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Synthesis of [D_5-Ethyl]-tamoxifen; A Mechanistic Probe of Tamoxifen Induced Hepatic DNA Adduct Formation

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SUMMARY

Tamoxifen which incorporates a fully deuterated ethyl group, [D_5-ethyl]-tamoxifen, has been synthesised in order to probe the mechanism of tamoxifen induced hepatic DNA adduct formation. The pentadeuteroethyl group was introduced into the tamoxifen structure by treatment of the ketone precursor 1-[4-(2-chloroethoxy)phenyl]-2-phenylethanone, as its sodium enolate, with [D_5]-iodoethane.

Keywords: [D_5-Ethyl]-tamoxifen, Tamoxifen, DNA-adduct, Metabolism, Kinetic Isotope Effect.

Abbreviations: tamoxifen = (Z)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene; [D_5-ethyl]-tamoxifen = [3,3,4,4,4-$^2H_5$]-(Z)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene.

INTRODUCTION

Tamoxifen **1** has been shown to cause liver cancer in rats[1], which is believed to be a consequence of covalent DNA adduct formation[2]. This has important implications when tamoxifen, which is commonly used for the treatment of breast cancer[3], is to be used on a regular basis by a large number of healthy women as a breast cancer preventative agent (prophylactic)[4]. Although the mechanism by which tamoxifen forms covalent DNA adducts is poorly understood[2,5], we have developed a hypothesis which explains the ability of tamoxifen to form electrophilic alkylating agents which are capable of DNA adduct formation, following hepatic oxidative metabolism[6]. The key step in this process is deduced to be cytochrome P-450 mediated α-oxidation of the ethyl group in tamoxifen.

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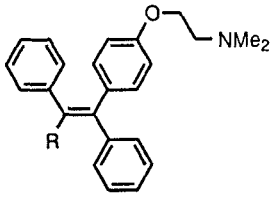
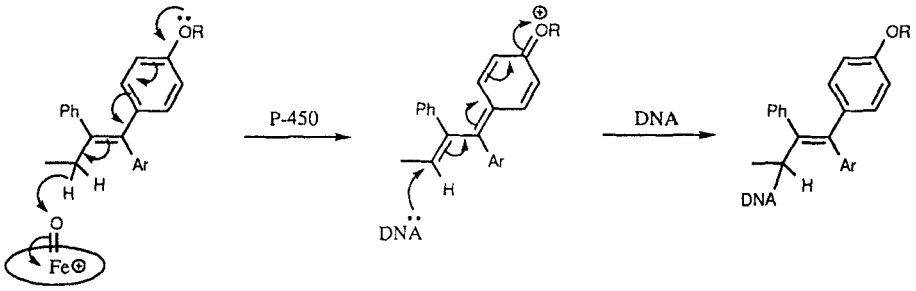


