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Synthesis and X-ray Analysis of Dihydrotetrabenazine, a Metabolite of Tetrabenazine

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The dihydrotetrabenazine (DTBZ) was synthesized by reduction of tetrabenazine with sodium borohydride in ethanol. Crystals suitable for X-ray analysis were obtained from a mixed solution of dichloromethane and ethanol in a five-to-one volume ratio. The crystal is monoclinic, space group $P2_1/c$ with crystallographic parameters: $a = 15.0129(14)$ Å, $b = 12.5677(12)$ Å, $c = 9.7715(9)$ Å, $\beta = 98.556(2)^\circ$, $\mu = 0.078\ mm^{-1}$, $V = 1823.1(3)$ Å^3, $Z = 4$, $Dc = 1.164\ g/cm^3$, $F(000) = 696$, $T = 296(2)$ K. The X-ray analysis and the chiral HPLC show that the DTBZ prepared by our method consists of (2R,3R,11bR) and (2S,3S,11bS) enantiomers.

Keywords Chiral center; dihydrotetrabenazine; enantiomer; tetrabenazine

Introduction

Tetrabenazine (TBZ) is a clinically used drug and it is a relatively safe and effective treatment for a wide variety of hyperkinetic movement disorders, particularly tardive dyskinesia (TD) and chorea. Dihydrotetrabenazine (DTBZ) has been identified as the pharmacologically active metabolite of TBZ. In mammalian, TBZ is rapidly and extensively metabolized by reduction of the 2-keto group, producing DTBZ [1,2]. DTBZ is a high-affinity ligand for the vesicular monoamine transporter 2 (VMAT2) in rodent and human brain neurons [3]. Therefore, imaging VMAT2 in the brain with radiolabeled DTBZ or its derivatives provides a measurement reflecting the integrity of monoaminergic neurons, and so as to monitor or diagnose neurodegenerative disorders such as Parkinson's, Alzheimer's, Tourette's, and Huntington's diseases [4]. In view of this, DTBZ and its derivatives have recently been labeled with carbon-11 and fluorine-18 radioisotopes and used for in vitro and in vivo studies of the VMAT2 in animal and human brain. For example, ^{11}C-DTBZ [5–7], ^{18}F-FE-DTBZ [8], and ^{18}F-FP-DTBZ [9–11] have been prepared as the positron emission tomography (PET) imaging agents targeting VMAT2. In order to synthesize and examine the novel radiolabeled DTBZ's derivatives, namely ^{18}F-FB-DTBZ, gram quantities of DTBZ were

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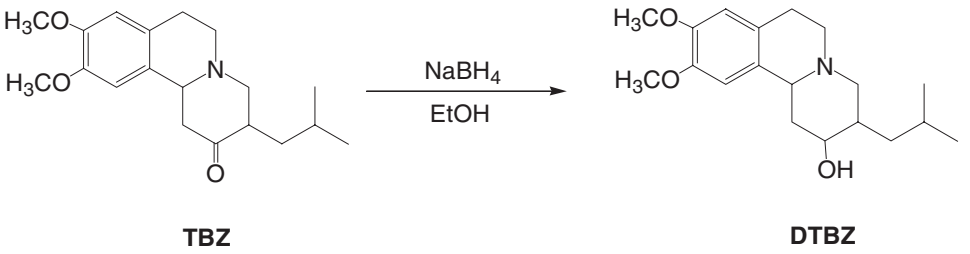

TBZ DTBZ

Scheme 1. Synthesis of DTBZ.

required. Reported herein is the synthesis of DTBZ as shown in Scheme 1. The objective product was characterized by ^{1}H NMR, IR, and MS.

The DTBZ has three chiral centers at C-2, C-3, and C-11b as shown in Fig. 1. Therefore, in theory, there are eight isomers of the DTBZ. X-ray diffraction and chiral HPLC were employed to investigate the structural groups of the isomers of the DTBZ prepared by our group.

