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Synthesis and Analysis of the X-ray structure of a Schiff base compound;trans-*N*,*N*'-bis-(phenylmethyl)cyclohexane1,2-diamine

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ABSTRACT: *N,N'-bis(Phenylmethyl)Cyclohexane-1,2-diamine crystallizes in the orthorhombic Pbcn space group with unit cell parameters, a = 19.931(14) Å, b = 9.266(6) Å, c = 9.352(6) Å and z = 4. The crystal structural data were collected using the Enraf-Nonius CAD4 X-ray diffractometer with monochromatic Kα radiation of Cu (λ = 1.54187 Å). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares procedures to the final R1 of 0.062, using SHELXL programs. The 3D-crystalline network of this compound is stabilized essentially by weak CH/pi type hydrogen bonds. Furthermore, the biological study of this Schiff base compound revealed no antibacterial activities but has antifungal activities with significant antioxidant properties.*

Keywords: *Schiff base; X-ray structure; CH/pi hydrogen bonds; Biological activities.*

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I. INTRODUCTION

Schiff bases synthesized from the reaction between any primary amine and an aldehyde or a ketone [1], are shown to have significant importance and a wide range of applications. Schiff bases are recognized by the presence of an imine or azomethine group (C=N), formed by the condensation reaction between an amine and a carbonyl compound. Azomethine or imine groups are present in various natural derived or artificial compounds [2, 3] and such compounds are shown to have significant biological effects [4, 5]. In reality, Schiff bases are known to exhibit a broad range of biological activities including anti-fungal, anti-bacterial, anti-malarial, anti-proliferative, anti-inflammatory, anti-viral and anti-pyretic properties [6-9]. As an extension of our study on the structural characterization of diamine schiff base compounds, the synthesis and analysis of the solid-state structure of our compound will be presented.

II. EXPERIMENTAL

2.1. General

The reference bacterial strains and the hospital strain used in this research were provided by the Pasteur Institute Center of Abidjan and the Microbiology Laboratory of Swiss center of Scientific Research of Côte d'Ivoire. For aseptic conditions, a laminar flow hood (CYTAIR, France) was essential. Other technical materials used in our study include: a bacteriological incubator (JOUAN type: EB 170 EL SANS), an autoclave (Trade Raypa, Model AE-75BRY Spain), microplates with U-shaped bottom cups (12 x 8 rows), and a precision electronic balance (AG 204 Delta Range). The ^{1}H and ^{13}C NMR analyzes were all carried out at Laboratoire de Pharmacognosie de la Faculté de Pharmacie of Châtenay-Malabry (France) on a Bruker-Avance apparatus 200 MHz.Mass spectroscopy was performed at Laboratoire de Pharmacognosie de la Faculté de Pharmacie of Châtenay-

Malabry (France). Mass spectra in ESI were recorded on one device: TOF LCT Premier (WATERS) coupled with HPLC Alliance 2695 (Waters). The software used for data analysis is the Masslynx. Infrared (IR) spectra were recorded on a Bruker Vector 22 device. The single-crystal X-ray diffraction study method were carried out at the Institut de Chimie des Substances Naturelles (Gif sur Yvette, France) using a CAD4 Enraf-Nonius diffractometer equipped with a rotating copper anode, whose Cu-Kα line (λ = 1.54187 Å) is selected by a graphite monochromator.

2.2. Synthesis of N, N'-bis(phenylmethylene)cyclohexane-1,2-diamine (1-CD)
Benzaldehyde (0.4 mmol) and cyclohexane-1, 2-diamine (0.2 mmol) were dissolved in ether (30 mL) and the mixture was continuously stirred at room temperature for three days to give a resulting light brown precipitate. The precipitate was filtered through suction, washed several times in ethanol and air dried. The resultant brownish powder was recrystallized several times in 95 % ethanol to obtain the pure colorless *N*,*N*'-bis(phenylmethylene)cyclohexane-1,2-diamine product (Rf: 0.71 in hexane/acetone (50; 50), yield: 90%, mp 102.6°C), showed in Fig. 1. A significant amount of the colorless single crystals product was obtained by the slow evaporation of hexane solvent after 7 days. ^{1}H NMR (200 MHz m/z, $CDCl_3$) ppm 8.21 (s, 2H), 7.41 (d, 4H, J ¼ 2.2 Hz), 7.33 (d, 4H, J ¼ 2.2 Hz), 7.29 (m, 2H), 3.37 (m, 2H), 1.80 (s, 6H), 1.49 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) ppm 160.9, 136.4, 131.1, 128.3, 127.8, 73.7, 32.9, 24.4. Mass (ESI) (relative intensity): 291[M+H, 100] $^{+}$.

