Specific Labelling of Putrescine Dihydrochloride by Heterogeneous Hydrogenation with Deuterium or Tritium Gas in Dimethyl Sulfoxide.

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SUMMARY

A series of putrescine dihydrochlorides specifically labelled at the 2 and 3 positions have been prepared by hydrogenation of olefinic and acetylenic precursors with deuterium or tritium gas. The olefinic precursor was synthesized by a modification of the Delépine reaction, and the acetylenic compound in a new, high-yielding (85%), chemoselective manner. Hydrogenation of the dihydrochloride salts of the precursors was conducted under an atmosphere of T_2 or D_2 gas, in the presence of Pd-C catalyst, with DMSO as solvent. Features of this experimental design were that labelling was the final synthetic step, and that removal of the solvent after hydrogenation yielded a pure, crystalline product. ^1H, ^2H and ^3H NMR spectra of the products were recorded immediately after their labelling, and the level of incorporation and positions of the stable and radioactive hydrogens were determined.

Keywords: Putrescine Dihydrochloride, Synthesis, Labelling, Deuterium and Tritium NMR.

Introduction

Putrescine dihydrochloride of high specific activity, tritiated specifically at the 2 and 3 positions was required for biological studies. Putrescine, a member of a group of important polyamines which are widely distributed among living forms, is formed by decarboxylation of the amino acid ornithine as a result of bacterial fermentation of proteins. It is an essential growth factor for a number of microorganisms (*e.g. H. parainfluenzae* and *A. nidulans*) and stabilizes membranous structures. It is

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also a follicle stimulating factor releasor in man, and an essential intermediate in the biosynthesis of spermidine and spermine. These materials are present in significant amounts in ribosomes and appear to be important to their structure and function.[1]

Putrescine has previously been labelled with tritium at different positions, by a number of methods, for use in radioimmunoassay and autoradiographic studies in biological systems.[2,3,4] Labelling procedures have included tritiation of succinonitrile in ethanolic HCl,[5,6] thermal decarboxylation of ornithine (-COOT),[6] and hydrogenation of 1,2-dicyanoethene and 1,4-diamino-2-butene with HT using PtO_2 and Pd-C as catalysts.[7,8] Putrescine labelled with deuterium and tritium at the 2 and 3 positions has recently been used as a probe for the biosynthesis of the pyrrolizidine alkaloid, retrorsine, and the stereochemistry of a number of intermediate enzymatic processes has been established.[8,9]

All of the tritiation procedures to date have employed protic solvents, usually water. This has the major disadvantage that the solvent contains readily exchangeable protons which dilute the tritium pool with hydrogen, thereby reducing the maximum attainable specific activity.[5,8] Indeed, the published procedures have yielded putrescine with low to moderate activity, the levels obtained varying with the type and volume of solvent used. An additional problem is that the catalysts used in most procedures are capable of inducing exchange between HTO and the substrate, so that tritium is incorporated in unwanted and unsuspected positions. In summary, tritium labelling of this compound has not been investigated in an aprotic solvent, and there is little proof of the specificity of labelling procedures published to date.

The most expedient way to obtain specifically tritium labelled putrescine at high specific activity appeared to be to prepare the olefinic and acetylenic precursors, and tritiate them with T_2 in an aprotic solvent, in the presence of a heterogeneous catalyst. The precursors were to be converted to their dihydrochloride salts before tritium labelling, so that the final product could be isolated as the crystalline, tritium labelled putrescine dihydrochloride. A major advantage of this method would be that chemical manipulation of the radiolabelled product is minimized. Hence, the primary aims of this investigation were to synthesise the unsaturated precursors with high chemical yield, to effect their isotopic labelling with tritium gas in an aprotic solvent, and thereby to obtain high specific activity putrescine dihydrochloride uniquely labelled at the 2 and 3 positions. The specificity of the labelling was then to be confirmed by ^3H NMR.

