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Synthesis, crystal structure, and protonation behaviour in solution of the recently-discovered drug metabolite, N^1,N^{10}-diacetyltriethylenetetramine

Kathrin A. Wichmann [a,b], Tilo Söhnel [a], Garth J.S. Cooper [b,c,d,e,*]

[a] School of Chemical Sciences, Faculty of Science, University of Auckland, Auckland 1010, New Zealand
[b] School of Biological Sciences and Maurice Wilkins Centre for Molecular Biodiscovery, Faculty of Science, University of Auckland, Auckland 1010, New Zealand
[c] Centre for Advanced Discovery and Experimental Therapeutics, NIHR Manchester Biomedical Research Centre, Central Manchester University Hospitals NHS Foundation Trust, York Place, Manchester M13 9WL, UK
[d] School of Medicine, The University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom
[e] Department of Pharmacology, Medical Sciences Division, University of Oxford, Mansfield Road, Oxford OX1 3QT, United Kingdom

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ABSTRACT

N^1,N^{10}-diacetyltriethylenetetramine (DAT) is a recently-discovered major *in vivo* metabolite of triethylenetetramine (TETA), a highly-selective Cu^{II} chelator currently under clinical development as a novel first-in-class therapeutic for the cardiovascular, renal and retinal complications of diabetes mellitus. Characterisation of DAT is an integral aspect of the pharmacological work-up required to support this clinical development programme and, to our knowledge, no previous synthesis for it has been published. Here we report the synthesis of DAT dihydrochloride (DAT·2 HCl); its crystal structure as determined by X-ray single-crystal (XRD) and powder diffraction (XRPD); and protonation constants and species distribution in aqueous solution, which represents the different protonation states of DAT at different pH values. The crystal structure of DAT·2 HCl reveals 3D-assemblies of alternating 2D-layers comprising diprotonated DAT strands and anionic species, which form an extensive hydrogen-bond network between amine groups, acetyl groups, and chloride anions. Potentiometric titrations show that $HDAT^+$ is the physiologically relevant state of DAT in solution. These findings contribute to the understanding of TETA's pharmacology and to its development for the experimental therapeutics of the diabetic complications.

1. Introduction

Linear polyamines such as 1,4-diaminobutane, N-(3-aminopropyl)-1,4-diaminobutane or N,N′-bis-(3-aminopropyl)-1,4-diaminobutane have a wide range of biological activities. They are of pharmacological interest in human therapeutics, particularly in respect to cell growth, enzymatic modulation, membrane stabilisation, DNA interactions, metal chelation and associated antioxidant roles [1]. We previously reported that treatment through oral administration of the related synthetic linear polyamine salt, triethylenetetramine dihydrochloride, $(H_2TETA)(Cl)_2$, causes profound improvement in left ventricular structure and function of hearts previously damaged by diabetes in rodents and humans, and also suppresses/normalises renal function in an animal model of diabetic nephropathy. We have subsequently shown that these effects are likely to be related to its positive therapeutic actions on both mitochondrial function as well as several pathways implicated in tissue inflammation, fibrosis and oxidative stress [2]. $(H_2TETA)(Cl)_2$ is also a known pharmaceutical copper-chelating agent used in the pharmacotherapy of Wilson's disease [3].

We recently developed a new liquid-chromatographic mass-spectrometry method to measure the TETA content of human plasma and urine. Thereby, we identified the two main metabolites of TETA, N^1-monoacetyltriethylenetetramine (MAT) and N^1,N^{10}-diacetylatedtriethylenetetramine (DAT) (Fig. 1), the latter of which is novel [4].

The polyamine family, to which the TETA and its metabolites belong, are ambivalent and multidentate ligands, which can form a variety of open-chain, macrocyclic and three-dimensional architectures. In their protonated forms, they can act as binding agents for a wide range of anionic species by forming significant hydrogen bonds and electrostatic interactions, whereas with cations their interactions are mainly by strongly coordinated bonds [5].

As is known, TETA can exist in various protonation states with different anionic species such as bromide, iodide and oxoanions in aqueous solution [6]. Therefore we determined the protonation and complexation behaviour of its metabolite forms, MAT and DAT.

The synthetic route, the first crystal structure of DAT·2 HCl, and the species distribution and protonation constants in solution, are

* Corresponding author. Address: Level 4, Thomas Building, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. Tel.: +64 9 923 7239; fax: +64 9 373 7045.
E-mail addresses: gjs.cooper@gmail.com, g.cooper@auckland.ac.nz (G.J.S. Cooper).

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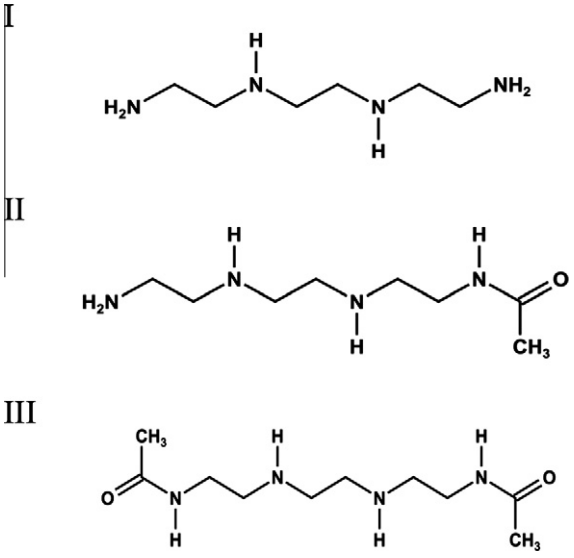

Fig. 1. Molecular structure of (I) TETA, (II) MAT and (III) DAT.

described herein. This synthetic route provides an authentic standard for the calibration of analytical methods, and also for the pharmacological study of the therapeutic response to TETA treatment. These findings are relevant to the experimental pharmacology and applications of TETA salts in the pharmacotherapy of the diabetes-related complications, including cardiovascular disease, diabetic nephropathy and diabetic retinopathy.

