REDUCTION OF IMINIUM ETHERS WITH SODIUM BORO[^{3}H]HYDRIDE. PREPARATION OF TRITIUM LABELLED CLOMIPHENE AND NITROMIPHENE (CI 628)**

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SUMMARY

The preparation of [^{3}H]-clomiphene and [^{3}H]-nitromiphene from amido precursors in a two-step reaction sequence utilizing NaB^3H_4 is described. The radiochemical yield and specific activity of the former are 34% and 16 mCi/mmol and those of the latter were 42% and 20 mCi/mmol, respectively. The method described is of micromolar scale designed for maintaining cost economy in the purchase of expensive radiolabelled reagents while yielding final products of sufficient radioactivity for in vitro and in vivo metabolism studies.

Key Words: Clomiphene, nitromiphene, NaB^3H_4, triethyloxonium tetrafluoroborate, Reacti-Vial† System.

INTRODUCTION

The triarylethylene antiestrogens remain the most significant of the nonsteroidal estrogen antagonists. Of these, tamoxifen 1 and clomiphene 2 have been used successfully in the suppression of estrogen receptor positive breast cancer and the induction of ovulation in subfertile women,

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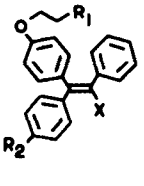
†Reacti-Vial, trade mark of Pierce Chemical Company.

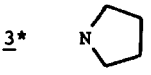
**Systematic chemical names: clomiphene, (E,Z)-1-[4-(2-dimethylamino-ethoxy)-phenyl]-1,2-diphenyl-2-chloroethene; nitromiphene, (E,Z)-1-[4-(2-(N-pyrrolidinyl)ethoxy)phenyl]-1-(4-methoxyphenyl)-2-nitro-2-phenyl-ethene.

0362-4803/84/090857-07$01.00

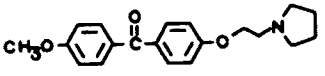
Received March 26, 1984
Revised May 11, 1984

respectively.[1,2] Studies of the biotransformation of 1[3] and 2[4] have revealed the production of metabolites with bioactivity equal to or greater than the parent compound.[5,6] Another triarylethylene which has displayed



	R_1	R_2	X
1	$N(CH_3)_2$	H	C_2H_5
2*	$N(C_2H_5)_2$	H	Cl
3*		OCH_3	NO_2

*Only the "trans" (E) isomers are shown.



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antiestrogenic properties and has been employed as a molecular probe to study the mechanisms of action of estrogens is nitromiphene (CI 628, 3).[7-9] Although one metabolite of 3 has been isolated from uterine tissue,[10] no formal investigation of its metabolism has been reported. Thus we began studies of the *in vitro* fate of 3, which had been labelled at the carbon atom bearing the nitro group with carbon-14.[11] During the course of these studies, we discovered that 3 underwent oxygen-independent ethylenic bond cleavage to yield the non-labelled benzophenone 4 and an as yet unidentified polar metabolite retaining the radiolabel.[12] In an attempt to obtain a clearer understanding of the metabolic profile of 3, which appears to be unique among triarylethylene antiestrogens, we sought radiolabel 3 in such a way that the radiolabel would be retained after biotransformation to 4. We accomplished this by a method of tritiation which was also applicable for radiolabelling 2. We report in this paper the syntheses of [^{3}H]-2 and [^{3}H]-3 and as previously in the synthesis of the carbon-14 labelled congeners[11] utilized micromolar scale in order to maintain cost economy in the purchase of radiolabelled reagents. Within the protocol for monitoring the

biotransformation of xenobiotics, the specific activities generated by this procedure are optimal.

EXPERIMENTAL

Materials and Methods

[^{3}H]-Sodium borohydride (specific activity 48 mCi/mmol) was purchased from Amersham Corporation and triethyloxonium tetrafluoroborate (1.0 M solution in methylene chloride) from Aldrich Chemical Company. Lactam 5 was prepared as described elsewhere.[13] Benzene and triethylamine were purified by distillation from calcium hydride and potassium hydroxide, respectively. 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. A positive-pressure dry nitrogen atmosphere was introduced through a stainless steel 22 gauge x 1.5 inch hypodermic needle via two 3 inch segments of Tygon tubing separated by a glass 3-way stopcock assembly. 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 Bausch and Lomb Spectronic 2000 Spectrometer System with ethanol as solvent. Radioactivity was measured in a Beckmann LS 7500 Liquid Scintillation System. Infrared spectra were measured on a Perkin-Elmer 467 Spectrophotometer.

1-[4-(2-(N-(1-oxoethyl)-N-ethyl)ethoxy)phenyl]-1,2-diphenyl-2-chloroethene (6). To a chilled, stirring suspension of N-desethylclomiphene[4] (0.32 g, 0.847 mmol), anhydrous Na_2CO_3 (0.5 g), and acetonitrile (5 mL), was added dropwise a solution of acetyl chloride (0.12 mL, 1.68 mmol) in acetonitrile (2 mL). The reaction mixture was stirred at room temperature for 90 min, concentrated in vacuo, and the dark brown residue dissolved in ether (50

mL). The etheral solution was successively washed with 10% HCl (10 mL), aqueous saturated $NaHCO_3$ (10 mL), 10% NaOH (10 mL), and water (3 x 10 mL) and dried (Na_2SO_4). The residue left after evaporation of solvent was chromatographed on silica gel (10 g, Baker, 60-200 mesh) with benzene-triethylamine (30:1) to afford 6 (0.11 g, 31%) as a colorless oil: IR (neat) 1645 cm^{-1}, (C=O). This was used directly in the preparation of [^{3}H]-2.

