Absolute Configuration of (+)-α-Dihydrotetrabenazine, an Active Metabolite of Tetrabenazine

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ABSTRACT Chiral column liquid chromatography and enantiospecific enzymatic hydrolysis were utilized to separate the enantiomers of α- and β-dihydrotetrabenazine and α-9-O-desmethyldihydrotetrabenazine, three benzo[a]quinolizines derived from the amine-depleting drug tetrabenazine. An X-ray crystal structure analysis of (–)-α-9-O-desmethyldihydrotetrabenazine gave an absolute structure of that compound as the 2*S,* 3*S,* 11b*S* isomer. Therefore, (–)-α-dihydrotetrabenazine also has the 2*S,* 3*S,* 11b*S* absolute configuration. (+)-α-Dihydrotetrabenazine, the single biologically active isomer from the metabolic reduction of tetrabenazine, thus has the absolute configuration of 2*R,* 3*R,* 11b*R.* For further in vitro and in vivo studies of the vesicular monoamine transporter, it is now possible to use the single enantiomer of radiolabeled α-dihydrotetrabenazine. *Chirality 9:59–62, 1997.*

KEY WORDS: resolution of enantiomers; chiral column chromatography; enzyme; X-ray crystallography

INTRODUCTION

Tetrabenazine, used clinically for the management of movement disorders, functions to deplete brain monoamine levels by inhibition of the vesicular monoamine transporter type 2 (VMAT2).[1] In rodents and humans, tetrabenazine is rapidly and extensively metabolized by reduction of the 2-keto group, producing α- and β-dihydrotetrabenazine[2] (Fig. 1). These alcohols also have high in vitro affinity for the VMAT2, and are likely the pharmacologically active agents in the mammalian brain.[3] α-Dihydrotetrabenazine and related benzo[a]quinolizines have recently been labeled with tritium and carbon-11 radioisotopes and used for in vitro and in vivo studies of the VMAT2 in animal and human brain.[4,5]

As part of our study of the structure-activity relationship for the binding of benzo[a]quinolizines to the VMAT2[6] we determined the relative configurations of α- and β-dihydrotetrabenazines, demonstrating that these compounds (and, indirectly, tetrabenazine) were racemic mixtures of two enantiomers. After completing resolution of the enantiomers on a chiral HPLC column, we then reported that the in vitro binding of α-dihydrotetrabenazine was stereospecific, with a high binding affinity (K_i = 0.97 nM) only for the (+)-isomer.[7] At that time, we were unable to determine the absolute configuration, and thus report here syntheses, resolution and X-ray crystallographic studies which allow the assignment of the absolute configuration of (+)-α-dihydrotetrabenazine.

MATERIALS AND METHODS

Chemicals were purchased from Aldrich Chemical Co. and are reagent grade unless otherwise noted. Procine pancreatic lipase (Altus 3) and ChiroCLEC™-PC dry (Altus 20) were purchased from Altus Biologic Inc. (Boston, MA). Silica gel for column chromatography (70–230 mesh ASTM) and silica gel thin layer chromatography plates were purchased from Merck Co. (±)-α-Dihydrotetrabenazine was prepared by hydride reduction of tetrabenazine (Fluka Chem. Co.) as previously described.[6] Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer PE-241 polarimeter, using the sodium line.

*Synthesis of (±)-α-9-O-Desmethyldihydrotetrabenazine (2,9-Dihydroxy-3-Isobutyl-10-Methoxy-1,2,3,4,6,7-Hexahydro-11b*H*-Benzo[a]quinolizine)*

α-Dihydrotrabenazine was selectively demethylated at the 9-methoxy group using sodium hydride/*N*-methyl aniline/HMPA[12]. *N*-Methylaniline (7.89 g, 74 mmol) was added dropwise at 65°C to a stirred suspension of sodium

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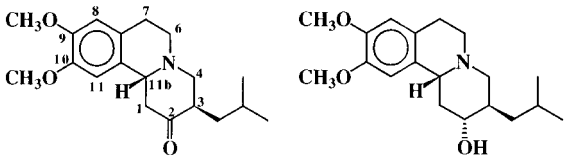

Fig. 1. Structures of tetrabenazine and dihydrotetrabenazine. For simplicity, a single enantiomer of each (3*R,* 11b*R*-tetrabenazine and 2*R,* 3*R,* 11b*R*-dihydrotetrabenazine) are shown.

