Tetrandrine as Host Partner in Molecular Recognition Phenomenon. Experimental and Theoretical Study on the Complexation of Tetrandrine with Calcium Cation

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Abstract

The X-Ray structure of tetrandrine was compared with the results obtained by molecular modeling "in vacuo" with MM3, AM1, 3-21G and DFT methods. As tetrandrine is well-know calcium channel blocker, the potentiometric measurements of the complexation between tetrandrine and Ca^{2+} cation has been performed. The formation of the complexe 1:1, experimentally found, has been simulated by MM3 procedure method.

**Keywords**. Tetrandrine; channel blocker; complexation; potentiometry; MM3; AM1; 3-21G;DFT.

1 INTRODUCTION

Tetrandrine (6, 6', 7, 12 - tetramethoxy -2, 2' - dimethyl – berbaman; TET) is a bis-benzylisoquinoline alkaloid extracted from the root of Stephania tetrandrae S. Moore. It is used as drug in oriental traditional medicine oriental [1]. TET has various biological activities: cardiovascular, anti-inflammatory and antitumoural. These activities are related to its calcium antagonist properties and they have been studied in previous papers [2-6]. The mechanism of action is not completely understood. We have recently showed, by various spectroscopic methods and potentiometric titration, that TET forms complexes with calcium and magnesium ions and this may explain his biological activity [7-10].

TET a natural cyclophane-like structure show a dimer of two benzylisoquinoline subunits condensed in a head to head, tail to tail fashion by two ether bridges, forming a small central cavity which may accommodate cationic species (Fig.1).

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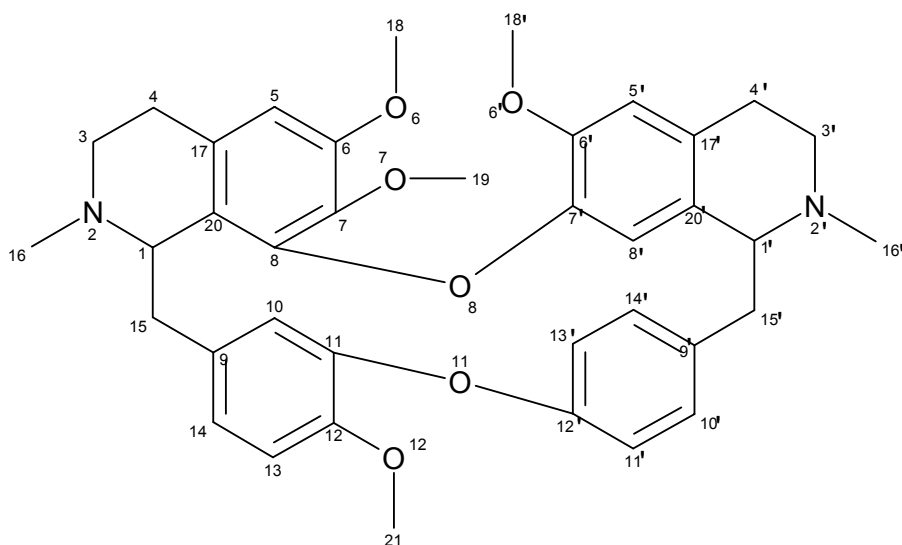
Figure 1. Molecular structure of TET with the numbering scheme

RMN and X-ray studies of TET have demonstrated that the structure has similar conformations both in solution and in solid state [11,12]. This paper reports our study on the structure of tetrandrine by MM3, AM1, 3-21G and DFT procedure methods, the potentiometric measurement concerning the complexation between tetrandrine and Ca^{2+} cation and the simulation of the complexation by MM3 methods.

2 MATERIALS AND METHODS

2.1 Computer Software

The molecular modeling was performed on a PC with a Genuine Intel Family 6 Model 7601 processor with the CAChe (**C**omputer **A**ided **Che**mistry) program and the MM3 Augmented force field, AM1 semiempiric, 3-21G ab-initio and DFT methods.

The lowest energy structure was found by geometry optimization with the Conjugate gradient algorithm, with the RMS gradient of 0.001 kcal/mol·Å. The energy map was obtained by molecular mechanics with the MM3 Augmented force field when calcium ion are approaching on an axe of 10 Å, with a step of 1 Å, perpendicular to TET cavity in its center.