Preparation of [^{3}H]-2 and [^{3}H]-3. General Procedure. To a stirring solution of the amide (0.105 mmol) in methylene chloride (0.4 mL) was added via syringe 115 uL of the triethyloxonium tetrafluoroborate reagent (0.115 mmol). The reaction mixture was stirred for 20 h. The vial cap was removed and the reddish-brown mixture was concentrated under a jet of dry nitrogen. Absolute ethanol (0.1 mL) was added and the vial was resealed. A solution of NaB^3H_4 (10 mg, 0.263 mmol, 12.5 mCi) in absolute ethanol (0.5 mL) was added via ten 50 uL injections. When the intensity of the gaseous evolution (ethane gas generated from unreacted $Et_3O^{+-}BF_4$) had subsided, the silicon-teflon seal was quickly replaced and the reaction mixture was applied to two preparative TLC plates and eluted with benzene-triethylamine (9:1). Product amine and unreacted amide were extracted from their respective bands with diethyl ether. Products 2 and 3 chromatographed with authentic samples in benzene-triethylamine (9:1) and chloroform-methanol-ammonia (95:5:0.5).

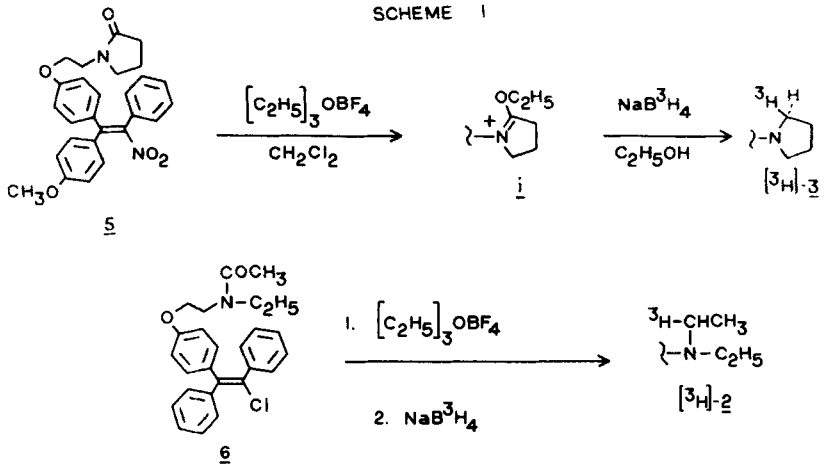
The radiochemical purity and specific activity of 2 were 98% and 16 mCi/mmol, respectively. The radiochemical yield was 34% and the chemical yield was 50% (4 mg) based on correction for unreacted 6 (17 mg). The radiochemical purity and specific activity of 3 were 92% and 20 mCi/mmol. The radiochemical yield was 43% and the chemical yield was 52% (14 mg) based on correction for unreacted 5 (21 mg).

Determination of Specific Activity of [^{3}H]-2 and [^{3}H]-3. This was carried out by application of the method of Katzenellenbogen, Tatee, and Robertson,[16] using the molar absorptivity of 2 (12.2 $mM^{-1}cm^{-1}$ at 294 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

The impetus behind the specific manner in which [^{3}H]-3 was synthesized lay in the ready availability in this laboratory of lactam 5. Previously, in anticipation of the structures of possible metabolites of 3, we had prepared 5 and in fact have identified it as a metabolite.[13] With the need for an alternative site of radioactivity in 3, we then envisaged 5 as a candidate for retro-tritiation to [^{3}H]-3. After a survey of methods for the selective reduction of lactams and amides in the presence of nitro and olefinic groups, and of those tritiating reagents commercially available, we chose the method of Borch.[14] By this method, lactams are activated toward reducing agents by conversion to lactim ethers using triethyloxonium tetrafluoroborate (e.g. i, Scheme I). Such ethers undergo reduction to the corresponding cyclic tertiary amines by treatment with $NaBH_4$.[14,15] Thus, lactam 5 was sequentially treated with triethyloxonium tetrafluoroborate and NaB^3H_4 (specific activity, 48 mCi/mmol) to afford [^{3}H]-3 in 52% chemical yield (Scheme I). Similarly, [^{3}H]-2 was prepared from acetamide 6 in 15% chemical yield. Respective specific activities were 20 and 16 mCi/mmol, which are appropriate for biotransformation studies. Higher specific activities, necessary for receptor binding experiments, could presumably be achieved using NaB^3H_4 which is available in specific activities up to 20 Ci/mmol.

SCHEME I



This two-step procedure for reducing lactams and amides is very clean, essentially affording only unreacted amide and the product amine. Our products were easily separated by preparative TLC and their zones on chromatograms detected with ultraviolet light (254 nm).

Statistically, metabolic N-dealkylation of 2 [ethyl-1-^{3}H] to N-desethyl-clomiphene and *in vivo* oxidation of 3 [pyrrolidine-2-^{3}H] to 5 would result in 50% loss of the tritium from each metabolite. However, use of these in suitable combination with their respective [^{14}C]-labelled counterparts should obviate any difficulties associated with quantitation of these metabolites or others arising from side chain alteration of 2 and 3.

ACKNOWLEDGEMENT

This research was supported by the National Institutes of Health, grant CA 28928-03. We would also like to thank Ms. W. Hall, who prepared the manuscript.

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