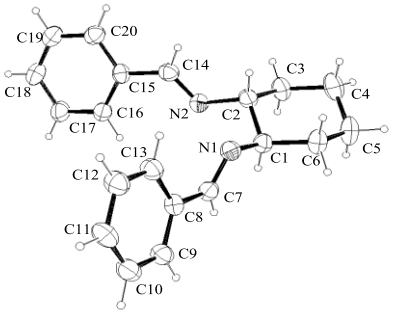


Figure 1: Molecular structure of 1-CD with atomic numbering scheme. (Displacement ellipsoids are drawn at 20% probability level).

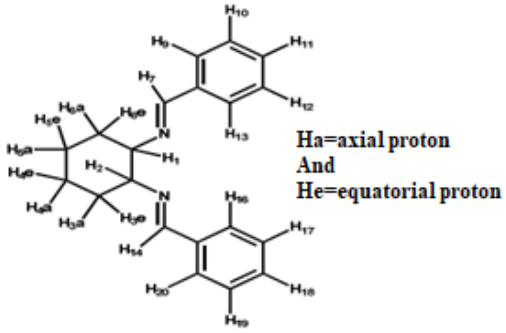


Figure 2: Compound 1-CD

2.3. X-ray crystal determination Single crystals of the schiff base compound (1-CD) were subjected to X-ray diffraction studies. Three dimensional intensity data of the solid-state structure were collected with the help of an Enraf-Nonius CAD4 diffractometer using graphite monochromatized Cu-Kα radiation (λ= 1.54187 Å). The structure was resolved by direct methods and refined on F^2 by full-matrix least-squares procedures using the SHELXL programs [10]. All the non-hydrogen atoms were refined using isotropic and later anisotropic thermal parameters. The hydrogen atoms were included in the structure factor calculation at idealized positions by using a riding model, but not refined and the images were created with ORTEP [11]. The X-ray Crystallographic data are presented in Table 1.

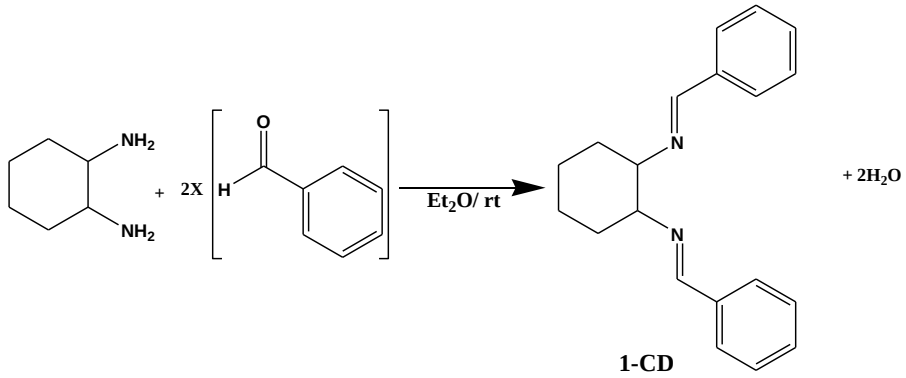
Table 1: Crystal data collection and structure refinement parameters.

