The purification of triethylenetetramine and its dihydrochloride for the treatment of Wilson's disease

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A method for the purification of triethylenetetramine and its dihydrochloride for use in the treatment of Wilson's disease is reported.

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The dihydrochloride of the well known chelating agent *N*,*N*′-bis(2-aminoethyl)-1,2-ethanediamine[1a,b] (triethylenetetramine; trien) (**1**) was introduced for the treatment of Wilson's disease,[2a-e] a rare hereditary copper storage disorder, by J. M. Walshe in 1968.[3] The therapeutic benefits of triethylenetetramine dihydrochloride (trien·2HCl; British Approved Name and International Non-Proprietary Name Modified, Trientine Dihydrochloride; United States Adopted Name, Trientine Hydrochloride[4]) for patients with Wilson's disease who are intolerant of the drug of choice, D-penicillamine, have been confirmed,[5a-e] and its mode of action for removing Cu(II) *in vivo*[6] and *in vitro*[7] discussed.

This paper describes a preparative route to trien·2HCl from technical grade triethylenetetramine, which can be used as a basis for the large-scale production of the drug, and which also allows the isolation of the three main impurities in technical grade triethylenetetramine,[8a-c] [polyamines **2**, **3** and **4**] for use as chromatographic standards for the analysis of the drug substance. The method reported here is also suitable for the purification of technical grade triethylenetetramine for preparative work with this ligand.

Two of the minor impurities in technical grade triethylenetetramine,[8a-c] *N*-(2-aminoethyl)ethane-1,2-diamine and *N*-(2-aminoethyl)piperazine, are available commercially. Impurities **3** and **4** have been characterised previously in technical grade triethylenetetramine by GLC-MS[8a] and IR spectroscopy.[8c] The analysis of amine **2**, but not amines **3** and **4**, in pharmaceutical grade trien 2HCl is described in the US Pharmacopeia.[9]

Discussion

Purification of technical grade triethylenetetramine usually relies on fractional precipitation or recrystallisation of trien·2HCl[10a-g] or trien·4HCl[11a-e] to remove the impurities **2**, **3** and **4**. Rigorous chromatographic evidence for the absence of these impurities in the purified product is often lacking.

In view of the probable pharmacological and toxicological significance of impurities **2**, **3** and **4**,[5a, 12a,b] and the need for a route suitable for the large scale production of the drug, technical grade triethylenetetramine was purified *via* a trien hydrate[13a-c] before conversion into the dihydrochloride.

Acidification of an aqueous slurry of the hydrate with concentrated hydrochloric acid to pH 7.8 gave trien·2HCl chromatographically free (GLC, TLC) from piperazines **3** and **4**, and containing a trace (<0.2 % by TLC) of **2**. Nearly all the residual **2** was removed as its trihydrochloride at this stage as described previously.[10b] The preferential protonation of the two terminal amino-groups in **1** has been established previously by ^{13}C NMR.[14a,b]

Concentration and fractional distillation of mother liquors left after the removal of trien hydrate gave two main fractions containing 29% **3** (plus 70% **1**, **2** and **4**) and 84% **4** (plus 13% **1**, **2** and **3**) respectively. Treatment of each fraction with concentrated hydrochloric acid to below pH 1.0 afforded the tetrahydrochloride of the piperazine derivatives **3** and **4**, respectively. Purification of these two salts took advantage of the difference in solubilities of the hydrochlorides of **1**, **2**, **3** and **4** in various combinations of aqueous ethanol or aqueous methanol.

The identities of the hydrochlorides of **1**, **2**, **3** and **4** isolated from technical grade triethylenetetramine were checked by ^{13}C NMR. After treating each salt with 5 M NaOH, the structures of the distilled bases **1**-**4** were confirmed by ^{13}C NMR and CI mass spectroscopy.

Experimental

Elemental analyses were carried out by Butterworth Laboratories Ltd., Teddington, Middlesex. Melting points were determined on a Townson & Mercer capillary tube melting point apparatus and are corrected. TLC was performed on Merck or Analtech silica gel plates using 28% m/v aqueous NH_3–ether–acetonitrile–ethanol (50:23:17:10) as the mobile phase and 0.3% m/v ninhydrin in butan-1-ol–acetic acid (100: 3) for detection. This method separated **1–4** (R_F 0.3, 0.05, 0.6,

