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1-Substituted Phenothiazine Derivatives (1)

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Several new 1-substituted phenothiazine derivatives have been synthesized and characterized. A 1-carboxythionine dye is described.

Phenothiazine derivatives have been intensively investigated owing to their properties as drugs and dyes. The 2,3 and 10-substituted derivatives of phenothiazine are well characterized since they are the products of the Friedel-Crafts reaction (2), nitration (3), and acylation (4) of phenothiazine, respectively. In contrast, the relatively few known 1-substituted phenothiazines have been prepared almost exclusively by ring closure reactions (5) rather than substitution reactions. Exceptions are the preparation of 1-carboxyphenothiazine (6), its derivatives (7), and certain intramolecular acylation reactions (8). Our work is an attempt to extend the chemistry of 1-substituted phenothiazine derivatives, and to prepare 1-substituted thionine dyes.

The carboxylic acid produced when phenothiazine was metalated and allowed to react with carbon dioxide was assigned the structure 1-carboxyphenothiazine (II) (5a,6). This assignment has been further substantiated by our synthesis of 1-methylphenothiazine (III) from II by reduction with a mixture of lithium aluminum hydride and aluminum chloride [mixed hydrides reduction (9)]. III was purified and isolated by preparative vapor phase chromatography. When lithium aluminum hydride and II were allowed to react without aluminum chloride present, the expected alcohol, 1-hydroxymethylphenothiazine (IV) was isolated. The alcohol (IV) was also reduced to the methyl compound (III) by the mixed hydrides technique.

Attempts to convert the alcohol (IV) to a halomethylphenothiazine with hydrochloric, hydrobromic or hydriodic acid, methanesulfonyl chloride or triphenylphosphite dibromide (10) all gave the same ether, 1,1'-(oxydimethylene)diphenothiazine (V) instead of the expected halo-compound. The synthesis of V required only a catalytic amount of acid.

An attempt to produce the acid chloride of II with thionyl chloride in benzene resulted in the formation of a dark red dichloro acid chloride (VI). Phenothiazine derivatives have been shown to undergo nuclear chlorination when refluxed with thionyl chloride (7a, 11). Refluxing ethanol converted VI into ethyl dichlorophenothiazine-1-carboxylate (VII).

Kehrmann's method (3) was used to prepare 3,7-dinitrophenothiazine sulfoxide (VIII). Nitration of 1-carboxyphenothiazine (II) yielded an analogous dinitro derivative, 1-carboxy-3,7-dinitrophenothiazine sulfoxide (X). The assignment of the nitro groups to the 3,7-positions in X is based on spectral evidence. The electronic spectrum of XIV, the *aci*-nitro sodium salt of 3,7-dinitrophenothiazine sulfoxide, [λ max 483 (4.50), 343 (3.66), 262 (3.95) and 225 mμ (4.30)] is very similar to that of XI, the di-sodium salt of X [λ max 500 (4.47), 352 (3.87), 265 (4.01) and 225 mμ (4.30)]. In addition, the catalytic reduction of the triethylammonium salt of X, followed by the air oxidation of XII, the unisolated diamino intermediate, gave triethylammonium 3,7-diaminophenazathionium hydroxide-1-carboxylate (XIII), a dye with a visible spectrum [λ max 596 (4.31), 555 sh (4.06), 285 (4.32) and 231 mμ (4.31)] almost identical to that of thionine (IX) [λ max 599 (4.56), 562 sh (4.35), 282 (4.48) and 235 mμ (4.13)]. The air oxidation of XII to the dye (XIII) required seven to nine days for completion (no change in ε with time). Neither the addition of 30% hydrogen peroxide nor saturation of the solution with oxygen gas caused a noticeable increase in the rate of oxidation. While ferric chloride was a very effective oxidant, the dye products produced were difficult to purify and contained higher percentages of chlorine than were expected. The rate of oxidation of the intermediate diamino compound, judged by the rate of blue dye formation, appeared to be considerably greater for thionine (IX) than the 1-substituted thionine (XIII).

