Relationships between the Molecular Structures and Stabilities of Functional Dyes with a Phenothiazine Skeleton

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10-*N*-Methylcarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine (MCDP) is one of the functional dyes which are applied clinically as diagnostics. MCDP spontaneously decomposes to methylene blue while increasing the undesirable background of diagnosis. Understanding the mechanisms of spontaneous decomposition is essential for the molecular design of better functional dyes. X-Ray analyses of two derivatives of MCDP, 10-*N*-methylthiocarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine and 10-*N*-benzylthiocarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine, have been undertaken. Based on the three-dimensional structures the structure-property relationships between them were analysed.

10-*N*-Methylcarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine (MCDP) is one of the functional dyes which are applied clinically as diagnostics. MCDP (**I**) is used to measure the activity of monoamine oxidase in blood. In the presence of peroxidase and hydrogen peroxide **I** converts into methylene blue, and the blue color is developed. An effective conversion is essential for the sensitivity and accuracy of the diagnosis. In the aqueous solution, however, **I** also spontaneously decomposes to methylene blue in the absence of peroxidase. It increases the background value. A high background is undesirable for an accurate analysis. To suppress the spontaneous decomposition and maintain a low background various derivatives of **I** were synthesized and the stability measured. Understanding the relationships between the molecular structures and the properties is essential in the molecular design of better dyes. To disclose the relationships we have been undertaking an X-ray analysis of **I**[1] and its derivatives. In the present paper we report on X-ray analyses of two derivatives, 10-*N*-methylthiocarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine (**II**) and 10-*N*-benzylthiocarbamoyl-3,7-bis(dimethylamino)-10*H*-phenothiazine (**III**), as well as the structure-property relationships between them. The chemical structures of **I**, **II**, and **III** are shown in Chart 1.

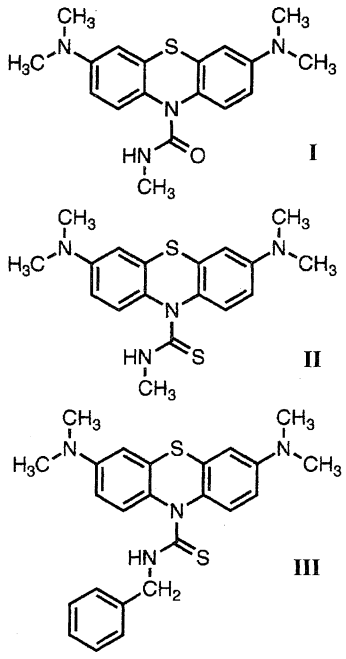

Chart 1.

Results and Discussion

ORTEP II[2] drawings of the molecular structures of **II** and **III** are shown in Figs. 1 and 2 with the atomic numbering.

The phenothiazine rings of both molecules take butterfly forms, as in **I**. The dihedral angles between the two phenyl rings are 49.9(1), 52.6(2), and 41.5(4)° in **II**, **III**, and **I**, respectively. The three compounds take a similar conformation as a whole under different crystalline fields, indicating that the conformation is relatively rigid. Therefore, the conformation of the three compounds in solution may be essentially identical with that in the crystal form. The parathiazine rings in **II** and **III** take a boat conformation, as in **I**. The torsion angles in the ring indicate that the boat form of **I** is more flattened than those of **II** and **III**. The C1a, C4a, C5a and C8a atoms constitute planes with mean deviations from the planes of 0.010(4), 0.002(3), and 0.003(3) Å in **I**, **II**, and **III**, respectively. The deviations of the S9 and N10 atoms from the least-squares planes are 0.582(4) and 0.460(4), 0.601(3) and 0.513(3), and 0.635(3) and 0.518(3) Å in **I**, **II**, and **III**, respectively. The order of flatness of the boat conformation is **I** > **II** > **III**. The C4a–S9–C8a angles in **I**, **II**, and **III** are 99.1(1), 98.5(1), and 97.6(2)°, respectively. Although the

