Reversible Formation of Cyclic 1,4-Benzothiazine Oligomers

Francesco Chioccara

Dipartimento di Chimica Organica e Biologica, Università di Napoli,
Via Mezzocannone 16, I-80138 Napoli, Italy

Ettore Novellino*

Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli,
Via L. Rodinò 22, I-80138 Napoli, Italy

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1,4-Benzothiazine **2**, generated *in situ* by mild hydrolysis of aminoacetal **3**, readily undergoes aldolization to give mainly two pairs of diastereoisomeric trimers and tetramers, having the gross structure **5** and **6** respectively. In strongly acidic media the oligomers are depolymerized to give monomeric 2*H*-1,4-benzothiazine (^{1}H nmr evidence) which, at slightly acidic or neutral *p*H, is converted to a mixture of the same trimers and tetramers. These results provide an improved background to look into the biosynthesis of phaeomelanins which are known to originate by polymerization of 1,4-benzothiazine intermediates.

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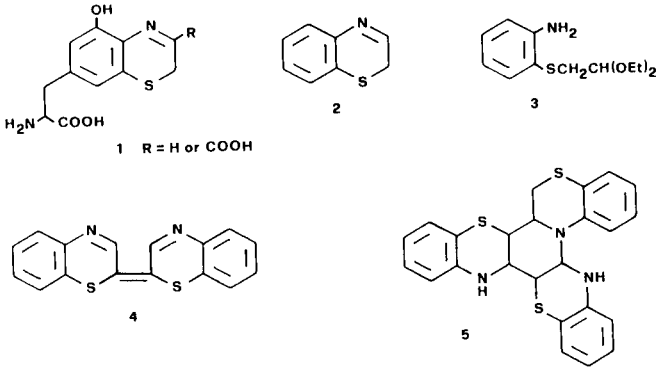
A number of earlier articles [1] have shown that phaeomelanins, the pigments responsible for the reddish colour of hair in various mammals, including man, are formed by oxidative polymerization of 1,4-benzothiazine intermediates such as **1**. In order to gain information about the further stages of the biosynthesis, which presumably requires no enzymatic pathway, we undertook an examination of the chemical behaviour of the parent heterocyclic system. In fact 1,4-benzothiazine **2** is an elusive molecule and a previous attempt [2] to obtain it by treatment of the aminoacetal **3** with hydrochloric methanol, resulted in the formation of the conjugated dimer **4**, arising from a facile, acid catalyzed coupling of **2** by the action of atmospheric oxygen.



On the other hand, in a short note [3], we reported that mild hydrolysis of the same acetal **3** by aqueous hydrochloric acid, in the absence of air, affords a mixture of polymeric products, the major one being formulated as the cyclic trimer **5**.

A careful re-examination of the reaction evidenced the formation of four main products having R_f 0.63, 0.51, 0.45 and 0.37 on silica gel (eluent benzene). These were identified as two pairs of diastereoisomeric trimers and tetramers of the 1,4-benzothiazine, generated *in situ* by hydrolysis of **3**. The most abundant product (R_f 0.63, 32%), corresponding to the previously described ones, was formulated as the trimer **5a** on the basis of the following evidence.

The mass spectrum showed M^+ at m/e 447 ($C_{24}H_{21}N_3S_3^+$) and significant fragments ions at m/e 298 ($C_{16}H_{14}N_2S_2^+$) and 149 ($C_8H_7NS^+$, base peak) formed by loss of one and two benzothiazine units, respectively. A NH absorption was present in the ir spectrum at 3360 cm^{-1} (broad) and the UV spectrum exhibited maxima at 234, 266 and 311 nm. The ^{1}H nmr spectrum (deuteriochloroform) showed the presence of an ABX system (partially overlapped with other signals) attributable to the cyclic -CH_2CH- grouping and a typical low field doublet due to the methine linked to two nitrogen atoms. The two dimensional ^{13}C-^{1}H shift correlated spectrum (HETCOR, Figure 1) enabled us to assign all the signals due to protonated carbons in the ^{13}C nmr spectrum showing, besides aromatic carbons, six sp^3-carbons assigned as in the Table. Carbons 1 and 3 were placed adjacent to the NH groups on the ground of deuteration effect [4] (*ca.* 0.08 ppm). Such a small variation in chemical shift could be clearly observed by partial D-exchange, resulting in the splitting of the original singlets.