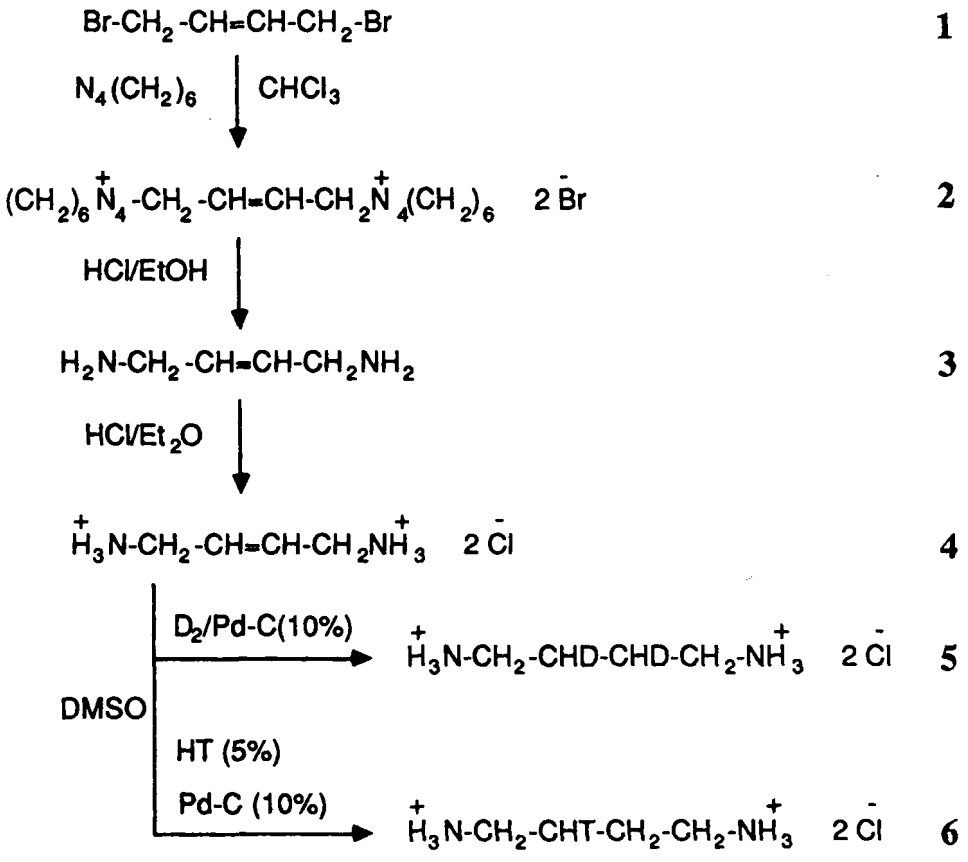
Results and Discussion

Dimethyl sulfoxide was the solvent of choice for labelling for the following reasons. Firstly, the

dihydrochloride salts of the unsaturated precursors and putrescine were soluble in DMSO or water, but in no other common solvents. Since the hydrogens of water are readily exchangeable under the hydrogenation conditions used, and DMSO is only slowly labelled,[10] DMSO was the obvious choice. We may note here that the use of DMSO as a solvent in heterogeneous hydrogenation reactions is rare.

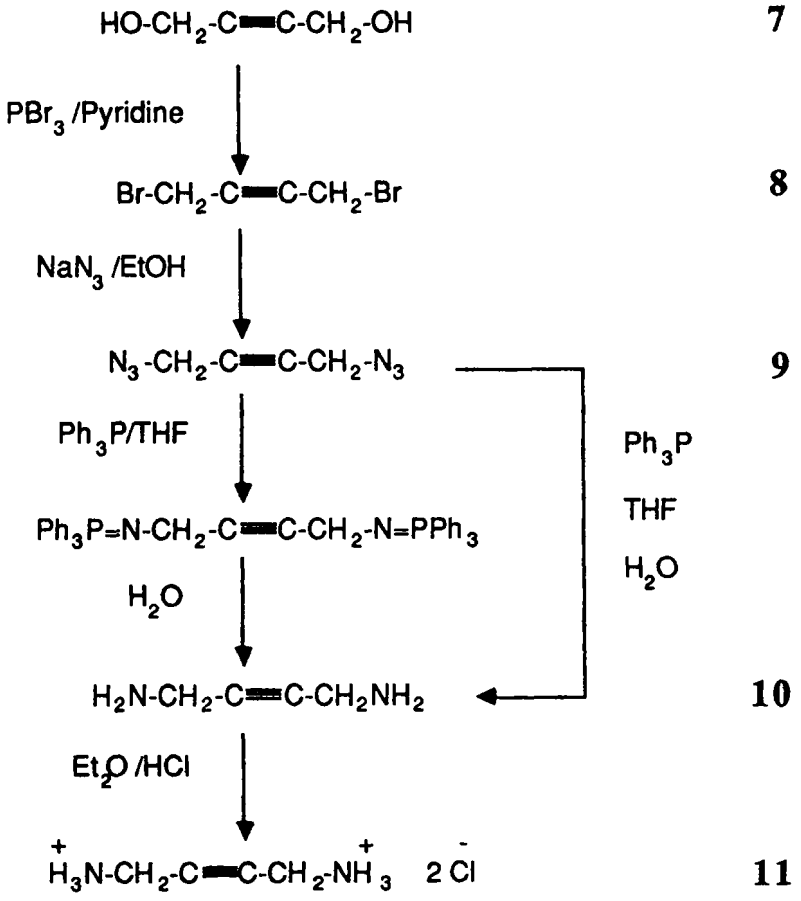
There are a number of procedures in the literature for the preparation of the precursors, 1,4-diamino-2-butene[11-18] and 1,4-diamino-2-butyne,[19-24] as their free bases and/or dihydrochloride salts. All of these methods have their own disadvantages, including low chemical yields, lengthy synthetic steps, or tedious and difficult purifications. In particular, in the preparation of 1,4-diazido-2-butene by the reaction of azide ion with 1,4-dibromo-2-butene, 3,4-diazido-1-butene is also produced. In fact, these allylic azides are formed in equlibrium mixture by rearrangement, and chromatographic separation of one of the isomers leads to rapid equlibration to the isomeric mixture.[18] Consequently, we prepared 1,4-diamino-2-butene (Scheme I) by a modification of the