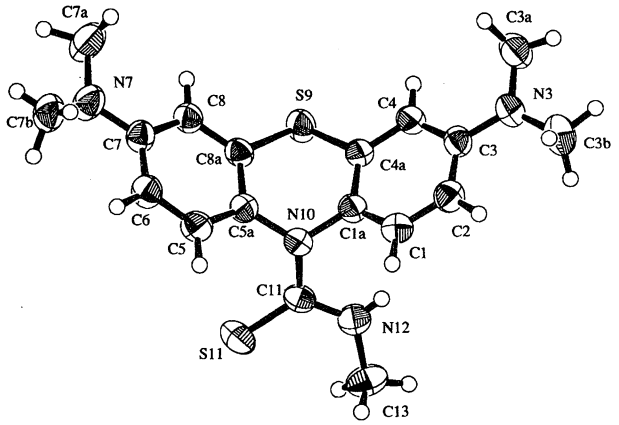

Fig. 1. ORTEP II drawing of **II** with atomic numbering, representing heavy atoms as 50% probability ellipsoids and H atoms as spheres of arbitrary radii.

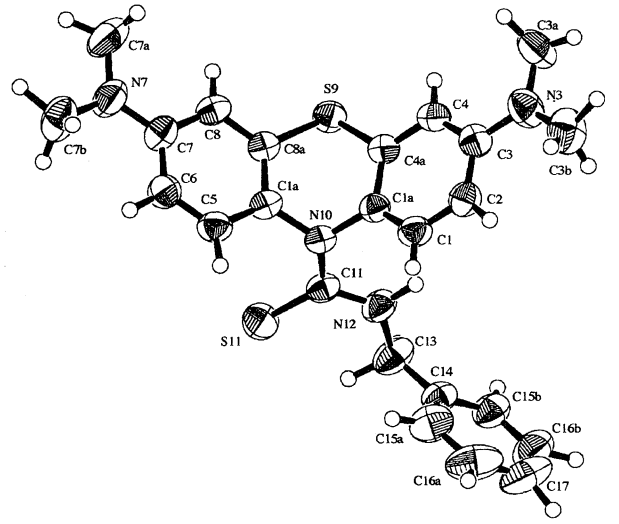

Fig. 2. ORTEP II drawing of **III** with atomic numbering, representing heavy atoms as 50% probability ellipsoids and H atoms as spheres of arbitrary radii.

C1a–N10–C5a angles in **II** and **III** are similar, they are significantly smaller than that of **I** (116.3(2)°). The endocyclic S–C bonds in the parathiazine ring are symmetric in **I** and **II**, but are highly asymmetric in **III**. Although in **I** and **III** the angles facing the carbonyl and thiocarbonyl groups are smaller, in **II** the angle facing the thiocarbonyl group is larger than the other. The N12–C11 bond is remarkably shorter than the N10–C11 bonds in the three compounds. The bond in **II** is especially short. The carbonyl and thiocarbony groups are twisted from the N10 plane with the torsion angles of C5a–N10–C11–O11 being 15.0(4) in **I**, C5a–N10–C11–S11 being 11.5(4) in **II**, and C5a–N10–C11–S11 being 18.4(5)° in **III**. The terminal N–C(ϕ) bonds are symmetrical in **I** and **II**, but are significantly asymmetric in **III**. The sums of the C–N–C bond angles around the N3 and N7 atoms indicate that the N3 atom in **I** (the sum is 356.1(2)°) and the N7 atom in **II** (the sum is 353.9(3)°) take non-planar configurations, and that other terminal nitrogen atoms take a planar configuration.