(1) R = CH_3CH_2- Tamoxifen

(2) R = CD_3CD_2- [D_5-Ethyl]-tamoxifen

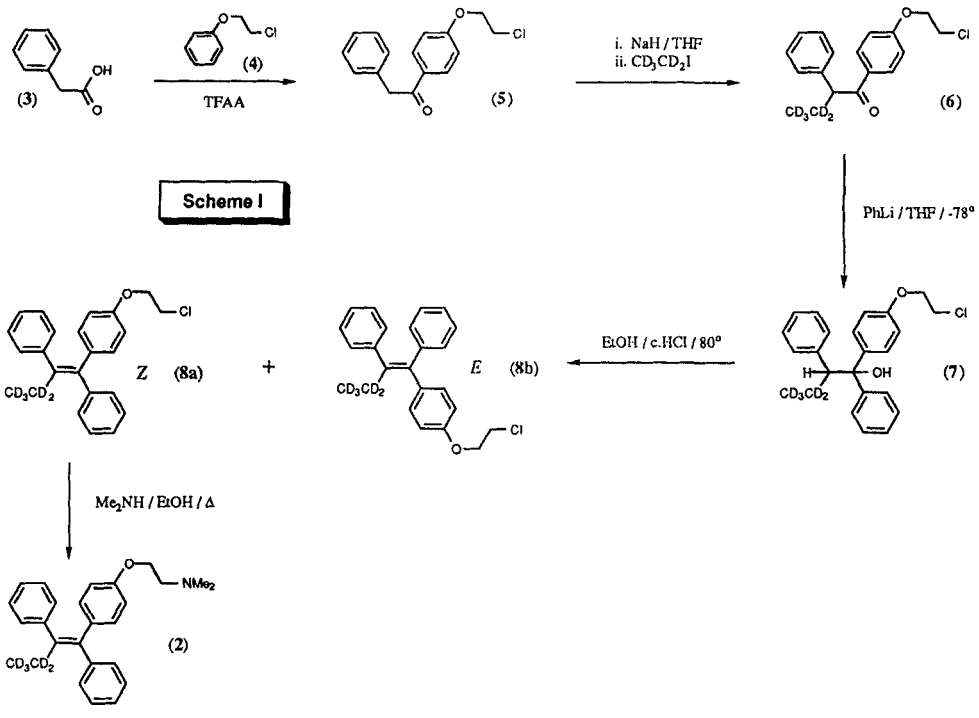
Mechanistically this is thought to proceed via hydride abstraction by the high oxidation state iron-oxo species [Fe^+=O] of the cytochrome P-450 enzyme responsible for the α-hydroxylation of tamoxifen. This generates an electrophilic allylic carbocation, which is resonance stabilised in part by the alkoxy ring substituent as shown (Fig 1). In reactions where C-H bond cleavage is the rate determining step replacement of hydrogen by deuterium at the site of cleavage produces a retardation in reaction rate as a result of a primary deuterium kinetic isotope effect[7]. Importantly, this kinetic isotope effect has been previously observed in cytochrome P-450 mediated hydroxylation reactions of alkyl groups, where hydrogen is replaced by deuterium at the site of oxidation[8]. Thus, a tamoxifen derivative which incorporates a deuterated ethyl group would allow the relative abundance of DNA adducts compared with tamoxifen to be measured by ^{32}P-postlabelling experiments[2]; here we report on the preparation of [D_5-ethyl]-tamoxifen **2**.

Figure 1: Postulated mechanism of covalent DNA adduct formation by tamoxifen[6]



RESULTS AND DISCUSSION

The strategy employed for the synthesis of **2** involved preparing the key intermediate deuterated 1,2-diaryl-1-butanone **6**, since the pentadeuteroethyl group could be introduced by C-alkylation of a suitable 1,2-diarylethanone enolate anion, and the corresponding unlabelled compound 1-[4-(2-chloroethoxy)phenyl]-2-phenyl-1-butanone is a versatile tamoxifen synthon which has been employed in the synthesis of a variety of tamoxifen analogues[9,10] (Scheme I).



The requisite 1,2-diarylethanone, that is 1-[4-(2-chloroethoxy)phenyl]-2-phenylethanone **5**, was prepared by Friedel-Crafts acylation of 2-chloroethoxybenzene **4** with readily available phenylacetic acid **3**, using trifluoroacetic anhydride as condensing agent[9]. This afforded **5** by a more direct approach than that reported previously[11]. The deuteroethyl group was then introduced by enolisation of **5** with sodium hydride followed by C-alkylation of the ketone with [D_5]-iodoethane. Sodium hydride was used for this enolisation so as to avoid possible dehydrochlorination of the chloroethoxy side-chain, which can occur with stronger bases[9]. The remainder of the route essentially followed that established for the synthesis of tamoxifen[12] and other substituted analogues[13]. This involved treatment of the 1,2-diaryl-1-butanone **6** with phenyllithium to give the intermediate tertiary alcohol **7**. Dehydration of this alcohol produced the triphenylbutene **8** as a mixture of geometric isomers **8a** and **8b**. Tamoxifen itself refers solely to the *Z*-isomer **1**, the *E*-isomer having contrasting agonistic properties, and it is therefore important to prepare [D_5-ethyl]-tamoxifen **2** as the pure *Z*-isomer also. Fortuitously, it was found that the *Z*-isomer of the triphenylbutene precursor (**8a**) could be obtained pure by crystallisation from isopropanol, provided the solution was sufficiently dilute. In the final step reaction of **8a** with dimethylamine in ethanol afforded the target molecule [D_5-ethyl]-tamoxifen **2**.

