

2*H*-1,4-Benzothiazines from the Reaction of 2-Aminoethanethiol  
with *Nascent* 4-Methyl-*o*-benzoquinone (I).

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4-Methyl-*o*-benzoquinone, generated oxidatively, reacts readily with 2-aminoethanethiol giving 5-hydroxy-7-methyl-3,4-dihydro-2*H*-1,4-benzothiazine (I) or 5-oxo-7-methyl-8-(2-hydroxy-4-methylphenoxy)-3,5-dihydro-2*H*-1,4-benzothiazine (VIII), depending on the conditions under which the reaction is performed. Chemical and spectral (uv, ir, nmr and ms) evidence for the assigned structures, as well as the course of the above mentioned reaction is described.

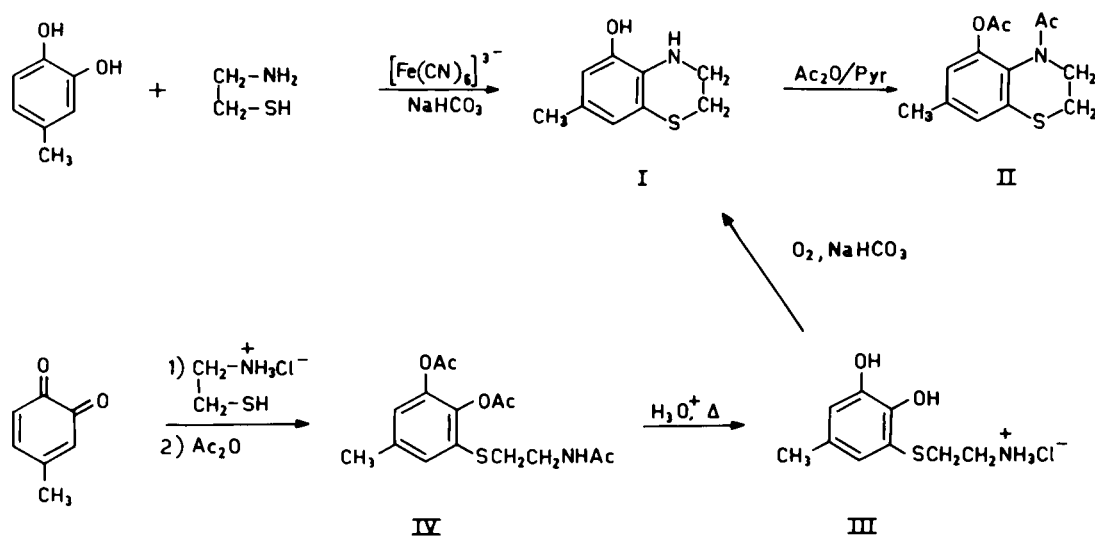
A study of the behaviour of compound VIII in the presence of acids and alkalis is also reported.

Reactions between thiols and *o*-quinones are of considerable significance in many biological processes which include the mode of attachment of melanin pigments to proteins (2,3) and the formation of phaeomelanins (4). We now report the results of an extensive investigation of the products obtained by oxidation of 4-methylcatechol in the presence of 2-aminoethanethiol. This study closely parallels that of the reaction between *o*-quinones and cysteine (5) the relevance of which to the chemistry and biogenesis of phaeomelanins has already been discussed (6,7).

The oxidation was carried out preferably by dropwise addition of a potassium ferricyanide solution to a stirred

mixture of 4-methylcatechol and an excess of 2-aminoethanethiol (8) in aqueous sodium bicarbonate. Under controlled conditions, oxidation of the mixture gave an almost colourless solution which showed an absorption maximum in the ultraviolet region at 307  $\mu$ . Micro-analytical data and mass spectrometry of the colourless product which was obtained as needles (I), m.p. 160-162° (50% yield) indicated an empirical formula of  $C_9H_{11}NOS$ . When treated with acetic anhydride in the presence of pyridine, I yielded a diacetyl derivative (II), the ir spectrum (carbon tetrachloride) of which showed no absorption in the NH/OH stretching region and two carbonyl bands at 1770 ( $-OCOCH_3$ ) and 1681 ( $=NCOCH_3$ )  $cm^{-1}$ .

Scheme I



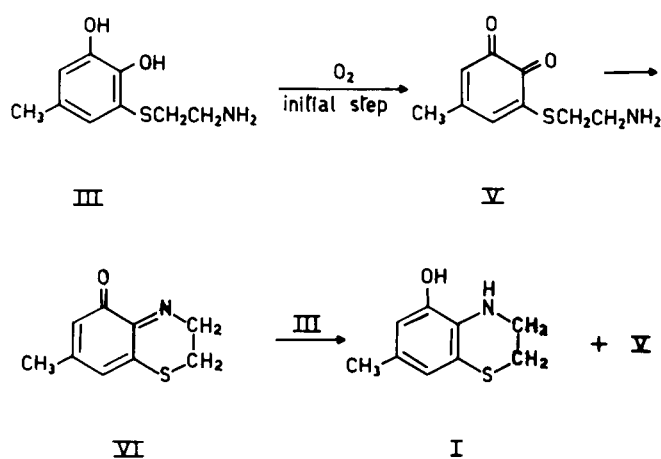
These data, combined with nmr spectra (see experimental section) indicated 5-hydroxy-7-methyl-3,4-dihydro-2*H*-1,4-benzothiazine as the structure for compound I.