2.2 Potentiometric measurements

The pH-metric titrations were performed in pH range 4.5-11.0 using a MOLSPIN automatic titration system with a micro combination pH electrode (Mettler-Tledo). Titrations were performed in triplicate and the SUPERQUAD computer program [13] was used for calculations of stability constans (β_{pqr}=[$M_pH_rL_q$]/ [M]$_p$[H]$_r$[L]$_q$). Standard deviations quoted refer to random errors only. They are, however, a good indications of the importance of a particular species in the equilibrium.

The pH electrode was calibrated using two standard buffer solutions and all calibrations and titrations were carried out under a CO_2-free argon atmosphere, to avoid any contact with carbon dioxide in a glass vessel (10 cm$_3$) thermostatted at 25 ± 0.1°C, and an ionic strength of 0.10 mol dm^{-3} KCl. The concentrations of the metal–ion and ligand solutions were maintained between 2.0 and 1.0 mmol dm^{-3}. A CO_2-free 0.100 mol dm^3 NaOH solution was used as the titrant to minimize ionic strength change during the titration.

Tetrandrine were obtained from Aldrich and used without further purification. Solutions were prepared in water/acetonitrile 60/40 (v/v). The following solutions were prepared and titrated potentiometrically against standard carbonate-free sodium hydroxide 0.10 mol dm^{-3} solutions (prepared and standardized against standard potassium hydrogen phthalate). Titrations of HCl solution in KCl were done daily to calculate the standard electrode potentiel E0 and the dissociation constant for water/acetonitrile 60/40 (PKw= -14.33). The used concentrations are: (a) 0.001 mol dm$_3$ HCl; (b) a+0.0005 mol dm^{-3} of tetrandrine; (c) b+0.00025 mol dm^{-3} of metal ion solution.

3 RESULTS AND DISCUSSION

a) *Structure of tetrandrine by MM3, AM1, 3-21G and DFT procedure methods.*

In this theoretical study, we are taken in consideration the X-Ray structure of tetrandrine published in literature [12]. The geometrical data obtained by MM3, AM1, 3-21G and DFT methods are presented in tables 1, 2 and 3. We are given only the bonds, angles and dihedral angles concerning the interior cage (a macrocyle with 18 atoms) defined by H(10), O(8), C(7'), C(8') and the inner surface of the phenyl ring C(9'), C(10'), C(11'), C(12'), C(13') and C(14'). The atoms O(8) and O(11) define on axe in the plane of the cavity.

Table 1. Bond length in TET molecule.

Bond	Bond length (Å)				
	X-ray	MM3	AM1	3-21 G	DFT
C20-C1	1.532	1.517	1.510	1.525	1.506
C1-C15	1.544	1.548	1.547	1.551	1.53
C15-C9	1.519	1.509	1.486	1.490	1.514
C8-O8	1.405	1.365	1.391	1.384	1.370
O8-C7'	1.393	1.369	1.392	1.367	1.395
C11-O11	1.379	1.367	1.393	1.394	1.359
O11C12'	1.401	1.362	1.397	1.393	1.37
C9'-C15'	1.508	1.511	1.485	1.512	1.487
C15'-C1'	1.546	1.549	1.549	1.564	1.541
C20'-C1'	1.520	1.514	1.510	1.521	1.497

The largest deviation is - 0.041 Å.

Table 2. Bond angles in TET molecule.

Bond angles	Bond angles (degrees)				
	X-ray	MM3	AM1	3-21 G	DFT
C8-C20-C1	120.5	119.1	119.7	119.17	120.20
C20-C1-C15	108.8	109.1	107.5	108.97	109.66
C1-C15-C9	114.3	112.8	112.8	112.69	115.36
C15-C9-C10	118.7	118.6	119.1	118.91	118.68
C10-C11-O11	124.0	121.4	122.1	121.89	123.79
C11-O11-C12'	116.3	120.2	113.7	118.40	117.34
O11-C12'-C13'	119.7	120.6	121.2	120.82	120.71
C14'-C9'-C15'	120.8	120.6	119.9	120.61	118.61
C9'-C15'-C1'	115.0	114.8	113.2	113.96	115.79
C15'-C1'-C20'	110.7	111.1	109.8	111.41	111.10
C1'-C20'-C8'	119.9	120.7	119.8	120.58	120.44
C8'-C7'-O8	115.7	116.6	113.9	117.47	117.24
C7'-O8-C8	121.8	125.6	119.0	126.17	122.11

The largest deviation is -3.8 degrees.

Table 3. Dihedral angles in TET molecule.