The conversion rates to methylene blue are different at different pH values and from compound to compound. The conversion involves a cleavage of the N10–C11 bond. The different substituents are attached at the C11 atom in **I**, **II**, and **III**; they should significantly influence the chemical characteristics of the C11 and N10 atoms. Therefore, the cleavage might ultimately be controlled by the geometrical and electronic characters of the atoms. The sp^3 character of the N10 atom was elucidated from the geometry around the atom. The sums of the C–N distances are 4.260(6), 4.251(6), and 4.271(7) Å in **I**, **II**, and **III**, respectively. The sums of the C–N–C angles are 357.4(3), 358.4(3), and 356.1(5)° in **I**, **II**, and **III**, respectively. The deviations of the N10 atom from the plane defined by the C1a, C5a, and C11 atoms are 0.125(4), 0.101(2), and 0.164(3) Å in **I**, **II**, and **III**, respectively. These data indicate that the order of the sp^3 character is **III** > **I** > **II**. Since at pH = 8.0 the order of the conversion rate for these three compounds is **III** > **I** > **II**, the breaking of the N11–C11 bond at pH = 8.0 can be considered to be governed by a nucleophilic attack at the carbonyl carbon atom by OH^- ions. The phenothiazine rings take non-planar structures, and the order of the magnitude of the dihedral angles between the phenyl rings is **III** > **II** > **I**. The distorsion of the central parathiazine ring makes the S···N transannular distance short. The S···N transannular distances are 2.946(2), 2.953(2), and 2.980(2) Å in **III**, **II**, and **I**, respectively. These non-bonded distances are significantly shorter than the sum of the van der Waals radii (3.4 Å) of the nitrogen and sulfur atoms. The sulfur and nitrogen atoms correspond to bow and stern positions in the boat conformation. Therefore, the lone pairs from these two atoms could cooperatively attract a proton. Alternatively, transannular interactions activate an electrophilic attack at the nitrogen atom by the proton. The order of the rate of conversion to methylene blue at pH = 6.0 is **III** > **II** > **I**, which is in good agreement with the order of the proximity of the sulfur and nitrogen atoms in the parathiazine ring and the flatness of the boat conformation of the ring.

The reactivities of the derivatives of the MCDP are different at different pH's. The present analysis showed that the molecular structures have a geometrical prerequisite for reactions under different pH conditions. Under alkaline conditions, the sp^3 character if responsible for a nuclephilic attack on the carbonyl carbon atoms by the OH^- ion. However, under acidic conditions the protrudent sulfur and nitrogen atoms possibly lead to an electrophilic attack of protons at the nitrogen atoms. The present results provide very useful information for the molecular design of new functional dyes which could be stable at a certain pH.

Experimental

X-Ray Analysis. Crystallographic data are summarized in Table 1. Single crystals of **II** and **III** were obtained from an ethanol solution. The intensity data were collected on an Enraf–Nonius CAD-4 Turbo diffractometer with graphite-monochromated Cu $K\alpha$ radiation using the ω–2θ scan technique. The structures were solved by direct methods (SAPI91[3] and DIRDIF[4]) and refined by a full-

Table 1. Crystallographic Data of **II** and **III**

Compound	**II**	**III**
Molecular formula	$C_{18}H_{22}N_4S_2$	$C_{24}H_{26}N_4S_2$
Formula weight	358.52	434.62
Crystal color	Ice blue	Ice blue
Crystal size/mm^3	0.3×0.2×01	0.5×0.4×0.4
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$	$P\bar{1}$
a/Å	7.3665(5)	10.1757(4)
b/Å	18.201(1)	10.4955(4)
c/Å	13.849(1)	11.6492(5)
α	—	98.499(3)
β	103.482(6)	99.140(3)
γ	—	108.089(3)
V/Å^3	1805.6(2)	1141.6(1)
Z	4	2
d_{calcd}/g cm^{-1}	1.319	1.264
λ/Å	1.54178	1.54178
Temperature/K	291	291
μ(Cu $K\alpha$)/cm^{-1}	27.16	22.43
$2\theta_{max}$/deg	139.8	139.8
Reflection measured	3671	4419
R_{int}	0.044	0.117
Reflection used ($I>3.0\sigma(I)$)	2234	3312
No.variables	306	376
R	0.045	0.062
R_w	0.054	0.064

Table 2. The final Atomic Coordinates (×10^5 for sulfur atoms and ×10^4 for other atoms) and Equivalent Isotropic Displacement Parameters (B_{eq}/Å^2) for **II**