Previous work on the reaction between *o*-quinones and cysteine (5) suggested that the formation of dihydrobenzothiazine I could be explained by the intermediacy of 3-(2-aminoethylthio)-5-methylcatechol (III), which, under reaction conditions (alkaline oxidizing medium), underwent ring closure. In order to get evidence for the intermediacy of III, 4-methyl-*o*-benzoquinone was allowed to react with a large excess of 2-aminoethanethiol under acidic conditions. After treatment of the reaction mixture with acetic anhydride a crystalline triacetyl derivate, was isolated which was assigned structure IV. The position of attachment of the acetylaminoethylthio residue was established by its nmr spectrum (deuteriochloroform), which showed in the aromatic region a pair of doublets centered at 7.03 and 6.71  $\delta$ , corresponding to two *meta*-coupled ring protons. Hydrolysis of the acetyl groups from IV with dilute hydrochloric acid afforded phenolic derivative III as crystalline hydrochloride.

As expected, in the presence of oxygen the free base was unstable, being readily converted into the dihydrobenzothiazine I in 78% yield.

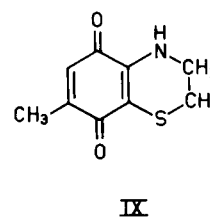
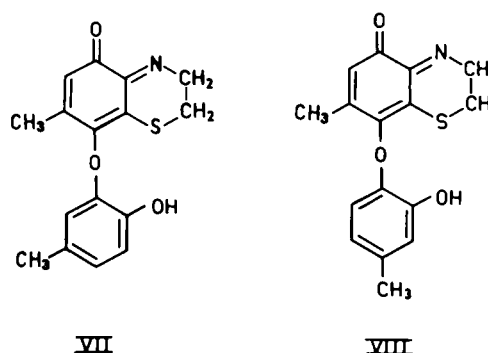
Presumably, the cyclization reaction proceeds *via* the initial formation of the quinone V (Scheme 2) which undergoes an intramolecular condensation producing as

Scheme 2



intermediate the quinonimine VI. Reduction of VI by catechol derivative III then gives dihydrobenzothiazine (I) and *o*-quinone (V). Such a cyclic sequence of reactions is consistent with the observation that the total oxygen absorbed during the cyclization reaction corresponded only to 0.32 atoms/molecule of substrate.

With the above background available we then investigated the nature of the products formed when the oxidation of 4-methylcatechol and 2-aminoethanethiol was performed by using an excess of potassium ferricyanide. Examination of the ether extracts of the reaction mixture indicated the presence of two coloured products (A and B) which were separated by column chromatography on polyamide powder. The molecular formula ( $\text{C}_{16}\text{H}_{15}\text{NO}_3\text{S}$ ) of the less polar fraction (A), which was yellow-orange in colour ( $\lambda_{\text{max}}$  (ethanol), 449, 281 and 232  $\text{m}\mu$ ;  $\log \epsilon$  3.32, 3.83, 4.29), suggested that compound A was derived from the reaction of two molecules of 4-methylcatechol and one molecule of 2-aminoethanethiol. Spectral properties of compound A were consistent with two isomeric structures (VII and VIII)



differing in the position of a methyl group in the benzene ring. The nmr spectrum of compound A (Fig. 1), which accounted for all the 15 protons, did not afford information on the type of substitution in the hydroxymethylphenoxy residue, since the three aromatic protons appeared as a broad singlet at 6.66  $\delta$ .

The position of the methyl group was then established by reduction of compound A with sodium and pyridine which afforded *m*-cresol along with 4-methylcatechol. The formation of *m*-cresol indicated that a 2-hydroxy-4-methylphenoxy residue was present in the molecule and this provided evidence for structure VIII.