Crystal data	**Parameters**
Empirical formula: $C_{20}H_{22}N_2$	10578 observed reflexions
Formula weight: 290.39	1466 independantereflexions
Temperature : 293K	100 parameters, F(0 0 0) : 624
X-ray: Cu Kα ; λ = 1.54197Å	Index ranges (h, k, l) -23~15 ; -10~10 ; -10~10
Crystal system: Orthorhombic	Theta range for data collection: 4.4°-≤ θ ≤ -69.8°
Space group : Pbcn	Absorption coefficient (μ) : 0.50 (mm-1)
a =19.931 (14)Å	Goodness of fit : 0.92
b =9.266 (6)Å	Refinement on F^2
c =9.352 (6)Å	Final R indices [I >2σ(I)]: R1 = 0.062 wR2 = 0.131
α = β = γ =90°	R indices (all data) : R1 = 0.225 wR2 = 0.162
V = 1722,17(18)Å^3	Maximum; minimum Δρ (eÅ-3) : 0.14 ; -0.20
D_{calc} = 1.120Mg m^{-3}	Enraf-Nonius CAD-4 diffractometer
Z = 4	Structure determination: SHELXS 97

Where $w = 1/[\sigma^2(Fo^2) + (0{,}1216P)^2]$ with $P = (Fo^2 + 2Fc^2)/3$

III. RESULTS AND DISCUSSION

3.1. Chemistry The schiff base compound N,N'-bis(phenylmethylene)cyclohexane-1,2-diamine, was synthesized by the condensation of benzaldehyde and cyclohexane-1,2-diamine in Diethyl ether under nitrogen at room temperature (scheme 1), followed by slow evaporation of the solvent (hexane/acetone) after 3 days to obtain colorless single crystals. The mass spectrum (ESI) of the 1-CD compound shows a peak at m/z 291 $[M+H]^+$, which makes it possible to propose $C_{20}H_{22}N_2$ as the formula.



Scheme 1: Synthesis of *N*,*N*'-bis(phenylmethylene)cyclohexane-1,2-diamine.

The IR spectrum shows a characteristic narrow band at 1642 cm^{-1}, corresponding to the azomethine (imine) functional group (C = N). The multiple bands between 3060-2857 cm^{-1} correspond to C-H elongation vibrations of the cyclohexane moiety and the aromatic nuclei. The ^1H NMR spectra of 1-CD reveal the presence of aromatic protons between [7.32-7.41] ppm. There is also the presence of unsinglet at δH 8.21 ppm corresponding to protons H7 and H14 in figure 2. The removal of these protons is due to the presence of the imine group. Protons H1 and H2 on the aromatic ring have chemical shift δH 3.37 ppm. These are also slightly deshielded by the proximity of the nitrogen atoms. In addition, four other protons in the axial positions have chemical shift; δH 1.49 ppm (H3a, H4a, H5a, H6a) and the protons in the equatorial position (H3e, H4e, H5e, H6e) have chemical shift; δH = 1.80 ppm, which indicates that protons in the equatorial positions are more shielded than those in the axial positions. From the ^{13}C NMR spectra, the presence of the aromatic system is confirmed by the existence of the resonance structure with chemical shifts ranging from 120 - 140 ppm. The signals observed at δC 160.9 ppm and 160.1 ppm correspond to the carbon atoms in the imine group (C7 and C14 respectively). The carbon atoms on the cyclohexane ring have chemical shifts at δC1 (75.5 ppm), δC2 (74.6 ppm), δC3 (33.6 ppm), δC4 (25.2 ppm), δC5 (25, 1 ppm) and δC6 (33.7 ppm) as confirmed in literature [ref]. The structure of the schiff base compound (1-CD) has been fully analyzed using both theoretical calculation data and experimental data, which are in complete agreement. The concordance of the spectral data (MS, IR, ^1H NMR, ^{13}C NMR) and the comparative table, allow us to predict the structure of the 1-CD to be N,N'-bis(phenylmethylene)cyclohexane-1,2-diamine.

3.2. Crystal Structure of the Semicarbazone 1-CD

Some geometric parameters such as bond lengths, valence angles and torsion angles are grouped in Table 2. Since the asymmetric unit of this compound consists of a half-molecule, the Table 2 contains only one half of the values.