EXPERIMENTAL

Anhydrous tetrahydrofuran (THF) was freshly prepared by distillation from potassium using benzophenone indicator. 2-Chloroethoxybenzene **4** was prepared as previously described[13], and phenylacetic acid **3** was obtained from Aldrich Chemical Co. Ltd. IR spectra were recorded on a Perkin-Elmer 1720X FT-IR spectrometer, NMR spectra recorded on a Bruker AC 250 instrument with TMS as internal standard, and mass spectra (MS, electron impact, 70 eV) on a VG7070H spectrometer with a VG2235 data station. High resolution mass spectra (HRMS) were calibrated against perfluorokerosene. Melting points were determined using a Reichert hot stage and are uncorrected. Chromatography refers to column chromatography on silica gel (Merck 15111) with the eluant indicated applied at a positive pressure of 0.5 atm. Ether refers to diethyl ether. 'Tiny-clave' refers to a Buchi mini autoclave.

1-[4-(2-Chloroethoxy)phenyl]-2-phenylethanone (**5**). To a stirred solution of phenylacetic acid **3** (77.6 g, 0.57 mol) in trifluoroacetic anhydride (85 ml, 0.60 mol), cooled on an ice-water bath, was added 2-chloroethoxybenzene **4** (99.2 g, 0.63 mol) and the reaction flask fitted with a calcium chloride guard tube. The cooling bath was removed and after stirring at room temperature for 24 h the reaction mixture was poured into water (500 ml), and triturated with a glass rod until crystallisation commenced. The crude product was collected on a sinter, washed with water (5 l), and dried *in vacuo* over P_2O_5. Recrystallisation from light petroleum (bp 80-100 °C) afforded the title compound **5** as off-white crystals (84 g, 53 %), mp 105-109 °C, of sufficient purity for the following step. A second recrystallisation from ethylacetate afforded white crystals, mp 111-113 °C (Lit[11]. mp 105-107.5 °C). IR (film) υ/cm^{-1} 1674 (C=O). ^{1}H-NMR ($CDCl_3$) δ 3.84 (2H, t, J 5.8 Hz, $OCH_2\underline{CH_2}$), 4.24 (2H, s, $\underline{CH_2}CO$), 4.29 (2H, t, J 5.8 Hz, OCH_2CH_2), 6.95 (2H, d, J 9.0 Hz, Ar-H, *ortho*, to OR), 7.33-7.25 (5H, m, Ph-H), 8.01 (2H, d, J 9.0 Hz, Ar-H, *meta*, to OR). MS *m/z* 274 (M^+).

[3,3,4,4,4-$^2H_5$]-1-[4-(2-Chloroethoxy)phenyl]-2-phenyl-1-butanone (**6**). To a stirred suspension of sodium hydride (0.67 g, 28 mmol) in anhydrous THF (30 ml) was added the ketone **5** (5.52 g, 20 mmol) in portions whilst cooling on an ice-water bath. After 30 min, [D_5]-iodoethane (1.9 ml, 24 mmol) was added via syringe and the cooling bath removed. The reaction mixture was stirred at ambient temperature for an additional 3 h, then partitioned between ether (75 ml) and water (75 ml). The aqueous phase was extracted further with ether (2 x 50 ml), and the combined organic extracts washed with water (50 ml), dried ($MgSO_4$), and concentrated. Chromatography, on elution with

hexane-dichloromethane (1:1), afforded the title compound **6** (5.5 g, 89%) which gave white crystals from light petroleum (bp 80-100 °C), mp 71.5-73.5 °C. IR (KBr disc) υ/cm^{-1} 2217 (C-D), 1688 (C=O). ^{1}H-NMR ($CDCl_3$) δ 3.81 (2H, t, J 5.8 Hz, $OCH_2C\underline{H}_2Cl$), 4.24 (2H, t, J 5.8 Hz, $OC\underline{H}_2CH_2Cl$), 4.38 (1H, s, C<u>H</u>CO), 6.88 (2H, d, J 9.0 Hz, Ar-<u>H</u> *ortho* to OR), 7.30-7.18 (5H, m, Ph-<u>H</u>), 7.96 (2H, d, J 8.9 Hz, Ar-<u>H</u> *meta* to OR). MS *m/z* 307 (M^+).