Experimental

Materials and Physical Measurements

The TBZ was synthesized according to literature methods [12,13]. All other solvents and reagents were of analytical grade and were used without further purification. IR spectra in the range 4000–400 cm^{-1} were obtained from samples in the form of KBr tablets using a Bruker Tensor27 infrared spectrometer. MS spectra were recorded with a Waters Platform ZMD 4000 mass spectrometer. Proton NMR spectra were obtained on a Bruker Avance III 400 MHz Digital NMR Spectrometer in $CDCl_3$. A Bruker CCD APEX2 diffractometer was used for the X-ray structure study.

Synthesis of Dihydrotetrabenazine (DTBZ)

Tetrabenazine (2.92 g, 9.20 mmol) was dissolved in anhydrous ethanol (240 mL) with heating. The resulting solution was stirred and cooled to room temperature; then sodium borohydride (3.50 g, 92 mmol) was added. The reaction mixture was stirred at room temperature for 12 h under nitrogen. The reaction solvent was removed under reduced

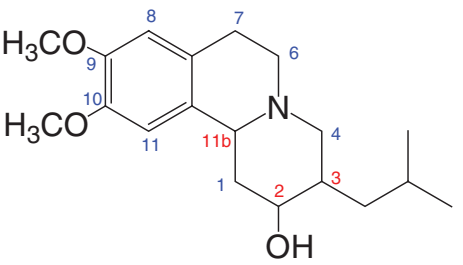

Figure 1. Structure of numbered DTBZ.

pressure. The residue was partitioned between dichloromethane (50 mL) and water (50 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 25 mL). Organic extracts were combined and concentrated in vacuum. The crude product was purified by crystallization with methanol giving DTBZ (1.50 g) as colorless crystals in a moderate yield (51%). MS, *m/z*: 320.1 $[M+H]^+$; 342.1 $[M+Na]^+$. IR (KBr, cm^{-1}): 3138; 2952; 2911; 1609; 1512; 1463. ^1H NMR ($CDCl_3$, 400 MHz), δ: 6.70 (s, 1H), 6.60 (s, 1H), 3.86 (s, 6H), 3.45–3.37 (m, 1H), 3.16–2.99 (m, 4H), 2.68–2.42 (m, 3H), 2.03–1.97 (t, 1H), 1.81–1.68 (m, 2H), 1.64–1.47 (m, 3H), 1.12–1.05 (m, 1H), 0.97–0.93 (dd, 6H).

The colorless single crystal of DTBZ was grown from a mixed solution of dichloromethane and ethanol in a five-to-one volume ratio by slow evaporation at room temperature.

X-ray Crystallography

A single crystal of the DTBZ with dimensions 0.23 × 0.20 × 0.17 mm was chosen for X-ray diffraction study. The data were collected on a Bruker APEX2 diffractometer equipped with graphite-monochromatic Mo-Kα radiation ($\lambda = 0.71073$ Å) by using ψ and ω scan mode at 296(2) K. In the range of $1.37^\circ < \theta < 25.00^\circ$, a total of 9900 reflections were collected, of which 3201 were independent ($R_{int} = 0.0380$) and 2699 were observed with $I > 2\sigma(I)$.

The structure was solved by direct and difference Fourier map methods with *SHELXS-97* [14]. Non-hydrogen atoms were refined by full-matrix least-squares techniques on F^2 with anisotropic thermal parameters, using *SHELXL-97* [15]. All H atoms were allowed to ride on their parent atoms at distances of 0.93 Å (C—H aromatic), 0.96 Å (C—H methyl), 0.97 Å (C—H methylene), and 0.98 Å (C—H methine) with U_{iso}(H) values of 1.2–1.5 times U_{eq} of the parent atoms.

