorporated. Benzene and tetrahydrofuran were dried by distillation from calcium hydride and lithium aluminum hydride, respectively. Triethylamine was purified

by distillation from potassium hydroxide. All other solvents were of analytical reagent quality. All reactions were carried out in clear 1.0 mL capacity Pierce Reacti-Vials with teflon/silicone disc closures. Dry nitrogen was vented through two stainless steel 22 gauge x 1.5 inch hypodermic needles, one attached to the nitrogen source and the other to a drying tube with tygon tubing. Heating was most conveniently provided by suspending the reaction vial $\leq$ 1.5 cm above a Fisher Thermix set at a temperature adjustment of 2. Analytical thin layer chromatography was performed using 0.2 mm silica gel-coated plastic sheets containing F-254 indicator (Merck). Preparative thick layer chromatography was carried out using 1.0 mm silica gel glass-backed 20 x 20 cm plates with F-254 indicator (Analtech). Spots and bands were visualized under 254 nm ultraviolet light. Ultraviolet spectra were measured on a Baush and Lomb Spectronic 2000 Spectrometer System with ethanol as solvent. Radioactivity was measured in a Beckman LS 7500 Liquid Scintillation System.

EXPERIMENTAL

1-[4-(2-Diethylaminoethoxy)phenyl]-1, 2-diphenyl-2-[^{14}C]-ethene (6). A reaction vial containing magnesium pieces (6.3 mg, 0.259 mmol, diameter < 1 mm) and a stirring triangle was flame-dried and set aside in a desiccator to cool. A solution of [methylene-^{14}C] benzyl chloride (18.8 mg, 0.25 mCi) in diethyl ether (0.2 mL) was introduced into the vial, a small iodine crystal was added, and the mixture was gently refluxed for 1 h. During this time 12 uL of unlabelled benzyl chloride (total amount of benzyl chloride is 32 mg, 0.253 mmol) was injected in order to maintain a consistant dissolution of the magnesium while fresh diethyl ether was periodically injected to maintain a suitable volume (0.2 mL). A solution of 4-(2-diethylaminoethoxy) benzophenone[9] (4, 55.1 mg, 0.185 mmol) in tetrahydrofuran

(0.3 mL) was slowly injected into the Grignard reagent. The mixture was stirred while gently heating for 16 h and then was quenched with saturated ammonium chloride solution (33 uL). The suspension was stirred for 30 min. and filtered through a pad of super-cel. The pad was washed with tetrahydrofuran (6 x 15 mL) and the filtrate concentrated. Examination by TLC (benzene-triethylamine, 9:1) showed that the crude material was predominately the precursor carbinol (5) and some unreacted ketone. This was dissolved in methanol (0.5 mL), 3 drops of 10% HCl was added, and the reaction mixture was stirred for 2.5 days. The crude mixture was streaked at the base of two preparative TLC plates and eluted with benzene-triethylamine (9:1). After two developments, the desired product 6 (41.6 mg) and unreacted 4 (7.1 mg) were extracted from their respective bands with diethyl ether. Yield of 6 based on correction for unreacted 4 was 69%.

1-[4-(Diethylaminoethoxy)phenyl]-1, 2-diphenyl-2-chloro-2-[^{14}C] ethene (2). A reaction vial containing a solution of 6 (41.6 mg, 0.112 mmol) in diethyl ether (0.10 ml) was chilled in an ice bath. Ethereal hydrogen chloride (0.8 mL) was added and the milky suspension was stirred for 15 min. The ether and excess hydrogen chloride was evaporated away with a stream of dry nitrogen leaving the white hydrochloride salt. This was dissolved in chloroform (0.5 mL), N-chlorosuccimide (16.4 mg, 0.123 mmol) was added, and the reaction mixture was gently heated for 40 h. The reaction mixture was streaked at the base of two preparative TLC plates and eluted with chloroform-methanol-ammonia (95:5:0.5). Extraction of the product band with diethyl ether yielded 36 mg of a yellow oil. This was rechromatographed with benzene-triethylamine (9:1) to afford pure 2 (34 mg, 74%) as a pale yellow oil. The product cochromatographed with commerically available clomiphene (Sigma) in benzene-triethylamine (9:1), benzene-piperidine (9:1), and chloroform-methanol (9:1)

as well as exhibiting the characteristic ultraviolet spectrum (λ max 297 nm). The radiochemical purity was shown to be 98%. The specific activity was 0.53 mCi/mmol and overall chemical and radiochemical yields were 45% and 53%, respectively.

