THIAPHYSOVENOL PHENYLCARBAMATES: X-RAY STRUCTURES OF BIOLOGICALLY ACTIVE AND INACTIVE ANTICHOLINESTERASE AGENTS

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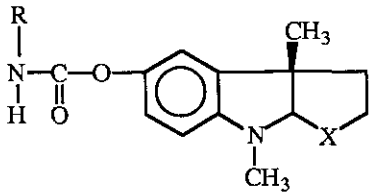
Abstract - The X-ray structure of the biologically active phenylcarbamate (4), and the biologically inactive phenylcarbamate (5) of the thiaphysovenol series, reveal that the two aromatic ring systems are differently oriented towards each other. The absolute configuration of both compounds, determined by using the anomolous scattering from the sulfur atoms, was found to be (3aS-cis).

The natural alkaloids physostigmine (1) and physovenine (2), and the synthetic analog thiaphysovenine (3) shown in Figure 1, are potent inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) *in vitro* (Table 1). The short half life of 1 *in vivo* (Table 1) is a serious drawback to its therapeutic uses and attempts were made to improve on it by chemical modification of the carbamate group.

Replacing the methyl group with longer aliphatic alkyl groups, such as heptyl [1], or octyl [2], has led to analogs which were similarly potent inhibitors of AChE and BChE, but were considerably longer acting when evaluated *in vivo* [3]. Phenylcarbamates of (-)-eseroline [4,5] and of (-)-physovenol [6], also were found to be long acting inhibitors of the enzymes, and inhibited AChE and BChE with high specificity which depended on the substitution in the phenyl group of the phenylcarbamate moiety [5]. A similar behavior was shown by phenylcarbamates of the thiaphysovenol series [7], with a sulfur atom instead of an oxygen atom in the C-ring of 2. High specificity for the inhibition of AChE was noted for the phenylcarbamate(4),whereas the 2', 4', 6'-

trimethylphenyl carbamate analogue (5) was found to be largely inactive against both enzyme preparations (Figure 1, Table 1). To collect additional information on the two analogs we decided to compare the highly crystalline carbamates (4) and (5) by X-Ray analysis. Besides getting concise information regarding the atomic distances, and the relative positioning of the two aromatic systems relative to the O11-C12-O13-N14 link, it was hoped that the X- ray analysis, using the anomolous scattering of the sulfur atom, would support the proposed (3aS-cis) absolute configuration for both 4 and 5. This configuration was anticipated since both compounds were derived from (-)-eseroline [9]. We now report the results of this investigation.



1 Physostigmine	R = CH_3, X = N-CH_3
2 Physovenine	R = CH_3, X = O
3 Thiaphysovenine	R = CH_3, X = S
4 Thiaphysovenol phenylcarbamate	R = Ph, X = S
5 Thiaphysovenol 2',4',6'-trimethylphenylcarbamate	R = 2',4',6'-tri-CH_3-Ph, X = S

Figure 1: Carbamate Analogues of Thiaphysovenol

	Compound	AChE	BChE	Duration of Action
1	(-)-Physostigmine	27.9 ± 2.4	16.0 ± 2.9	Short (30 min)
2	(-)-Physovenine	27.1 ± 0.8	3.7 ± 1.4	Short
3	(-)-Thiaphysovenine	9.9 ± 1.0	0.6 ± 0.1	Short
4	(-)-Thiaphysovenol phenlycarbamate	27.2 ± 7.1	1657.1 ± 353.8	Long (8 hours)
5	(-)-Thiaphysovenol -2',4',6'-trimethyl-phenylcarbamate	>10,000	>10,000	None

1) Values are means (±SE) of 4 or more separate assays, undertaken in duplicate, measured as described in ref. 7.

2) Duration of action was measured in rats. Tremor, a centrally mediated action of cholinesterase inhibitors, was monitored after administration of compounds 1-5 (2 mg/kg i.v.). Hexamethonium bromide (5 mg/kg, i.p.) and atropine methyl bromide (4 mg/kg, s.c.) were prior administered to inhibit peripheral cholinergic actions. Additionally, plasma samples were removed and later assayed for AChE activity to confirm cholinesterase inhibition during tremor.