Results and Discussion

Synthesis of Dihydrotetrabenazine (DTBZ)

The DTBZ was synthesized through procedures described in the literature [16]. However, the synthesis steps were improved on the basis of the literature. The literature method was as follows. The reaction mixture was stirred at 0°C–3°C for 3 h under N_2. Our approach was that the mixed solution was stirred at room temperature for 12 h under nitrogen. Therefore, convenient operation was achieved through the slight optimization of reaction conditions.

Spectroscopic Characterization

The infrared spectra of the DTBZ exhibits a broad band of large intensity at 3138 cm^{-1}, which is attributed to the stretching vibration of the hydroxyl radical (—OH). The DTBZ shows strong absorption bands at 2952 and 2911 cm^{-1}, with a shoulder at approximately 2900 cm^{-1} due mainly to the C—H stretching vibration of the methyl and methylene groups present in the DTBZ molecule. The C=C skeletal vibration of the benzene ring in the DTBZ molecule appears at 1609, 1512, and 1463 cm^{-1}.

The ^1H NMR spectrum of the DTBZ in $CDCl_3$ solution shows two singlet peaks for the benzene ring hydrogen atoms (—PhH) at δ 6.70 and 6.60 ppm. The hydrogen atoms of the methoxy groups (—OCH_3) bonded to the aromatic ring in the DTBZ is observed in

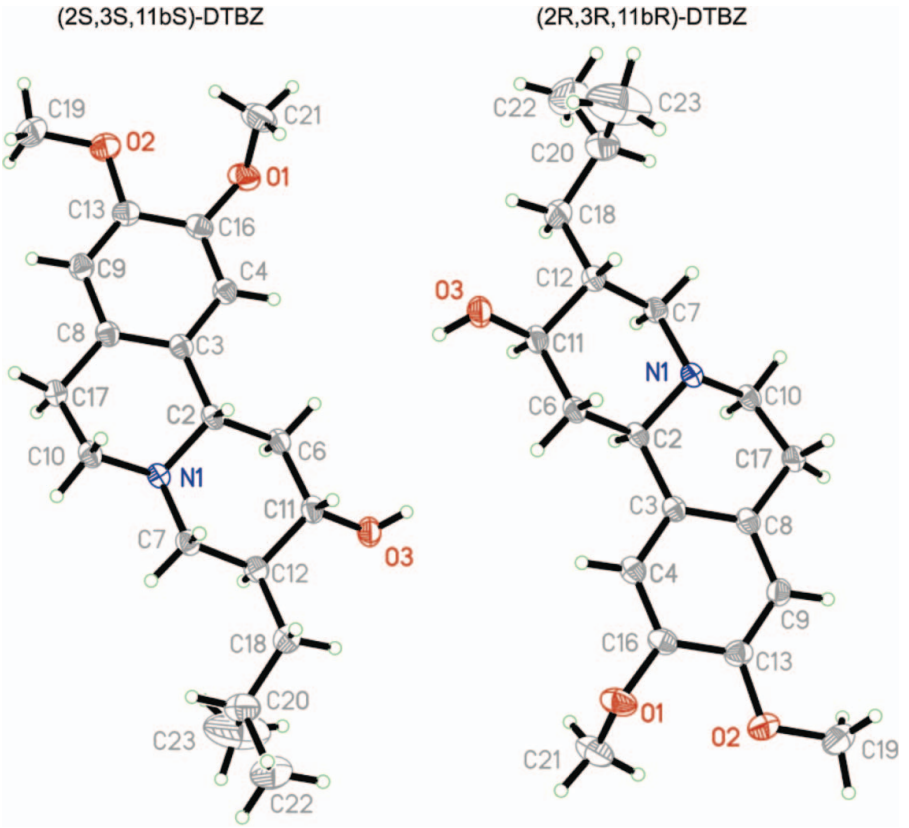

Figure 2. The molecular structures of DTBZ enantiomers; 30% probability ellipsoids are shown.

the ^{1}H NMR spectrum as a singlet, exhibiting at δ 3.86 ppm. The two doublets at δ 0.97∼ 0.93 ppm is assigned to the hydrogen atoms of the methyl groups (−CH_3). The hydroxyl radical (−OH) is at δ 2.03–1.97 ppm. On the other hand, the signals referred to the hydrogen atoms of the methylene and methine groups (−CH_2R; −CHR_2) appear at 3.45–3.37, 3.16–2.99, 2.68–2.42, 1.81–1.68, 1.64–1.47, and 1.12–1.05 ppm respectively.

