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

I. The Synthesis of Carbon-14 Labelled Tamoxifen ('Nolvadex'*)

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SUMMARY

The preparation of [^{14}C]tamoxifen from [U-^{14}C]bromobenzene is described. Several preparations of the compound have been made with overall radiochemical yields of 12% - 14%, and specific activities 1.3 to 10.1 mCi/mmol have been obtained.
The purification procedures used, including the separation of the cis-trans isomers by preparative thin layer chromatography, are also described.

Key Words: Tamoxifen, carbon-14, cis-trans isomers, photochemical, synthesis

INTRODUCTION

Tamoxifen (ICI 46,474 'Nolvadex'*), one of a series of triphenyl ethylenes, is the trans isomer of 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene. It has shown anti-oestrogenic behaviour in some laboratory animals (1) The pharmacological action is believed to occur as a result of competition with oestrogens for binding sites on target organs and the compound has also shown selective uptake in human uterine tissue (7).

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It has been used successfully for the treatment of advanced breast cancer (2, 3). Clinical experience has shown tamoxifen to have a superior tolerance to that found with other forms of hormonal therapy. It has also been administered to patients with anovulatory infertility (4).

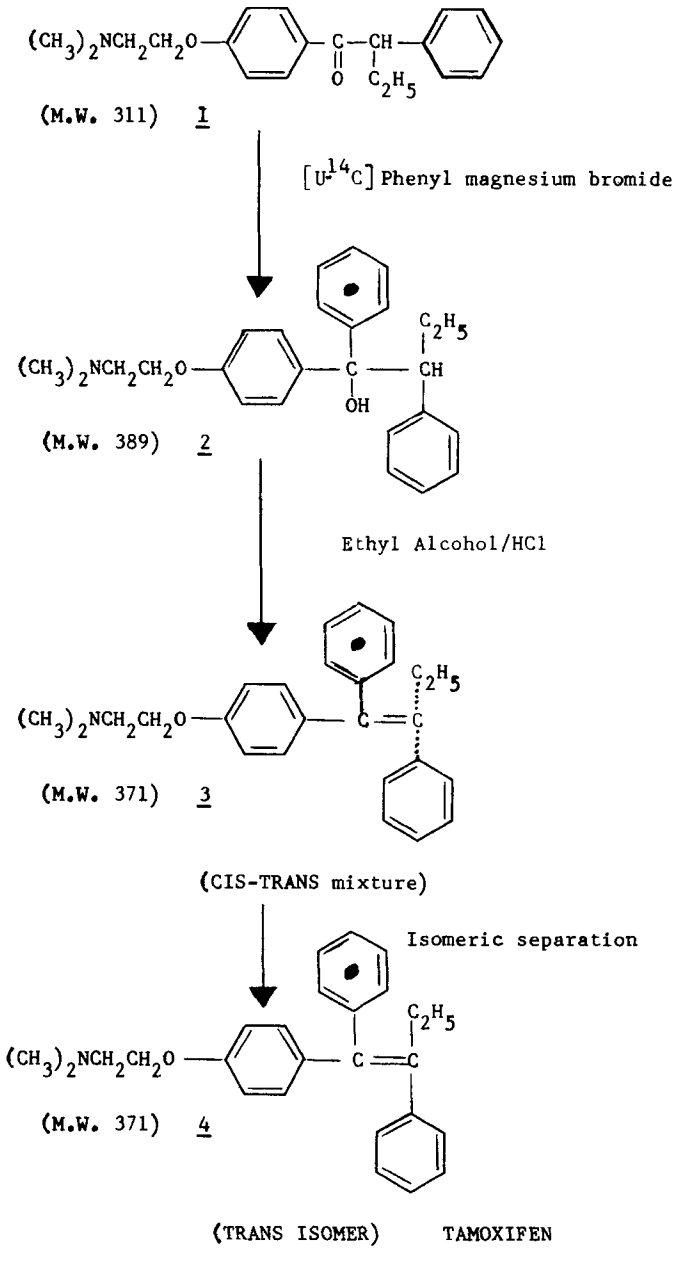
In order that absorption, distribution, metabolism and excretion studies could be investigated, the compound has been prepared isotopically labelled with carbon-14 or tritium at various molar specific activities. A study of its metabolism in laboratory animals employing carbon-14 labelled material has been carried out and has shown that 80 - 90% of the radioactive dose was eliminated in the faeces over a period of 10 - 12 days, in four animal species (5). The metabolism of tamoxifen in female patients has also been investigated (6).

