SYNTHESIS OF A TRITIUM LABELLED ANTIHISTAMINIC DRUG [^{3}H]-N,N-DIETHYL-2-[4-(PHENYLMETHYL)PHENOXY]-ETHANEAMINE • HCl

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SUMMARY

A tritium labelled antihistamine, N,N-diethyl-2-[4-(phenyl-methyl)phenoxy]- ethaneamine • HCl (DPPE, 4) was synthesized to investigate its binding characteristics to intracellular histamine receptors (H_{IC}) and for further studies with specific ligands of H_{IC} to determine their potential effects on cell proliferation. A palladium catalyzed reduction of an activated carbonyl between the two phenyl groups of N,N-diethyl-2-[4-(benzoyl)phenoxy]-ethaneamine • HCl (DBPE, 3) with 100% tritium gas was successful, where 55% of the theoretical activity (specific activity 31.6 Ci/mmol) was obtained.

Key words: Tritium, antihistamine, histamine, H_{IC} receptor, platelets

INTRODUCTION

Compound 2 (Figure 1) binds with high affinity to anti-estrogen binding sites, while its affinity for estrogen binding sites is not significant at reasonable concentrations[1,2,3]. The contribution of both anti-estrogen binding sites and the growth inhibitory activity of the triphenylethylene derivative tamoxifen (TAM, 1) and 2 on human breast cancer cells has

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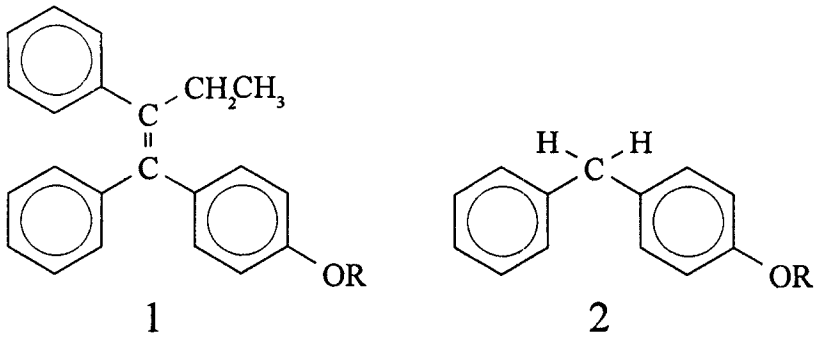

Figure 1. The structures of TAM (1) and DPPE (2).

been established[4]. Further studies indicate that 2 binds selectively to intracellular histamine receptors (H_{IC}) and inhibits the binding of histamine to that site[5]. The binding of histamine to H_{IC} sites is known to mediate platelet aggregation and also to somehow participate in cell proliferation[5]. Compound 4, a tritiated analogue of 2 (Figure 2) was synthesized to facilitate further study of intracellular mechanisms of histamine binding sites, and also to aid the development of new H_{IC} ligands.

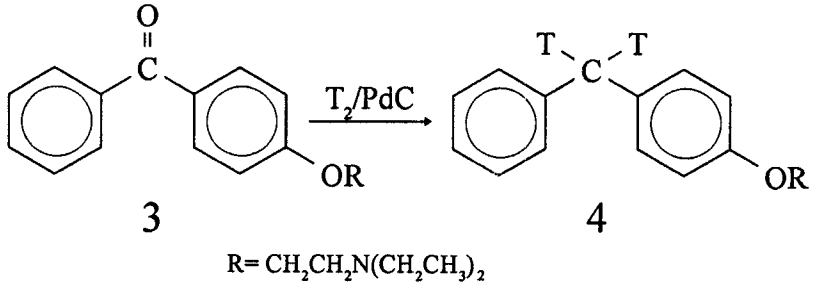

Figure 2. Synthesis of [^{3}H]DPPE (4) from DBPE (3).

RESULTS AND DISCUSSION

The radioactive labelling of a pharmaceutical compound often has limitations determined by subsequent pharmacological assays. In this case the specific activity (S.A.) of 4 must be sufficiently high, due to the small number of H_{IC} receptors in most biologic

preparations. Also the conformation of compound 2 should not be changed by the labelling, and of course the resulting label should be as stable as possible. For our purposes a S.A. greater than 20 Ci/mmol was required.