Crystal Structure

The DTBZ crystallized in the monoclinic system, space group $P2_1/c$ with crystallographic parameters: a = 15.0129(14) Å, b = 12.5677(12) Å, c = 9.7715(9) Å, β = 98.556(2)°, μ = 0.078 mm^{-1}, V = 1823.1(3) Å^3, Z = 4, Dc = 1.164 g/cm^3, F(000) = 696, T = 296(2) K. The molecular structure of DTBZ is shown in Fig. 2 and a perspective view of the crystal packing in the unit cell is shown in Fig. 3.

There are three rings in the structure of DTBZ, namely benzene ring and two nitrogen-containing six-membered rings. They do not share a common plane. The benzene ring is a plane conformation while the other two rings are chair conformations.

The DTBZ synthesized by our team is a racemic form. According to the molecular ellipsoid graph of DTBZ in Fig. 2, the single crystal of DTBZ consists of (2R,3R,11bR) and (2S,3S,11bS) enantiomers. The crystal data and refinement details are listed in Table 1.

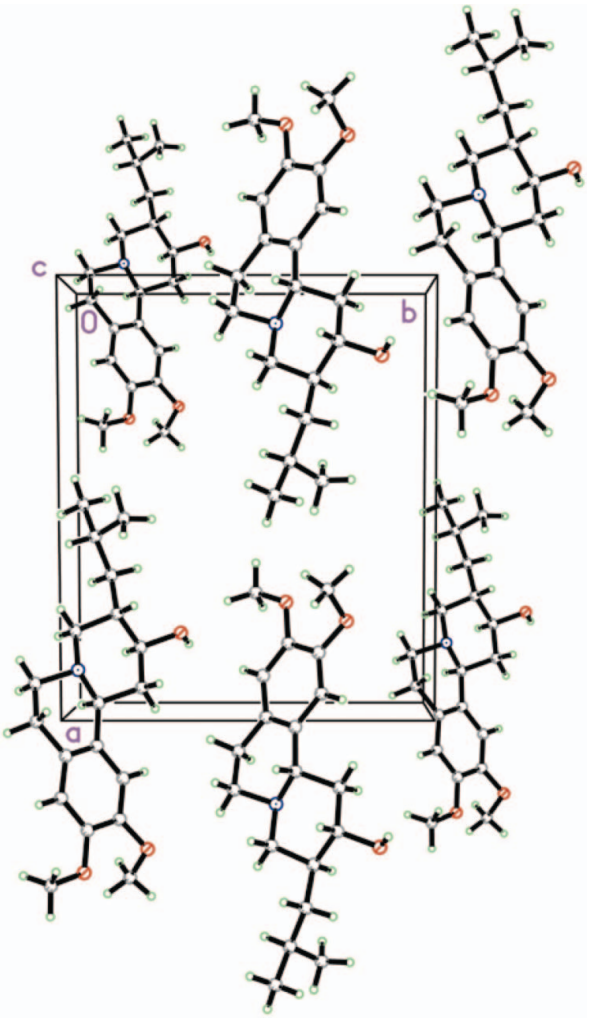

Figure 3. Packing of the DTBZ molecules viewed along the *c* axis.

The selected bond lengths and bond angles are listed in Table 2. The interatomic distances of conjugated C—C bonds in the benzene ring vary from 1.368(3) Å to 1.407(3) Å. Those of other C—C bonds range from 1.441(7) Å to 1.531(3) Å. The bond lengths of C—N and C—O single bonds vary in the range of 1.467(2) to 1.481(2) Å and 1.364(3) to 1.425(3) Å, respectively. These data are in accordance with the typical bond lengths in organic compounds [17].