hydride (2.677 g; 111 mmol) in dry xylene (20 ml) and hexamethylphosphoramide (13.20 g; 74 mmol). After 15 min (±)-α-dihydrotetrabenazine (11.900 g, 37.3 mmol) suspended in 10 ml xylene was added dropwise with stirring. The suspension was stirred 48 h at 65°C. The reaction mixture was hydrolyzed with 5% HCl (50 ml) and extracted several times with ether to remove unreacted starting material. The aqueous phase was filtered through a glass frit, and to the clear brown solution was added HCl (conc.) dropwise to precipitate the product. The solid was then dissolved in methanol/NH_4OH to obtain the free base of the crude product. The resulting solid was recovered by filtration and recrystallized several times from small volumes of methanol. Yield of (±)-α-9-O-desmethyldihydrotetrabenazine was 3.444 g (30%). The product was ⩾98% pure as determined by HPLC (Inertsil C_8; UV 240 nm; 1:5.5 CH_3CN:10 mM ammonium acetate, pH 4.5; R_t 12 min) and was identical by HPLC and ^{1}H-NMR to a sample of (±)-9-desmethyl-α-dihydrotetrabenazine synthesized stepwise from 3-benzyloxy-4-methoxybenzaldehyde.[2] m.p. 187–190° (lit.[2] 192.5–193°). ^{1}H nmr (δ ppm, CD_3OD): 6.84 (1H,s,H-8), 6.67 (1H,s,H-11), 4.43 (1H,d,12Hz,H-11b), 3.87 (3H,s,CH_3-10), 3.71 (1H,m,H-6), 3.63 (2H,m,H-4, 2), 3.33 (1H,m,H-7), 3.26 (1H,m,H-6), 3.00 (2H,m,H-4, 7), 2.89 (1H,m,H-1), 1.99 (1H,m,H-3), 1.77 (3H,m,H-1, 1′, 2′), 1.15 (1H,ddd, 16Hz,8Hz,8Hz,H-1′), 1.00 (3H,d,7.5Hz, CH_3-3′), 0.97 (3H,d,7.5Hz, CH_3-3′). ^{13}C nmr (δ ppm, CD_3OD): 148.4 (C-9), 147.7 (C-10), 125.2 (C-11a), 124.5 (C-7a), 116.1 (C-8), 109.4 (C-11), 72.0 (C-2), 62.6 (C-11b), 58.6 (C-4), 56.6 (OCH_3), 51.9 (C-6), 40.4 (C-3), 39.9 (C-1), 39.2 (C-1′), 27.1 (C-7), 26.2 (C-2′), 24.9 (C-3′), 21.9 (C-3′). Anal. (HRMS) Calcd. 305.1991; Found 305.1977. Specific rotation [α] = 0.

Resolution of the Enantiomers of (±)-α-Dihydrotetrabenazine, (±)-β-Dihydrotetrabenazine, and (±)-9-O-Desmethyl-α-dihydrotetrabenazine by Chiral Chromatography

High performance liquid chromatography (HPLC) resolutions of the isomers of (±)-α-dihydrotetrabenazine, (±)-β-dihydrotetrabenazine, and (±)-9-O-desmethyl-α-dihydrotetrabenazine were done using a preparative HPLC column [Chirex 3014: ((S)-val—(R)-1-(α-naphthyl)ethylamine), 20 × 250 mm: Phenomenex], eluted with 60:30:9.5:0.5 hexane: 1,2-dichloroethane:ethanol:trifluoroacetic acid at a flow rate of 7 ml/min. Isolated products were re-injected until pure by analytical HPLC analysis, determined using an analytical (4.6 × 250 mm) Chirex 3014 column and the above solvent mixture at a flow rate of 1 ml/min. To obtain the best separation each injection contained no more than 30 mg, and fractions were pooled to obtain sufficient quantities (>300 mg) of both the (–)- and (+)-isomers for further chemical or biological characterization.

For resolution of (±)-α-9-O-desmethyldihydrotetrabenazine, the first enantiomer eluting was the (–)-isomer, ⩾99% pure, molecular rotation $M\lambda^t$ = –152.7. This material was recrystallized from ethanol, and was used to grow the crystals which were suitable for X-ray structure determination (see following section). The (+)-isomer eluted second, and was obtained in ⩾98% enantiomeric purity.