$H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$

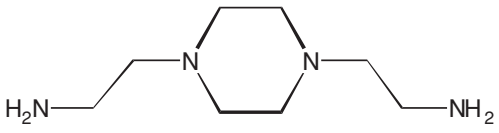
1

N,*N*′-bis(2-aminoethyl)-1,2-ethanediamine

$(H_2NCH_2CH_2)_3N$

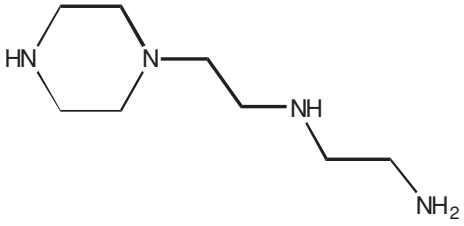
2

N,*N*-bis-(2-aminoethyl)-1,2-ethanediamine



3

1,4-piperazinediethanamine



4

N-[2-(1-piperazinyl)ethyl]-1,2-ethanediamine

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0.5 respectively) with a detection limit of 0.1 μg for each component. GLC was carried out on a Pye instrument (series 104) with a glass column (6 ft × 3 mm i.d.) packed with 20% Carbowax 20M plus 5% KOH on Chromosorb W (80–100 mesh) operated at 220 °C, with nitrogen as carrier gas (60 ml min^{-1}) and flame ionisation detection. Hydrochlorides of **1**–**4** were dissolved in 2% m/v aqueous KOH, and free bases in water, before GLC examination. This procedure separated **3** and **4** from either **1** or **2**, but **1** and **2** co-eluted as observed previously.[8a] ^{13}C NMR spectra were recorded on a Bruker WP80SY spectrometer operated in the proton-noise decoupled mode at 20.15 MHz. Samples were dissolved in D_2O (MSD isotopes; 99.8 atom %), and 1,4-dioxane (δ_C 67.7 with respect to Me_4Si[14a]) was employed as internal standard. Mass spectra were determined on a 70-70F VG Micromass double focusing spectrometer using chemical ionisation (isobutane). A.R. concentrated hydrochloric acid (Fisons) and reagent grade solvents were used throughout. Technical grade triethylenetetramine was supplied by The Dow Chemical Co.

*Triethylenetetramine [*N,N'-*bis(2-aminoethyl)-1,2-ethanediamine]* (**1**): Technical grade triethylenetetramine (1.18 kg, approx. 8.1 mol), (an azeotropic mixture containing, by GLC and TLC, *ca* 75% **1**, 5% **2**, 10% **3**, and 10% **4**), was mixed with water (262 ml, 14.6 mol]) and seeded with a few crystals of trien hydrate (obtained from a small-scale preparation[13a-b]). The precipitated trien hydrate was collected and washed successively with tetrahydrofuran and with diethyl ether. M.p. 44 °C (lit.,[13a] 47 °C), triethylenetetramine content (colorimetric assay) 91.9%. The trien hydrate was slurried with water (500 ml), cooled, and acidified with concentrated hydrochloric acid to pH 7.8. Rotary evaporation of the aq. solution (below 60 °C, 12 mm Hg) and treatment of the residue with cold 95% ethanol (3 l) afforded the dihydrochloride of **1** (854 g, 48%). M.p. 122–123 °C (decomp.) (from boiling 95% ethanol) (lit.,115–118 °C[10b]; 118–122 °C [10d]) ^{13}C NMR: δ_C 39.3, 47.2, and 47.7. TLC showed <0.2% **2**. Calc. for $C_6H_{20}Cl_2N_4$: C, 32.9; H, 9.2; N, 25.6; Cl, 32.4. Found: C, 33.0; H, 9.1; N, 25.7; Cl, 32.0%. Basification (5 M NaOH) and distillation *in vacuo* gave **1** as a colourless oil. B.p. 115 °C (1.0 mmHg). ^{13}C NMR: δ_C 41.1 ($–CH_2NH_2$), 48.9 ($–CH_2NH–$), and 51.9 ($–CH_2NH$). *m/z* 147 (MH^+, 100%), 116 (13), 87 (63), and 73 (35). One component by capillary GLC (examined as its t-butyldimethylsilyl derivative[15] on a 25 m × 0.32 mm SE 52 fused silica column, f.i.d., temperature programmed 100–280 °C). TLC showed <0.2% (**2**). Calc. for $C_6H_{18}N_4$: C, 49.3; H, 12.4; N, 38.3. Found: C, 49.3; H, 12.7; N, 38.6 %.

N,N-*Bis(2-aminoethyl)-1,2-ethanediamine* (**2**): Products insoluble in boiling 95% ethanol from several preparations of trien·2HCl were combined and recrystallised from hot methanol–water (68:32) to give the trihydrochloride of **2**,[10b] m.p. >300 °C. ^{13}C NMR: δ_C 37.8 and 51.0. One component by TLC and GLC. Calc. for $C_6H_{21}C1_3N_4$: C, 28.2; H, 8.3; N, 21.9; Cl, 41.6. Found: C, 28.3; H, 8. 2; N, 22.1; Cl, 41.2 %. Basification (5 M NaOH) and distillation *in vacuo* gave **2** as a colourless oil, b.p. 110 °C (0.5 mmHg). ^{13}C NMR: δ_C 39.1 ($NH_2CH_2–$) and 57.5 ($–CH_2N<$); *m/z* 147 (MH^+), 130 (147–NH_3, 30) and 116 (38). One component by TLC and GLC. Calc. for $C_6H_{18}N_4$: C, 49. 3; H, 12.4; N, 38.3. Found C, 49.05; H, 12.9; N, 38.4 %.