The more polar fraction (B) had a molecular formula of  $\text{C}_9\text{H}_9\text{NO}_2\text{S}$  and displayed absorption maxima (methanol) at 591, 273 and 232  $\text{m}\mu$  ( $\log \epsilon$  3.18, 4.05, 4.13). Compound B behaved as a quinone on reduction and its infrared spectrum showed a sharp absorption in the N-H

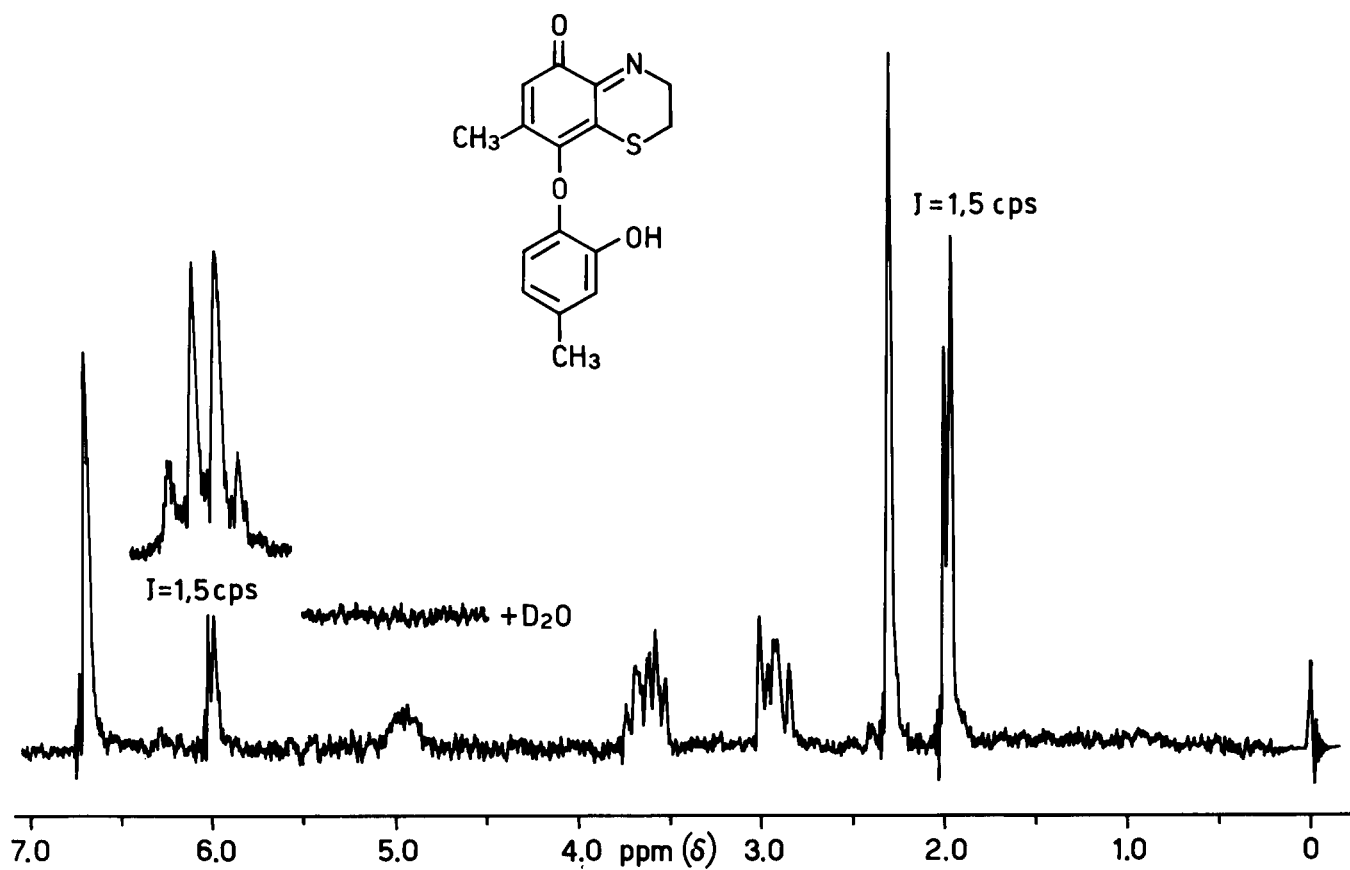


Fig. 1 - Nmr spectrum of compound VIII in deuteriochloroform.