Synthetic pathways

In order to maximize S.A. it is often wise to introduce tritium as the last step in the synthesis. Evidently a good position for labelling in 2 is the carbon atom between the two aromatic groups or on the aromatic ring itself. At first we tried catalytic hydrogenation of the carbonyl group of 3 with PtO_2[6,7] but this resulted in reduction of the aromatic rings to cyclohexane. Catalytic hydrogenation of a dithioacetal protected carbonyl with Raney nickel[8] was only partially successful (30% of the substrate was obtained) and hydrogen atoms were introduced from the solvent (water). Because the efficiency of reaction was not good and the preparation of Raney nickel/T_2O is not very convenient the method was excluded. Although the ring-labelled 2 has been synthesized by Brandes et al[9], with S.A. of 35.6 Ci/mmol, the bromination-debromination method was found to be inferior due to nonselective bromination of 2, where even the methylene carbon between the two phenyl groups was brominated to some extent. Also, we expect that a label on the methylene carbon would be more stable than labelling on the aromatic rings. Catalytic hydrogenation of the carbonyl group of 3 with 10% Pd/C was not successful at room temperature, but we finally succeed when the reaction temperature was increased to 45°C. All preliminary reaction products were verified by ^{1}H- and ^{13}C-NMR.

Characterization of the products

The unlabelled reference and substrate compounds (2 and 3) were characterized by MS and NMR (^{1}H and ^{13}C) spectroscopy. The tritiated analogue 4 was characterized by NMR (^{1}H and ^{3}H) and compared with 2. Specific radioactivity of the product was measured by liquid scintillation counting (LSC), radio-HPLC and ^{3}H-NMR.

Characterization of unlabelled reference (compound 2).

The yield of 2 was 70% of the theoretical, and purity of the product was determined to be 90% by ^{1}H-NMR. The material decomposed at 150-153^{o}C.

Mass spectrometry: m/z 283 (8%,M), 91 (5%), 86 (100%).

^{1}H-NMR: chemical shift (ppm) 7.24 (2H, m), 7.17 (5H, m), 6.94 (2H, d), 4.31 (2H, t), 3.91 (2H, s), 3.58 (2H, t), 3.33 (4H, q), 1.35 (6H, t).

^{13}C-NMR: chemical shift (ppm) 155.73 (s), 141.25 (s), 134.64 (s), 130.10 (d), 128.77 (d), 128.45 (d), 126.05 (d) 114.64 (d), 62.74 (t), 50.57 (t), 47.47 (t), 41.00 (t), 8.83 (t)

Characterization of substrate (compound 3).

The yield of 3 was 50% of the theoretical and purity of the product was determined to be 98% by ^{1}H-NMR. The material decomposed at 132-134^{o}C.

Mass spectrometry: m/z 297 (5%,M), 105 (14%), 86 (100%).

^{1}H-NMR: chemical shift (ppm) 7.83 (2H, m), 7.72 (2H, m), 7.62 (1H, m), 7.52 (2H, m), 7.16 (2H, m), 4.49 (2H, t), 3.67 (2H, t), 3.34 (4H, q), 1.40 (6H, t.)

^{13}C-NMR: chemical shift (ppm) 195.3 (s), 160.83 (s), 137.94 (s), 132.47 (d), 132.16 (d), 131.23 (s), 129.64 (d), 128.30 (d), 114.30 (d), 62.98 (t), 50.55 (t), 47.60 (t), 8.76 (t)

Characterization of tritiated product (compound 4).

The radiochemical yield of the tritiation reaction, as calculated by HPLC, was 16.2 mg (83%). The S.A. of radiochemically pure product was 37.7 Ci/mmole measured by liquid scintillation counting, 27.7 Ci/mmole by radio-HPLC and 30.7 or 30.3 Ci/mmole by NMR depending on the basis of calculations (Table 1).

^{1}H-NMR: same pattern of peaks as in compound 2 except chemical shift 3.91 (2H, s), 3.85 (H,T, doublet which another peak is under 3.91 singlet) which pattern of peaks was also found in ^{3}H-NMR spectrum.