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

Bond lengths	Angles (Å, °)	Bond lengths	Angles (Å, °)
C1-C2	1.531(6)	C_7=N_1-C_1	118.1 (3)
C2-C3	1.518(4)	N_1-C_1-C_6	109.1 (3)
C3-C4	1.520(4)	C_6-C_1-C_2	110.8 (2)
C4-C5	1.528(8)	N_1=C_7-C_8	122.3 (3)
N1-C1	1.469(4)	C_1-C_6-C_5	111.6 (3)
C7=N1	1.269(4)	C_6-C_5-C_4	110.3 (3)
C7-C8	1.465(4)	C_9-C_8-C_{13}	118.3 (3)
C8-C9	1.381(4)	C_9-C_8-C_7	121.6 (3)
C8-C13	1.392(4)	C_{13}-C_8-C_7	120.1 (3)
C11-C10	1.374(4)	C_{10}-C_9-C_8	120.7 (3)
C12-C11	1.356(5)	C_{11}-C_{12}-C_{13}	120.8 (4)
C12-C13	1.373(5)	C_{12}-C_{13}-C_8	120.2 (4)
N1-C7-C8-C13	17.15(4)	C_7- N_1-C_1-C_2	129.93(4)
N1-C7-C8-C9	-161.34(4)	C_7- N_1-C_1-C_6	-108.92(3)

In our previous publication, we reported the crystal structure of *N,N'*-bis (3-nitrophenylmethylene) cyclohexane-1,2-diamine compound [12]. The C-C bond lengths of the aromatic ring in 1-CD (Table 2), show a great similarity with *N,N'*-bis (3-nitrophenylmethylene) cyclohexane-1,2-diamine. With an average value of about 1.375 Å, these bonds are in conformity with the values proposed by Allen et al [13]. The valence angles associated to these aromatic rings do not also undergo major deformations and have values around 120° characteristic of the benzene ring. Like many schiff bases [14-17], the two azomethine functions present in our schiff base compound (1-CD), with C=N bond lengths of 1.269(4) Å are significantly shorter than the literature value (quote value if possible) [16]. In addition, the bond angle; C1-N1=C7 [118.1(3)°], and N1= C7-C8 ([122.3(3)°]) (Table 2) are congruent with their SP^2 hybridization characters. The cyclohexane C-C-C bond angles seen between 110.3 (3)° and 111.6(3)° indicate that our structure adopts a non-planar conformation in which the carbon atoms respect their SP^3 hybridizations. The fact that the sum of torsional angles; C7-N1-C1-C2 and C7-N1-C1-C6 is about 21°, is another proof of the non-planarity of cyclohexane ring. Indeed, the conformational analysis of the structure obtained, clearly shows that the cyclohexame ring adopts the more stable chair conformation as oppose to its boat counterpart [18-20]. In this chair conformation, the substituents preferentially adopt an equatorial position, which indicates according to conventional stereo descriptors [21] that our compound (1-CD) is the trans isomer and the absolute configuration of asymmetric carbons is R. Therefore the single crystal X-ray diffraction characterization shows that this compound is a pure trans (1R 2R) enantiomer, in contrast to racemic cis and trans cyclohexane α-diamine mixture used for its synthesis.

The value of the torsional angle N1-C7-C8-C13 observed, indicates that the mean planes formed by the six carbon atoms C8 to C13 of aromatic nucleus on the one hand and the four central atoms C8, C7, N1, C1 that bridge the two cycles on the other hand, are not coplanar. The various mean planes are defined in Table 3 for the rest of the description of this structure.

Table 3: Mean planes

Plane	Atoms defining each plane
I	C2, C3, C5, C6
II	C8, C9, C10, C11, C12, C13
III	C1, N1, C7, C8
IV	C2, N2, C14, C15
V	C15, C16, C17, C18, C19, C20

Table 4: Dihedral angles between mean planes (°)