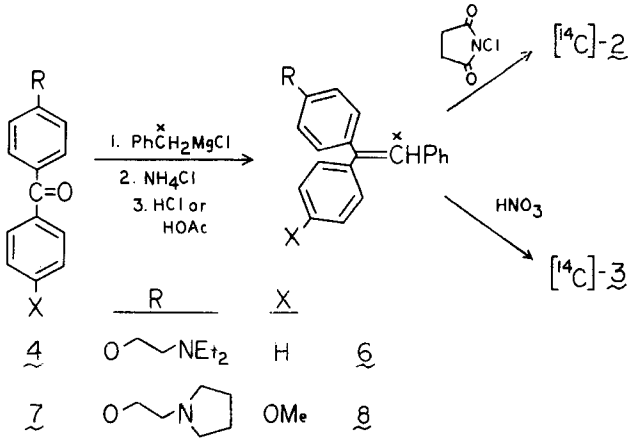
1-[4-(2-(N-Pyrrolidinyl)ethoxy)pheny]-1-(4-methoxyphenyl)-2-nitro-2-phenyl-2[^{14}C] ethene (3). This was prepared by the method of Black et al[10] with the scalar modification employed in the previously described synthesis of [^{14}C]-2. Thus the tertiary alcohol precursor was prepared from 6.9 mg of magnesium pieces, [methylene-^{14}C] benzyl chloride (0.25 mCi), an excess of unlabelled benzyl chloride and 70.3 mg of 7. Purification of the crude product by preparative TLC (benzene-triethylamine, 9:1) afforded an amber, viscous oil (98 mg). This was dissolved in acetic acid (0.7 mL), 90% nitric acid (20 uL) was added, and stirring at room temperature was continued for 6 h. The yellow reaction mixture was applied to two preparative TLC plates and the majority of the acetic acid was removed under a stream of cold air (hot air may cause decomposition of product!). The product was eluted with benzene-triethylamine (9:1), extracted with ethyl ether, and rechromatographed with the same solvent system to afford 67.5 mg (70%) of a yellow semi-solid which cochromatographed with authentic 3 in benzene-triethylamine (9:1), benzene-piperidine (9:1), and chloroform-methanol-ammonia (95:5:0.5). The radiochemical purity was 98% and the specific activity was calculated to be 0.23 mCi/mmol. The overall radiochemical yield was 26%.

Determination of Specific Activity of [^{14}C]-2 and -3. This was carried out by application of the method of Katzenellenbogen, Tatee, and Robertson[11], using the molar absorptivity of 2 (11.6 $mM^{-1}cm^{-1}$ at 297 nm) and 3 (15.5 $mM^{-1}cm^{-1}$ at 282 nm) to determine the amounts of these compounds in samples of measured radioactivity.

RESULTS AND DISCUSSION

While compound 1 has been prepared in labelled form by a variety of procedures,[12-14] less information is available about methods for 2 and 3. Synthesis of [^{14}C]-2 has not been reported, although its use in clinical and animal studies of its disposition has.[15] A method for tritiation of 3 ortho to its methoxy group has been reported.[11] The present compounds were prepared with the objective of positioning the label in such a way that it will be retained fully in known or suspected metabolites resulting from routes of biotransformation other than ethylenic bond cleavage. Preparation of these compounds, patterned after the way in which the unlabelled drugs are prepared, is summarized in the Scheme. The carbinol precursors of 6 and 8 were dehydrated, in turn, with HCl and *in situ* with a mixture of nitric and acetic acids.

SCHEME I



Preparative TLC, with detection/identification of product zones on chromatograms carried out under ultraviolet light (254 nm), proved to be a convenient, efficient method for obtaining materials of high chemical and radiochemical purity. While these compounds are not of sufficient specific activity for detailed pharmacodynamic studies, they are of such for in vitro and in vivo biotransformation studies.

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9. Compound 4 was prepared in 80% yield by a procedure previously published by this research group [J. Med. Chem. 25, 1056 (1982)]. It distilled ($b_{0.01}$ 171.5-173.5°C) as a colorless oil [Chem. Abstr. 54: 5581e (1960), $b_{0.7}$ 200-204°C].
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