Scheme I

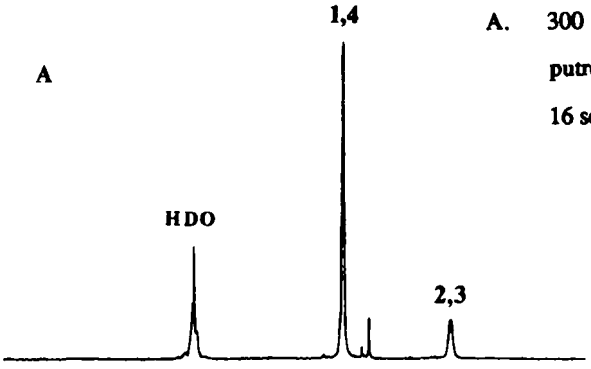


Delépine reaction for primary amines, based upon quaternisation of hexamethylenetetramine with the desired alkenyl dihalide (1). Subsequent decomposition of the hexamine salt (2) with ethanolic hydrochloric acid gave the diamine (3), in high yield. Other fragments of the hexamethylenetetramine ring form ammonia and diethyl formal.[25] The diamine was then converted to the dihydrochloride salt (4) by addition to a solution of ethereal HCl. Catalytic deuteriation (D_2) and tritiation (5% T_2 in H_2) over 10% Pd-C, with purified, dry DMSO as the solvent, gave [2,3-$^2H_2$] (5) and [2-^{3}H] putrescine dihydrochloride (6). The integrity of the products was confirmed by mass spectrometry and ^{2}H NMR for 5, and ^{3}H NMR for 6.

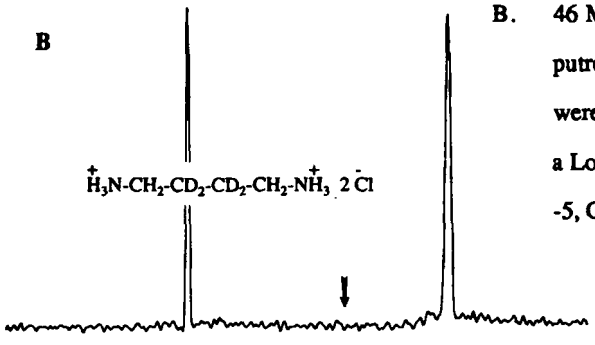
Scheme II



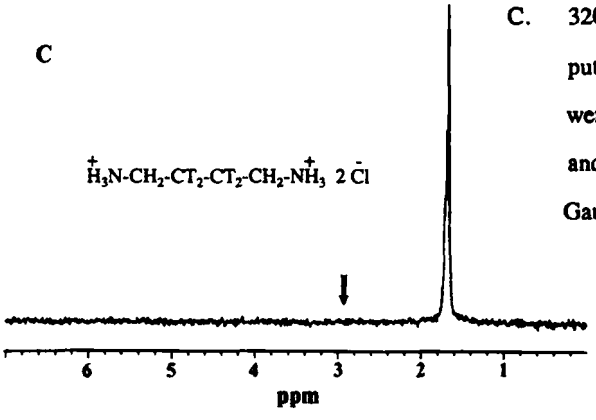
In contrast to 1,4-diazido-2-butene, 1,4-diazido-2-butyne is a stable product. Therefore, we chose to synthesise the 1,4-diamino-2-butyne as shown in Scheme II. 1,4-Dihydroxy-2-butyne (7) was dibrominated in the presence of PBr_3 and pyridine, and the dibromo product (8) was converted to



A. 300 MHz ^1H NMR spectrum of [2,2',3,3'-$^2H_4$] putrescine dihydrochloride in D_2O, acquired in 16 scans, and treated by 1 Hz line broadening.



B. 46 MHz ^2H NMR spectrum of [2,2',3,3'-$^2H_4$] putrescine dihydrochloride in H_2O. 200 scans were acquired, and the data were treated with a Lorentzian-Gaussian window function (LB -5, GB 0.05).



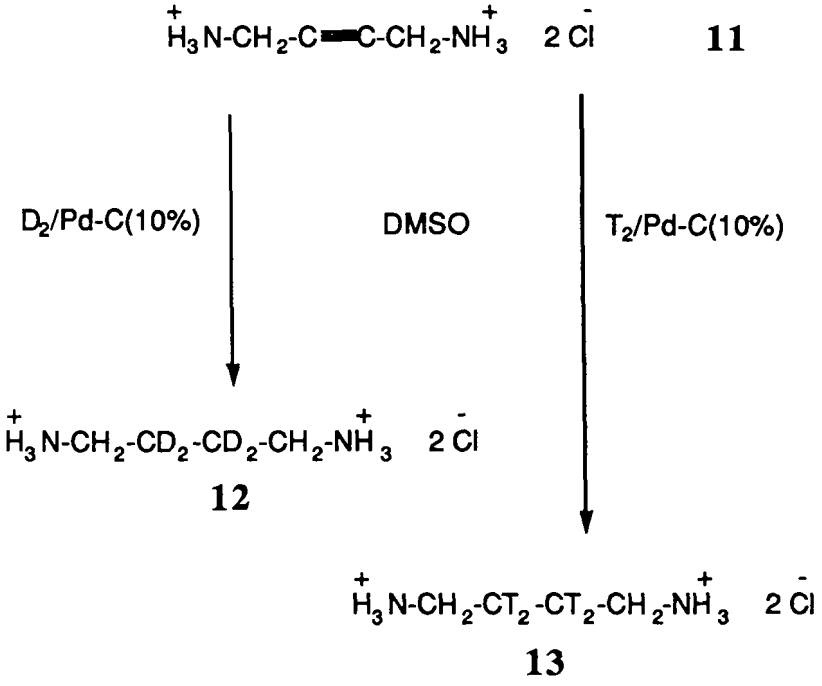
C. 320 MHz ^3H NMR spectrum of [2,2',3,3'-$^3H_4$] putrescine dihydrochloride in D_2O. 140 scans were taken with broadband proton decoupling, and the data were treated with a Lorentzian-Gaussian window function (LB -2, GB 0.05).

