THE PREPARATION OF CARBON-14 AND TRITIUM LABELLED 1-[4-(2-DIMETHYLAMINO-ETHOXY)PHENYL]-1, 2-DIPHENYL-1-BUTENE [ICI 46,474, Tamoxifen (Nolvadex*)] AND THE SEPARATION OF CIS-TRANS ISOMERS

2. The Synthesis of Tritium Labelled Tamoxifen (Nolvadex*)

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SUMMARY

The preparation of tritiated tamoxifen generally labelled, and also specifically labelled in two separate positions is described. The generally tritiated material was prepared by an acid catalysed exchange reaction which produced labelled material of low molar specific activity [4.7 mCi/mmol]. Both specifically tritiated products were derived from tritium labelled intermediates which were used in synthetic procedures to produce tritiated tamoxifen. [G-^{3}H]Bromobenzene was used to incorporate the label in the 1-phenyl ring, and tamoxifen with a specific activity of 401 mCi/mmol was obtained. The reductive tritiation of a 3, 5-dibromo precursor and subsequent synthesis gave tamoxifen with a specific activity of 19.5 Curies/ mmol.

Key Words: Tamoxifen, tritium, cis-trans isomers, synthesis, stability.

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INTRODUCTION

Tamoxifen, the trans isomer of 1-[4-(2-dimethylaminoethoxy) phenyl]-1, 2-diphenyl -1-butene is a member of a class of compounds, the non-steroidal anti-oestrogens, derived from triphenyl ethylene. Although the pharmacology of tamoxifen in experimental animals is complex, showing both species and target organ variations in agonist (oestrogenic) and antagonist (anti-oestrogenic) activity(1), it is predominantly anti-oestrogenic in humans. For this reason, and because of its relative freedom from side effects, tamoxifen is widely used in the therapy of advanced breast cancer(2). The clinical application of the drug has provided the impetus for an extensive investigation into its mechanism of action and also for its use as a molecular probe for investigations of oestrogen dependent responses in target organs. In Part 1 of this paper the synthesis of [^{14}C]tamoxifen is described. It was prepared for absorption, distribution, metabolism and excretion studies but its specific activity [10.1 mCi/mmol] was too low for the quantitative measurements required for studies involving changes at receptor binding sites in selected target organs. A specific activity of 10 Ci/mmol was regarded as the minimum required for these investigations, and tritium was therefore the isotope of choice. Three approaches to the preparation of the tritiated product were explored.

ROUTE I

This involved an acid catalysed exchange reaction using a tritiated phosphoric acid-boron trifluoride complex(3), to produce a randomly labelled tritiated product with a specific activity of 4.7 mCi/mmol.

ROUTE 2

[G-^{3}H]Bromobenzene was used as the labelled precursor to introduce the tritium label into the tamoxifen molecule at the same location, and by the same synthetic pathway as described for the [^{14}C]tamoxifen in Part 1 under this title. A specific activity of 401 mCi/ mmol was obtained.

The [^{3}H]tamoxifen obtained from both Route 1 and Route 2 was used for metabolic and other studies not involving receptor site binding.

ROUTE 3

1-[3, 5-Dibromo-4-(2-dimethylaminoethoxy) phenyl]-1, 2-diphenyl-1-butanol, (4) was prepared from α-ethyl-4-hydroxydesoxy benzoin (1). The product was then reductively dehalogenated with tritium to give the tritiated alcohol (5) which responded to a dehydration procedure to give the cis-trans mixture of 1-[4-(2-dimethylaminoethoxy)-[3,5-^{3}H]phenyl]-1, 2-diphenyl-1-butene (6). The isolation of pure trans isomer (tamoxifen) was carried out by the chromatographic separation described previously in Part 1, for the ^{14}C labelled product.