There are two different patterns of the stacked arrangements in general aromatic systems for π–π interactions: offset face-to-face and edge-to-face packing. The distance between aromatic ring centroids with the range of 3.3–3.8 Å is the valid value of π–π interactions in both the patterns [18]. The shortest distance between two benzene ring centroids is 5.891 Å in the crystal stacking of DTBZ as shown in Fig. 4. Obviously, in the crystal packing, there is no π–π interaction, and the molecules of DTBZ are mainly stabilized by Van der Waals forces and hydrogen bonds.

Table 1. Crystallographic data and structure refinement details

CCDC deposit number	831592
Compound name	Dihydrotetrabenazine
Empirical formula	$C_{19}H_{29}NO_3$
Formula weight	319.43
Temperature (K)	296 (2)
Wavelength (Å)	0.71073
Crystal size (mm)	$0.23 \times 0.20 \times 0.17$
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	15.0129(14)
b (Å)	12.5677(12)
c (Å)	9.7715(9)
α (°)	90.00
β (°)	98.556(2)
γ (°)	90.00
V (Å^3)	1823.1(3)
Z	4
D_{ca}.(g·cm^{-3})	1.164
F(000)	696
Absorption coeff. (mm^{-1})	0.078
θ range (°)	1.37–25.00
Index ranges	$-17 \leq h \leq 14$; $-14 \leq k \leq 14$; $-8 \leq l \leq 11$
Reflections collected	9900
Independent reflections	3201 [$R_{int} = 0.0380$]
Observed reflections	2699
Data/restraints/parameters	3201/18/214
Goodness-of-fit on F^2	1.255
R, wR indices [$I > 2\sigma(I)$]	0.0603, 0.1799
R, wR indices (all data)	0.0740, 0.2024
Largest diff. peak and hole (e·Å^{-3})	0.734, −0.377

Table 2. Selected bond lengths (Å) and angles (°)

	Bond lengths		
C2–N1	1.481(2)	C11–O3	1.415(2)
C16–O1	1.379(2)	C3–C4	1.396(3)
C2–C3	1.521(3)	C2–C6	1.523(3)
C12–C18	1.531(3)	C20–C22	1.497(5)
	Bond angles		
C16–O1–C21	114.08(18)	C7–N1–C2	109.60(14)
C16–C4–C3	122.51(19)	O3–C11–C12	109.15(16)
C8–C17–C10	112.95(17)	C6–C11–C12	110.55(15)
C12–C18–C20	117.5(2)	C22–C20–C23	109.5(4)

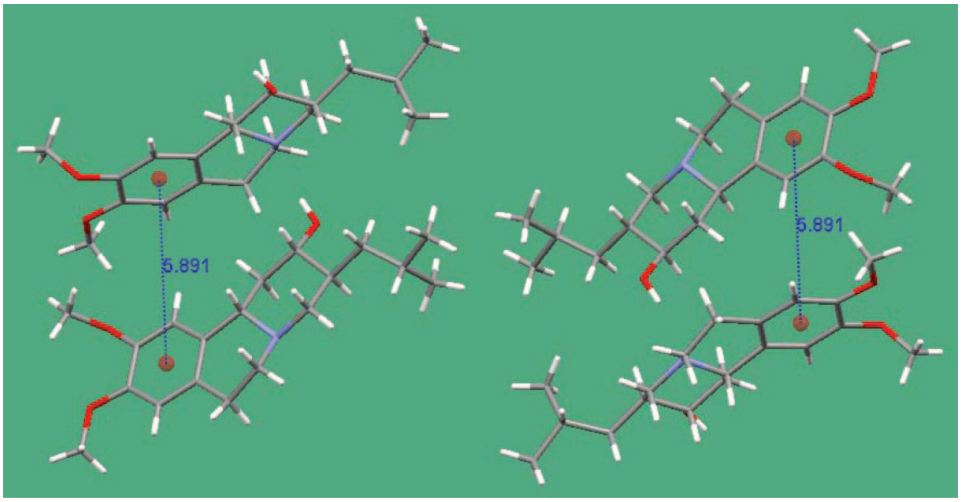

Figure 4. The shortest distance between two benzene ring centroids in the crystal stacking of DTBZ.