Figure 1: NMR spectra of labelled putrescine dihydrochloride products. Spectra were obtained at 24°C, with the samples spinning.

1,4-diazido-2-butyne (**9**) by the action of NaN_3.[26] This product was then chemoselectively reduced by triphenylphosphine in THF[27,28] to afford the free diamine (**10**) in one step with 86% yield. 1,4-Diamino-2-butyne was converted to the dihydrochloride salt (**11**) as described above, and was used as the precursor for several hydrogenation reactions.

Deuteriation of 1,4-diamino-2-butyne.2HCl with D_2 and Pd-C (10%) in DMSO gave [2,2,3,3,-$^2H_4$] putrescine.2HCl (**12**, Scheme III). The proton NMR spectrum of the product (Figure 1A) showed hydrogen in the 2 and 3 positions to the extent of *ca.* 24% of the added hydrogen (*i.e.* deuterium plus hydrogen). This observation was supported by the mass spectrum of the sample, which contained peaks for all the multi-deuteriated species, D_4-D_1, with D_3 the most abundant. The ^2H NMR spectrum (Figure 1B) has a resonance at 1.6 ppm, but no peak at 2.9 ppm, showing that all the deuterium was added to the 2,3 positions (*i.e.* there was very little exchange into the 1 and 4 positions).

Scheme III



Tritiation of the acetylenic precursor under the same conditions afforded [2,2,3,3-$^3H_4$] putrescine.2HCl (**13**) with a specific activity of 3480 GBq mmol^{-1} (94.1 Ci mmol^{-1}). Radio-HPLC analyses of this ^3H-labeled butane as its *N,N'*-dibenzamide derivative[20] showed a radiochemical purity of better than 98%. At the same time the purity of the cold butene and butyne precursors as

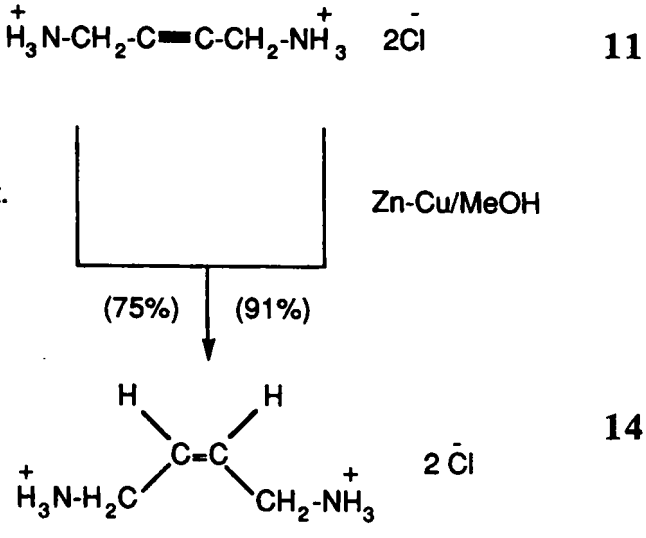
their *N,N'*-dibenzamide derivatives was confirmed, and comparison with the hot chromatogram (above) showed that none of the unreduced intermediates remained after the tritiation reaction. For this sample the ^{1}H NMR showed a peak at 1.6 ppm with an intensity of *ca.* 25% relative to the other broad singlet (2.95 ppm, 100%), which confirms the dilution of isotopic source by other factors, as observed in the deuterium experiment. The ^{3}H NMR spectrum (Figure 1C) showed a multiplet centred at 1.61 ppm, which arises from the range of multi-tritiated species predicted by the analogous deuterium experiment. Less than 0.5% of the tritium was in the 1 and 4 positions of the product, demonstrating the specificity of the labelling procedure. It is also clear that the problem of isotopic dilution of the tritium pool is not totally resolved simply by employing an aprotic solvent. The same tritiation experiment on a sample of the butyne which had been dissolved and freeze-dried from D_2O gave the same specific activity, and the same proportion of hydrogen in the 2,3 positions as compound **13**. Therefore, it seems unlikely that the labile amino hydrogens of this compound contributed a significant dilution factor in the experiment. Although the catalyst was extensively evacuated before hydrogenation reactions, hydrogen contribution from the catalyst still seems to have been the major source of dilution, and this is the subject for further investigation.

Preparation of the acetylenic compound suggested the possibility of synthesizing the isomeric *trans* 1,4-diamino-2-butene[29] and *cis* 1,4-diamino-2-butene,[30,31] with hydrogenation leading to the *meso* and *dl* stereoisomers, respectively, of putrescine dihydrochloride (Scheme IV). The first synthesis of *cis* 1,4-diamino-2-butene.2HCl (**14 a**), using the Lindlar catalyst, gave both the desired product (75%) and the fully hydrogenated compound. An alternative synthesis (**14 b**) using zinc-copper couple in boiling MeOH[31] gave the *cis* compound in 91% yield. The *trans* isomer (**15**) was synthesized by $LiAlH_4$ reduction of 1,4-diazido-2-butyne (**9**) and conversion to the dihydrochloride salt. Tritiation of *cis* 1,4-diamino-2-butene.2HCl under very similar conditions to the acetylenic precursor gave [2,3-$^3H_2$] putrescine.2HCl as the *meso* stereoisomer (**16**) with a specific activity of *ca.* 1200 GBq mmol^{-1} (32.4 Ci mmol^{-1}, Scheme V). The same reaction with the *trans* isomer afforded [2,3-$^3H_2$] putrescine.2HCl as the mixture of enantiomers (**17**) with a specific activity of 1700 GBq mmol^{-1} (46 Ci mmol^{-1}). The difference in specific activities for these two products is significant, and it suggests that the stereochemistry of the precursors plays a role in the activity obtained (*i.e.* the isotopic dilution) for each compound.