[3,3,4,4,4-$^2H_5$]-(Z)-1-[4-(2-Chloroethoxy)phenyl]-1,2-diphenyl-1-butene (8a). A solution of phenyllithium (11 mmol) in THF was freshly prepared by the addition of *n*-butyllithium (1.6 M; 6.9 ml, 11 mmol) in hexane to a solution of bromobenzene (1.73 g, 11 mmol) in anhydrous THF (10 ml), under an argon atmosphere, and cooled to -78 °C on an acetone-cardice bath. The phenyllithium solution was then introduced, via a cannula, to a solution of the ketone **6** (3.08 g, 10 mmol) in anhydrous THF (20 ml), also under an argon atmosphere and cooled to -78 °C. The reaction mixture was allowed to attain ambient temperature, quenched by the addition of water (5 ml), and then partitioned between ether (75 ml) and water (75 ml). The aqueous phase was acidified by the addition of conc. HCl, and extracted further with ether (2 x 50 ml). The organic extracts were combined, washed with water (75 ml), dried ($MgSO_4$), and concentrated to give the intermediate tertiary alcohol (**7**) as an oil. This oil was then dissolved in ethanol (40 ml), conc. HCl (12 ml) added, and the solution heated under reflux for 3 h. After allowing to cool the mixture was made alkaline by the addition of sodium hydroxide solution (5 M), and then partitioned between ether (75 ml) and water (75 ml). The aqueous phase was further extracted with ether (2 x 50 ml). The organic extracts were combined, washed with saturated sodium bicarbonate solution (50 ml), water (2 x 50 ml), brine (50 ml), dried (Na_2CO_3), and concentrated to give the crude product as a mixture of geometric isomers **8a** and **8b**. Crystallisation of the resultant oil from isopropanol at a concentration of 50 g ml^{-1} afforded white crystals (1.16 g, 32%), mp 109-110 °C. ^{1}H-NMR[14] showed this to be the pure Z-isomer 8a. IR (KBr disc) υ/cm^{-1} 2222 (C-D), 1606 (C=C). ^{1}H-NMR ($CDCl_3$) δ 3.74 (2H, t, J 5.9 Hz, $OCH_2C\underline{H}_2Cl$), 4.10 (2H, t, J 5.9 Hz, $OC\underline{H}_2CH_2Cl$), 6.56 (2H, d, J 8.7 Hz, Ar-<u>H</u> *ortho* to OR), 6.79 (2H, d, J 8.7 Hz, Ar-<u>H</u> *meta* to OR), 7.36-7.10 (10H, m, Ph-<u>H</u>). MS *m/z* 367 (M^+).

[3,3,4,4,4-$^2H_5$]-(Z)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene, [D_5-Ethyl]-tamoxifen (2). In a Buchi 10 ml 'tiny-clave' was placed **8a** (1.00g, 2.72 mmol) and dimethylamine (~33% soln. in ethanol; 5.4 ml, 30 mmol). The 'tiny-clave' was sealed and heated with stirring at 80 °C for 16 h. After allowing to cool the reaction mixture was partitioned between ether (50 ml) and water (50 ml) containing aqueous sodium hydroxide (2M; 1 ml). This was further

extracted with ether (3 x 50 ml), the organic phases combined, washed with water (2 x 100 ml), then brine (100 ml), dried (Na_2CO_3), and concentrated to an oil. Chromatography, eluting with ether-methanol 10:1, followed by crystallisation from light petroleum (bp 80-100 °C) afforded the title compound **2** as white crystals (0.904 g, 89 %), mp 98-100 °C. IR (KBr disc) υ/cm^{-1} 2224 (C-D), 1610 (C=C). ^{1}H-NMR ($CDCl_3$) δ 2.29 (6H, s, $N\underline{Me}_2$), 2.65 (2H, t, J 5.8 Hz, $OCH_2C\underline{H}_2NMe_2$), 3.93 (2H, t, J 5.8 Hz, $OC\underline{H}_2CH_2NMe_2$), 6.56 (2H, d, J 8.8 Hz, Ar-H, *ortho* to OR), 6.77 (2H, d, J 8.8 Hz, Ar-H, *meta* to OR), 7.39-7.07 (10H, m, Ph-H). HRMS Found: *m/z* 376.2579; $C_{26}H_{24}D_5NO$ requires 376.2563.

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