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CRYSTAL STRUCTURES OF TWO TRIMERS OF 1,4-BENZOTHIAZINE

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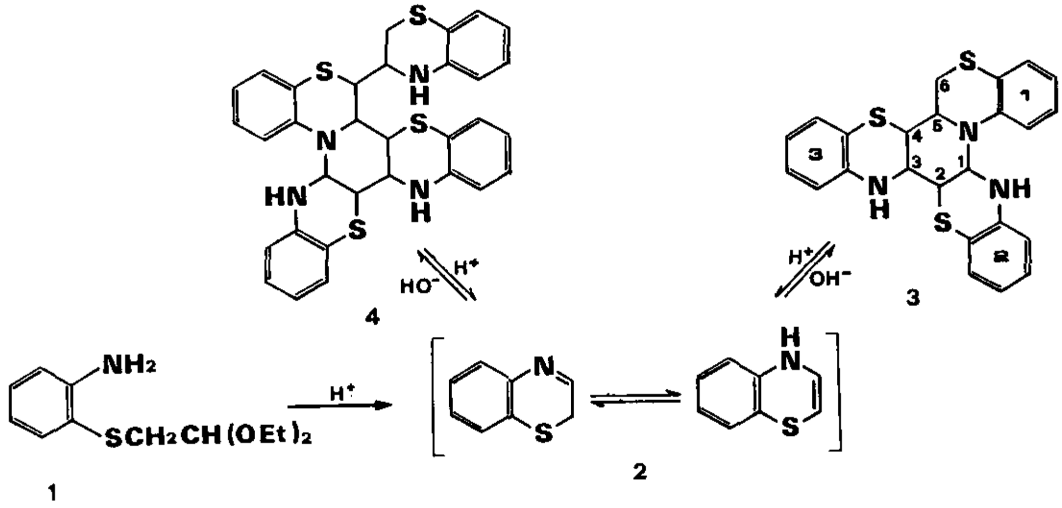
Abstract– Crystal and molecular structures of two diastereoisomeric trimers of 1,4-benzothiazine, the parent system of the key intermediate in biosynthesis of phaeomelanins, were unambiguously determined by nOe's experiments and X-ray analysis. The relative configuration of the asymmetric centers has been determined. The two compounds show inverted configurations at the chiral atom C(4) leading to different overall shapes of the molecules.

We have previously reported[1] that 2H-1,4-benzothiazine **2**, generated in situ, under nitrogen, by mild hydrolysis of aminoacetal **1**, undergoes aldol type polymerization to give mainly a mixture of two pairs of diastereoisomeric trimers and tetramers, having the gross structures **3** and **4** respectively.

These results both account for the failure of previous attempts[2] to obtain unsubstituted 1,4-benzothiazine and are remarkable in relation to phaeomelanins biosynthesis.

Phaeomelanins, the pigments responsible for the reddish colour of hairs in mammals, including man, are believed to be formed by polymerization of 1,4-benzothiazine intermediates[3].



On the grounds of our results, the further stages of biosynthesis, which probably does not require enzymic assistence, could be hypothesized to involve aldol condensation of 1,4-benzothiazine units more than oxidative coupling at positions 2 and 8, as suggested[4].

In this contest, we attempt to elucidate completely the stereochemistry of the two trimers obtained by us, by nOe's experiments and X-ray crystallography. It is noteworthy that trimers **3** were both obtained in well formed crystals by slow evaporation of the solvent ($CHCl_3$) while the two tetramers failed to crystallize.

In order to gain informations on the stereochemistry of the compound **3a**, a heteronuclear ^{13}C-^{1}H shift correlated 2D-nmr spectrum via ^{2}J and ^{3}J (COLOC) was performed[5]. This experiment, which was run in pyridine-d_5 (compound **3a** showed the maximum solubility in this solvent), displayed in the contour plot a series of correlation peaks which fully agree with structure **3a**.