In agreement with the proposed structure **5**, when the trimer was dissolved in acidic media (*e.g.* hydrochloric acid/methanol) in the presence of air, the solution rapidly turned violet and work-up of the reaction mixture gave the conjugated dimer **4** in 35% yield. This latter could derive by oxidative coupling at C-2 of 1,4-benzothiazine, initially formed by depolymerization of the trimer **5** in acidic environment. Supporting evidence for this view derived by monitoring the ^{1}H nmr spectrum of a solution of the

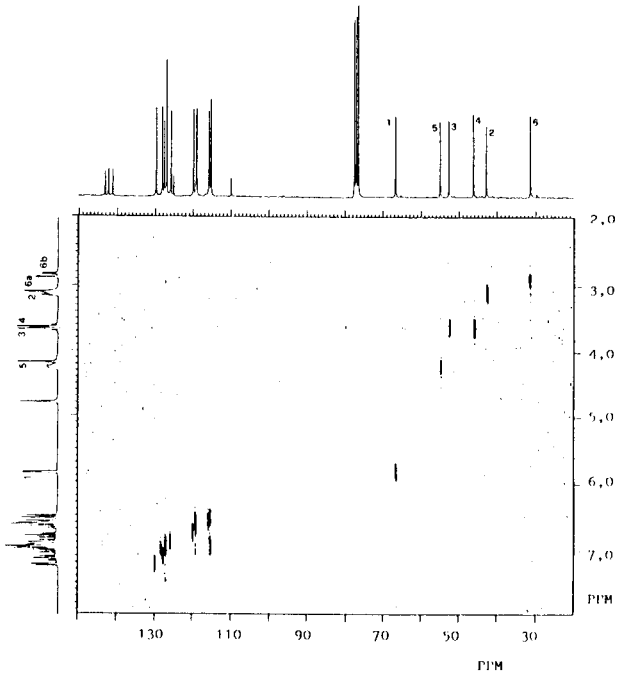


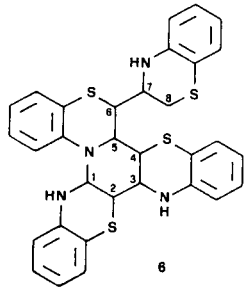
Figure 1. Contour plot of the ^{13}C-^{1}H heterocorrelated 2D-nmr (Bruker WM-250) spectrum of **5a** in deuteriochloroform which showed the interrelation of protonated carbons with the pertinent proton(s).

trimer in trifluoroacetic acid, resulting after a few minutes in a triplet at δ 8.64 (1H) and a doublet at δ 4.04 (2H), attributable to H-3 and H-2 protons respectively of 1,4-benzothiazine in the protonated imine form.

As expected, rapid neutralization of the solution led to the formation of a mixture of polymeric products analogous to the one obtained by hydrolysis of the acetal **3**, thus evidencing an equilibrium among benzothiazine monomeric and oligomeric forms.

The structural elucidation of the trimer **5a** facilitated the analysis of the other products formed in the reaction and particularly of compound **5b** having R_f 0.51. Examination of the chemical and spectral properties of this latter, strictly resembling those of **5a**, led to the conclusion that the two compounds are diastereoisomers, both corresponding to the gross structure **5**.

NOe difference experiments failed to give conclusive information on the relative stereochemistry, so we are unable to specify the relative arrangement of the protons in the molecule containing five chiral carbons.



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More interestingly, from the analysis of the remaining two major products (R_f 0.45 and 0.37, **6a** and **6b** respectively), it was concluded that they are diastereoisomeric tetramers of 1,4-benzothiazine, formulated as **6**.

The two compounds gave almost identical mass spectra with very weak molecular ions at m/e 596 and diagnostic fragments at m/e 447, 298 and 149 formed by successive loss of benzothiazine units. The ^{13}C nmr spectra showed, in addition to aromatic carbons, eight sp^3 hybridized carbons (seven methines and one methylene) assigned as in the Table.

Table

^{13}C Chemical Shifts (δ in ppm, Deuteriochloroform) of sp^3 Carbons in Benzothiazine Trimers **5a** and **5b** Tetramers **6a** and **6b** [a]

Carbon	1	2	3	4	5	6	7	8
5a	66.8	42.9	52.8	46.3	55.0	31.4	– –	– –
5b	66.6	41.3	51.0	47.1	55.5	32.8	– –	– –
6a	66.8	42.5	52.8	46.6	53.2	46.2	50.0	28.7
6b	67.0	42.3	52.8	44.6	53.2	46.6	52.7	28.4

[a] The order of all atoms, determined by DEPT experiments, were consistent with assignments.