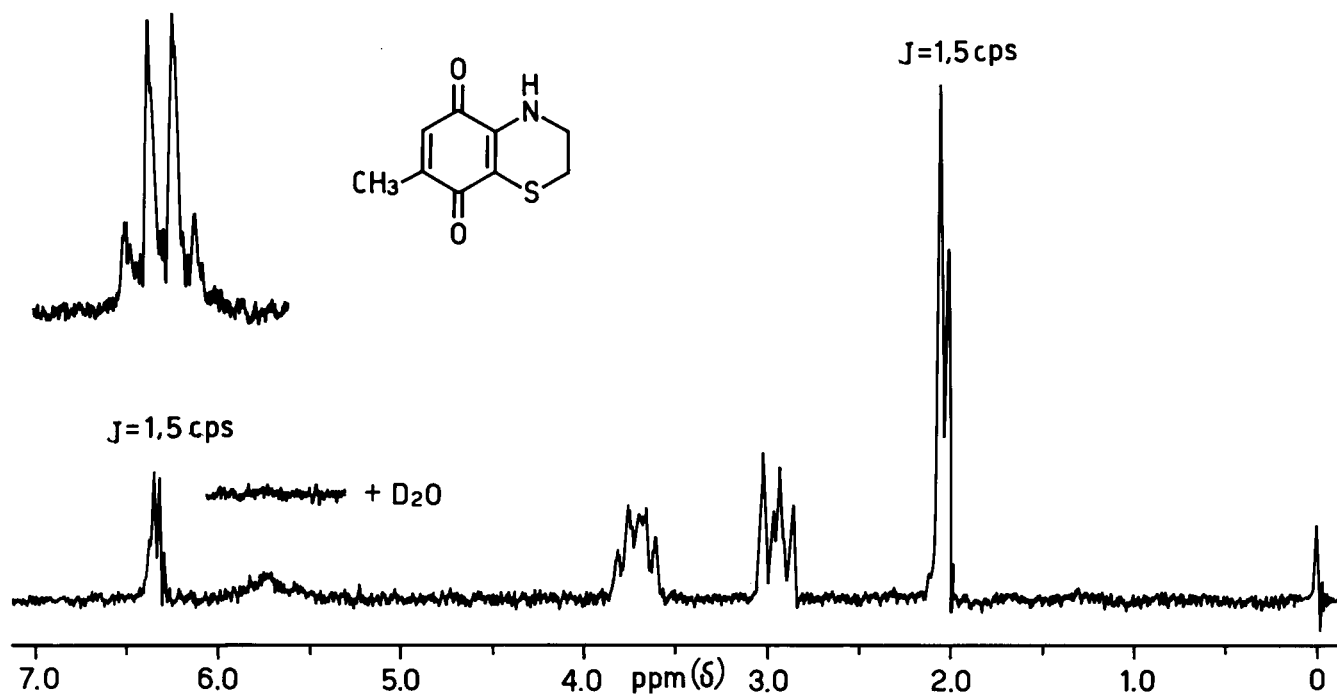


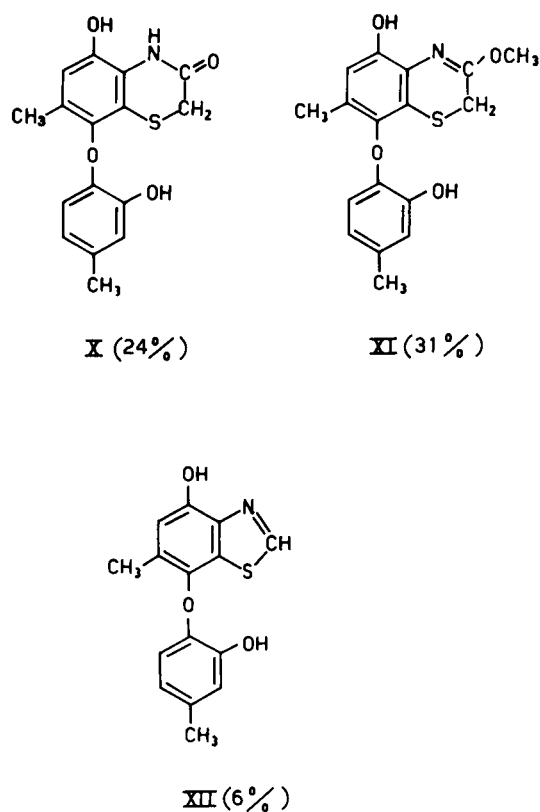
Fig. 2 - Nmr spectrum of compound IX in deuteriochloroform.

stretching region. The product was recovered unchanged after treatment with acetic anhydride under various conditions. The close similarity of its nmr spectrum (Fig. 2) with that of compound VIII indicated 7-methyl-3,4,5,8-tetrahydro-2*H*-1,4-benzothiazine-5,8-dione (IX) as the structure for compound B.

It is to be noted that both VIII and IX were obtained in very low yields (3.6% and 2.2%, respectively). The poor yields can be accounted for in part by the fact that under the reaction conditions (excess potassium ferricyanide) most of the 2-aminoethanethiol is readily oxidized to the corresponding disulfide so that only a fraction of the original amount of the thiol may react with 4-methyl-*o*-benzoquinone, yielding probably as an intermediate the dihydrobenzothiazine I by a route similar to that proposed above. This supposition was substantiated by the oxidation of compound I in the presence of 4-methylcatechol which afforded better yields of VIII (24%) as well as IX (33%).

Failure to oxidize compound I to IX in the absence of 4-methylcatechol suggested that the latter product was probably an artifact arising from VIII by hydrolysis of the enoether group during the isolation procedure. In accordance with this view, compound VIII was found to be particularly sensitive to acid, being rapidly hydrolysed at room temperature to give IX along with 4-methylcatechol.