	I	II	III	IV	V
I	0	62,04	79,57	83,04	82,33
II		0	17,76	75,81	77,56
III			0	68,73	75,81
IV				0	17,76
V					0

The relative orientations of the different mean planes are presented in Table 4. Analysis of this table shows that the planes IV and V with dihedral angles of 83.04° and 82.33° respectively, are practically perpendicular to plane I. The plane II deviates the most from the median plane of the cyclohexane chair conformation with a deviation angle of 62°. While planes III and IV, which connect the two aromatic rings of the structure to cyclohexane deviate by an angle of 68.73°, the aromatic nuclei (planes II and V) tend to move away and define between them a greater angle of 77.56°. This result is in contrast to that observed in the case of N,N'-bis (3-nitrophenylmethylene)cyclohexane-1,2-diamine compound [12] in which the two aromatic rings tended to approach one another with a smaller dihedral angle of about 63°. In addition, the main plane containing planes II and III on the one hand and planes IV and V on the other hand, form between them a dihedral angle of about 73°.

In the dimer showed in Figure 3 in which the two molecules 1 (x, y, z) and 2 (½-x, ½-y, ½+z) are symmetric with regards to the 2 fold screw axis parallel to [0 0 1] direction and located at (¼, ¼, 0), the hydrogen atom H8 of molecule 1 points to the aromatic nucleus of molecule 2 with an average distance of 3.231Å in Table 5. This C-H...π (arene) interaction, although weaker in strength compared to those reported by other authors [22-24], their combine effect contribute significantly to the stability of the 3-D crystal network in this direction.

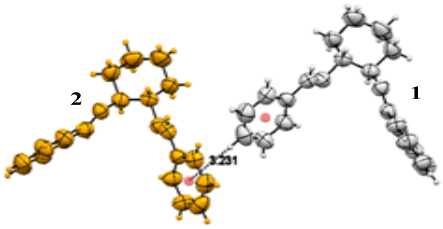


Figure 3: C-H…π (arene) interaction between 1 and 2.

Table 5: Intermolecular interaction (Å, °).

D-H•••A	D-H	H•••A	D•••A	D-H•••A
C8-H8…πi	0.93	3.231	4.083	163.28
Symmetry code (i) : ½ - x, ½ - y, ½ + z				

In view of the molecules packing showed in Figure 4 and Figure 5, and analysis of intermolecular interaction describe above, it seems easy to note that the molecules inside the crystal are organized in an infinite chain along the crystallographic axis "a". Inside each cluster, stability is achieved by the weak hydrogen C-H...π-arene bonds in addition to conventional Van der Waals type interactions. Furthermore, stability between the different clusters is essentially achieved by the combine effect of the classical Van der Waals interactions.

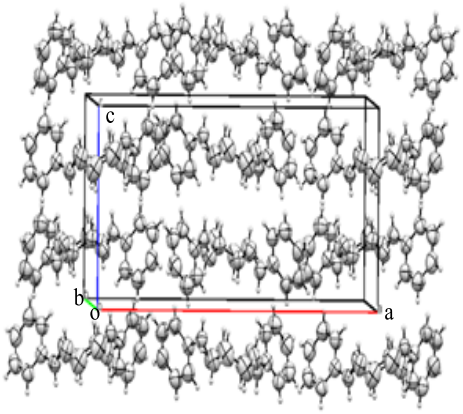


Figure 4: View of crystal packing

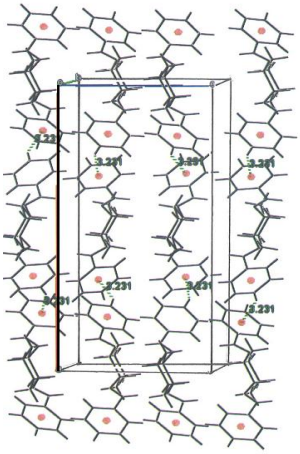


Figure 5: View of crystal network with C-H…π interactions.

3.3. Antibacterial Activity of the Semicarbazone

Biological activities, such as the antibacterial screening effects were performed on our schiff base compound (1-CD), and the results are presented in Table 6.

Table 6: Mean diameters (mm) of zone of inhibition and value of minimal inhibitors concentrations (MICs) of the compounds 1-CD.