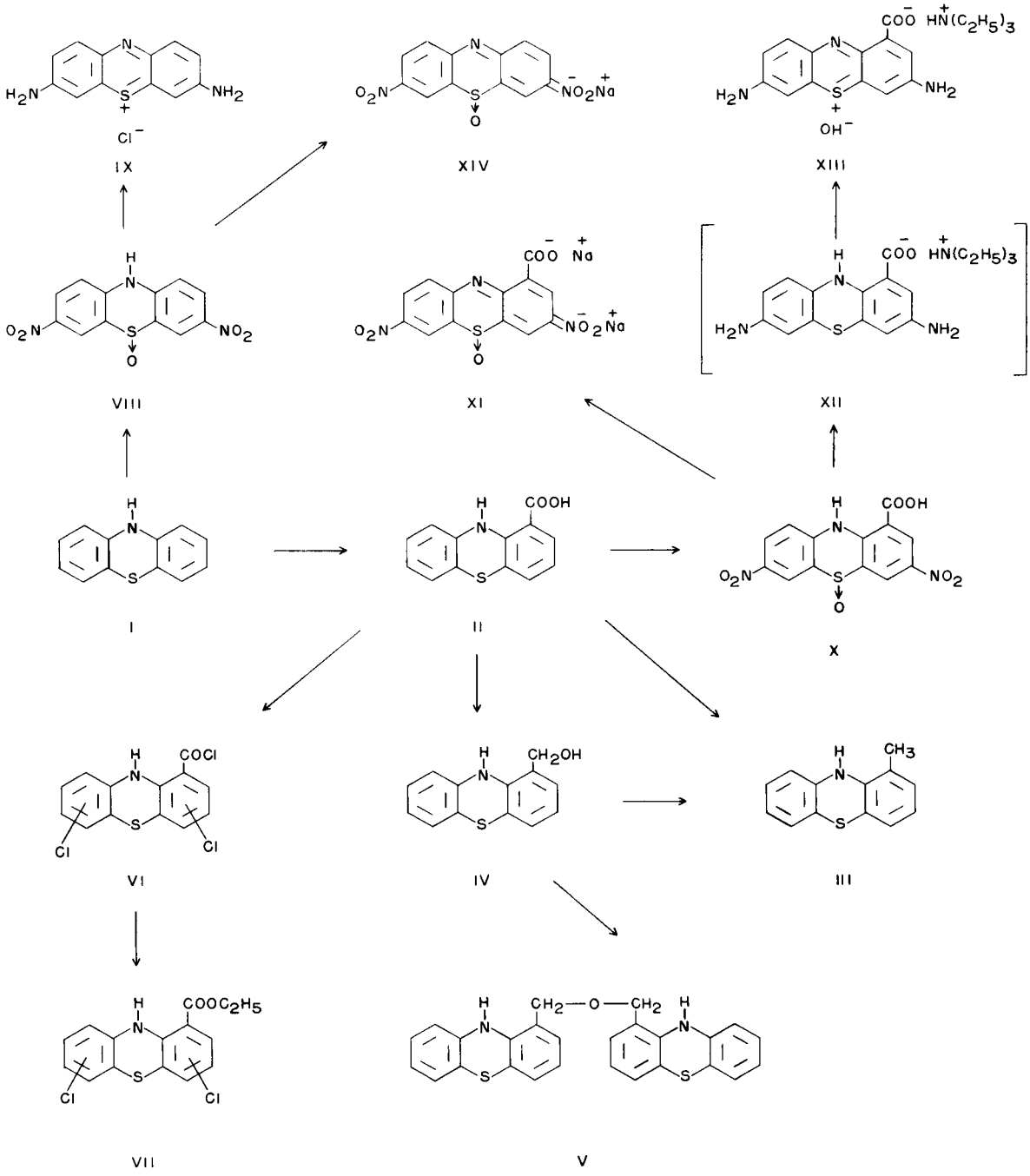
These data indicate that (a) a carboxyl group in the 1-position on phenothiazine does not alter the positions of nitration relative to unsubstituted phenothiazine, (b) nuclear chlorination as well as acyl halide formation occurs when II is treated with thionyl chloride, and (c) the 1-halomethylphenothiazines are very sensitive toward nucleophilic displacement reactions.