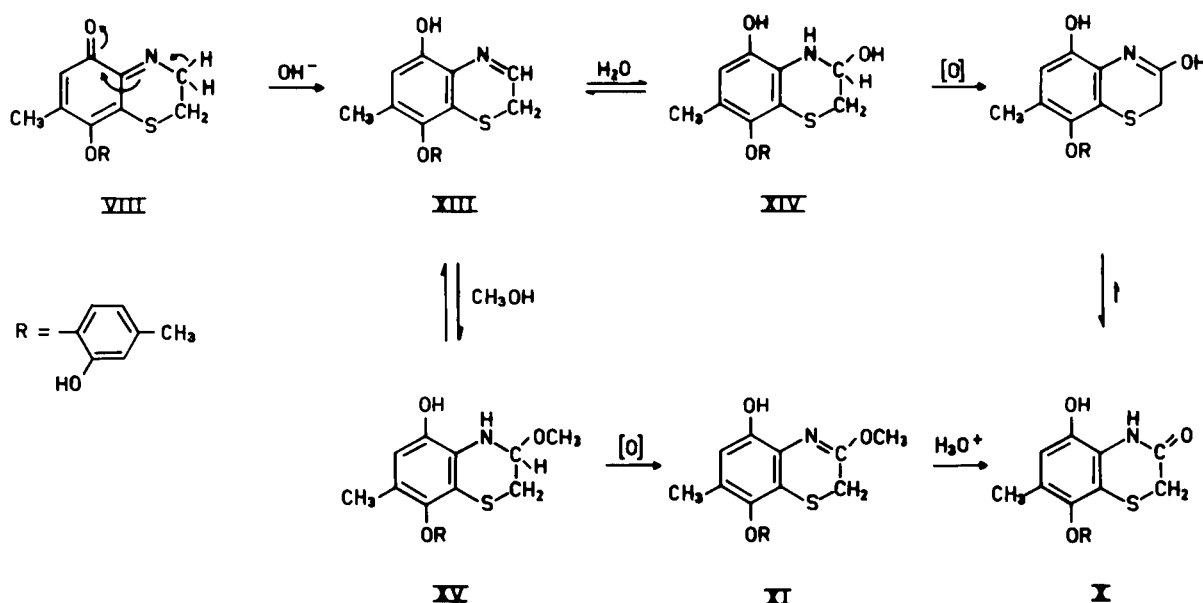
Of particular interest for the chemistry of 2*H*-1,4-benzothiazines are the reactions which took place when base was added to a methanolic solution of the quinonimine VIII in contact with air. Under these conditions VIII afforded three colourless compounds which were characterized by spectral analyses and shown to have



structures X, XI and XII (see experimental section).

The origin of X and XI from VIII probably involves a base catalysed rearrangement to produce intermediate benzothiazine derivative XIII, which can react with either moisture to give amino alcohol derivative XIV or with

Scheme 3



methanol to give methoxy derivative XV. Air oxidation of XIV gives the amide X while oxidation of XV gives XI. The amide X may also be partially derived by hydrolysis of XI (during the work up procedure). The origin of the benzothiazole XII from base-catalysed oxidation of VIII is obscure since it involves a ring contraction which, although already observed in the chemistry of 2*H*-1,4-benzothiazines (9-11), has not yet been clarified.

#### EXPERIMENTAL

The melting points were taken with a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Infracord 137E, uv spectra with a Bausch and Lomb (Spectronic 505) spectrophotometer and nmr spectra with a Varian A-60A spectrometer. Chemical shifts and coupling constants are expressed in ppm from TMS and in c/s respectively; br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. To some solutions deuterium oxide was added to check on exchangeable protons. Mass spectra and exact mass measurements were measured by the direct insertion technique with an A.E.I. MS 902 spectrometer (70 eV and 50  $\mu$ a) with a source temperature ca. 200°. Microanalyses were carried out by E. Thommen, Department of Organic Chemistry, University of Basel, Switzerland. The elemental composition of compounds IX, XI and XII was obtained by high resolution mass spectrometry. Thin-layer chromatography (TLC) and preparative thick-layer chromatography (PLC) were carried out on F<sub>254</sub> silica (E. Merck, A.G., Germany) and all solvents used for development and for elution were redistilled. Proportions given for mixed solvents are by volume. Paper chromatography were run on Whatman No. 1 paper (descending technique) in *n*-butanol-acetic acid-water (60:15:25; BAW). As spraying reagents, the following were used: 2% ninhydrin in acetone; 3% ferric chloride in ethanol; 2% ceric sulfate in 2 *N* sulfuric acid; diazotized sulphanilic acid followed by *N* sodium hydroxide (DZA).

Reaction of 2-Aminoethanethiol with *N*ascent 4-Methyl-*o*-benzoquinone: Isolation of 5-Hydroxy-7-methyl-3,4-dihydro-2*H*-1,4-benzothiazine (I).