For separation of the (±)-α-dihydrotetrabenazine, the enantiomers eluted in the same order as for α-9-O-desmethyldihydrotetrabenazine. The molecular rotation for the (+)-isomer was $M\lambda^t$ = 84.6.

Finally, the HPLC column was used for the resolution of the enantiomers of (±)-β-dihydrotetrabenazine, where the (+)-isomer eluted first: the molecular rotation for the (–)-isomer was $M\lambda^t$ = –174.5.

Synthesis of (±)-α-Dihydrotetrabenazine Acetate Ester (2-Acetoxy-3-Isobutyl-9,10-Dimethoxy-1,2,3,4,6,7-Hexahydro-11bH-Benzo[a]quinolizine)

A solution of (±)-α-dihydrotetrabenazine (1 g, 3.13 mmol) in acetic anhydride (20 ml) was heated to reflux for 5 h. The solvent was then removed by a stream of N_2 to provide a dark oily residue. The oil was then purified by silica gel column chromatography (EtOAc) to provide crude ester, which was crystallized from EtOAc. The reaction yield was almost quantitative. HRMS: calcd 361.2253; found 361.2255. The product was used for the enzymatic resolution (below) without further purification.

Enzymatic Resolution of (+)-α-Dihydrotetrabenazine

The Altus #3 (26.684 g) was poured into 750 ml of sodium phosphate buffer solution (0.12 M, pH = 7) and stirred at room temperature for 20 min. The substrate ester ((±)-α-dihydrotetrabenazine acetate, 738 mg, 2.043 mmol) dissolved in acetone (85 ml) was then slowly added. The reaction mixture was stirred at room temperature for one hour, then transferred to cold room (4°C) for 17 d. The enzyme was then filtered out (celite). The clear aqueous solution was adjusted to pH = 8 and extracted with CH_2Cl_2 (3 × 150 ml). The combined organic extracts were concentrated and separated by silica gel column chromatography (EtOAc eluent) to provide (–)-ester (250 mg, containing a small amount of the (+)-ester) and (+)-α-dihydrotetrabenazine (143 mg, 0.448 mmol) which was ⩾99% pure by analytical chiral HPLC analysis.

X-Ray Crystal Structure Analysis

A sample of crystalline (–)-α-9-O-desmethyldihydrotetrabenazine ($C_{18}H_{28}O_3N$ + CF_3CO_2-CH_3CH_2OH) was mounted in a thin-walled capillary to preserve the lattice solvent. Crystallographic data was collected at room temperature on a S/N/S automated R3 diffractometer with monochromated Mo radiation. Absorption corrections were applied in the basis of psi scans. The structure was solved using the programs of SHELXL-93.[8] To determine

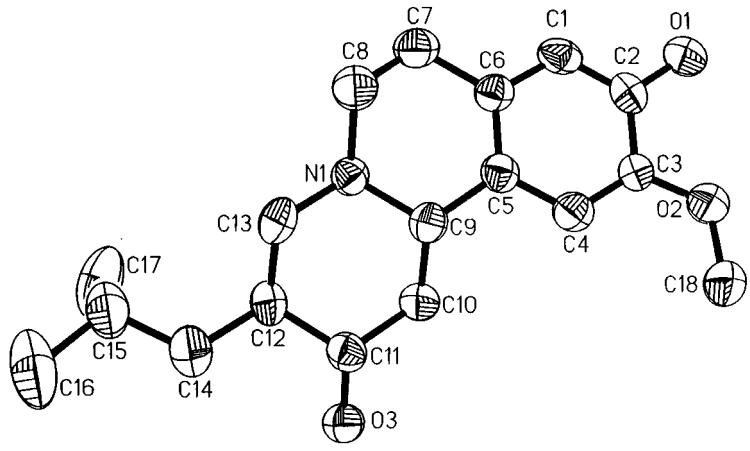

Fig. 2. Diagram of (–)-α-9-O-desmethyldihydrotetrabenazine with thermal ellipsoids representing 50% probability. The absolute configurations at atoms C9, C11, and C12 (corresponding to the 11b, 2, and 3-positions of the tetrabenazine structure shown in Fig. 1) are all *S*.

absolute configuration, the entire sphere of hkl data was collected and compared. In SHELXL-93, the absolute configuration parameter is near zero when the configuration assignment is correct, and near +1 when the configuration is wrong. For this data set, the absolute structure parameter refined to –0.4 ± 0.3 for the assignment shown in Figure 2. When the opposite configuration was tested, the parameter was +1.3 ± 0.3.