Calculations of S.A. of 4 based on NMR data. Products of hydrogen isotope exchange reactions often contains a mixture of various labelled species. The significant possibilities

in this case were molecules that had two tritium atoms, one tritium and one hydrogen atom or two hydrogen atoms. Since the S.A. of a species containing one tritium atom would have 28.76 Ci/mmole and corresponding species containing two tritium atoms would have a S.A. of 57.52 Ci/mmole, the molar fractions of each component must be calculated to determine the actual S.A. of the entire product. Due to the fact that each isotopomer has a different chemical shift in NMR spectrum, the integrals from ^{3}H-NMR spectroscopy are directly proportional to the number of tritium nuclei in a molecule, as are the number of hydrogen nuclei in ^{1}H-NMR spectroscopy. When the molar fractions of existing isotopomers are resolved in a mixture, then the S.A. can be calculated by multiplying each mole fraction to its specific activity and summing the results[10].

Mole fractions of isotopomers were calculated by combining information from the proton coupled tritium spectrum (Figure 3) and the tritium-coupled proton spectrum (Figure 4) and also by combining the information of the inverse gated proton decoupled tritium spectrum (Figure 5) and the tritium-coupled proton spectrum of compound 4.

In the tritium spectrum the integral of peak -CT_2 (which includes a half of the -CTH doublet) has an integral value of 801. The integral of one half of the -CTH doublet peak is 251, and so the value which is proportional to the total amount of the -CT_2 isotopomer in the mixture is 550. The amount of the -CTH isotopomer is proportional to the integral value of 502. Since isotopomers containing only a -CH_2 group do not appear in the ^{3}H-NMR we consider the ^{1}H-NMR spectrum. By excluding the integral of one side of -CHT doublet from the integral of the -CH_2 singlet (Figure 3) we calculate an integral of 42.59. The mole fraction of -CH_2 can now be calculated, since the value of the -CTH integral from the tritium spectrum represents the same number of molecules as the integral value of -CHT integral from the proton spectrum. Hence, we have mole fractions for -CT_2 (27.9%), -CTH (51.0%) and -CH_2 (21.1%) and the S.A. of 4 can be calculated (Table 1).

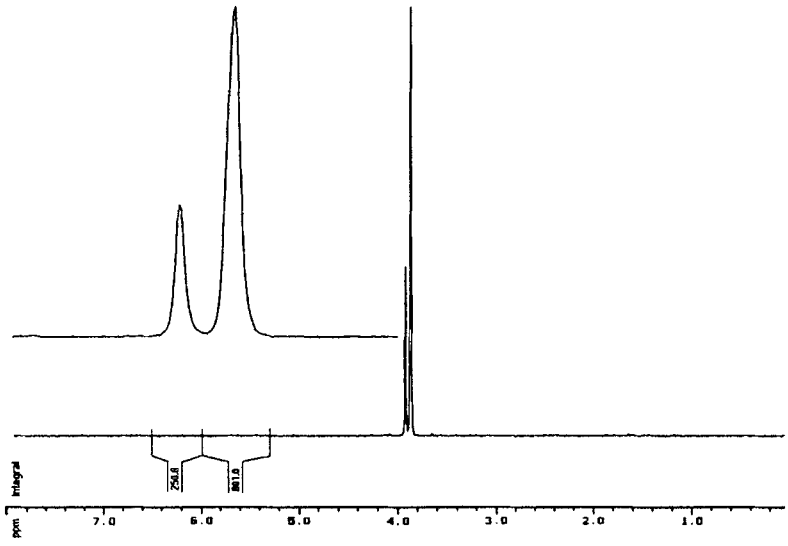

Figure 3. The proton coupled ^{3}H-NMR spectrum of [^{3}H]DPPE (4).

Another way to make these calculations is to take the values for -CT_2 and -CTH from the inverse gated ^{1}H-decoupled tritium spectrum (Figure 5). The integral value for -CH_2 and S.A. can then be calculated as just described. A comparison of these two methods are detailed in Table 1.