The tritiated tamoxifen prepared by this route had a molar specific activity of 19.5 Curies/mmol and proved suitable for mechanistic studies. It was shown to compete with [^{3}H]oestradiol for specific receptor sites, using human breast cancer cells maintained in long term tissue culture (4), and also in human and rat mammary carcinomas investigated by in vitro studies (5). In both the immature calf and rat uterus it has been shown to bind directly to 8S cytosol oestrogen receptors, and it has been suggested that [^{3}H]tamoxifen and [^{3}H]oestradiol may bind to two different receptor sites of the same protein (6).

Jordan et al (7) have studied [^{3}H]tamoxifen binding to rat uterine estrogen receptors and concluded that there are a number of binding sites for both tamoxifen and oestradiol and that tamoxifen has a lower affinity for the oestrogen receptor sites. In addition, the interactions with [^{3}H]tamoxifen and the cytoplasmic binding sites from both ER+ and ER- human mammary carcinoma biopsies have been investigated (8). It was concluded from these studies that under in vitro assay conditions tamoxifen was bound by both the oestrogen receptor and a high affinity binding site which is distinct from the oestrogen receptor site. Studies of the binding of [^{3}H] 17β-oestradiol and [^{3}H]-tamoxifen to the cytosol oestrogen receptors of the anterior pituitary of female rats indicated that the interactions were at common receptor sites and suggested possible conformational changes of the receptor in the presence of tamoxifen (9). In the chick oviduct [^{3}H]tamoxifen behaves as a pure antagonist of 17β-oestradiol (10).

Tritiated tamoxifen at 19.5 Curies/mmol has been found to show enhanced photochemical and autoradiolytic stability by storing as a solution in AR methanol at -20°C in the absence of light.

MATERIALS

Tritiated water and [G-^{3}H]bromobenzene were purchased from Amersham International Ltd. The plates used for chromatography (TLC; preparative TLC) were prepared from Merck Silica GF, Merck Alumina GF, or were obtained from Anachem Manufacturing Ltd. The thickness of the silica film was 0.25 mm for analytical determinations and 0.5 mm for all preparative uses. The ether used was commercially available diethyl ether, which was additionally dried over sodium wire.

Sulphur free toluene (May and Baker Ltd.) was used without further purification. All other solvents were either of analytical reagent quality or were redistilled. For radiochemical purity and specific activity determinations, the samples prepared in standard 20 ml glass vials of low potassium content were counted on a Packard Tri Carb Liquid Scintillation Spectrophotometer model 3320. The 2,5-diphenyl oxazole (P.P.O.) and 1, 4-bis (4-methyl-5-phenyl oxazole) benzene (DMPOPOP) were purchased from Packard Instruments Ltd., Wembley. Naphthalene, (scintillation grade) was obtained from Thorn Electrics Ltd. The photographic film used for autoradiographic studies was either KODAK 'Kodirex' X-ray, or Agfa-Gevaert Structurix film. Kodak Royal Blue X-ray film was used for all fluorographic examinations.

THIN LAYER CHROMATOGRAPHY (TLC)

The systems used throughout this work were: -

System A; Silica GF developed with benzene;triethylamine [90:10]

System B; Silica GF developed with benzene;dicyclohexylamine [90:10]

System C; Alumina GF developed with toluene;triethylamine [95:5]

System D; Silica GF developed with cyclohexane;triethylamine [95:5]

System E; Silica GF developed with ethanol (74 OP);ammonia solution (SG 0.880);water [80:4:5]

System F; Silica GF developed with n-butanol;glacial acetic acid;water [40:10:5]

System G; Silica GF developed with toluene;triethylamine [90:10]

System H; Silica GF developed with toluene;ethyl acetate [75:25]

With the exception of systems C and H all chromatographic separations were developed under light-proof conditions. Systems A and G are interchangeable.