Atom	x	y	z	B_{eq}
S(9)	123780(1)	31745(4)	86166(6)	4.22(2)
S(11)	99420(1)	35400(6)	50290(6)	4.99(2)
N(3)	12329(1)	5707(2)	10280(2)	5.03(7)
N(7)	17423(4)	1892(2)	7179(2)	5.04(7)
N(10)	11842(3)	4061(1)	6786(2)	3.58(6)
N(12)	8933(4)	4539(2)	6161(2)	4.62(7)
C(1)	12007(4)	5258(2)	7652(2)	4.01(7)
C(1a)	12015(4)	4500(2)	7667(2)	3.55(7)
C(2)	12094(4)	5659(2)	8505(2)	4.08(7)
C(3)	12266(4)	5307(2)	9427(2)	3.95(7)
C(3a)	12728(6)	5348(3)	11230(3)	5.5(1)
C(3b)	12046(8)	6482(3)	10235(4)	6.5(1)
C(4)	12406(4)	4540(2)	9447(2)	3.94(7)
C(4a)	12253(4)	4140(2)	8581(2)	3.45(6)
C(5)	14326(5)	3431(2)	6165(2)	4.08(7)
C(5a)	13236(4)	3504(2)	6852(2)	3.49(6)
C(6)	15719(5)	2919(2)	6280(2)	4.20(8)
C(7)	16076(4)	2431(2)	7092(2)	3.87(7)
C(7a)	17925(7)	1477(3)	8086(3)	6.7(1)
C(7b)	18868(6)	1960(3)	6638(3)	5.4(1)
C(8)	14998(4)	2516(2)	7791(2)	3.74(7)
C(8a)	13613(4)	3044(2)	7680(2)	3.48(7)
C(11)	10237(4)	4072(2)	6033(2)	3.49(7)
C(13)	7262(5)	4716(3)	5408(3)	5.4(1)

Table 3. The Final Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (B_{eq}/Å^2) for **III**

Atom	x	y	z	B_{eq}
S(9)	8609(1)	4396(1)	8293(1)	4.31(2)
S(11)	9199(1)	7515(1)	5650(1)	5.58(3)
N(3)	4200(4)	0093(4)	6439(3)	5.9(1)
N(7)	8879(4)	8852(4)	11023(3)	6.3(1)
N(10)	7369(3)	5550(3)	6419(2)	3.90(7)
N(12)	7919(4)	4875(4)	4644(3)	4.88(8)
C(1)	5280(4)	3457(4)	5591(4)	4.5(1)
C(1a)	6550(4)	4141(4)	6375(3)	3.66(8)
C(2)	4493(4)	2121(4)	5600(4)	4.5(1)
C(3)	4986(4)	1439(4)	6429(3)	4.3(1)
C(3a)	4749(7)	−599(6)	7272(6)	6.9(2)
C(3b)	2851(6)	−608(6)	5672(6)	6.9(2)
C(4)	6253(4)	2151(4)	7229(3)	4.2(1)
C(4a)	7040(4)	3489(4)	7224(3)	3.64(8)
C(5)	7505(4)	7659(4)	7790(4)	4.5(1)
C(5a)	7757(4)	6420(4)	7582(3)	3.81(8)
C(6)	7865(4)	8442(4)	8918(4)	4.7(1)
C(7)	8493(4)	8054(4)	9894(3)	4.6(1)
C(7a)	9479(9)	8410(7)	12025(5)	9.1(2)
C(7b)	8485(6)	10067(6)	11263(5)	6.9(1)
C(8)	8693(4)	6768(4)	9683(3)	4.3(1)
C(8a)	8349(3)	6004(4)	8540(3)	3.65(8)
C(11)	8134(4)	5911(4)	5558(3)	4.00(9)
C(13)	8606(5)	5000(6)	3624(4)	5.9(1)
C(14)	7549(4)	4342(4)	2480(3)	4.4(1)
C(15a)	6355(5)	4697(6)	2189(4)	6.2(1)
C(15b)	7761(5)	3365(5)	1668(4)	5.3(1)
C(16a)	5421(6)	4091(7)	1105(5)	8.1(2)
C(16b)	6840(6)	2778(6)	600(4)	7.3(2)
C(17)	5653(6)	3113(7)	311(5)	8.3(2)