Configuration of Dihydrotetrabenazine

In theory, there are eight isomers of the DTBZ, namely (2R,3R,11bR), (2S,3S,11bS), (2R,3R,11bS), (2S,3S,11bR), (2R,3S,11bR), (2S,3R,11bS), (2S,3R,11bR), and (2R,3S,11bS). The DTBZ was synthesized by using racemic TBZ as raw material, which was a racemic mixture of (3R,11bR)-TBZ and (3S,11bS)-TBZ. Therefore, the DTBZ prepared by our method contains a maximum of four isomers. The X-ray diffraction of the single crystal indicated that the precipitated crystal contains (2R,3R,11bR) and (2S,3S,11bS) enantiomers. As the single crystal X-ray diffraction analysis of the sample is only in a crystal, the result is not universal. Therefore, the analysis of DTBZ was investigated on a chiral HPLC column (Phenomenex Chirex (S)-Val and (R)-NEA; 250 × 4.6 mm) using

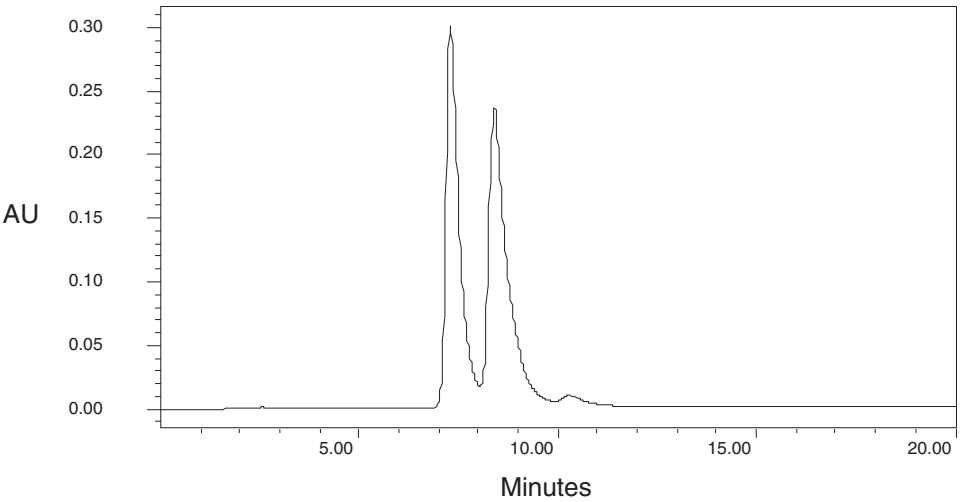

Figure 5. The chiral HPLC analysis of DTBZ.

isocratic 95% A/5% B at 1.0 mL/min with ultraviolet (UV) detection at 280 nm with solvent A being hexane/1,2-dichloroethane (2:1) and solvent B being 0.1% trifluoroacetic acid (TFA) ethanol solution. The result is shown in Fig. 5. By this means, we can determine that our DTBZ consists of two isomers. Finally, we can infer that the DTBZ obtained from our group consists of (2R,3R,11bR) and (2S,3S,11bS) enantiomers.

Conclusions

The DTBZ was characterized by ^{1}HNMR, IR, MS, and X-ray diffraction. By means of X-ray diffraction and chiral HPLC, we confirmed that the DTBZ prepared by our method consists of (2R,3R,11bR) and (2S,3S,11bS) enantiomers.

Supplementary Material

CCDC-831592 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing at data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223−336033.

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