Crystal data are presented in Table 1, and the final atomic coordinates are given in Table 2. The molecular geometry and crystallographic labeling are shown in Figure 2.

TABLE 1. Crystallographic data for $[(C_{18}H_{28}O_3N)^+(CF_3CO_2)^- \cdot (CH_3CH_2OH)]$

Formula	$C_{22}H_{34}F_3NO_6$	Formula weight	465.50 amu
a	9.438(2) Å	space group	monoclinic $P2_1$
b	12.953(3) Å	T	22°C
c	10.070(2) Å	λ	1.54178 Å
β	92.40(2) °	Goodness of fit	1.039
V	1230.0(5) $Å^3$	ρ(calc)	1.257 g cm^{-3}
Z	2	μ	8.93 cm^{-1}
transmission coefficients 0.940–0.592			
R	0.045 for $I > 2\sigma(I)$; 0.049 for all 2486 data		
wR	0.120 for $I > 2\sigma(I)$; 0.123 for all 2486 data		
refinement method: full-matrix least-squares on F^2			

RESULTS AND DISCUSSION

Starting from commercially available tetrabenazine, it is possible to synthesize a large number of derivatives through reduction, demethylation, and organometallic addition reactions.[1,6] Cleavage of the methoxy groups of such compounds, either using the basic conditions reported here or using acidic conditions such as boron trihalides,[9] are low to moderate yield reactions requiring separation of the desired product from numerous impurities. Assignment of the isolated product as the 9-O-desmethyldihydrotetrabenazine, rather than the 10-O-desmethyl compound, was done on the basis of an X-ray crystal structure of the racemic mixture (data not shown), and verified by comparison with a sample of authentic 9-O-desmethyldihydrotetrabenazine synthesized from 3-benzyloxy-4-methoxybenzaldehyde.[2] Demethylation of tetrabenazine using boron tribromide yields primarily the 10-desmethyl isomer as determined by NMR and X-ray crystallographic analysis (data not shown), rather than the 9-desmethyl isomer as originally reported.[9] Although a moderate yield reaction, the one-step demethylation of α-dihydrotetrabenazine using the sodium hydride/N-methylaniline/HMPA method was considerably easier than the multi-step total synthesis.

Through the use of 2-D NMR techniques it has been determined that all of these compounds, and tetrabenazine itself, are a mixture of enantiomers with the identical relationship of configurations at C-3 and C-11b.[6,10] The biological activity of α-dihydrotetrabenazine resides in the (+)-isomer (K_i = 0.97 nM), with a very low affinity in vitro for the other enantiomer (2200 nM).[7] Two methods were developed for the resolution of enantiomers of these benzo[a]quinolizines. Application of chiral column HPLC provides, using repetitive injections, a suitable method for resolution and purification of sufficient amounts of resolved isomer for further use: the resolved (+)-α-9-O-desmethyldihydrotetrabenazine, for example, serves as the precursor for the synthesis of (+)-α-[^{11}C]dihydrotetrabenazine (via alkylation with [^{11}C]methyl iodide) which is used in imaging of human brain monoamine vesicular transporters using positron emission tomography.[11] As an alternative method of synthesis of the resolved isomer, we also examined the enantiospecific hydrolysis of an acetate ester of (±)-α-dihydrotetrabenazine using porcine pancreatic lipase (Altus 3). This enzyme selectively deacetylated the (+)-ester to provide a mixture of (+)-α-dihydrotetrabenazine and unhydrolyzed ester (enriched in the (–)-isomer). After separation of alcohol and ester on a silica gel column, pure (+)-α-dihydrotetrabenazine could be obtained. Although this was a suitable method for also obtaining useful quantities of pure resolved isomer, attempts to scale the enzyme synthesis 10-fold were less successful.