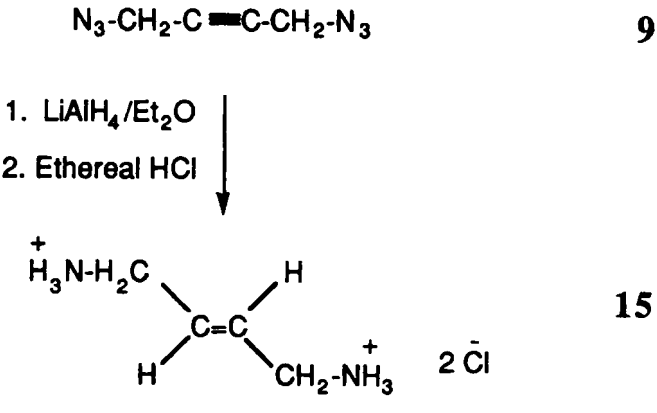
In many cases the chirality of a carbon means that chemical shifts for tritons on the adjacent carbon are distinct, but in this case analysis of the ^{3}H NMR spectra of the products (**16** and **17**) was inconclusive due to the chemical shift degeneracy of the 2 and 3 positions, and because less than 100% T was added across the double bonds.

Scheme IV

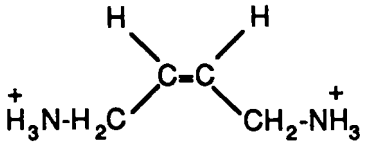
A. Cis Isomer



B. Trans Isomer

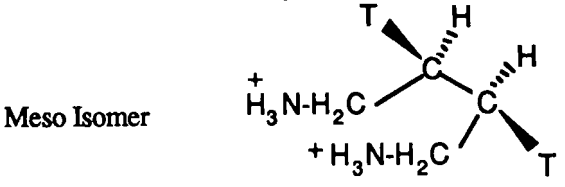


Scheme V

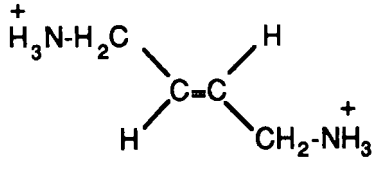


2 $\bar{C}l$ **14**

T_2/Pd-C(10%)/DMSO

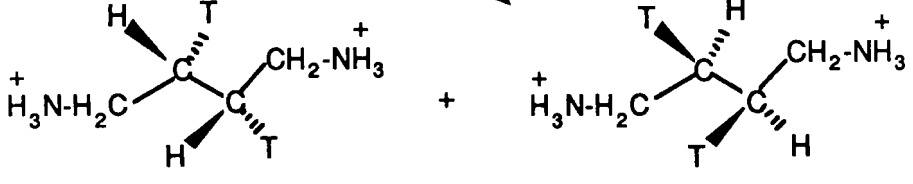


Meso Isomer 2 $\bar{C}l$ **16**



2 $\bar{C}l$ **15**

T_2 /Pd-C(10%)/DMSO



2 $\bar{C}l$ Mixture of Enantiomers 2 $\bar{C}l$ **17**

Conclusions

We report new and high yielding syntheses for two important precursors for the labelling of putrescine dihydrochloride. The labelling procedures developed in this work afford tritiated or deuteriated products in the last step of synthesis, which facilitates the labelling and ease of handling of these highly active compounds.

Dimethyl sulfoxide was effective as the solvent for the hydrogenation experiments with deuterium and tritium since it is aprotic, polar, and only slightly labelled under the reaction conditions. Freeze-drying of the solvent furnished a pure crystalline product at the end of each experiment. In short, DMSO was found to be an excellent medium for the hydrogenation of these dihydrochloride salts.

By the application of proton, deuterium and tritium NMR spectroscopy, the orientation of isotope incorporation was readily monitored in the reaction products, and dilution of isotopic source was easily detected and quantitated. In none of the labelling experiments was more than 0.5% of tritium or deuterium found in the 1,4-positions of the molecule. Hence, we have been able to prepare specifically labelled [2,3-$^{2}H_{2}$], [2,3-^{3}H], [2,2,3,3-$^{2}H_{4}$], [2,2,3,3-$^{3}H_{4}$], *meso* and *dl* [2,3-$^{3}H_{2}$] putrescine.2HCl in an aprotic solvent (DMSO) using acetylenic and *cis* and *trans* olefinic precursors.

Acknowledgements

Our interest in the synthesis of radioactive putrescine was stimulated by discussions with Dr H.J. Segall, Associate Professor of Toxicology, School of Veterinary Medicine, University of California, Davis. This research was supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant P41 RR01247-05, and by the Department of Energy under Contract DE-AC03-76SF00098.