EXPERIMENTAL

Melting points are corrected. Elemental analyses were carried out by Galbraith and Carol K. Fitz laboratories. Infrared spectra were determined in KBr pellets on a Perkin-Elmer Model 30 spectrophotometer. Visible and ultraviolet spectra were recorded with a Cary Model 14 spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were obtained with a Varian A-60 spectrometer. Vapor phase chromatographic analyses (v.p.c.) were determined on an Aerograph Model A-350 chromatograph with automatic temperature programming. Rf values reported are for heat activated thin-layer chromatographic substrates, 250 microns thick.

Phenothiazine (I).

A solution of commercial phenothiazine in benzene was refluxed with dry, activated charcoal. Filtration and evaporation of the solvent gave a gray-white solid, m.p. 181-182° [lit. (12) m.p. 185°] which was 100% pure by v.p.c. analysis.



1-Carboxyphenothiazine (II).

The procedure of Gilman, *et al.* (6), gave a 64% yield of II, m.p. 267-268° dec. [lit. (6) m.p. 264-264.5°] on a 0.2 molar scale; ν max 3289 (NH); 1667 (COOH) 3000-2400, 905 cm^{-1} (OH); R_f 0.1 (silica gel-methanol). The methyl ester of II, m.p. 111-113.5° [lit. (6) m.p. 113-113.5°] was prepared with methanol and sulfuric acid; ν max 1689 cm^{-1} ($COOCH_3$); R_f 0.8 (silica gel-methanol).

1-Methylphenothiazine (III).

Method A.

Aluminum chloride (6.60 g., 0.05 mole) was dissolved in 50 ml. of dry ether. The solution was filtered and added dropwise to a stirred suspension of 1.90 g. (0.05 mole) of lithium aluminum hydride in 250 ml. of dry ether. This ether suspension was used to extract 4.90 g. (0.02 mole) of 1-carboxyphenothiazine by the Soxhlet technique (13) for 24 hours. First water (50 ml.), then 6 *N* sulfuric acid (40 ml.) was added dropwise with stirring. The ether layer was removed and the aqueous layer was extracted with three 250 ml. portions of ether. The ether extracts were combined, dried over magnesium sulfate, and evaporated *in vacuo* to yield 2.43 g. of a

tan solid, m.p. 95-115° to a cloudy liquid. Preparative v.p.c. (6 ft. 10% SE-30 on 60-80 mesh diatoport, 60 p.s.i. helium at 10 cc./minute, temperature programmed 100-300°) gave white crystals, retention temperature 270°, m.p. 136-137° [lit. (5a) m.p. 137-138°]. N.m.r. in deuteroacetone (14) showed peaks at 2.24s [3] (CH_3) and 6.6-7.2m [8] (aromatic and NH).

Method B.

Aluminum chloride (0.66 g., 5 mmoles) was dissolved in 10 ml. of dry ether and the solution was dropped into a stirred suspension of 0.19 g. (5 mmoles) of lithium aluminum hydride in 5 ml. of dry ether. After 5 minutes, a solution of 1.15 g. (5 mmoles) of 1-hydroxymethylphenothiazine in 10 ml. of dry ether was added dropwise over a 15 minute period. A gummy precipitate resulted. After 0.5 hour, 15 ml. of water, 5 ml. of 6 *N* sulfuric acid, and 25 ml. of ether were added in that order. The ether layer was separated, dried over magnesium sulfate, and evaporated to dryness to yield 0.40 g. of white solid, m.p. 108-112° to a cloudy liquid. The infrared spectrum of this product was identical with that of the material obtained by Method A.

1-Hydroxymethylphenothiazine (IV).

1-Carboxyphenothiazine (4.90 g., 0.02 mole) was extracted in a Soxhlet apparatus for 24 hours with 250 ml. of dry ether containing 2.75 g. (0.07 mole) of lithium aluminum hydride. After cooling the suspension to room temperature, 100 ml. of water was cautiously added with stirring. A solution of 10 ml. of concentrated sulfuric acid in 40 ml. of water was then added. The ether layer, which separated upon addition of 50 ml. of ether, was removed and the aqueous layer extracted with 100 ml. of ether. The ether extracts were combined, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. The resulting light yellow solid was dissolved in 100 ml. of methanol, boiled with charcoal, and filtered. This procedure was twice repeated before the hot solution was diluted with 200 ml. of hot water. After refrigeration, 2.90 g. (63%) of white needles were obtained, m.p. 100-100.5°; ν max 3356 (OH and NH); 1004 cm^{-1} (CO); n.m.r. in deuterochloroform (14) 2.32s [1.3] (OH), 4.56s [2.3] (CH_2) and 6.3-7.1m [8.0] (aromatic, NH).