To a well-stirred solution of 4-methylcatechol (0.124 g.) and 2-aminoethanethiol hydrochloride (0.240 g.) in 2% sodium bicarbonate (50 ml.), a 2% potassium ferricyanide solution (40 ml.) was added dropwise over a period of 1 hour. After the addition, the reaction mixture was extracted with ethyl acetate and the organic layer washed with 0.5 *M* borate buffer (pH 8.5) and then with water. The crude material, obtained by evaporation of the solvent, was purified by PLC on silica with benzene-methanol (95:5) (Rf 0.80; DZA as spraying reagent) yielding I (0.090 g.), white needles (from ethanol), 160-162°; mol. wt. 181 (mass spectrometry); uv (ethanol);  $\lambda$  max 307  $\mu$ m (log  $\epsilon$  3.39); nmr (DMSO-*d*<sub>6</sub>):  $\delta$  2.03 (3H, s, -CH<sub>3</sub>), 2.95 (2H, m, -CH<sub>2</sub>-N=), 3.45 (2H, m, -CH<sub>2</sub>-S-), 4.76 (1H, br s, =NH, deuterium oxide-exchangeable), 6.16 (1H, d, J  $\sim$  1, aromatic proton), 6.25 (1H, d, J  $\sim$  1, aromatic proton), 8.86 (1H, br s, -OH, deuterium oxide-exchangeable).

*Anal.* Calcd. for C<sub>9</sub>H<sub>11</sub>NOS: C, 59.66; H, 6.12; N, 7.73; S, 17.66. Found: C, 59.23; H, 6.30; N, 7.65; S, 17.41.

#### Diacetyl Derivative of I (II)

A mixture of I (0.100 g.), acetic anhydride (10 ml.) and

anhydrous pyridine (1 ml.) was allowed to stand at room temperature for 24 hours. Removal of the solvent left an oil which was purified by PLC on silica (benzene-ether 80:20) to give 4-acetyl-5-acetoxy-7-methyl-3,4-dihydro-2*H*-1,4-benzothiazine (II) (0.087 g.), m.p. 73-74° (from water); mol. wt. 265 (mass spectrometry); uv (methanol),  $\lambda$  max 233, 263  $\mu$ m (log  $\epsilon$  3.35, 3.31); nmr (carbon tetrachloride),  $\delta$  1.88 (3H, s, =NCOCH<sub>3</sub>), 2.18 (3H, s, -OCOCH<sub>3</sub>), 2.30 (3H, s, -CH<sub>3</sub>), 2.68 (2H, m, =NCH<sub>2</sub>-), 3.20 (2H, m, -S-CH<sub>2</sub>-), 6.60 (1H, d, J = 1.8, aromatic proton), 6.90 (1H, d, J = 1.8, aromatic proton).

*Anal.* Calcd. for C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub>S: C, 58.86; H, 5.70; N, 5.28; S, 12.07. Found: C, 58.73; H, 5.93; N, 5.46; S, 12.08.

Reaction of 2-Aminoethanethiol with 4-Methyl-*o*-benzoquinone: Isolation of 3,4-Diacetoxy-5-(2-acetylaminoethylthio)toluene (IV).

To a stirred solution of 2-aminoethanethiol hydrochloride (1.70 g.) in 60 ml. of ethanol-0.2 *N* hydrochloric acid mixture (50:50 v/v), an ethanolic solution of 4-methyl-*o*-benzoquinone (1.22 g.) was added dropwise over a period of ca. 3 hours. After the addition, the reaction mixture was concentrated under reduced pressure to about 30 ml. and extracted with ethyl acetate. The aqueous layer was analyzed by paper chromatography (eluent BAW) and showed one principal spot (Rf = 0.63), which gave a positive test with ferric chloride (green colour) and with ninhydrin (red-purple colour). The oil obtained after evaporating the aqueous layer was dissolved in acetic anhydride (30 ml.) and pyridine (5 ml.) and the mixture allowed to stand at room temperature for 24 hours. Removal of the solvent left an oil (1.25 g.) which was purified by chromatography on a silica column (high grade silica gel 0.05-0.2 mm, Merck) developed with chloroform-methanol (99:1) to give IV (0.81 g.), prisms, m.p. 112-113°, mol. wt. 325 (mass spectrometry); uv (methanol),  $\lambda$  max 253  $\mu$ m (log  $\epsilon$  3.91); ir (chloroform),  $\nu$  max 3367 (-NHCOCH<sub>3</sub>), 1770 (-OCOCH<sub>3</sub>), 1667 (-NHCOCH<sub>3</sub>) cm<sup>-1</sup>; nmr (deuteriochloroform),  $\delta$  1.66 (3H, s, =NCOCH<sub>3</sub>), 2.10 (3H, s, -CH<sub>3</sub>), 2.18 (6H, s, -OCOCH<sub>3</sub>), 2.86 (2H, m, =NCH<sub>2</sub>-), 3.13 (2H, t, J = 5.5, -SCH<sub>2</sub>-), 6.71 (1H, d, J = 1.9, aromatic proton), 7.03 (1H, d, J = 1.9, aromatic proton).