matrix least-squares method. Anisotropic thermal parameters were used for all non-hydrogen atoms, whereas isotropic thermal parameters were used for all hydrogen atoms. The atomic scattering factors were taken from International Tables for Crystallography.[5] Corrections for secondary extinction were applied. The final coefficients were 7.251×10^{-6} and 4.374×10^{-5} for **II** and **III**, respectively. All of the calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.[6] The atomic parameters of **II** and **III** are summarized in Tables 2, 3, 4, and 5, respectively. #

Measurement of Rate of Conversion to Methylene Blue. Each 6 mg of **I**, **II**, and **III** was dissolved in 1 ml of *N*,*N*-dimethylformamide (DMF). Two different buffer solutions were prepared as follows: 0.1 M succinate buffer (1 M = 1 mol dm^{-3}) at pH = 6.0 and 0.1 M phosphate buffer at pH = 8.0. Each buffer contained 0.1% Triton X-100 and 10 U ml^{-1} of horse-raddish peroxidase. A DMF solution of the dyes was added to each buffer solution, and the optical density at λ_{max} (666 nm) of the methylene blue was monitored every 60 s up to 1800 s. Within these ranges the OD vales increased linearly and linear regression functions were obtained. The slopes (OD value/s) of the functions at pH = 6.0 were 7.5(5), 9.5(5), and 11.1(5) for **I**, **II**, and **III**, respectively. The values at pH = 8.0 were 16.4(5), 13.0(5), and 23.6(5) for **I**, **II**, and **III**, respectively. The estimated standard deviations are shown in parentheses and the OD

#The tables of the anisotropic temperature factor for non-hydrogen atoms and the $F_o - F_c$ are deposited as Document No. 69020 at the Office of the Editor of Bull. Chem. Soc. Jpn.

Table 4. Selected Bond Lengths (l/Å), Angles (ϕ/°) and Torsion angles (τ/°) of **II**

		Length/Å			Length/Å
S(9)	C(4a)	1.760(3)	S(9)	C(8a)	1.765(3)
S(11)	C(11)	1.666(3)	N(3)	C(3)	1.378(4)
N(3)	C(3a)	1.435(5)	N(3)	C(3b)	1.426(5)
N(7)	C(7b)	1.443(4)	N(7)	C(7a)	1.438(5)
N(7)	C(7)	1.380(4)	N(10)	C(1a)	1.439(3)
N(10)	C(5a)	1.430(4)	N(10)	C(11)	1.382(3)
N(12)	C(11)	1.325(4)	N(12)	C(13)	1.452(4)
C(1a)	C(4a)	1.399(4)	C(5a)	C(8a)	1.395(4)

			Angle/deg				Angle/deg
C(4a)	S(9)	C(8a)	98.5(1)	S(9)	C(4a)	C(4)	120.5(2)
C(3)	N(3)	C(3b)	120.6(3)	S(9)	C(4a)	C(1a)	119.1(2)
C(3)	N(3)	C(3a)	120.3(3)	N(10)	C(5a)	C(5)	123.1(3)
C(3a)	N(3)	C(3b)	119.1(3)	N(10)	C(5a)	C(8a)	118.7(2)
C(7b)	N(7)	C(7)	119.7(3)	N(7)	C(7)	C(6)	121.0(3)
C(7b)	N(7)	C(7a)	115.3(3)	N(7)	C(7)	C(8)	122.1(3)
C(7a)	N(7)	C(7)	118.9(3)	S(9)	C(8a)	C(5a)	119.0(2)
C(1a)	N(10)	C(5a)	114.7(2)	S(9)	C(8a)	C(8)	120.4(2)
C(1a)	N(10)	C(11)	121.5(2)	S(11)	C(11)	N(10)	122.9(2)
C(5a)	N(10)	C(11)	122.2(2)	S(11)	C(11)	N(12)	121.8(2)
C(11)	N(12)	C(13)	124.4(3)	N(10)	C(11)	N(12)	115.3(3)
N(10)	C(1a)	C(4a)	118.4(3)	N(3)	C(3)	C(2)	121.1(3)
N(3)	C(3)	C(4)	121.5(3)				