Dihedral angle	Dihedral angles (degrees)				
	X-ray	MM3	AM1	3-21 G	DFT
C8-C20-C1-C15	-77.9	-77.4	-79.5	-74.57	80.50
C20-C1-C15-C9	165.8	171.1	162.5	164.76	-166.21
C1-C15-C9-C10	-74.5	-80.7	-93.7	-105.45	75.62
C14-C9-C15-C1	109.0	100.9	88.0	73.97	-105.14
C9-C15-C1-C2	-69.0	-64.2	-71.2	-70.95	68.19
C7-C8-O8-C7'	51.7	64.2	56.1	34.44	-48.95
C20-C8-O8-C7'	-130.5	-120.3	-130.3	-148.01	133.19
C8-O8-C7'-C6'	24.6	20.3	33.5	41.64	-25.31
C8-O8-C7'-C8'	-159.0	-161.7	-152.7	-140.64	159.84
O12-C12-C11-O11	1.3	-1.5	3.6	0.39	1.52
C12-C11-O11-C12'	-176.5	-169.5	-148.0	-152.33	173.93
C10-C11-O11-C12'	5.6	10.5	35.5	28.25	-8.12
C11-O11-C12'-C13'	79.4	74.3	55.9	62.37	-78.68
C11-O11-C12'-C11'	-103.4	-105.2	-127.1	-116.14	102.61
O8-C7'-C8'-C20'	179.2	179.0	-175.8	179.36	179.55
C8'-C20'-C1'-C15'	-80.2	-88.2	-87.7	-85.61	79.41
C20'-C1'-C15'-C9'	62.9	59.3	66.1	65.08	-65.63
C1'-C15'-C9'-C14'	-112.8	-105.4	-108.9	-101.93	113.34
C10'-C9'-C15'-C1'	69.1	76.9	72.0	77.73	-83,41
C9'-C15'-C1'-N2'	-175.1	-176.8	-169.9	-172.50	170.41

The largest deviation is –29.9º and may be explained by the possibility of rotation of the methoxy substituent. The dihedral angle involved is C10-C11-O11-C12'.

Indeed, the numerical data prove that the differences in geometrical parameters are insignificants. There are no evident modifications between the solid state and the in vacuo form of tetrandrine.

b) potentiometric study on the complexation between tetrandrine and Ca^{2+} cation.

The SUPERQUAD computer program [13] was used to refine the protonation constant of tetrandrine. The pK_a values obtained from potentiometric data are listed in Table 4.

Table 4. The pK_a values of tetrandrine.

Ligand	Species	Log β	pK_a
Tetrandrine	LH2	16.17	7.35
	LH	8.82	8.82

These values are obtained at 25±0.1°C and I=0.1 mol/dm^{-3} in water/acutonitrile 60/40.

To determine the stoichiometry and stability constants of complex, which take place between the ligand and calcium ions $Ca(ClO_4)_2$, the solutions including metal ions, the ligands and acid have been titrated with standard NaOH solution. The metal to ligand molar ratio used in titration exceed the ratio 1:2 respectively. The best-fit models based on the potentiometric data calculations are given in Table 5.

Table 5. Constants of formation TET-Ca^{2+}.

Log β (CaHL)	Log β (CaL)	Log β (CaH$_{-1}$L)
12.57 (0.14)	4.97 (0.08)	-5.36 (0.20)