Of the pure resolved compounds which were prepared,

TABLE 2. Atomic coordinates (×10^4) and equivalent isotropic displacement parameters (A^2 × 10^3)[a]

	x	y	z	U (eq)
C (1)	122 (5)	1435 (3)	8178 (4)	49 (1)
C (2)	−940 (4)	1764 (3)	8932 (4)	49 (1)
O (1)	−2234 (3)	1313 (2)	8819 (3)	60 (1)
C (3)	−667 (4)	2588 (3)	9805 (4)	45 (1)
O (2)	−1801 (3)	2844 (2)	10555 (3)	62 (1)
C (4)	636 (4)	3051 (3)	9857 (4)	48 (1)
C (5)	1709 (4)	2723 (3)	9052 (3)	42 (1)
C (6)	1468 (4)	1886 (3)	8226 (3)	43 (1)
C (7)	2618 (5)	1490 (4)	7365 (4)	55 (1)
C (8)	4067 (5)	1854 (3)	7817 (5)	54 (1)
N (1)	4008 (3)	3003 (2)	7992 (3)	40 (1)
C (9)	3112 (4)	3298 (3)	9139 (4)	45 (1)
C (10)	2971 (4)	4461 (3)	9133 (4)	50 (1)
C (11)	4398 (4)	5007 (3)	9241 (4)	45 (1)
O (3)	4213 (3)	6097 (2)	9252 (3)	52 (1)
C (12)	5363 (4)	4658 (3)	8151 (4)	46 (1)
C (13)	5417 (5)	3477 (3)	8145 (6)	50 (1)
C (14)	6837 (5)	5140 (4)	8309 (6)	62 (1)
C (15)	7918 (5)	4861 (4)	7321 (5)	74 (1)
C (16)	9322 (7)	5390 (7)	7700 (9)	114 (2)
C (17)	7371 (8)	5086 (7)	5890 (6)	108 (2)
C (18)	−1586 (7)	3619 (4)	11551 (6)	71 (1)
O (4)	4545 (5)	2861 (4)	4869 (3)	105 (1)
O (5)	2845 (4)	3767 (2)	5673 (3)	73 (1)
C (19)	3561 (6)	3455 (3)	4771 (4)	63 (1)
C (20)	3157 (10)	3828 (6)	3387 (6)	112 (2)
F (1)	3293 (11)	3173 (6)	2497 (4)	264 (5)
F (2)	1984 (6)	4274 (5)	3212 (5)	178 (2)
F (3)	4032 (9)	4557 (7)	3070 (7)	253 (4)
O (6)	6317 (5)	1868 (3)	3215 (4)	93 (1)
C (21)	7318 (11)	1477 (7)	4081 (11)	171 (4)
C (22)	8077 (10)	2111 (8)	4932 (8)	147 (3)

[a]U(eq) is defined as one-third of the trace of the orthogonalized Uij tensor.

we were successful at growing good crystals from the (–)-α-9-O-desmethyldihydrotetrabenazine, and thus the determination of absolute configuration was done on that compound. The crystal structure determined is shown in Figure 2, and can be assigned as the 2*S,* 3*S,* 11b*S* isomer. As (–)-α-9-O-desmethyldihydrotetrabenazine can be converted to the corresponding (–)-α-dihydrotetrabenazine by simple O-methylation of the phenol group, the absolute configuration of (–)-α-dihydrotetrabenazine (the inactive isomer) is also 2*S,* 3*S,* 11b*S,* and thus the configuration of the high affinity (+)-α-dihydrotetrabenazine is 2*R,* 3*R,* 11b*R* (as depicted in Fig. 1). The resolution of the desmethyl compound, α-9-O-desmethyldihydrotetrabenazine, has also allowed us to prepare the single active enantiomer (+)-2*R,* 3*R,* 11b*R*-dihydrotetrabenazine in both tritium and carbon-11 labeled forms,[7] providing the optimal radioligands for in vitro and in vivo studies of the vesicular monoamine transporter type 2.

Finally, as α-dihydrotetrabenazine is obtained from tetrabenazine by a simple hydride reaction which produces the chiral center at C-2 but does not racemize the carbon centers at C-3 and C-11b, these studies would suggest that tetrabenazine itself may consist of active (3*R,* 11b*R*) and inactive (3*S,* 11b*S*) enantiomers. The relative biological activities of the enantiomers of tetrabenazine do, however, remain to be verified.

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