ROUTE I

TRITIATION BY ACID CATALYSED EXCHANGE REACTION

The tritiation reagent employed in these reactions was $TH_2PO_4.BF_3$. It was conveniently prepared by the stoichiometric admixture of phosphorus pentoxide and tritiated water to produce tritiated phosphoric acid which was then converted into the tritiation complex by saturation with boron trifluoride gas. When formed, the complex was a liquid of high density and for safety was stored in polyethylene containers since it rapidly attacks glassware. The mechanism of the reaction is believed to involve the transfer of a triton from the complex to the organic molecule. The cation obtained then loses a proton leaving the organic molecule with one or several tritium atoms incorporated, if the process is repeated on other aromatic hydrogens within the molecule. The reactions with tamoxifen were all carried out in light-proof containers in view of the photochemical effect which promotes some conversion to the cis isomer of tamoxifen (ICI 47,699). A sample of tamoxifen which had been purified by repeated recrystallisation was used for the tritiation.

EXPERIMENTAL

1-[4-(2-Dimethylaminoethoxy)phenyl]-1, 2-trans-diphenyl-1-butene,(tamoxifen) (250 mg) was stirred in diethyl ether (sodium dried) at ambient temperature and under light-proof conditions for 48 hours with the tritiation complex, $TH_2PO_4.BF_3$ (2.304 g). The specific activity of the complex was 911 mCi per gram. On completion of the reaction, the mixture was cooled in an ice-water bath prior to basification with a 50% aqueous solution of sodium hydroxide, followed by extraction with diethyl ether (5 x 50 ml). The ether extracts were combined and washed with water (3 x 10 ml) before drying over anhydrous magnesium sulphate for 24 hours. After filtration, the ether extract and

washings were evaporated to dryness under reduced pressure in a light-proof flask at ambient temperature (Crude Chemical Yield 140 mg; 56%).

Examination by TLC

The residue was examined by TLC using systems E and F, and in each case only one component was seen when visualised under U.V. 254 nm. The product corresponded in R_f to reference tamoxifen indicating that there had not been any major molecular breakdown of the tamoxifen molecule due either to acid catalysis or to radiolytic decay in the presence of 2.1 curies of tritium. TLC examination using system A produced the characteristic 'figure of eight' configuration showing the presence of a mixture of the isomers in the ratio of 60:40 trans-cis. Since the reaction had been carried out under light-proof conditions, the energy required for production of cis isomer from the pure trans material may have been provided by the curie levels of tritium.

Separation of the cis-trans mixture

An attempt was made to separate the tritiated isomeric mixture by isotopically diluting with pure unlabelled trans isomer (100 mg) and recrystallising the mixture serially five times from petroleum ether (Br 40 - 60°C). TLC examination with system A still showed the presence of some cis material indicated by the 'figure of eight' configuration produced. The recrystallisation liquors were therefore combined and evaporated to dryness, and the isomers separated by Method 1 which is described in Part 1 of the paper. After removal of the silica band corresponding to the trans isomer (tamoxifen), the product was isolated from the silica by soxhlet extraction using AR methanol as the refluxing solvent. The methanolic extracts were centrifuged to remove small traces of silica before evaporating to dryness under reduced pressure from a water bath at 35 - 40°C in the absence of light. The residue obtained was recrystallised from petroleum ether (Br 40 - 60°C) to yield 60.5 mg of a white crystalline solid (tamoxifen).

The purity of product was determined by TLC employing systems A, E and F. When viewed under UV 254 nm and after chromatography, using systems E and F, only material corresponding in Rf to reference tamoxifen, was detected. System A showed no 'figure of eight' configuration indicating that a good isomeric separation had been achieved. Plate segmentation, and segmental counting in a toluene based phosphor gave a minimum radiochemical purity of 96.3%. The specific activity was 12.7 μCi/mg [4.7 mCi/mmol]. The product was also examined autoradiographically employing the fluorographic technique described by Luthi and Wasser(11). The plates used were prepared from Merck Silica GF (15 g) which had been ball-milled for 16 hours with an equal weight of anthracene in ethanol (100 ml). Prior to use, the plates were dried at 30°C for 24 hrs. A series of concentrations of the tritiated product were applied to the plate which was referenced with the mixture of isomers and then developed with system A. The plates after drying were covered with Kodak Royal Blue X-ray film, enclosed in a hinged light-proof aluminium container and packed in "dry ice" at -70°C for 16 hrs. The fluorograph obtained was developed as for a normal autoradiograph and the required isomer was the only component detected.