Anal. Calcd. for $C_{13}H_{11}NOS$: C, 68.09; H, 4.84; N, 6.12; S, 13.98. Found: C, 68.29; H, 4.92; N, 5.82; S, 14.01.

1,1'-(Oxydimethylene)diphenothiazine (V).

One drop of concentrated hydrochloric acid solution was added to a stirred, filtered solution of 0.57 g. (2.5 mmoles) of pure 1-hydroxymethylphenothiazine in 10 ml. of glacial acetic acid.

After stirring for 18 hours at room temperature, the suspension was filtered. The resulting solid was washed well with 150 ml. of filtered ether, and dried to yield 0.50 g. (80%) of tan solid m.p. > 320°. V became a gum in n.m.r. solvents.

Anal. Calcd. for $C_{26}H_{20}N_2OS_2$: C, 70.87; H, 4.57; N, 6.36; S, 14.54. Found: C, 71.00; H, 4.14; N, 6.52; S, 14.48.

Dichlorophenothiazine-1-carbonyl chloride (VI).

A suspension of 1.00 g. (4 mmoles) of 1-carboxyphenothiazine in 5.0 ml. of dry benzene and 2.0 ml. (3.2 g. 270 mmoles) of thionyl chloride was refluxed for 1 hour. The resulting precipitate was filtered, washed with 25 ml. of benzene, and dried to yield 0.87 g. of a red solid, m.p. 213-215° dec. Recrystallization from benzene gave red needles, with no increase in the melting point; λ max (THF) 458 (ϵ, 5,800), 300 (ϵ, 5,000), 248 mμ (ϵ, 34,000); ν max 3280 (NH); 1690 cm^{-1} (COCl).

Anal. Calcd. for $C_{13}H_6Cl_3NOS$: C, 47.20; H, 1.82; N, 4.24; Cl, 32.17. Found: C, 47.77; H, 2.08; N, 4.33; Cl, 32.41.

Ethyl dichlorophenothiazine-1-carboxylate (VII).

Dichlorophenothiazine-1-carbonyl chloride (0.61 g. 1.8 mmoles) was stirred at reflux with 38 ml. of absolute ethanol for 2 hours. The cloudy, yellow solution was filtered. The bright yellow solid which precipitated immediately was filtered and dried to give 0.54 g. (86%) of yellow needles, m.p. 152-155°. Recrystallization from ethanol raised the melting point to 156.5-157.5°; ν max 3247 (NH), 1681 cm^{-1} (COOR).

Anal. Calcd. for $C_{15}H_{11}Cl_2NO_2S$: C, 52.94; H, 3.24; Cl, 20.84; N, 4.12; S, 9.41. Found: C, 53.04; H, 3.45; Cl, 20.86; N, 4.19; S, 9.65.

3,7-Dinitrophenothiazine sulfoxide (VIII).

VIII, m.p. 258-265° dec. [lit. (3) m.p. *ca.* 260° dec.] was prepared in 82% yield on a 10 mmole scale by Kehrmann's procedure (3). Extraction with acetone reduced the yield by 16% and raised the melting point to 268-269° dec.; ν max 3247 (NH); 1515, 1325, 743 (NO_2). Solution of VIII in 0.1 *N* sodium hydroxide solution gave XIV, the *aci*-nitro salt (15).

3,7-Diaminophenazathionium chloride (Thionine) (IX).

3,7-Dinitrophenothiazine sulfoxide (0.50 g., 1.64 mmoles) and 0.10 g. of platinum oxide were heated with 100 ml. of methanol on a steam bath for 15 minutes. The suspension was cooled to 30° and hydrogenated in a Parr apparatus for 2.3 hours. Upon filtration, the light green solution immediately became purple (air oxidation). After standing overnight, the solvent was evaporated *in vacuo* to yield 0.35 g. of purple crystals. The crystals were added to 375 ml. of methanol, and a solution of 7.0 ml. of hydrogen chloride in methanol (0.1 g./ml.) was added. The solution was filtered and the filtrates evaporated *in vacuo* to give 0.24 g. (56%) of a dark maroon solid, m.p. > 320°; R_f 0.08 (silica gel-methanol). A qualitative visible spectrum (ethanol) of the product was identical with that of a commercial sample of thionine (82% pure). The quantitative spectral data (aqueous 10^{-5} molar) (15) was very similar to that reported for thionine (16).