Ca(II) complex formation constant (log β) of tetrandrine has been determinated at 25±0.1°C and I=0.1 mol/dm^{-3} in water/acutonitrile 60/40.
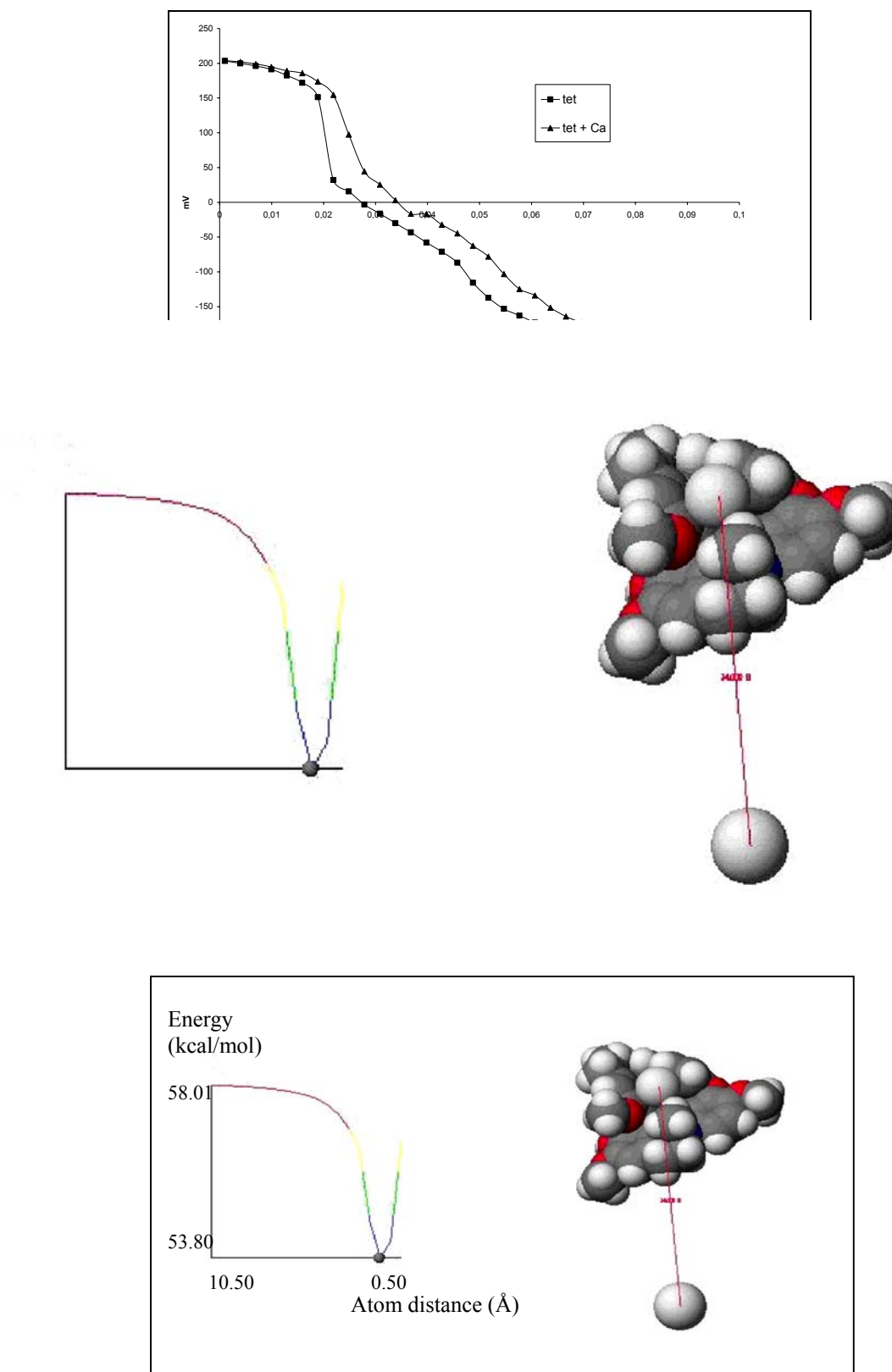
Figure 3. The variation of the potential energy when Ca^{2+} is docking in the cavity of TET

The conformation of TET is changing when the calcium ion is approaching the cavity, and

we can observe a reduction of the total energy with approximate 4.21 kcal mol^{-1}.

Considering these data and the obtained experimental results we propose a structure with an eight coordinated calcium ion (with the four ether oxygen atoms (O(8), O(11), O(12) and O(6')), the two oxyanions of the perchlorate couter-ion and two waters molecules).

The obtained bond lengths in the complex are as follows: d(O8…Ca^{2+})=2.8Å, d(O11…Ca^{2+})=2.74Å, d(O12…Ca^{2+})=2.79Å, d(O6'…Ca^{2+})=2.65Å, d(ClO$_4^-$…Ca^{2+})=2.50-2.52 Å and d(O$_2$H…Ca^{2+})=2.65-2.73Å respectively.

4 CONCLUSIONS

1. The theoretical geometries of tetrandine obtained by MM3, AM1, 3-21G and DFT methods are similar to that experimentally determined by X-Ray analysis.
2. The potentiometric measurements on the complexation between tetrandrine and Ca2+ cation indicate the formation of 1:1 complex.
3. Using MM3 augmented parameters by a docking of Ca2+ into tetrandrine, the structure of the complex 1:1 has been achieved.
4. This paper could be considered as a contribution to the explanation of the role of tetrandrine as calcium channel blocker.

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