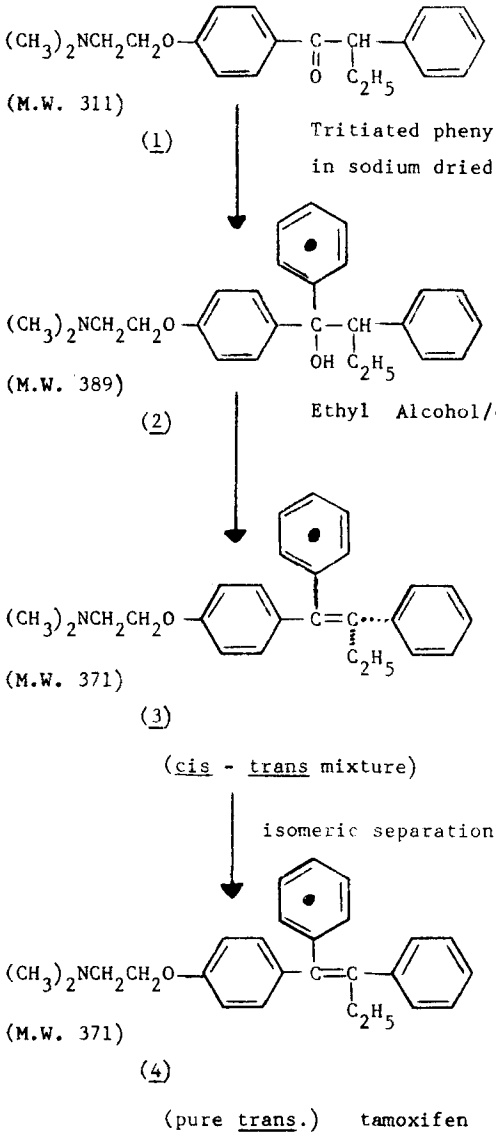
CIS ISOMER (ICI 47,699)

The silica band containing the tritiated cis isomer of 1-[4-(2-dimethylamino-ethoxy)phenyl] 1, 2-diphenyl-1-butene (ICI 47,699) was also removed from the plate and isolated by the same method as that described above for the trans isomer (tamoxifen), when 19.4 mg of a white crystalline solid were obtained. The product was examined by TLC, eluting systems A and F, and examination of the plates under UV 254 nm showed the required product with the same R_f as reference cis isomer; no impurities were detected. Plate segmentation followed by liquid scintillation counting gave radiochemical purities of 98.6% on system A and 98.7% on system F.

ROUTE 2

Scheme 1

SYNTHETIC PATHWAY



· Indicates the position of the Tritium label.

ROUTE 2

EXPERIMENTAL

The preparation of 1-[4-(2-Dimethylaminoethoxy) phenyl]-1-[^{3}H]phenyl-2-trans-(phenyl)-1-butene (4)

All the apparatus was dried at 80°C under reduced pressure, prior to use.

For the preparation of tamoxifen tritiated in the 1-phenyl ring, [G-^{3}H]bromobenzene was used and the synthetic pathway followed was similar to that described in Part 1 of this paper for the preparation of [^{14}C]tamoxifen, where the isotopic label was located in the same position (Scheme I).

The [G-^{3}H]bromobenzene was dissolved in diethyl ether (sodium dried), and maintained over molecular sieve prior to use. The specific activity was 490 mCi/mmol and 173 mg (1.1 mmol) were used to form the Grignard reagent (phenyl magnesium bromide), which was then reacted with 1-[4-(2-dimethylaminoethoxy) phenyl]-2-phenyl-1-butanone (I) (154.5 mg; 0.5 mmol). The product, 1-[4-(2-dimethylaminoethoxy) phenyl]-1-[^{3}H]phenyl-2-(phenyl)-1-butanol (2) was purified by preparative TLC on alumina GF plates and developed with system C.