				Angle/deg					Angle/deg
C(1a)	C(4a)	S(9)	C(8a)	36.4(3)	C(4a)	S(9)	C(8a)	C(5a)	−36.7(3)
S(9)	C(8a)	C(5a)	N(10)	−0.8(4)	C(1a)	N(10)	C(5a)	C(8a)	48.6(4)
C(4a)	C(1a)	N(10)	C(5a)	−48.9(3)	S(9)	C(4a)	C(1a)	N(10)	1.3(4)

Table 5. Selected Bond Lengths (l/Å), Angles (ϕ/°) and Torsion Angles (τ/°) of **III**

		Length/Å			Length/Å
S(9)	C(8a)	1.778(4)	S(9)	C(4a)	1.754(3)
S(11)	C(11)	1.673(4)	N(7)	C(7)	1.375(4)
N(7)	C(7b)	1.453(6)	N(7)	C(7a)	1.435(7)
N(3)	C(3b)	1.419(6)	N(3)	C(3)	1.393(5)
N(3)	C(3a)	1.439(6)	N(10)	C(5a)	1.437(4)
N(10)	C(1a)	1.443(4)	N(10)	C(11)	1.391(4)
N(12)	C(11)	1.341(4)	N(12)	C(13)	1.473(5)
C(5a)	C(8a)	1.375(5)	C(1a)	C(4a)	1.397(5)

			Angle/deg				Angle/deg
C(8a)	S(9)	C(4a)	97.6(2)	S(9)	C(8a)	C(8)	119.3(3)
C(7)	N(7)	C(7b)	120.4(4)	S(9)	C(8a)	C(5a)	118.5(3)
C(7)	N(7)	C(7a)	121.4(4)	N(10)	C(1a)	C(1)	122.8(3)
C(7b)	N(7)	C(7a)	117.5(4)	N(10)	C(1a)	C(4a)	118.0(3)
C(3b)	N(3)	C(3)	122.0(4)	N(3)	C(3)	C(2)	120.4(4)
C(3b)	N(3)	C(3a)	118.7(4)	N(3)	C(3)	C(4)	121.9(4)
C(3)	N(3)	C(3a)	119.3(4)	S(9)	C(4a)	C(1a)	119.4(3)
C(5a)	N(10)	C(1a)	114.1(3)	S(9)	C(4a)	C(4)	121.3(3)
C(5a)	N(10)	C(11)	120.6(3)	S(11)	C(11)	N(10)	122.3(3)
C(1a)	N(10)	C(11)	121.4(3)	S(11)	C(11)	N(12)	123.3(3)
C(11)	N(12)	C(13)	124.5(4)	N(10)	C(11)	N(12)	114.4(3)
N(10)	C(5a)	C(4a)	119.5(3)	N(12)	C(13)	C(14)	111.0(4)
N(7)	C(7)	C(6)	122.4(4)	C(13)	C(14)	C(15b)	119.9(4)
N(7)	C(7)	C(8)	120.7(4)				

				Angle/deg					Angle/deg
C(5a)	C(8a)	S(9)	C(8a)	−38.4(3)	C(8a)	S(9)	C(4a)	C(1a)	38.4(3)
S(9)	C(8a)	C(1a)	N(10)	−0.1(4)	C(4a)	C(1a)	N(10)	C(5a)	−48.5(4)
C(1a)	N(10)	C(5a)	C(8a)	48.7(4)	N(10)	C(5a)	C(8a)	S(9)	0.0(5)

values are in an arbitrary scale.

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