Experimental

Tritium gas was purchased from Oak Ridge National Laboratory, and contained 97.9% T_2, with the largest contaminant being DT (1.76%). Deuterium gas was purchased from the Liquid Carbonic Company, and contained 99.7% D_2. Starting materials **1** and **7** were purchased from Aldrich Chemical Co., and re-purified prior to use. DMSO was distilled from calcium hydride immediately prior to use. All melting points were determined on an electrothermal apparatus and are reported uncorrected. Infrared spectra were recorded on a Perkin Elmer 1320 IR spectrometer. ^{1}H, ^{2}H and ^{3}H NMR spectra were recorded in D_2O or H_2O, on an IBM AF-300 NMR spectrometer. HPLC

analyses of the butane, butene and butyne as their *N,N'*-dibenzamide derivatives were performed by using a Waters C-18 radial pak column with a mobile phase of MeOH/H_2O (45/55, 3 mL/min). Mass peaks were observed by UV detection at 230 nm on a HP 1040A diode array spectrophotometer, and radioactivity measurements were made with a Berthold HPLC flow detector, using a Lithium glass scintillant cell with an efficiency of *ca.* 0.05%. Tritiated samples were counted with a Packard 2002 liquid scintillation counter. Mass spectra of deuteriated products were measured at 10 eV with an A.E.I. MS-12 Mass Spectrometer. All micro and mass spectrometric analyses were carried out by the Analytical Laboratory, Chemistry Department, University of California, Berkeley.

1,4-Diamino-2-butene (3). Hexamethylenetetramine (18 g, 128 mmol) was added to 150 mL of $CHCl_3$ and heated to 50 °C to almost full dissolution. 1,4-Dibromo-2-butene (21.4 g, 100 mmol) was dissolved in 20 mL of $CHCl_3$ and was added dropwise over 30 min to the previous solution (the reaction is exothermic). A white precipitate was formed which was cooled at room temperature, filtered, and dried in air to give **2**: 49 g, 146 mmol; mp 153 °C. To this salt was added 48 mL of concentrated HCl and 145 mL of EtOH (96%) and the mixture was heated to 80 °C. After 10 min a white precipitate formed. The upper layer (EtOH) was removed by distillation and the precipitate (NH_4Cl) was filtered: 25 g, 467 mmol. It was suspected that some of the desired product may be occluded within this precipitate, so it was set aside for later treatment. The filtrate was re-treated with a mixture of concentrated HCl/EtOH (16 mL/58 mL, 96%), heated to 90 °C, and the solvent layer once again removed by distillation. The remaining mixture was evaporated on a water bath and the residue added to 10 mL of NaOH (50%) at -10 °C. The solution was warmed to room temperature and left for 6 h, after which the upper layer was distilled under reduced pressure (35-40 mmHg). The distillate (6 g) was cooled, treated with solid KOH (2 g), and again distilled under vacuum to give compound **3**: 4 g, 46.5 mmol (43%); bp 91-92 °C (45 mmHg). The original precipitate of NH_4Cl (25 g, 467 mmol) was treated with 100 mL of NaOH (50%) and heated to 50 °C for 15 min. The resulting precipitate (NaCl) was filtered off and the filtrate was twice extracted with ether (50 mL, 2 h). Evaporation of the solvent gave a brown oil which was fractionated to yield **3**: 3 g, 34.8 mmol (32%); bp 95-96 °C (50 mmHg). Thus the total yield of this reaction was improved from the literature value of 39%[16] to 75%.

1,4-Diamino-2-butene dihydrochloride (4). Magnesium sulfate (5 g, 41.3 mmol) was added to 50 mL of anhydrous ether and the mixture was shaken for 5 min and filtered. Concentrated HCl (2 mL) was added to the ether and the solution was stirred. **3** (1 mL) was added dropwise and the solution was stirred for 1 h. A white precipitate was formed as the dihydrochloride salt **4** which was

filtered, dried and kept in a dessicator: 1.7 g, 10.2 mmol (89%): mp 300-310 °C. TLC R_f 0.5 (EtOH/NH_4OH, 8/3); IR (KBr) 3800, 2915, 1640 cm^{-1}; ^1H NMR δ 3.6 (m, 4 H), 5.9 (m, 2 H). Anal. Calcd for $C_4H_{12}Cl_2N_2$: C, 30.2; H, 7.6; Cl, 44.7; N, 17.6. Found: C, 30.1; H, 7.7; Cl, 44.9; N, 17.3.

[2,3-$^2H_2$] Putrescine dihydrochloride (5). 1,4-diamino-2-butene dihydrochloride (**4**, 50 mg, 0.3 mmol) was dissolved in 4 mL of DMSO, Pd-C (10%, 150 mg) was added, and the substrate was hydrogenated under one atmosphere of D_2 for 3 h. The rapid uptake of deuterium gas was complete after 2 h, as evidenced by the total pressure in the reaction cell remaining constant over the final hour. The catalyst was filtered off and the filtrate was freeze-dried to give **5** as a white powder. Water (2 mL) was added and the mixture was refreeze-dried to give white crystals of **5**: 47 mg, 0.3 mmol (92%); mp 273 °C; TLC R_f 0.2 (EtOH/NH_4OH, 8/3); ^1H NMR δ 1.61 (m, *ca.* 2 H), 2.95 (m, 4 H); ^2H NMR (H_2O) δ 1.60 (s, B); Mass spectrum *m/z* 90,73(100%),61,44,36.