The product (2) isolated, (83.9 mg) (crude yield based on (I) 43.4%) was dehydrated by dissolving in ethyl alcohol 74 OP (1.9 ml) to which concentrated hydrochloric acid (0.06 ml) had been added, and refluxing the mixture for 3 hours under light-proof conditions. On completion of the reaction, the mixture was diluted with water (10 ml), cooled to ambient temperature and basified with an aqueous solution of sodium hydroxide (20% ^{w}/v) before extraction with diethyl ether (2 x 15 ml; followed by 6 x 10 ml). The ether extracts were combined, washed with water (2 x 10 ml) and dried over anhydrous magnesium sulphate.

After filtration, the extract together with the washings, were evaporated to dryness under reduced pressure at 35°C in light-proof conditions. This produced 61.4 mg of a viscous oil. Examination by TLC (system A) and visualisation under UV 254 nm showed the characteristic 'figure of eight' configuration for a cis-trans mixture of the isomers (3) together with two minor impurities. That all the components seen were radiolabelled, was confirmed by autoradiography.

The cis-trans mixture isolated was purified by preparative TLC as described in method 2 (Part 1 of this paper). This gave 42.3 mg of the trans isomer, tamoxifen. (Stage yield 52.9%).

The product was recrystallised from petroleum ether [Br 60-80°C]. After cooling at 0-4°C for 2 hrs the white crystalline solid was centrifuged to separate, washed with ice-cold petroleum ether and dried under reduced pressure, at ambient temperature in the absence of light. Yield 17 mg. [Overall yield based on ketone = 9.2%; based on bromobenzene 4.2%].

Purity criteria of 1-[4-(2-Dimethylaminoethoxy)phenyl]-trans-1-[^{3}H]phenyl-2-phenyl)-1-butene (4) (tamoxifen)

A series of concentrations of the tritiated product were applied to a silica GF plate referenced with tamoxifen and eluted with solvent system B in a light-proof tank. Examination under UV 254 nm showed only one component, of identical R_f value to that of the reference material. The plate was autoradiographed for 16 hrs. The autoradiograph was then used to "map" the plate which was segmented and counted. The radiochemical purity was determined at 98.4%.

Elemental analysis showed C, 83.3; H, 7.8; N, 3.5; $C_{26}H_{29}ON$ required C, 84.1; H, 7.8; N, 3.6.