A distinguishing feature of the ^{1}H nmr spectra, recalling the trimer ones, was the low field 1H doublets at δ 5.96 and 5.98 (for **6a** and **6b** respectively, J = 4 Hz) attributable to the methine groups linked to two nitrogen atoms. The uv spectra strictly resembled that of the trimers **5** and the ir spectra showed a broad absorption at 3360 cm^{-1} due to NH protons. Likewise the trimers **5**, the tetramers of 1,4-benzothiazine in strong acidic media undergo depolymerization which was evidenced both from the formation of the conjugated dimer **4** and, directly, from the ^{1}H nmr spectra of the trifluoroacetic acid solutions of tetramers.

This study provides evidence that 1,4-benzothiazine behaves somewhat similarly to other heterocyclic enamines [5] in giving reversible aldolization products. Such a behaviour is remarkable in relation to phaeomelanins biosynthesis which could proceed *via* aldol condensation of postulated benzothiazine intermediates **1** more than by suggested [6] oxidative coupling involving position 2 and 8.

EXPERIMENTAL

The sample of compound **5a** utilized for HECTOR experiments was prepared by dissolving 0.1 mmole of pure material in 0.5 ml of deuteriochloroform. Spectra were recorded on a WM-250 Bruker spectrometer operating at 62.9 (^{13}C) and 250.13 (^{1}H) MHz, respectively. The HECTOR experiments were performed using a Bruker microprogram according to

Bax and Morris [7]. The acquisition parameters were as follow: 90° pulse width 9.3 μs, duration of the 90° pulse from the decoupler coils 15.6 μs, spectral window in F_1 dimension 750.1 Hz, spectral window in F_2 dimension 8196.7 Hz, acquisition time 0.062 s, number of points 1024, number of transients 256. Delays were optimized for J_{C-H} values of 135 Hz for an average one bond coupling and 6 Hz for long range coupling ($\triangle_2 = 0.5 \cdot \triangle_1 = 0.25/J_{C-H}$).

The nOe spectra were recorded by means of nOe difference techniques.

1-(*o*-Aminophenylthio)-2,2-diethoxyethane) **3**.

The acetal **3** was prepared with a procedure analogous to the method previously described [8]. To a solution of *o*-aminothiophenol (12.5 g) in anhydrous dimethyl sulfoxide (30 ml), sodium (2.3 g) was added and was allowed to dissolve. Then bromoacetaldehyde diethyl acetal (19.7 g) was added and the solution was kept under stirring at room temperature for about 30 minutes until a copious, white precipitate was formed. The reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed several times with water, dried over anhydrous sodium sulphate and evaporated. Purification of the residue on a column of silica (2.6 x 45 cm) in benzene gave **3** (19.5 g, 81% yield) as pale yellow oil, homogenous on tlc.

1,4-Benzothiazine Trimers **5** and Tetramers **6**.

In a typical experiment aqueous 0.5 *M* hydrochloric acid (20 ml) was added to the aminoacetal **3** (140 mg), dissolved in ethanol (2 ml) and the solution was heated under a nitrogen atmosphere at 60° for 10 minutes. After cooling, the reaction mixture was extracted twice with chloroform and the combined extracts were washed with water and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure and fractionation of the brown oily residue by plc on silica in benzene afforded two pairs of benzothiazine trimers and tetramers having R_f 0.63, 0.51, 0.47 and 0.37. The less polar trimer (R_f 0.63, **5a**) formed prisms (30 mg, 32% yield), mp 222-224° dec from benzene/petroleum ether; ms: m/e 447 (M^+, 40), 323 (42), 298 (14) and 149 (100%); uv (methanol): λ max 234, 266 and 311 nm (log ε 4.64, 4.08 and 3.87); ir (chloroform): ν max 3360 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.4-6.5 (12H, cm, ArH), 5.88 (1H, d, J = 4 Hz, H-1), 4.80 (1H, s, removed by D-exchange, NH), 4.20 (1H, m, H-5), 3.66 (2H, m, H-3 and H-4), 3.14 (2H, m, H-2 and H-6a) and 2.85 (1H, q, J = 13 and 5 Hz, H-6b).

Anal. Calcd. for $C_{24}H_{21}N_3S_3$ (447.64): C, 64.40; H, 4.73; N, 9.39; S, 21.49. Found: C, 64.53; H, 4.74; N, 9.37; S, 21.51.