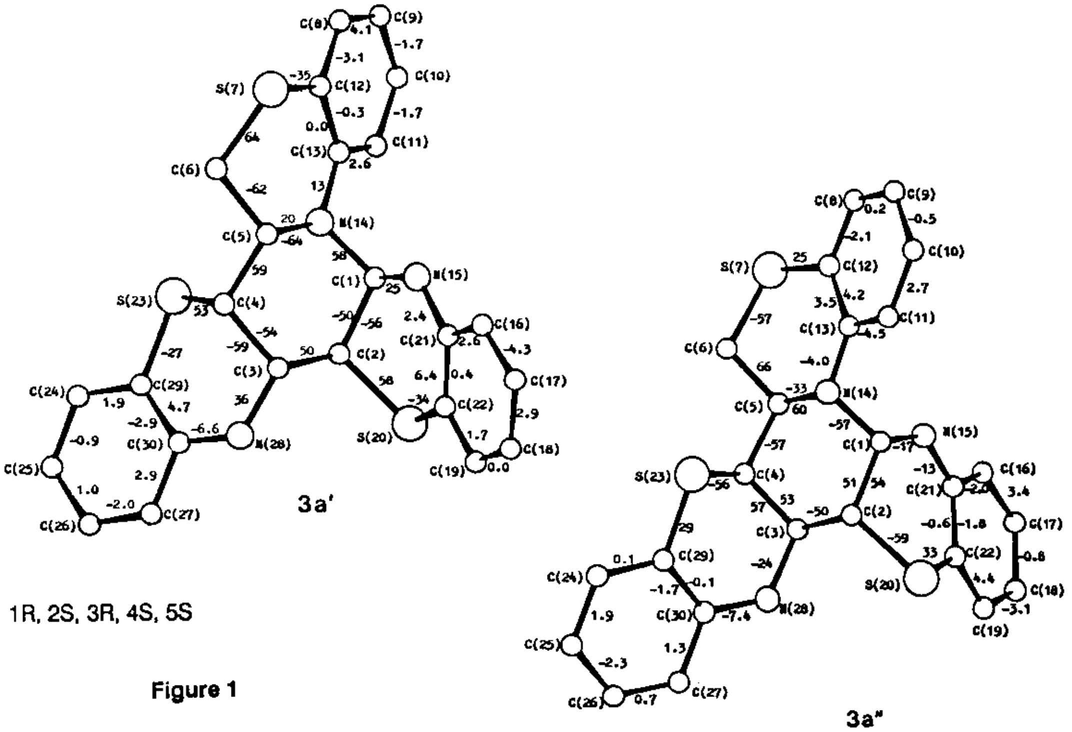
Unfortunately, a bad proton dispersion in the region of the aromatic resonances prevented a complete assignment of each signal to the pertinent carbon atom in the ^{13}C-nmr spectrum.

The overall relative stereochemistry of the compound **3a** could be deduced from nOe's experiments. Particularly significance was the positive nuclear Overhauser effects observed among H-5, H-4 and H-3 which indicated that these protons are on the same face of the molecule. Analogously the presence of nOe effect between H-1 and H-2 pointed to the *cis*-relationship between this couple of the protons, while the absence of any significative enhancement of H-2 by irradiation at the frequence of H-3 and *vice-versa* allowed us to assign the relative stereochemistry of the chiral centres C-2 and C-3.

Definitive proof for the correctness of the stereostructures **3** was gained from X-ray analysis. Crystal structures of the two diastereoisomeric compounds **3a** and **3b** were solved with the aim of achieving a detailed stereochemistry of these molecules. Compound **3a** crystallizes in the monoclinic space group $P2_1$, with two crystallographic independent molecules (**a'** and **a"**) which are enantiomerics. The two independent molecules show slightly different conformations (Fig. 1). The relative configuration of chiral atoms C(1), C(2), C(3), C(4), C(5) of molecule **3a'** is R, S, R, S, S, (or S, R, S, R, R), while molecule **3a"** shows an opposite configuration: S, R, S, R, R (or R, S, R, S, S).

The two molecules show slightly different conformations: while the central ring is in the chair conformation for both molecules with differences in the values of corresponding torsion angles less than 4 degrees, the three peripheral rings, *i.e.* those with two heteroatoms, are more flexible, and show distorted half chair conformations.

The difference in the conformations of corresponding rings in the molecules is probably due to the flexibility of the system of the six membered rings with two heteroatoms and a double bond, and probably led to more favourable packing interactions.



3a'

1R, 2S, 3R, 4S, 5S

3a"