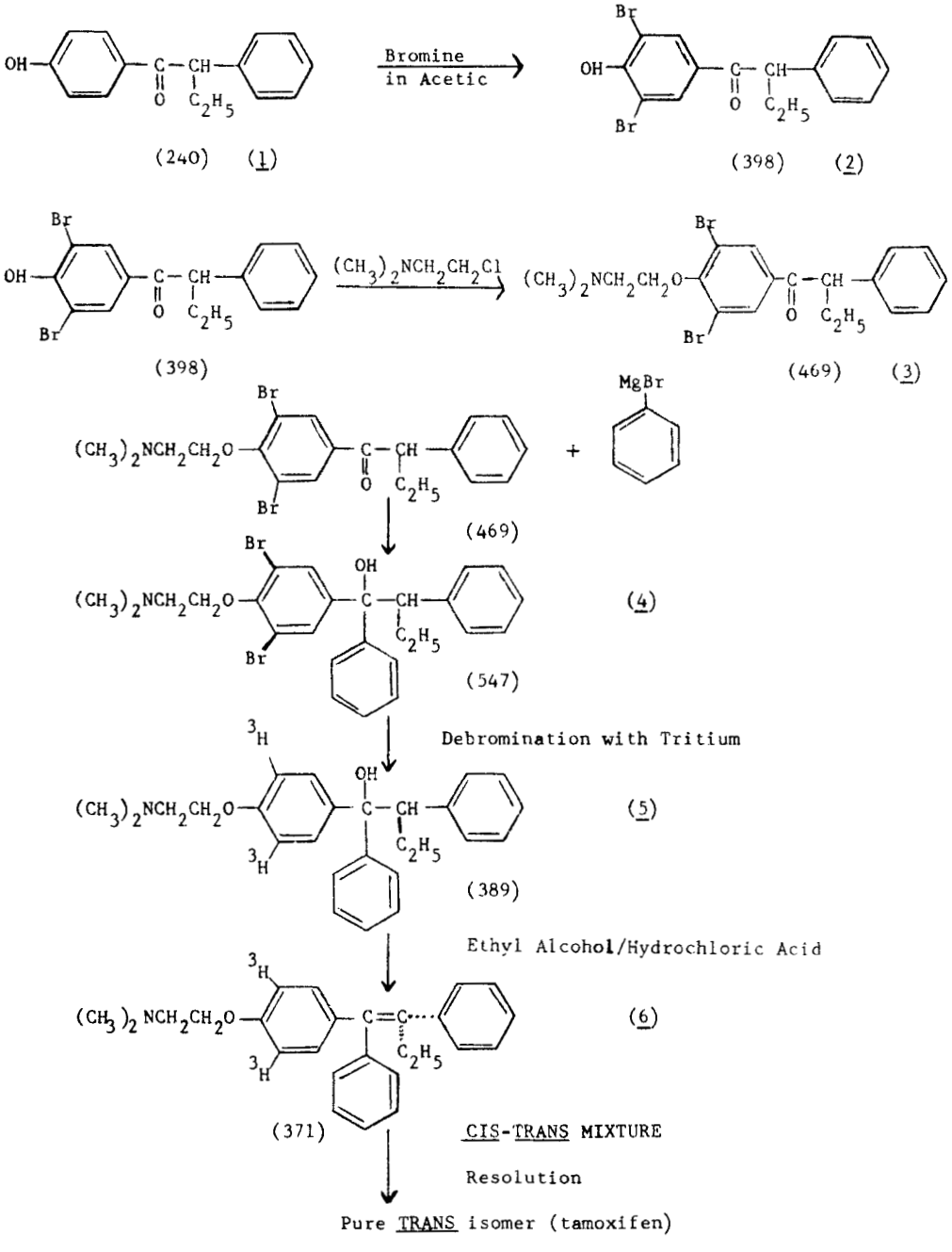
The product was also examined by gas-chromatography - mass spectrometry using an LKB 9000 Spectrometer. The column was 1% OV-1 on Gas Chrom Q (80 - 100 mesh), oven temperature 222°, and a helium gas flow at 30 ml min^{-1}. Only a single peak for the required product was seen.

Two methanolic solutions were prepared for specific activity determinations and triplicate determinations were carried out on each. The specific activity was shown to be 1081.4 $\pm$ 15.5 μCi/mg [401 mCi/mmol].

ROUTE 3

Scheme 2

SYNTHETIC PATHWAY



ROUTE 3

See Scheme 2.

EXPERIMENTAL

Preparation of 3,5-Dibromo-α-ethyl-4-hydroxydesoxy benzoin (2)

Bromine (3.0 ml) was added dropwise with rapid stirring to a solution of α-ethyl-4-hydroxydesoxy benzoin (1) (12.0 g, 0.05 mol) in glacial acetic acid (200 ml) and water (50 ml). When the reaction was complete as shown by TLC (system H) the solution was evaporated to dryness under reduced pressure. The residue was recrystallised from petroleum ether (Br 80-100°C) and 14.1 g of an off-white crystalline solid was obtained (Stage yield 70.9%). Elemental analysis showed C, 48.1; H, 3.4. $C_{16}H_{14}O_2Br_2$ requires C, 48.2; H, 3.5. The structure was confirmed by nuclear magnetic resonance spectroscopy.