Table 1: IC50 Values (nM) of Compounds 1 - 5 vs Human AChE and BChE and Duration of Action

EXPERIMENTAL

(-)-(3aS-cis)-Thiaphysovenol phenylcarbamate (4): mp 175-176°C (Et_2O), $[\alpha]_D$ = -268.8° (c = 0.8, $CHCl_3$) [7]. $C_{19}H_{20}N_2O_2S$, F.W. = 340.4, monolinic space group $P2_1$, a = 9.775(2), b = 9.705(2), c = 10.034(2) Å, β = 109.41(2), V = 897.8(3) Å3, Z = 2, ρcalc = 1.259 mg mm-3, λ(Cu Kα) = 1.54184 Å, μ = 1.70 mm-1, $F(000)$ = 360, T = 295°K.

A clear colorless irregularly shaped (0.22 x 0.35 x 0.42 mm) crystal was used for data collection on an automated Siemens R3m/V diffractometer equipped with an incident beam monochromator. Lattice parameters were determined from 25 centered reflections within $39 \leq 2\theta \leq 60°$. The data collection range of hkl was: $-1 \leq h \leq 10$, $-10 \leq k \leq 0$, $-10 \leq l \leq 10$, with $[(\sin\theta)/\lambda]\max = 0.54$. Three standards, monitored after every 97 reflections, exhibited random variations up to ±2.5% during the data collection. A set of 1449 reflections was collected in the $\theta/2\theta$ scan mode, with scan width $[2\theta(K_{\alpha 1}) - 1.0]$ to $[2\theta(K_{\alpha 2}) + 1.0]°$ and ω scan rate (a function of count rate) from 4.0°/min to 30.0°/min. There were 1310 unique reflections, and 1285 were observed with $F_o > 3\sigma(F_o)$. The structure, shown in Figure 2, was solved and refined with the aid of the SHELXTL system of programs [8]. The full-matrix least-squares refinement varied 221 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms, coordinates for the hydrogen bonded to the nitrogen atom, all other H atoms included using riding model [coordinate shifts of C applied to attached H atoms, C-H distance set to 0.96 Å, H angles idealized, $U_{iso}(H)$ set to 1.1 $U_{eq}(C)$ or, if methyl, 1.2 $U_{eq}(C)$]. Final residuals were $R = 0.029$ and $wR = 0.037$ with final difference Fourier excursions of 0.16 and –0.13 eÅ–3. Tables of atomic coordinates, bond distances and angles have been depositedwith the Cambridge Crystallographic Data Base. [9]

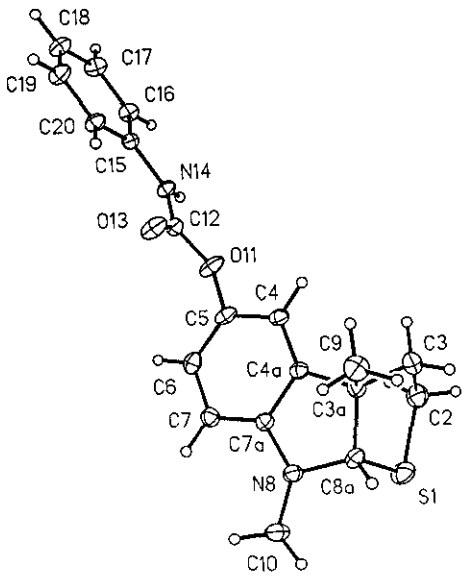


Figure 2: Molecular structure and numbering scheme for (3aS-cis)-thiaphysovenol phenylcarbamate (4). The figure is drawn using experimentally determined coordinates with the thermal ellipsoikds at the 20% probability level.