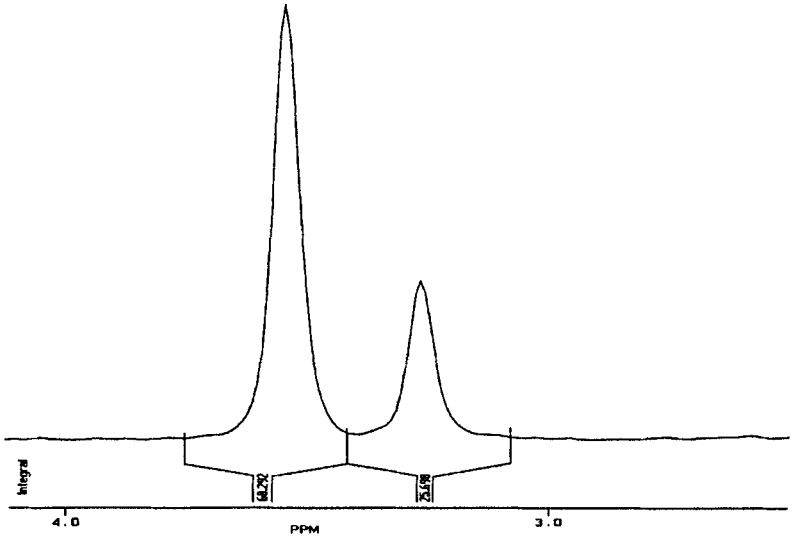

Figure 4. The tritium coupled ^{1}H-NMR spectrum of [^{3}H]DPPE (4).

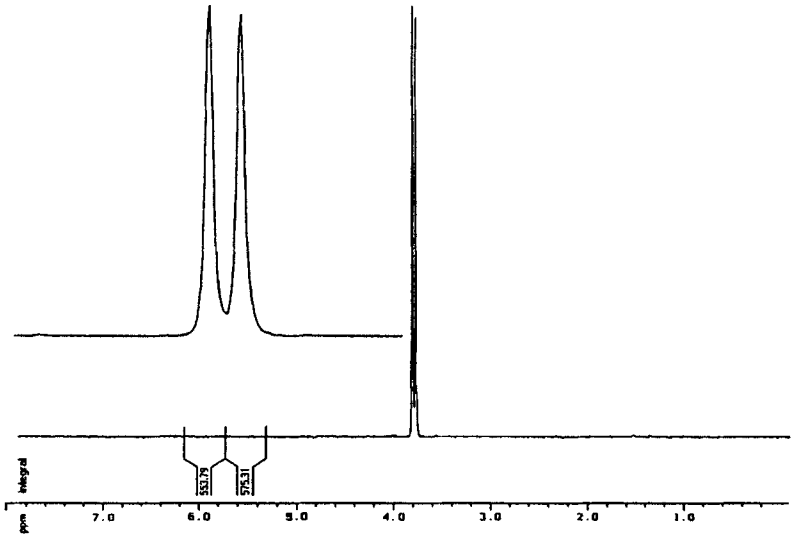

Figure 5. The inverse gated proton decoupled ^{3}H-NMR spectrum of [^{3}H]DPPE (4).

Table 1. Calculations of S.A. for [^{3}H]DPPE (4) based on NMR data.

Molecular species	Integral values [A]	[B]	Integral values for molefractions [A]	[B]
$-CT_2$	550	575	275	288
-CTH	502	554	502	554
$-CH_2$	416	459	208	230
			+ 985	+ 1072

Calculations:

$$\text{S.A.[A]: } \frac{275}{985}\cdot 57.52 + \frac{502}{985}\cdot 28.76 + \frac{208}{985}\cdot 0 = 30.72 Ci/mmol$$

$$\text{S.A.[B]: } \frac{288}{1072}\cdot 57.52 + \frac{554}{1072}\cdot 28.76 + \frac{230}{1072}\cdot 0 = 30.32 Ci/mmol$$

[A] = From ^{1}H coupled ^{3}H-NMR spectrum
[B] = From inverse gated ^{1}H decoupled ^{3}H-NMR spectrum

EXPERIMENTAL

4-Hydroxybenzophenone, 2-diethylamino ethylchloride • HCl and 10% Pd/C were all purchased from Fluka Chemicals, Buchs, Switzerland. 4-Hydroxydiphenylmethane was purchased from Aldrich, USA. All solvents were analytical grade or better.

Synthesis of N,N-Diethyl-2-[4-(phenylmethyl)phenoxy]-ethaneamine •HCl (DPPE, 2)

The unlabelled reference material was synthesized by a nucleophilic substitution of 4-hydroxydiphenylmethane (0.92 g, 5 mmol) to 2-diethylamino ethylchloride (0.86 g, 5 mmol of 2-diethylamino ethylchloride • HCl) under basic conditions (0.4 g NaOH + 1.2 ml H_2O) according the procedure of Poling et al.[11] Yield 1.12 g (70%).