Preparation of 3,5-Dibromo-4-(2-dimethylaminoethoxy)-α-ethyldesoxy benzoin (3)

The 3,5-dibromo-α-ethyl-4-hydroxydesoxy benzoin (2) (8.0 g) was converted to the lithium salt by dissolving in a solution of lithium hydroxide monohydrate (0.84 g) in water (6.0 ml), and azeotroping with toluene until all the water had been removed. A solution of dimethylaminoethyl chloride was added to the suspension of the lithium salt in toluene. The mixture was stirred and refluxed for 16 hours. After cooling in an ice-water bath, the mixture was filtered and the filtrate evaporated to dryness under reduced pressure from a water bath at 50°C. The residue was dissolved in diethyl ether and serially extracted with a 5% aqueous solution of acetic acid. The combined acid extracts were stirred with charcoal, filtered, and the acid solution basified with sodium hydroxide solution before serial extraction with diethyl ether (4 x 25 ml). The combined ether extracts were dried over anhydrous magnesium

sulphate, filtered and evaporated to dryness under reduced pressure. A clear oil was obtained (7.7 g) (Found C, 51.1; H, 5.1; N, 3.0. $C_{20}H_{23}Br_2NO_2$ requires C, 51.2; H, 4.9; N, 3.0.) This was equivalent to an 82% yield, and examination by TLC using system G showed only the required product when viewed under UV 254 nm. The structure was confirmed by proton nuclear magnetic resonance spectroscopy.

Preparation of 1-[3,5-Dibromo-4-(2-dimethylaminoethoxy) phenyl]-1,2-diphenyl-1-butanol (4)

A solution of phenyl magnesium bromide was prepared by a Grignard reaction from magnesium (720 mg) and bromobenzene (3.3 ml) dissolved in diethyl ether (sodium dried) (60 ml). The solution was added dropwise to a stirred solution of 3,5-dibromo-4-(2-dimethylaminoethoxy)-α-ethyldesoxy benzoin (3) (6.5 g) in sodium dried diethyl ether (60 ml). On completion of the addition the mixture was refluxed for 2 hours, cooled to ambient temperature in a cold water bath and the magnesium complex decomposed by the addition of a saturated aqueous solution of ammonium chloride. The ether extract was separated and the aqueous phase extracted with diethyl ether (2 x 50 ml). The combined ether extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The oily residue was recrystallised from petroleum ether (Br 80 - 100°C) to produce a white crystalline solid. Elemental analysis showed C, 57.0; H, 5.2; N, 2.3. $C_{26}H_{29}Br_2NO_2$ requires C, 57.0; H, 5.3; N, 2.6. The structure was also confirmed by proton nuclear magnetic resonance spectroscopy.

1-[3,5-Dibromo-4-(2-dimethylaminoethoxy)phenyl]-1,2 diphenyl-1-butanol (4) (150 mg) was subjected to the TR3 process for catalysed halogen-tritium replacement, at Amersham International Ltd. The preparation used tritium gas (35 curies) in the presence of a catalyst which on completion of the reaction was removed,

together with all the labile tritium present. The residue was dissolved in ethanol (25 ml), and shown to contain 8.34 curies of activity. The crude product was returned to ICI and examined by TLC (system C). Detection by UV 254 nm and autoradiography confirmed that there were two components, both tritium labelled, one of which corresponded in R_f to 1-[4-(2-dimethylaminoethoxy)-[3,5-^{3}H]phenyl]-1,2-diphenyl-1-butanol (5). The ethanol solution (25 ml) was evaporated to dryness under reduced pressure from a water bath at 35-40°C when a sticky product was obtained, crude yield 160 mg. Preliminary work had shown that the crude product could be dehydrated successfully.

Dehydration of 1-[4-(2-dimethylaminoethoxy)-[3,5-^{3}H]phenyl]-1,2-diphenyl-1-butanol (5)

A solution of the crude product (160 mg) in ethanol 74 OP (3.6 ml) containing concentrated hydrochloric acid (0.12 ml) was stirred and refluxed for two hours in a light-proof flask. The mixture was cooled by the addition of crushed ice, basified with an aqueous solution of sodium hydroxide (2.0 molar) before extracting with toluene (5 x 15 ml). The toluene extracts were combined, washed with water (2 x 2.0 ml) and dried for 16 hours over anhydrous magnesium sulphate. Examination by TLC (system A) showed the expected 'figure of eight' for the cis-trans mixture when viewed under UV 254 nm, together with other impurities. Autoradiography confirmed that all the products seen by the chromatographic separation were radioactive.