[2-^{3}H] Putrescine dihydrochloride (6). 1,4-Diamino-2-butene dihydrochloride (**4**, 25 mg, 0.15 mmol) was dissolved in 3 mL of DMSO and Pd-C (10%,100 mg) was added. The reaction mixture was kept under one atmosphere of HT (5% T). The gas uptake was complete after 2 h, but the reaction was continued for an additional hour. After 3 h, 1 mL of MeOH was injected into the reaction vessel to exchange the labile tritium. The catalyst was filtered off and the filtrate was freeze-dried to give **6** as a white powder. Water (2 mL) was added and the mixture freeze-dried again to furnish **6** as a white crystalline substance: 19 mg, 0.12 mmol (76%); specific activity 70 GBq $mmol^{-1}$; ^1H NMR δ 1.61 (m, 4 H), 2.95 (m, 4 H); ^3H NMR (^1H decoupled) δ 1.61 (s); ^3H NMR (^1H coupled) δ 1.66 (m).

1,4-Dibromo-2-butyne (8). 1,4-Dihydroxy-2-butyne (**7**, 5 g, 58 mmol) was added to a mixture of 45 mL of benzene and 5 mL of pyridine at 0 °C and stirred for 5 min. To this solution, phosphorous tribromide (13.7 g, 50 mmol) was added dropwise with stirring for 15 min in a slow stream of nitrogen. The temperature was maintained at 0 °C for 2 h and then allowed to rise gradually to room temperature over a period of 3 h. The reaction mixture was stirred overnight. Iced water was added and the mixture was extracted with ether (3 x 50 mL). The ethereal solution was washed with saturated brine and dried over $MgSO_4$ (2 g). The solvent was evaporated and the residue was fractionated to give **8** as a yellow oil: 10.5 g, 49 mmol (89%); bp 61-62 °C (1 mmHg); ^1H NMR δ 3.95 (s, 4H). This compound is a very strong and vesicant lachrymator.

1,4-Diazido-2-butyne (9). A solution of NaN_3 (12.5 g, 192 mmol), water (65 mL), **8** (5 mL) and ethanol (96%, 200 mL) was stirred for 24 h. Ethanol was removed by aspiration, and 150 mL of

water added. The reaction mixture was extracted with ether (4 x 100 mL), the ethereal solution was dried over $CaCl_2$ (25 g) and concentrated by evacuation. Compound **9** remained as a yellow liquid: 4.9 g, 36 mmol (77%); Lit. bp 26 °C (0.1 mmHg); IR (film) 2915, 2100 cm^{-1}; ^1H NMR δ 4 (s, 4 H). Owing to a violent explosion, the elemental analysis of the diazide was not completed.

1,4-Diamino-2-butyne (10). 1,4-Diazido-2-butyne (**9**, 3 g, 22 mmol) was dissolved in 100 mL of THF and triphenylphosphine (10 g, 138 mmol) in 25 mL of THF was added dropwise over a period of 10 min. The reaction mixture was stirred for 3 h, and TLC R_f 0.6 (CH_2Cl_2/THF, 1/1) revealed that the diazide had reacted completely. Water (5 mL) was added and the reaction mixture was stirred for 24 h. The organic solvent was evaporated and the insoluble triphenylphosphine oxide filtered off. The aqueous layer was made alkaline (NaOH) and extracted continuously by ether in a liquid liquid extractor for 10 h. The ether was then evaporated and the residue distilled. A colorless liquid was obtained: 1.68 g (86%); bp 110-112 °C (30 mmHg). The thick liquid immediately solidified to give **10**: mp 46-48 °C. The *N,N'*-dibenzamide derivative of the diamine was prepared, and after recrystallisation from hot ethanol yielded white crystals: mp 210 °C.

1,4-Diamino-2-butyne dihydrochloride (11). To avoid CO_2 absorption **10** was immediately converted to the dihydrochloride salt as follows: $MgSO_4$ (5 g, 41.3 mmol) was added to 50 mL of ethyl acetate and stirred for 5 min. To this mixture was added 2.5 mL of concentrated HCl and the solution was shaken for 5 min and filtered. Compound **10** (1 g, 6.4 mmol) was warmed and added dropwise to the filtrate and the mixture was heated for 1 h. A tar precipitate resulted, with the crude product yield: 1.6 g, 10.2 mmol (86%); mp 245 °C. The precipitate was filtered and recrystallized from DMSO: mp 255 °C; TLC R_f 0.8 (EtOH/NH_4OH, 8/3); ^1H NMR δ 2.6 (s, 6 H), 3.8 (s, 4 H). The analysis was calculated for $C_4H_{10}Cl_2N_2$, but showed the empirical formula for $C_6H_{16}Cl_2N_2SO$. This indicated that there was a complex of the desired compound **11** and one mole of DMSO. The sample was dried in a drying pistol for 12 h using xylene as the solvent, and this resulted in the removal of the trapped DMSO from the crystals. ^1H NMR δ 3.8 (s, 4 H); Anal. Calcd for $C_4H_{10}Cl_2N_2$: C, 30.6; H, 6.4; N, 17.8. Found: C, 30.4; H, 6.5; N, 17.5.