*Anal.* Calcd. for C<sub>15</sub>H<sub>19</sub>NO<sub>5</sub>S: C, 55.38; H, 5.89; N, 4.31; S, 9.84. Found: C, 55.54; H, 5.98; N, 4.30; S, 9.36.

#### Acid Hydrolysis of IV.

A suspension of IV (1.5 g.) in 3 *N* hydrochloric acid was refluxed for 6 hours under nitrogen current. The hydrolysate was evaporated to dryness *in vacuo* and excess hydrochloric acid was removed by adding a small amount of water to the residue and evaporating to dryness four successive times. The residue was crystallized from anhydrous ethanol to give white needles of III, m.p. 186-188°, M<sub>2</sub><sup>+</sup> 199; uv (water),  $\lambda$  max 292  $\mu$ m (log  $\epsilon$  3.52); nmr (deuterium oxide) (12),  $\delta$  2.10 (3H, s, -CH<sub>3</sub>), 3.06 (4H, m, -SCH<sub>2</sub>-CH<sub>2</sub>-N<sup>+</sup>, A<sub>2</sub>B<sub>2</sub> system), 6.71 (2H, br s, aromatic protons).

*Anal.* Calcd. for C<sub>9</sub>H<sub>14</sub>ClNO<sub>2</sub>S: C, 45.85; H, 5.98; N, 5.94; S, 13.60; Cl, 14.93. Found: C, 45.63; H, 5.84; N, 6.04; S, 13.65; Cl, 15.09.

#### Intramolecular Cyclization of III.

A stream of oxygen was bubbled into a solution of III (1 g.) in 2% aqueous sodium bicarbonate (50 ml.). After 20 minutes the mixture was extracted with ethyl acetate (150 ml. in 4 portions). The combined organic layers were dried with sodium sulfate and evaporated *in vacuo* to dryness. Recrystallization of the residue

(0.82 g.) from ethanol gave 0.6 g. of almost colourless crystals, m.p. 160-162°, which showed no melting point depression when mixed with a sample of 5-hydroxy-7-methyl-3,4-dihydro-2*H*-1,4-benzothiazine (1).

By using an apparatus similar to that described by Brackman and Havinga (13) it was found that the total oxygen absorbed during the cyclization reaction corresponded to 0.32 atom/molecule of substrate.

Reaction of 2-Aminoethanethiol with Excess *Nascent* 4-Methyl-*o*-benzoquinone: Isolation of VIII and IX.

To a stirred solution of 4-methylcatechol (0.124 g.) and 2-aminoethanethiol hydrochloride (0.170 g.) in 2% sodium bicarbonate (100 ml.) a 10% potassium ferricyanide solution (12 ml.) was added dropwise over a period of *ca.* 1 minute. The reaction mixture, obtained by repeating the above procedure ten times, was extracted with ether. The combined organic layers were washed with water, dried with sodium sulfate and after addition of 50 ml. of benzene concentrated under reduced pressure to a volume of about 15 ml. The concentrated solution was chromatographed (14) on a 2.5 x 8 cm silica column using benzene-chloroform (50:50) as eluent. The yellow-orange band, which emerged from the column after 50 ml. was further purified on a 3 x 50 cm polyamide (Macherey, Nagel and Co.) column developed with petroleum ether-benzene (50:50). Two main fractions were obtained; the less polar fraction, which emerged from the column after 280 ml., was concentrated *in vacuo* at 30° to a volume of about 15 ml. and kept overnight in the refrigerator to give VIII (0.055 g.), red-orange prisms, m.p. 128-129°, mol. wt. 301,  $M^+$  301.0776 ( $C_{16}H_{15}NO_3S$  requires: 301.0772); ir (carbon tetrachloride),  $\nu$  max 3363, 2915, 2842, 1666, 1640, 1610, 1488, 1449, 1429, 1374, 1339, 1316, 1282, 1238, 1205, 1152, 1124, 1093, 1038, 1009, 990, 940, 908, 898, 858  $cm^{-1}$ .

*Anal.* Calcd. for  $C_{16}H_{15}NO_3S$ : C, 63.78; H, 5.02; N, 4.65; S, 10.63. Found: C, 63.54; H, 5.10; N, 4.37; S, 10.78.

The more polar fraction, which emerged from the column after 520 ml., on concentration and after standing overnight in the refrigerator gave 0.044 g. of IX, deep blue needles, m.p. 165-166°, mol. wt. 195; ir (chloroform),  $\nu$  max 3333, 2915, 2849, 1667, 1639, 1585, 1493, 1449, 1420, 1374, 1353, 1312, 1285, 1250, 1175, 1157, 1053, 1010, 998, 914, 880, 860, 842  $cm^{-1}$ .

*Anal.* Calcd. for  $C_9H_9NO_2S$ : 195.0346. Found: 195.0353.

Reduction of VIII with Sodium in Pyridine.