Synthesis of N,N-Diethyl-2-[4-(benzoyl)phenoxy]-ethaneamine • HCl (DBPE, 3)

Synthesis of substrate for the tritiation reaction was made by a similar procedure for 2. The starting materials were 4-hydroxybenzophenone (0.99 g, 5 mmol) and 2-diethylamino ethylchloride • HCl (0.86 g, 5 mmol). Yield 0.80 g (50%).

Synthesis of [^{3}H] N,N-Diethyl-2-[4-(phenylmethyl)phenoxy]-ethaneamine • HCl ([^{3}H]DPPE, 4)

Synthesis of 4 was carried out in ethanol by catalytic reduction of the carbonyl group of 3 on 10% Pd/C with tritium gas. A hydrochloride salt of compound 3 (20.3 mg, 0.06 mmole) was dissolved in 2 ml of 95% ethanol in 15 ml reaction flask. The catalyst (8.6 mg 10% palladium on charcoal) was weighed and placed in a small carrier above the solvent. The flask was purged three times with nitrogen gas and degassed under ultra high vacuum (10^{-12} mmHg) by twice freezing the sample in liquid nitrogen and thawing it slowly to room temperature. After three more flushings with nitrogen gas, the 500 mmHg pressure of 100% tritium gas was generated and introduced into the reaction vessel by controlled heating of a uranium hydride source. The catalyst was added and the reaction vessel was heated to 45°C increasing the pressure inside reaction flask to nearly 700mmHg. After stirring for six hours the reaction was terminated and the tritium-contaminated solvent was removed by lyophilization. The residue was dissolved in 2 ml of methanol and filtered. The catalyst was washed five times with 1 ml aliquots of methanol, and five times with 1 ml aliquots of ethanol. Solvent was again removed by lyophilization before characterization of product.

Mass spectrometry

Electron impact mass spectra were measured at 70 eV electron energy by Trio-2-VG Masslab (Manchester, England) mass spectrometer, with on ion source temperature of 250°C. The sample was introduced with a glass capillary by a direct insertion probe, where the temperature was raised from 30°C to 400°C at the rate of 50°C/min.

NMR Spectroscopy

The ^{1}H- and ^{13}C-NMR spectra of 2 and 3 were recorded on a Bruker AM 400 WB spectrometer operating at 100.6 MHz and 400.1 Mhz, respectively. ^{3}H-NMR spectra of compound 4 were from a Bruker AF 300 operating at 320 MHz. The sample solutions were prepared in 5 mm tubes (CD_3OD) using TMS as a reference. ^{1}H- and ^{13}C-NMR spectra were acquired using 32 kW data points with resolution enhancement and zero filling to point resolution better than 0.1 Hz, and ^{3}H spectra using 16 kW data points.

Radio-HPLC

HPLC separation of the tritiated product was made with a Spherisorb LC-8 column (2.5 x 250 mm) with a mobile phase of acetonitrile:ammonium acetate (0.1M) pH 6.4, 80:20, at a flow rate of 1 ml/min. Detection was by UV detector at 226 nm and corresponding radioactivity was detected by a β-RAM® radioactivity detector. The S.A. of the product was calculated by relative proportions of UV detected signal from the radioactive product and an unlabelled reference.

CONCLUSIONS

The tritium labelled antihistamine N,N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethaneamine • HCl ([^{3}H]DPPE, 4) was synthesized by a palladium catalyzed reduction of the activated carbonyl group of N,N-diethyl-2-[4-(benzoyl)phenoxy]-ethaneamine • HCl (DBPE, 3) with 100% tritium gas. In ethanol, which has one exchangeable hydrogen atom, the

specific activity obtained was 37.7 Ci/mmole as measured by LSC, 27.7 Ci/mmole as measured by radio-HPLC, and 30.7 and 30.3 Ci/mmole measured by two NMR methods. The mean value of specific activities obtained correspond to 55% of the maximum theoretical activity, which is sufficient for our purposes. A higher S.A. might have been possible by using the free amine as a precursor in a nonprotic solvent (i.e. without exchangeable hydrogens), e.g. ethylacetate or dimethylforamide, and $LiAlT_4$.

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