The use of [U-^{14}C]bromobenzene produced tamoxifen with the label in the 1-phenyl ring. The overall radiochemical yields were of the order of 12 - 14% and labelled material has been synthesised with specific activities ranging from 1.3 mCi to 10.1 mCi per mmol. Tritiated material, which has been prepared with a wide range of molar specific activities and by several synthetic routes, will be described in a subsequent paper under this title.

MATERIALS

[U-^{14}C]Bromobenzene was purchased from Amersham International. Commercially available anhydrous diethyl ether was additionally dried over sodium wire. Sulphur free toluene (May and Baker Ltd.) was used without further purification. All other solvents used were either redistilled or of analytical reagent quality. The plates used for chromatography (TLC; preparative TLC) were prepared from Merck Silica GF or Merck Alumina GF and were 0.25 mm thick for analytical determinations and 0.5 mm for preparative purifications.

Synthetic Pathway



● Indicates the position of the CARBON-14 LABEL

All samples used for the determination of radiochemical purity and specific activity were counted on a Packard Tri Carb Liquid Scintillation Spectrophotometer model 3320 in standard glass screw-cap vials of low potassium content (Packard Instruments Ltd. Wembley). The 2,5-diphenyl oxazole (P.P.O.) and 1, 4-bis [2-(4-methyl-5-phenyloxazoyl)]-benzene (DM POPOP) were purchased from Packard Instruments Ltd., Wembley. Naphthalene (scintillation grade) was obtained from Thorn Electronics Ltd. For autoradiography, the photographic film used was Kodak 'Kodirex' X-ray film.

THIN LAYER CHROMATOGRAPHY (TLC)

Four systems were used throughout this work; these 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]

All chromatographic separations employing systems A, B and D were developed under light-proof conditions.

EXPERIMENTAL

The Preparation of 1-[4-(2-Dimethylaminoethoxy) phenyl]-1-[U-^{14}C] phenyl-2-phenyl-1-butanol (2)

All the apparatus used was dried at 80^{o}C under reduced pressure prior to use. [U-^{14}C]Bromobenzene (188.6 mg, 1.20 mmol) with a specific activity of 16.7 mCi/mmol was isotopically diluted with unlabelled bromobenzene (137.4 mg) and was then stirred with diethyl ether (sodium dried) (6.0 ml) in the presence of magnesium turnings (48 mg) which had been degreased prior to use by washing with warm toluene. The reaction was initiated catalytically with a crystal of iodine and was maintained under reflux conditions for three hours.

A solution of 1-[4-(2-dimethylaminoethoxy)phenyl]-2-phenyl-1-butanone (I) (309 mg, 0.99 mmol), in sodium dried ether (5.0 ml) was added dropwise over a period of 45 mins. The mixture was stirred for a further 2.5 hours. As the magnesium complex was deposited from the solution the maintainance of stirring was difficult.

After cooling in a cold water bath to ambient temperature, the reaction mixture was treated with a saturated solution of ammonium chloride to decompose the magnesium complex. The ether layer was removed and the product serially extracted from the aqueous phase with diethyl ether (6 x 15 ml). The ether extracts were combined, washed with distilled water (2 x 10 ml) and dried over anhydrous magnesium sulphate for 16 hours. The extract was examined by TLC against reference standards using system C.

Visualisation under U.V. 254 nm followed by autoradiography showed that the required alcohol (2) had been formed together with some impurities and that the chromatographic patterns detected by both methods were identical. Following filtration, the ether extract and washings were evaporated to dryness under reduced pressure at 40^{o}C. A sticky, off-white solid was obtained (399 mg).

Purification

Preliminary work had indicated that it was necessary to purify the alcohol (2) at this stage. Recrystallisation of the crude product from petroleum-ether (B.r. 60-80^{o}C) gave 265 mg of the purified alcohol (2) (Stage Yield 69%) [Recrystallisation Yield 66.4%]. Examination by TLC (system C) and inspection by U.V. 254 nm and autoradiographic methods showed that the product was the required material, with only trace impurities.