A mixture of VIII (0.100 g.), anhydrous pyridine (2 ml.) and sodium (0.170 g.) was heated to 120° for 3 hours. After cooling, the mass was diluted first with pyridine (3 ml.), then with a pyridine-water mixture (50:50 v/v) and eventually with water. After acidification with 6 *N* hydrochloric acid and centrifugation, the solution was extracted with ether (30 ml. in 4 portions). The combined extracts were evaporated to dryness and the residue, fractionated by PLC over silica with benzene-methanol (95:5) afforded 0.008 g. of *m*-cresol (Rf 0.35) and 0.010 g. of 4-methylcatechol (Rf 0.14), identified by chromatographic and ir comparison with authentic samples.

Acidic Cleavage of VIII.

To an ethanolic solution of VIII (0.100 g.) 5 ml. of 6 *N* hydrochloric acid was added dropwise. The solution, the colour of which rapidly turned from yellow to deep blue, was allowed to stand for 20 minutes at room temperature and then evaporated *in vacuo* to dryness. The residue was dissolved in chloroform and the resulting solution washed with water. PLC of the chloroform

extract on silica (eluent chloroform-methanol 95:5) afforded 0.034 g. of 4-methylcatechol and 0.049 g. of IX, identical in all respects, ir, m.p., mixed m.p. with that obtained above.

Oxidation of a Mixture of I and 4-Methylcatechol with Potassium Ferricyanide: Isolation of VIII and IX.

To a stirred mixture of 4-methylcatechol (0.050 g.) and I (0.050 g.) in 2% sodium bicarbonate (100 ml.) a 10% potassium ferricyanide solution (2.7 ml.) was added dropwise over a period of *ca.* 1 minute. Working up as in the above experiment gave 0.020 g. of VIII and 0.018 g. of IX, identical in all respects, uv, ir, with those obtained above.

Base-catalysed Oxidation of VIII: Isolation of X, XI and XII.

Compound VIII (0.200 g.) dissolved in benzene (3 ml.) was added to a well-stirred solution of potassium hydroxide (1 g.) in methanol (50 ml.). The solution was allowed to stand for 30 minutes at room temperature and then neutralized by adding acetic acid. After removal of the solvents, the residue was extracted with ethyl acetate and the resulting solution washed with aqueous sodium bicarbonate and water. The extract was evaporated and the products separated by PLC on silica with chloroform-methanol (95:5).

The band Rf 0.73 (0.070 g.), located with ceric sulfate, formed prisms (XI) from methanol, m.p. 180-182°, uv (methanol),  $\lambda$  max 288, 312 (shoulder)  $m\mu$  ( $\log \epsilon$  3.85, 3.74, uv (methanol + sodium hydroxide),  $\lambda$  max 292, 338  $m\mu$  ( $\log \epsilon$  3.86, 3.62), mol. wt. 331 (mass spectrometry). The nmr spectrum (deuteriochloroform) showed singlets at  $\delta$  2.13 and 2.26 (6H, Ar-CH<sub>3</sub>), 3.25 (2H, -S-CH<sub>2</sub>-), 3.91 (3H, -OCH<sub>3</sub>) and a complex series of signals at 5.9-7.0  $\delta$  [6H, four aromatic protons with two deuterium oxide-exchangeable protons (phenolic protons)].

*Anal.* Calcd. for  $C_{17}H_{17}NO_4S$ : 331.0878. Found: 331.0875.

The band Rf 0.27 afforded 0.012 g. of XII, m.p. 192-195°, uv (methanol),  $\lambda$  max 236, 265, 271, 287 (infl.), 313  $m\mu$  ( $\log \epsilon$  4.32, 3.78, 3.77, 3.66, 3.56); uv (methanol + sodium hydroxide),  $\lambda$  max 244, 282, 341  $m\mu$  ( $\log \epsilon$  4.39, 3.82, 3.61); mol. wt. 287. The nmr spectrum (deuterioacetone) showed two singlets at 2.20 and 2.25  $\delta$  (6H, Ar-CH<sub>3</sub>), a broad signal at 4.6  $\delta$  (2H, phenolic protons, deuterium oxide-exchangeable), a complex series of signals at 6.0-7.1  $\delta$  (4H, aromatic protons) and a sharp singlet at 9.03  $\delta$  (1H, benzothiazole-2 proton).

*Anal.* Calcd. for  $C_{15}H_{13}NO_3S$ : 287.0616. Found: 287.0643.

The band Rf 0.19 (0.051 g.) formed prism (X) from ethanol, m.p. 265-266°, undepressed on admixture with an authentic sample of 5-hydroxy-7-methyl-8-(2-hydroxy-4-methylphenoxy)-3,4-dihydro-2*H*-1,4-benzothiazine-3-one (5), and indistinguishable from it by ir, uv and mass spectrometry. The identification was confirmed by comparison of the properties of the dimethyl-derivative of X, m.p. 183-185°, with those of an authentic sample (5).

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