2. Experimental section

2.1. Materials, methods and synthesis

Synthesis of DAT·2 HCl: All reagents for the synthesis were commercially available and used as received. The DAT·2 HCl powder material was synthesised by CarboGen, Switzerland [7], by the following method. To a solution of 2-[3-(2-amino-ethyl)-2-phenyl-imidazolidin-1-yl]-ethylamine in THF/MeOH, a solution of acetic anhydride in THF was slowly added at $T = 0$ °C. Then a solution of concentrated HCl in THF was added at the same $T = 0$ °C. The precipitation of the dihydrochloride salt was completed by addition of isopropanol and the precipitate was collected by filtration. The pale yellow filter cake was washed with isopropanol and dried at 45 °C under reduced pressure until constant mass with purity of 72.88% by HPLC was obtained. Final purification was performed by re-crystallisation from wet MeOH (5% v/v water). Crude material was dissolved in warm MeOH, and the resulting, slightly cloudy and pale brown solution was further heated to reflux for 5 min. The solution was cooled to $T = 0$ °C over a period of 3 h; first crystallisation occurred at $T = 43$ °C. Solids were collected by filtration and washed with pre-cooled MeOH. The filter cake was dried at $T = 45$ °C with reduced pressure until a constant mass of white solid was obtained. Elemental analysis was as follows (calculated values): C: 32.23%, (32.28%), H: 7.69%, (7.79%), N: 18.68%, (18.82%), O: 5.92%, (5.38%), Cl: 35.31%, (35.73%); ion chromatography 97.85% (a/a), HPLC 99.86% (a/a) and ESI$^+$ Mass Spectroscopy 188 m/z corresponded to the free base [8]. Results of ^{1}H- and ^{13}C NMR were consistent with the structure (Figs. S1 and S2, Supplementary material),

2.2. Potentiometric titrations

Potentiometric measurements of DAT·2 HCl were performed in a carbonate-free 0.1 M KNO_3 aqueous solution. All solutions were prepared by using CO_2-free deionised water, and all reagents were of the highest analytical grade and used as received. The titrations were carried out under nitrogen atmosphere and by using an automatic microburette, and a Methrom pH meter with a combined pH glass electrode that was calibrated before each titration with standard buffer solutions. All titrations were performed at 20.0 ± 0.5 °C. To determine the protonation constants of DAT, a 10^{-3} M aqueous solution (ionic strength 0.1 M KNO_3) was titrated with 0.1 M NaOH. Triplicates were performed to confirm reproducibility of measured values. Data were analysed by using the computer program FITEQL 4.0 [9].

2.3. Crystal structure determination and refinement

DAT·2 HCl crystals sufficient for XRD were grown over a 4-week period by slow evaporation of an aqueous-powder solution.

Single-Crystal X-ray Crystallography. XRD measurements for DAT·2 HCl were performed on a CCD diffractometer (Siemens Smart) with graphite-monochromatised Mo Kα radiation, λ_{Mo} = 0.71073 Å. Data reduction was carried out using the SAINT [10] program. Semi-empirical absorption corrections were applied based on equivalent reflections using SADABS [11]. The structure solution and refinements were performed with the SHELXTL program package [12]. The crystal data and structural refinement details are given below in Tables 1 and 2. Figures were created using Diamond [13] and VESTA [14] visualisation software. All non-hydrogen atoms were identified after isotropic refinement of the initial solution. All H-atoms could be localised with difference Fourier analysis and could be fully optimised isotropically. Full matrix least-squares refinement on F^2 give R indices [$I > 2$sigma(I)] $R1 = 0.0257$, w$R2 = 0.0695$, and GOF = 1.101. The crystallographic data is also available from the Cambridge Crystallographic Data Centre (Fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk) as supplementary publication No. **CCDC 797519**.

The X-ray powder diffraction pattern was measured by using a STOE STADI-P diffractometer, (transmission setup, Ge(1 1 1) monochromator, Cu Kα_1 radiation, $\lambda = 1.54051$ Å, image plate detector). The samples were packed into flatbed sample holders, and data

Table 1
X-ray single crystal structure summary table.

Formula	$C_{10}H_{22}N_4O_2$ 2HCl
Molecular weight	303.23
Crystal system	monoclinic
Space group	$P2_1/n$ (No. 14)
Lattice constants	
a (Å)	9.4514(4)
b (Å)	4.7296(2)
c (Å)	16.5451(6)
β (°)	93.928(3)
V (Å^3)	737.85(5)
T (K)	85(2)
Z	2
Density, calculated (g cm^{-3})	1.356
$F(000)$	320
Absorption coefficient μ (mm^{-1})	0.441
Crystal size (mm)	0.24 × 0.16 × 0.08
$2\theta_{min}$, $2\theta_{max}$ (°)	0.997, 27.96
Number of reflections (collected)	9022
Number of reflections (independent)	1771
T (min, max)	0.9015, 0.9655
Goodness of fit on F^2	1.101
$R1$, w$R2$ (observed data)[a]	0.0257, 0.0695
$R1$, w$R2$ (all data)[a]	0.0307, 0.0729
ρ_{mm} (e/Å^3)	−0.178
ρ_{max} (e/Å^3)	0.391

[a] $R1 = \sum||Fo| - |Fc||/\sum|Fo|$; w$R2 = \{\sum[w(Fo^2 - Fc^2)^2]/\sum[w(Fo^2)^2]\}^{1/2}$.