Preparation of the cis-trans mixture of 1-[4-(2-Dimethylaminoethoxy) phenyl]-1-[U-^{14}C]phenyl-2-phenyl-1-butene (3)

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-[U-^{14}C]phenyl-2-phenyl-1-butanol (2) (265 mg) was dehydrated by dissolving in ethyl alcohol 740P IMS (Industrial

Methylated Spirit)(6.0 ml) to which concentrated hydrochloric acid (0.19 ml), was added. The mixture was stirred and heated under reflux in a light-proofed flask under an atmosphere of argon for 3 hours. The mixture was cooled, diluted with crushed ice, basified with 2N sodium hydroxide solution, and then extracted with toluene (5 x 15 ml). The combined toluene extracts were washed thoroughly with distilled water until the washings were alkali-free, and then dried over anhydrous magnesium sulphate in the absence of light.

The extract was examined by TLC (system A) under light-proof conditions. Visualisation under U.V. 254 nm showed the expected 'figure of eight' configuration for the cis-trans isomeric mixture of the required triphenyl ethylene, together with several more polar impurities. (See Plate I). It was confirmed by autoradiography of the TLC plate over a 16 hour period that all the products seen under U.V. 254 nm were carbon-14 labelled. The toluene extract, after filtration, was evaporated to dryness under reduced pressure (water bath temperature 40°C) in a light-proofed flask. A light oil was obtained comprising the cis-trans mixture (3), crude yield 244 mg (stage yield 96.6%).

Separation of the cis-trans mixture of 1-[4-(2-Dimethylaminoethoxy) phenyl]-1-[U-^{14}C]phenyl-2-phenyl-1-butenes. (3)

METHOD 1

The mixture of the cis-trans isomers (244 mg) (see Plate 1) was dissolved in AR methanol (8.0 ml) and equal aliquots applied as thin streaks to 18 Silica GF plates (0.5 mm silica layer and dimensions of 20 x 40 cm). All the plates had been pre-washed three times with the developing solvent (system A) before application of the mixture and elution in a light-proofed tank. The plates were dried in the absence of light and when examined under U.V. 254 nm the expected isomeric separation of the cis-trans mixture (3) was clearly seen on each plate together with six impurities of a more polar character.

Each plate was then carefully marked into three separate areas:

1) The lead band (tamoxifen; trans isomer)
2) The overlap area being a cis-trans mixture
3) The lag band (ICI 47,699; cis isomer)

The lead bands were removed from the plates and combined. The trans isomer was removed from the silica by extraction with AR methanol at ambient temperature in a light proofed container, and freed from the silica by filtration through a pad of High-Flow Supercell. The methanolic extracts together with the washings were evaporated to dryness in the absence of light, under reduced pressure, from a water bath at 40°C. An oily product (147 mg) was obtained.

Recrystallisation

The product (147 mg) was dissolved in boiling petroleum ether [Br 60 - 80°C] (5.0 ml) and the solution centrifuged to separate traces of insoluble material. The supernatant liquid was removed and evaporated to dryness in a stream of nitrogen. The residue was redissolved in petroleum ether (1.0 ml) and kept cold at 0 - 4°C for 16 hours. The product, a white crystalline solid, was centrifuged and washed with ice cold petroleum ether (2 x 0.3 ml) before drying under reduced pressure at ambient temperature for 16 hours. A white crystalline solid (108 mg) was obtained (Found: C, 83.9; H, 7.8; N, 3.8. $C_{26}H_{29}ON$ requires C, 84.1; H, 7.8; N, 3.8). This represents an overall chemical yield of 14.0%, a radiochemical yield of 14.7%, and a recrystallisation yield of 73.5%.

METHOD 2

Several labelled syntheses of [^{14}C]tamoxifen have been prepared over several years. On one occasion, owing to a change in the batch of silica used for the preparative TLC separation, it was not possible to obtain the 'figure of eight' configuration of the product showing cis-trans isomeric separation when the plate was developed in system A.

Subsequently, another system (B) was found after a considerable search, which achieved the desired cis-trans separation. When the lead band was removed and the product extracted from the silica by AR methanol, the methanolic extract on evaporation, produced a mixture of the required product in dicyclohexylamine. This occurs because the boiling point of dicyclohexylamine is considerably in excess of that for triethylamine and the amine is not removed when the methanolic extract is evaporated to dryness under reduced pressure at 30°C. The mixture of product and dicyclohexylamine was then separated by preparative TLC employing system D. The band corresponding to the required trans isomer was now removed from the silica and the pure product isolated as in Method 1. The results obtained were comparable, since on the same scale synthesis as described above the overall yield was 12%.