(-)-(3aS-cis)-Thiaphysovenol 2',4',6'-trimethylphenylcarbamate (5): mp 158-159°C (Hexane), $[\alpha]_D$ = -223.9° (c = 0.4, $CHCl_3$) [7]. $C_{22}H_{26}N_2O_2S$, F.W. = 382.5, tetragonal space group $P4_3$, a = 12.482(2), c = 13.756(2) Å, V = 2143.2(6) Å3, Z = 4, ρcalc = 1.185 mg mm-3, λ(Cu Kα) = 1.54184 Å, μ = 1.48 mm-1, $F(000)$ = 816, T = 295°K.

A clear colorless 0.15 x 0.20 x 0.30 mm crystal, in the shape of an irregular prism, was used for data collection on an automated Siemens R3m/V diffractometer equipped with an incident beam monochromator. Lattice parameters were determined from 25 centered reflections within $55 \leq 2\theta \leq 65°$. The data collection range of hkl was: $-13 \leq h \leq 13$, $0 \leq k \leq 13$, $-14 \leq l \leq 0$, with $[(\sin \theta)/\lambda]max$ = 0.54. Three standards, monitored after every 97 reflections, exhibited random variations with devs. up to ±2.5% during the data collection. A set of 3204 reflections was collected in the $\theta/2\theta$ scan mode, with scan width $[2\theta(K_{\alpha 1}) - 1.0]$ to $[2\theta(K_{\alpha 2}) + 1.0]°$ and ω scan rate (a function of count rate) from 10.0°/min to 30.0°/min . There were 1467 unique reflections, and 1362 were observed with $F_o > 3_\sigma(F_o)$. Data were corrected for Lorentz and polarization effects. An empirical absorption correction was also applied (maximum and minimum transmission factors were 0.836 and 0.683 respectively). The structure, shown in Figure. 3, was solved and refined with the aid of the SHELXTL system of programs [7] .

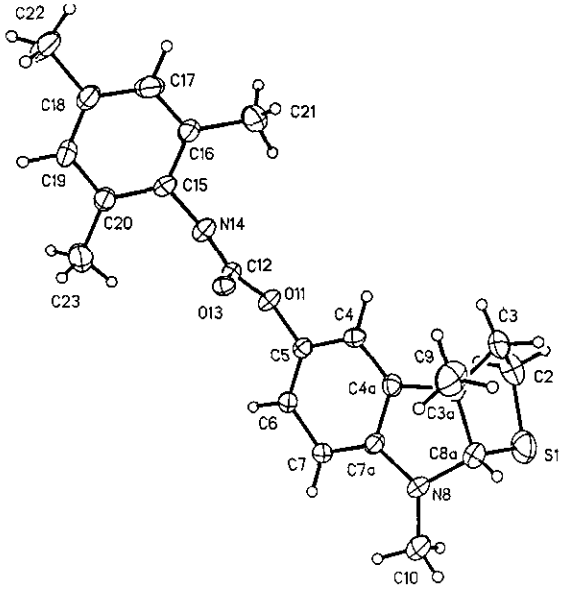


Figure 3: Molecular structure and numbering scheme for (3aS-cis)-thiaphysovenol 2',4',6'-trimethylphenylcarbamate (5). The figure is drawn using experimentally determined coordinates with the thermal ellipsoids at the 20% probability level.

The full-matrix least-squares refinement varied 248 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms, and coordinates only for the hydrogen atom on N14. All other H atoms included using a riding model [coordinate shifts of C applied to attached H atoms, C-H distance set to 0.96 Å, H angles idealized, $U_{iso}(H)$ set to 1.1 $U_{eq}(C)$ or, if methyl, 1.2 $U_{eq}(C)$] Final residuals were $R = 0.044$ and $wR = 0.052$ with final difference Fourier excursions of 0.17 and -.14 eÅ–3. Tables of atomic coordinates, bond distances and angles have been deposited with the Cambridge Crystallographic Data Base.2

DISCUSSION:

The O11-C12-O13-N14 moiety is planar in both molecules (4) and (5). In 4 it is coplanar with the phenyl ring of the carbamate and approximately perpendicular to the thiaphysovenol ring system (angle between the planes through the aromatic ring of the thiaphysovenol and phenyl ring is 91.6°). In 5, the O11-C12-O13 N14 plane is approximately perpendicular to both the thiaphysovenol ring system (interplanar angle with the aromatic ring = 76.3°) and the trimethyl phenyl ring (interplanar angle = 81.4°). The result of this double twist is that the