The other trimer (R_f 0.51, **5b**) formed a white powder (13 mg, 14% yield), from benzene/petroleum ether, mp 225-227° dec; ms: m/e 447 (M^+, 48), 323 (22), 298 (15) and 149 (100%); ^1H nmr (deuteriochloroform): δ 7.4-6.5 (13H, cm, ArH and 1 NH), 5.90 (1H, d, J = 3 Hz, H-1), 4.78 (1H, m, H-5), 4.1 (1H, bs, removed by D-exchange, NH), 3.92 (2H, m, H-2 and H-3), 3.80 (1H, m, H-4), 3.14 (1H, dd, J = 6 and 13 Hz, H-6a) and 2.98 (1H, dd, J = 6 and 13 Hz, H-6b); ir and uv spectra were identical with those of **5a**.

Anal. Calcd. for $C_{24}H_{21}N_3S_3$ (447.64): C, 64.40; H, 4.73; N, 9..39; S, 21.49. Found: C, 64.20; H, 4.71; N, 9.40; S, 21.46.

The more fast moving tetramer **6a** (R_f 0.45) crystallized from benzene in prisms (6 mg, 6% yield), mp 238-240° dec; ms: m/e 596 (M^+, 6), 447 (2), 298 (4) and 149 (100%); uv (methanol): λ max 242, 265 and 313 (log ε 4.61, 4.20 and 4.03); ir (chloroform): ν max 3360 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.3-6.5 (16H, cm, ArH), 5.96 (1H, d, J = 4 Hz, H-1), 4.8 (2H, bs, removed by D-exchange, 2 NH), 4.36 (1H, m, H-5), 4.20 (1H, bs, removed by D-exchange, NH), 3.88 (1H, t, J = 10 Hz, H-4), 3.74 (1H, t, J = 10 Hz, H-3), 3.48 (1H, m, H-7), 3.34 (1H, m, H-6), 3.15 (2H, m, H-2 and H-8a), 2.95 (1H, dd, J = 4 and 13 Hz, H-8b).

Anal. Calcd. for $C_{32}H_{28}N_4S_4$ (596.86): C, 64.40; H, 4.73; N, 9.39; S, 21.49. Found: C, 64.59; H, 4.74; N, 9.38; S, 21.44.

The other tetramer **6b** (R_f 0.37) was obtained from benzene/petroleum ether as a white powder (10 mg, 11% yield), mp 241-243° dec; uv, ir and mass spectra were virtually identical with those of **6a**; ^1H nmr (deuteriochloroform): δ 7.4-6.4 (16H, cm, ArH) 5.98 (1H, d, J = 4 Hz, H-1), 4.72 (1H, bs, removed by D-exchange, NH), 4.50 (1H, dd, J = 10 and 1 Hz, H-5), 4.1 (2H, bs, removed by D-exchange, 2 NH), 3.82 (1H, t, J = 10 Hz, H-4), 3.68 (1H, t, partially overlapped with adjacent signals, J = 10 Hz, H-3), 3.6 (2H, m, H-6 and H-7), 3.28 (1H, ABq, J = 13 and 3 Hz, H-8a), 3.10 (1H, dd, J = 10 and 4 Hz, H-2), 2.92 (1H, dd, J = 13 and 3 Hz, H-8b).

Anal. Calcd. for $C_{32}H_{28}N_4S_4$ (596.86): C, 64.40; H, 4.73; N, 9.39; S, 21.49. Found: C, 64.62; H, 4.71; N, 9.41; S, 21.43.

The ^1H nmr assignments for compounds **5b**, **6a** and **6b** derived from double resonance irradiation experiments. The ^{13}C nmr data are reported in the Table.

Depolymerization of **5** and **6** in Hydrochloric Acid-Methanol. Formation of **4**.

To the trimer **5a** (100 mg), dissolved in chloroform (4 ml), methanol (16 ml) and concentrated aqueous hydrochloric acid (4 ml) was added and the mixture was left at room temperature for 48 hours under a stream of air. The resulting, deep violet solution, was concentrated to a small volume under reduced pressure, diluted with water and extracted twice with chloroform. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous sodium sulphate and evaporated. Crystallization of the residue from toluene afforded *trans*-$\triangle^{2,2'}$-bi-(2*H*-1,4-benzothiazine) **4**, orange-red prisms (20 mg, 20% yield) mp 298-300°, identical in all respect with an authentical sample [2].

With a similar procedure by treatment of **5b**, **6a** and **6b** with hydrochloric methanol was obtained the dimer **4** in about 25% yield determined spectrophotometrically.

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