1,4-Piperazinediethanamine (**3**) *and* N-*[2-(1-Piperazinyl)ethyl]-1,2-ethanediamine* (**4**): The mother liquors obtained from a large-scale (25 kg) preparation of trien hydrate were evaporated *in vacuo* (100 mmHg), and the residue was quickly distilled *in vacuo*. A portion (approx. 4 kg) of the distillate [b.p. 102–115 °C (0.5 mmHg)] was slowly distilled *in vacuo* through a thermostatically heated glass column (2 ft × 1½") filled with Fenske helices[16] and connected to a 'Quickfit' sluice type reflux ratio head. The distillation was monitored by GLC and two fractions (a) [b.p. 90–95 °C (0.15 mmHg)] and (b) [b.p. 90–95 °C (0.15 mmHg)] collected. Fraction (a) 150 g containing 29% **3** and 70% **1**, **2**, and **4**] was dissolved in water (100 ml) and acidified with concentrated hydrochloric acid to below pH 1.0. Rotary evaporation *in vacuo*, treatment of the residue with cold ethanol and successive recrystallisations from boiling ethanol–water (1.5:1.0) and boiling methanol–water (1.2:1.0) afforded the tetrahydrochloride of **3** (47 g), m.p. >300 °C. ^{13}C NMR: δ_C 35.2, 50.9 and 53.9. One component by TLC and GLC. Calc. for $C_8H_{24}Cl_4N_4$: C, 30.2; H, 7.6; N, 17.6; Cl, 44.6. Found: 30.3; H, 7.7; N, 17.6; Cl, 45.55%. Basification (5M NaOH) and distillation *in vacuo* gave **3** as a colourless solid. B.p. 100 °C (0.3 mmHg), m.p. 34–36 °C. In a repeat experiment the m.p. was 53–56 °C (Reichert hot-stage microscope), (lit., 38–39 °C[17a], 40 °C[17b]). ^{13}C NMR: δ_C 38.4 ($NH_2CH_2CH_2$), 53.1 (ring $–CH_2–$), and 60.7 ($NH_2CH_2CH_2$); *m/z* 173 (MH^+, 100%.), 156 (173-NH_3, 13), and 142 (26). One component by GLC and TLC. Calc. for $C_8H_{20}N_4$: C, 55.8; H, 11.7; N, 32.5. Found: C, 55.7; H, 11.9; N, 32.9%. Fraction (b) (140 g containing 84% **4** and 13% **1**, **2** and **3**) was dissolved in water (100 ml) and acidified with concentrated hydrochloric acid to below pH 1.0. Rotary evaporation *in vacuo* and successive treatments of the residue with cold methanol and cold methanol–water (9:1) gave (after filtration) a solution which was rotary evaporated *in vacuo*. One portion of the residue was triturated with cold ethanol then dissolved in cold methanol–water (9:1) and the solution was treated dropwise with concentrated hydrochloric acid to precipitate the tetrahydrochloride of **4**. M.p. 155–156 °C (after washing with dry ether and extended drying *in vacuo*). On exposure to the atmosphere for periods of 2h to 2 weeks the m.p. changed to 77–79 °C. ^{13}C NMR: δ_C 36.6, 42.2, 43.2, 45.9, 50.4, and 54.1. TLC and GLC showed *ca* 0.5% **1**. Calc. for $C_8H_{24}Cl_4N_4$: C, 30.2; H, 7.6; N, 17.6; Cl, 44.6. Found: C, 29.8; H, 7.8; N, 17.2; Cl, 44.7%. The remainder of the residue from the initial aqueous methanolic treatment was basified (5 M NaOH) and distilled *in vacuo* to give **4** as a colourless oil, b.p. 110 °C (1.0 mmHg). ^{13}C NMR: δ_C 41.1 ($NH_2CH_2–$), 45. 2 (ring $–CH_2NH–$), 46.0 ($–NH_2CH_2CH_2N<$), 51.9 ($NH_2CH_2CH_2NH–$), 54.3 (ring $>NCH_2–$) and 58.4 ($–NHCH_2CH_2N<$); *m/z* 173 (MH^+, 100%), 113 (46), and 99 (52). TLC and GLC showed *ca.* 0.5% **1** and 0.5% **2**. Calc. for $C_8H_{20}N_4$: C, 55.8; H, 11.7; N, 32.5. Found: C, 55.8; H, 12.1; N, 32.8%.

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