1S, 2R, 3S, 4R, 5R

Figure 1

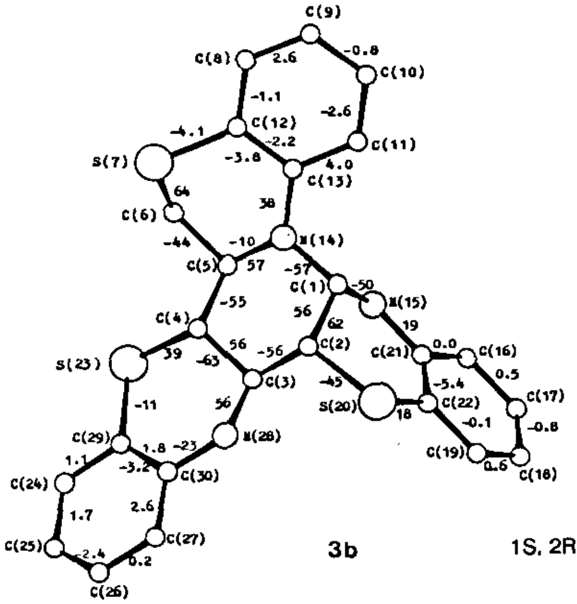
Table 1 – Crystallographic Data

parameter	compound **3a**	compound **3b**
mol. formula	$C_{24}H_{21}N_3S_3$	$C_{24}H_{21}N_3S_3$
mol.weight (amu)	447.63	447.63
crystal system	monoclinic	monoclinic
space group	$P2_1$	$P2_1/c$
$\underline{Z}$, molecule/unit cell	4	4
$\bar{a}$, Å	13.107(3)	9.515(1)
$\bar{b}$, Å	10.542(4)	18.401(4)
$\bar{c}$, Å	15.127(1)	12.496(5)
β, deg	99.57(1)	108.62(5)
V, Å^3	2061(1)	2073(1)
d (calcd.), g cm^{-3}	1.442	1.434
d (exptl.), g cm^{-3}	1.44	1.42
radiation, Å	CuKα,1.5418	MoKα, 0.71069
no. of indep. refls.	4134	3652
reflections with $I \geq 3\sigma(I)$	3775	1945
final R value	0.054	0.055
final weighted R value	0.060	0.058
temp., °C	ambient	ambient
crystallization solvent	$CHCl_3$	$CHCl_3$

Compound **3b** crystallizes in the centric space group $P2_1/c$; therefore the enantiomeric molecules are related trough the crystallographic centre of symmetry. The relative configuration of the chiral atoms C(1), C(2), C(3), C(4), C(5), is S, R, S, S, R(or R, S, R, R, S). There are one inversion of configuration on chiral atoms C(4) with respect to compound **3a**, previously described. This led to a different overall shape of the molecule (Fig. 2), in comparison with the molecule **3a**. In fact if we consider the orientation of the benzene rings with respect to each other, we find that in compound **3a** the dihedral angles between least squares planes defined by the benzene rings 1[C(8) ~ C(13)], 2[C(16) ~ C(21)] and 3[C(24) ~ C(29)], are the following: 61° mol **3a'**, (66° mol **3a"**) dihedral angle between planes 1 and 2, 86° mol **3a'** (99° mol **3a"**) angles between planes 1 and 3, 29° mol **3a'**(41° mol **3a"**), angles between planes 2 and 3. While the corresponding dihedral angles in the structure of **3b** are 110°, 53°, 104°, respectively. In compound **3b** the six membered central ring adopts a chair conformation, while the peripheral rings show a distorted half-chair conformation.

For both compounds, bond lenghts and angles are within the normal values previously reported, and the packing is achieved through Van der Waals interactions.

Figure 2



3b 1S, 2R, 3S, 4S, 5R

EXPERIMENTAL

The sample of compound **3a** utilized for COLOC experiments was prepared by dissolving 0.1 mmole of pure material in 0.5 ml of pyridine-d_5.

Spectra were recorded on a WM-250 Bruker spectrometer operating at 62.9 (^{13}C) and 250.13 (^{1}H) MHz, respectively .

The long-range heteronuclear correlation with polarization transfer was performed adjusting the fixed delays to give maximum polarization for J_{CH} = 6.25 Hz, leading to ^2J and ^3J spots in the same spectrum[4]. Determination of nOe experiments was performed on a Bruker WM-250 spectrometer in C_5D_5N solution with the aid of Aspect 2000 microprograms. The sample used for nOe measurements was previously degassed by bubbling Ar through the solutions for 40 min.

Crystals of the substances **3a** and **3b** were obtained by slow evaporation from $CHCl_3$ solution, *crystallographic data are reported in Table* 1.

Preliminary oscillation and Weissenberg photographs were taken to establish the crystal symmetry and space group. Determination of the cell constants and collection of the X-ray intensity data were performed on CAD4 Enraf-Nonius diffractometer of the Centro Interdipartimentale di Metodologie Chimico-Fisiche at the University of Naples, equipped with PDP8/E and PDP11/34 Digital computers. For the structure determination and refinement, the GX package[6] was used. Unit cell parameters were obtained by a least square procedure on the angular parameters of 25 high-angle reflections. The analysis of the peak profiles suggested an $\omega-2\theta$ scan. The total number of independent reflections and those used in the refinement [$I \geq 3\sigma(I)$] are given in Table 1.

All reflection were corrected for Lorentz and polarization effects.

The structures were solved using MULTAN[7]. The analysis of the E-map of the set of phases with the best combined figure of merit revealed the position of most of the non hydrogenoid atoms. The position of the remaining atoms was derived from subsequent difference Fourier maps. The refinement used a full matrix least-squares procedure, minimizing the quantity $\Sigma w(F_o^2-F_c^2)^2$ with weights w equal to $1/\sigma(F_o^2)$.

All heavy atoms were refined with anisotropic temperature factors. Hydrogen atoms were introduced in their stereochemically expected positions with isotropic temperature factors equal to the equivalent B factor of the atom to which each of them was linked.

Refinements were ended when the shifts in the atomic coordinates and anisotropic temperature factors for the heavy atoms were less than 1/5 and 1/3 of the corresponding standard deviations, respectively.

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