Purity Criteria of 1-[4-(2-Dimethylaminoethoxy) phenyl]-1-[U-^{14}C]phenyl-2-trans-phenyl-1-butene (4) (Tamoxifen)

A series of concentrations of the methanolic solution of the purified product were applied to two silica plates which were developed in solvent systems A and B, in light-proofed tanks, the plates were then dried and autoradiographed for 16 hours. The autoradiographs were used to 'map' the silica plates which were then segmented and counted. Triplicate determinations gave an average purity using system A of 99.6% $\pm$ 0.2%, and 99.2% $\pm$ 0.1% in system B. The chromatographic patterns detected by irradiation with U.V. 254 nm, and by autoradiographic procedures were identical. The product was also examined by gas chromatography - mass spectrometry using an LKB 9000 spectrometer. The gas chromatograph was operated under the following conditions:
Column 1% OV-1 on Gas-Chrom Q (80 - 100 mesh), oven temperature 222° and helium carrier gas flow at 30 ml/min $^{-1}$. A single peak for the required product was found and no impurities were detected. The specific activity of the final product was shown to be 27.3 $\pm$ 0.17μCi/mg [10.1 mCi/mmol].

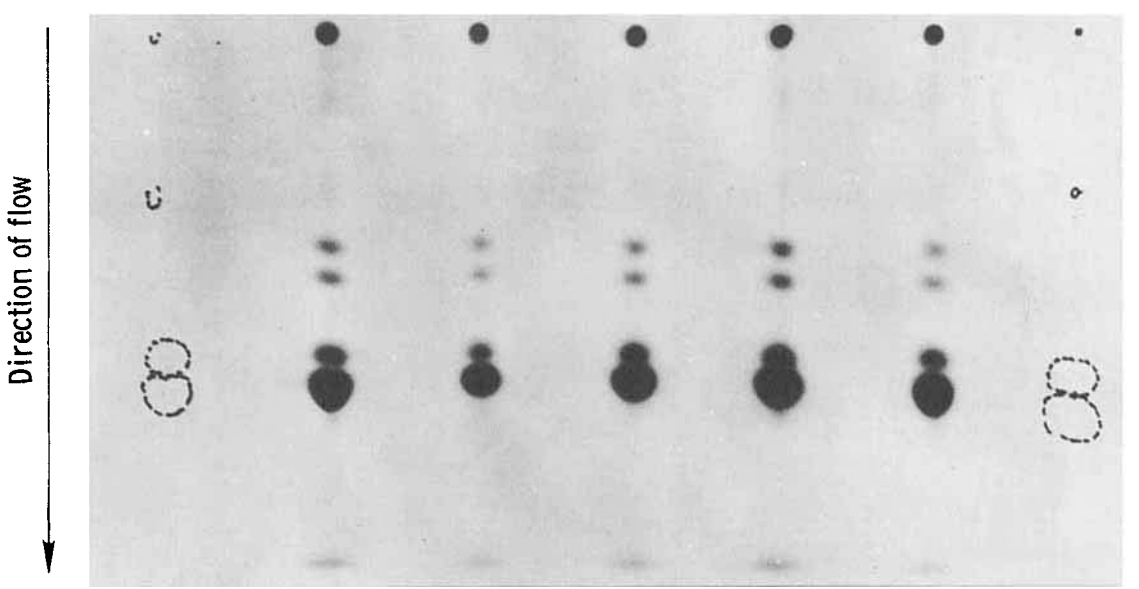


Plate 1. System A. Benzene - Triethylamine (90:10)

REFERENCES

1. HARPER, M.J.K. and WALPOLE, A.L. J. Endocrinol, 37: 83-90 (1967)

2. WARD, H.W.C. Brit. Med. Journal, 1: 13-14 (1973)

3. COLE, M.P., JONES, C.T.A. and TODD, I.D.H. Brit. J. Cancer, 25: 270-275 (1971).

4. KLOPPER, A. and HALL, M. Brit. Med. Journal 1: 152, (1971)

5. FROMSON, J.M., PEARSON, S. and Mrs S. BRAMAH, Xenobiotica, 3: No. 11, 693-709 (1973)

6. FROMSON, J.M., PEARSON, S. and Mrs S. BRAMAH. Xenobiotica, 3: No. 11, 711-714 (1973)

7. FROMSON, J.M. and SHARP, D.S. J. Obstetrics and Gynaecology, 81: 321-323 (1974)

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