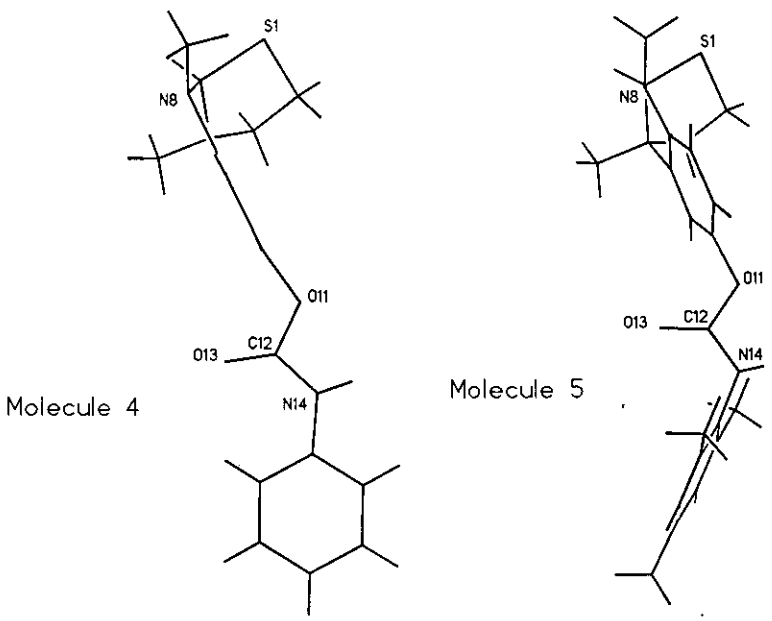


Figure 4: Comparison of the conformations of 4 and 5. Both molecules were drawn using the projection generated by calculating the best least-squares plane fit to atoms O11, C12, O13 and N14. Unlabelled terminal atoms are hydrogens.

aromatic rings are approximately gauche with respect to one another in 5 (angle between the two aromatic ring planes is 128.3°) rather than perpendicular as in 4. This difference in orientation between the aromatic ring systems is illustrated in Figure 4. Using the method suggested by D. Rogers [10] it was possible to determine the absolute configuration of both compounds using the anomolous scattering from the sulfur atom. The parameter η which multiplies all $\Delta F''$ values (imaginary component of the atomic scattering factors) refined to a value of 1.1(1) for molecule 4 and 1.1(2) for molecule(2)(a correct choice of enantiomer should give +1.0 for η, an incorrectly identified enantiomer should give a value of -1.0). The configuration at both C3a and C8a is 'S' in both compounds. In the crystal of 4 the 2-fold screw axis generates helical columns of molecules with each molecule in the column being linked to its two nearest neighbors by an N-H...O hydrogen bond (N-H = 0.84Å, H...O = 2.28Å, N...O =3.09Å and N-H...O = 161.2°). In 5 each molecule is also linked to its two nearest neighbors by an N-H...O hydrogen bond (N-H = 0.64Å, H...O = 2.24Å, N...O = 2.87Å and N-H...O = 170.6°), however, in this case the two closest molecules are generated by independent symmetry operations. In both 4 and 5 the hydrogen bonds are the only intermolecular approaches less than Van derWaals separations.

COMMENTS

Inspection of the X-ray structure of 4, and of physostigmine [1] measured earlier [11], suggests that in both compounds the carbonyl group of the esteratic site is readily available for nucleophilic attack by an OH group located on the enzyme [12]. This reaction in 5 might be seriously impaired by the presence of the two methyl groups attached to the ortho-positions of the phenyl substituent. Although conclusions drawn from solid state X-ray structures might not necessarily reflect the situation present in solution, we believe that the steric hindrance of the bulky methyl groups in 5 might be the reason for its inactivity.

ACKNOWLEDGEMENT

This work was supported in part by the Office of Naval Research, and NIDA.

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Received, 29th June, 1992