Purification

1. Preparative Thin Layer Chromatography

After filtration to remove the magnesium sulphate, the toluene extract and washings, were evaporated to dryness under reduced pressure in a light-proof flask. The residue was dissolved in AR methanol and the solution applied to six preparative silica TLC plates which were developed with system A in a light-proof tank. The bands corresponding in R_f to tamoxifen (trans isomer) were removed and the product extracted from the silica by stirring with AR methanol in a light-proof vessel. After filtration, the methanolic extract was evaporated to dryness under reduced pressure at ambient temperature; the residual pale yellow gum slowly crystallised.

2. Recrystallisation

The product was dissolved in petroleum ether (Br 60-80°C) (4.0 ml) and centrifuged to separate from a small amount of insoluble material which included traces of silica. The clear supernatant was evaporated to dryness in a stream of nitrogen and the residue was dissolved in petrol ether (1.0 ml). After cooling for 30 mins at 0°C a white crystalline product was produced. The mixture was centrifuged to separate and the product washed with ice cold petroleum ether (Br 60-80°C) (2 x 0.3 ml) before drying under reduced pressure at ambient temperature in the absence of light (Yield 30 mg).

The specific activity of the product was determined in triplicate on three separate solutions in methanol and shown to be 52.5 Mci/mg (19.5 Curies/mmol). A solution containing 0.94 mg in 100 ml AR methanol was prepared for purity and stability studies. A range of concentrations were applied to a silica GF plate, and each was overspotted with a reference solution of unlabelled tamoxifen in

AR methanol. The plates were referenced with the cis-trans tamoxifen mixture, and developed with system A. When viewed under UV 254 nm the unlabelled reference isomeric mixture showed the expected 'figure of eight' configuration, but the purified product showed only the trans isomer (tamoxifen) to be present and no impurities were detected. This was confirmed by autoradiography. Plate segmentation and counting of the chromatographic strip gave a minimum radiochemical purity of 91.5%.

STABILITY OF THE LABELLED TAMOXIFEN

Tamoxifen had not previously been prepared at such a high molar specific activity and hence no information as to its radiochemical stability either in solution or the solid state was available. The purified material isolated as a crystalline solid and stored at -20°C in absence of light showed a radiochemical purity of 91.5% on the day of preparation (day 1), by day 5 the purity had fallen to 84.4% and by day 7 there had been a further fall to 79.5%.

Stability of the solution

The methanolic solution of the labelled product (0.94 mg%) prepared from the recrystallised tritiated tamoxifen on day 1 was stored at -20°C in the absence of light and the radiochemical purity determined daily by the TLC method described. It was shown that the purity was unchanged over 7 days. The tritiated tamoxifen is therefore much more stable when stored as a methanolic solution at -20°C in the absence of light than in the solid state. The solid whose radiochemical purity had fallen by 12% in seven days was repurified by Method I described in Part I under this title.

The labelled tamoxifen isolated (26.2 mg) was then immediately dissolved in AR methanol (100 ml) and stored at -20°C in the absence of light. The purity of the tritiated product in solution was determined at various time intervals, and the results are shown in Table 1.

There is clearly, as expected, a retardation of autoradiolytic decay of the product when it is stored under these conditions as opposed to the solid state.

The product was examined by gas chromatography - mass spectrometry using an L.K.B. 9000 spectrometer. A single peak for the required product was found and no impurities were detected. The sample was also spiked with a solution of ICI 47,699 (cis isomer of tamoxifen) and the GC-MS spectrum obtained confirmed the authenticity of the product as trans isomer (tamoxifen).

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TABLE I

Determination of the Radiochemical Purity of tritiated Tamoxifen in methanol at -20°C in the absence of light

DAY	RADIOCHEMICAL PURITY %	ORIGIN IMPURITY %
1	94.2	2.0
14	93.7	1.96
24	95.1	1.64
38	95.2	1.7
52	91.4	2.2
69	92.6	2.3
94	91.9	2.5
134	90.7	2.2
181	91.5	2.8
241	88.0	3.34
302	82.4	5.3

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