The average diameters of the inhibition zones (mm)											Value of MICs (µg /mL)	
strains tested	*P.aeruginosa* CIP		*S.aureus* CIP		*S.aureus* sensitive		*E. coli* CIP		*S.aureus* ATTC		*S.aureus* CIP	*S.aureuss* sensitive
compounds	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2		
1-CD	0	0	0	0	0	0	0	0	0	0	-	-
witnesses	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3		
gentamicin	23	10	29	23	32	23	12	0	19	10	0.78	12.5
tetracycline	0	0	36	29	27	19	29	17	24	15	0.0976	12.5

Concentrations (µg/mL) C1=1500 ; C2=250 ; C3=25. *S. aureus*. Sensitive : *Staphylococcus aureus* susceptible to penicillin; *S. aureus*: *Staphylococcus aureus*; *P. eruginosa*: *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*. Analysis of the anti-bacterial effects (Table 6) indicate that the schiff base compound, 1-CD is insensitive to the strains of *Escherichia coli* 54127AF, *Pseudomonas aeruginosa* 103467 and *Staphylococcus aureus* ATTC 25923, even at very high concentration of 1500 µg/mL. This is in agreement to earlier reports from Sharma et al. [25].

3.4. Antifungal Activity of the Semicarbazone

The result of the anti-fungal activities of the compound, 1-CD are presented in Table 7. It is shown that, even at high concentrations of 1500 µg/mL, the compound 1-CD does not show any anti-fungal inhibitory activities against C. albicans (CIP).

Table 7: Mean diameters (mm) of inhibition zones and values minimal inhibitory concentrations (MIC) of the compounds tested.

The average diameters of the inhibition zones (mm)			**MIC values (µg/mL)**	**MIC values (µg/mL)**
Tested trains	*C. albicans* CIP	*C. glabrata*	*C. albicans* CIP	*C. glabrata*
Compounds	C1	C1		
1-CD	0	10	-	>1500
witnesses	C3	C3	-	-
Nystatin	11	0	50	-

Concentrations (µg/mL) C1 = 1500: C3 = 25

3.5. Antioxidant Activity of the Semicarbazone

The demonstration of the antioxidant power of the compounds was carried out according to the techniques of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Statistical analysis in the assay of the anti-radical activity by DPPH method, gave the results presented in Table 8.

Table 8: DPPH Inhibition Percentages of Tested Compounds

Compounds	Inhibition average(%) + standard deviation
1-CD	16.840 ± 0,663 (*)
Vitamin C	68.896 ± 7,540(****)
F	34.2852
P	< 0.001

Values are averages ± standard deviation, n = 3 (number of tests). Compounds with the same symbol (*) have their activities not very different from each other. (****): Best activity (***): Average activity (**): Low activity (*): Very low activity.

Analyzing the results on Table 8 above shows that the test compound has antioxidant properties. In addition, the value of the inhibition average percentage varies proportionately to its antiradical potential. This implies that, the higher this value, the stronger the antiradical potential and vice versa.Therefore, compound 1-CD shows a very low inhibitory average percentage (16,840 ± 0,663) towards DPPH.

IV. CONCLUSION

The single crystal x-ray diffraction analysis showed that compound 1-CD; *N*,*N*'-bis(Phenylmethyl) cyclohexane-1,2-diamine crystallizes in the orthorhombic Pbcn space group with unit cell parameters, a = 19.931(14) Å, b = 9.266(6) Å, c = 9.352(6) Å and z = 4. In addition, the antibacterial studies of compound 1-CD revealed its insensitive nature towards the strains of Escherichia coli 54127AF, *Pseudomonas aeruginosa* 103467 and *Staphylococcus aureus* ATTC 25923, even at very high concentration of 1500 µg/mL. Furthermore, 1-CD showed no antifungal inhibitory activities towards *C. albicans* (CIP) even at very high concentrations of 1500 µg/mL and a very low inhibitory average percentage (16.840 ± 0.663) towards DPPH.

ACKNOWLEDGEMENTS

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Supplementary Materials: Crystallographic data for the structural analysis have been deposited at the Cambridge Crystallographic Data Centre, CCDC No. 1542762. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ1E Z, UK (Fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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