[2,2,3,3-$^2H_4$] Putrescine dihydrochloride (12). 1,4-Diamino-2-butyne dihydrochloride (**11**, 5 mg, 0.03 mmol) was dissolved in 2.5 mL of DMSO and Pd-C (10%, 25 mg) was added. The reaction vessel was provided with a magnetic bar and was connected to the vacuum line, frozen in liquid nitrogen and flushed three times with nitrogen. It was then kept under one atmosphere of deuterium gas for 2.5 h at room temperature. The catalyst was then filtered and the filtrate was freeze-dried to give a white amorphous solid which was recrystallized as before to afford **12**: 4.7 mg,

0.03 mmol (90%); mp 275 °C; ^{1}H NMR δ 1.61 (s, 24%-^{1}H), 2.95 (m, 4 H); ^{2}H NMR (H_2O) δ 1.61 (s, 76%-^{2}H); MS *m/z* 75,74(100%),73,72,62,45.

[2,2,3,3-$^3H_4$] Putrescine dihydrochloride (13). 1,4-Diamino-2-butyne dihydrochloride (**11**, 6 mg, 0.04 mmol) was dissolved in 3 mL of DMSO and Pd-C (10%, 25 mg) was added as the catalyst. This compound was tritiated with T_2 for 2 h, and the reaction mixture was worked up as before to give **13**: 5 mg, 0.029 mmol (78%); mp 270 °C; specific activity 3480 GBq mmol^{-1}; ^{1}H NMR δ 1.60 (s, B, 25%-^{1}H), 2.95 (s, 4 H); ^{3}H NMR (^{1}H decoupled) δ 1.61 (m, 75%-^{3}H).

Cis-1,4-diamino-2-butene dihydrochloride (14).

a- 1,4-Diamino-2-butyne dihydrochloride (**11**, 16 mg, 0.1 mmol) was dissolved in 3 mL of ethanol and palladium on calcium carbonate (Lindlar catalyst, 10 mg) was added. The compound was hydrogenated under one atmosphere of hydrogen at room temperature and the rate of hydrogenation was monitored by TLC (EtOH/NH_4OH, 8/3). The starting material with R_f 0.8 was consumed in 20 min and two products were formed, putrescine dihydrochloride with R_f 0.2, and **14** with R_f 0.5. These two compounds were separated as their free bases by use of a small column. The *cis*-butene gave: 11.5 mg, 0.07 mmol (74%); mp 295-300 °C; ^{1}H NMR δ 3.7 (m, 4 H), 5.8 (m, 2 H).

b- 1,4-Diamino-2-butyne dihydrochloride (**11**, 100 mg, 0.64 mmol) was dissolved in 30 mL of MeOH and Zn-Cu couple,[31] prepared from zinc dust (2 g), was added and the mixture was refluxed. The rate of reduction was monitored by TLC (EtOH/NH_4OH, 8/3), which showed the product and no starting material after 6 h. The couple was removed by filtration and the solvent evaporated. A white precipitate remained which was not soluble in water. NH_4OH (3 mL) was added and the reaction mixture was stirred for 30 min. The solid dissolved and was extracted with 10 mL of ether for 3 h. Ethereal HCl (5 mL) was added, a white precipitate formed, which was then filtered and dried: 91 mg 0.57 mmol (90%); mp 292-300 °C; ^{1}H NMR δ 3.7 (m, 4 H), 5.8 (m, 2 H).

Trans-1,4-diamino-2-butene dihydrochloride (15). 1,4-Diazido-2-butyne (**9**, 1 g, 7.3 mmol) in 10 mL of dry ether was added to a solution of $LiAlH_4$ (380 mg, 10 mmol) in 25 mL of dry ether over a period of 10 min. After one hour of stirring and refluxing, TLC R_f 0.5 (EtOH/NH_4OH, 8/3) showed the product and a trace of starting material. Aqueous ethanol (5 mL) was added to destroy the excess $LiAlH_4$, the reaction mixture was filtered, and the filtrate evaporated. A yellow oil remained as the residue, which was fractionated under reduced pressure to give **15**: 0.45 g, 5.2 mmol (75%); bp 92-95 °C (45-50 mmHg); IR (film) 3850, 2900, 1610 cm^{-1}; ^{1}H NMR δ 3.5 (m, 4 H), 5.7 (m, 2 H). This compound was converted to the dihydrochloride salt by addition to a solution of ethereal HCl.

Meso [2,3-$^3H_2$] Putrescine dihydrochloride (16). *Cis*-1,4-Diamino-2-butene dihydrochloride (**14 b**, 5 mg, 0.03 mmol) was dissolved in DMSO (2.5 mL), Pd-C catalyst (25 mg) was added, and the compound was tritiated under an atmosphere of T_2 gas for 2 h. Labile tritium was removed and the sample worked up as for compound **6**, to yield **16**: 4.1 mg, 0.025 mmol (80%); mp 268 °C; specific activity *ca.* 1200 GBq mmol^{-1}; ^{1}H NMR δ 1.6 (m, 71%-^{1}H), 2.95 (m, 4 H); ^{3}H NMR (^{1}H decoupled) δ 1.68 (m, 29%-^{3}H).

(dl) [2,3-$^3H_2$] Putrescine dihydrochloride (17). *Trans*-1,4-diamino-2-butene dihydrochloride (**15**, 5 mg, 0.03 mmol) was dissolved in 2.5 mL of DMSO and was tritiated and worked up as above to give **17**: 4 mg, 0.024 mmol (79%); mp 270 °C; specific activity 1700 GBq mmol^{-1}; ^{1}H NMR δ 1.61 (m, 62%-^{1}H), 2.95 (m, 4 H); ^{3}H NMR (^{1}H decoupled) δ 1.65 (m, 38%-^{3}H).

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