1-Carboxy-3,7-dinitrophenothiazine sulfoxide (X).

A mixture of glacial acetic acid (500 ml.) and 1-carboxyphenothiazine (12.02 g., 0.05 mole) was heated on a steam bath for 2 hours in a 2-liter, 4-necked flask equipped with a mechanical stirrer, reflux condenser (Drierite tube), thermometer, and dropping funnel. The stirred solution was allowed to cool to room temperature and was then chilled to 5° with an ice bath. Concentrated nitric acid (94 ml., 113 g., 1.8 mole) was added to the stirred, cooled suspension (1-2 drops/second) over a period of 30 minutes. An immediate black coloration developed. When the addition was complete, the ice bath was removed and the solution was allowed to stir at room temperature for 30 minutes. The mixture was then cautiously heated on a steam bath for 1 hour (temperature 92°). The suspension was cooled to 35° and was poured into 500 ml. of water (stirring). The resulting brown precipitate was filtered and washed with 100 ml. of water. The brown solid was stirred with 135 ml. of acetone, filtered, and dried to give 5.02 g. of yellow product, m.p. 250° dec.

The original aqueous acidic filtrates were diluted with one liter of water resulting in precipitation of a brown solid. Addition of another two liters of water gave a third crop of solid after 2 days. The additional crops of brown solid were combined and extracted with 50 ml. of acetone to yield an additional 2.15 g. of yellow product, m.p. 247-249° dec., which had an infrared spectrum identical with that of the first crop [total yield 7.15 g. (41%)]. Two reprecipitations from 5% aqueous sodium hydroxide with dilute hydrochloric acid yielded a yellow solid, m.p. 260-262° dec.; ν max 3226 (NH); 2900-1800, 985 (OH); 1695 (COOH); 1527, 1333, 745 cm^{-1} (NO_2) (15).

Anal. Calcd. for $C_{13}H_7N_3O_7S$: C, 44.70; H, 2.00; N, 12.03; S, 9.17. Found: C, 44.8; H, 2.0; N, 11.9; S, 9.2.

X forms a red, hydrated mono-sodium salt, m.p. > 320°, when allowed to react with 1 equivalent of base; ν max 1626 cm^{-1} (COONa). The visible spectrum of the mono-sodium salt in dilute sodium hydroxide solution is quantitatively the same as that of X in the same solvent (15).

Anal. Calcd. for $C_{13}H_6N_3O_7NaS \cdot H_2O$: C, 40.10; H, 2.06; Na, 5.91. Found: C, 40.29; H, 1.96; Na, 5.07.

X forms a dark green, hydrated di-sodium salt (XI), m.p. > 320°, when it was allowed to react with 2 equivalents of base; ν max 1265-1235 cm^{-1} (NO_2^-) (17). The visible spectrum of XI is quantitatively the same as that of X in sodium hydroxide solution (15).

Anal. Calcd. for $C_{13}H_5N_3O_7Na_2S \cdot H_2O$: C, 37.96; H, 1.70; Na, 11.19. Found: C, 37.59; H, 2.26; Na, 11.02.

Triethylammonium 3,7-diaminophenazathionium hydroxide-1-carboxylate hemihydrate (XIII).

Triethylamine (0.25 g., 2.5 mmoles) was added to a suspension of twice recrystallized 1-carboxy-3,7-dinitrophenothiazine sulfoxide (0.87 g., 2.5 mmoles) in 150 ml. of methanol. After standing for 1 hour, the amber solution was filtered to remove a very small amount of undissolved yellow solid. Platinum oxide catalyst (0.25 g.) was added and the mixture was hydrogenated on a Parr apparatus at 25° for 3.5 hours. The dark brown solution was filtered and then exposed to the atmosphere for nine days. The resulting dark blue solution was filtered and evaporated *in vacuo* to yield 0.78 g. (78%) of purple crystals, m.p. softens 125°, 130° dec. The spectral data was similar to that of thionine (15); R_f 0.36 (silica gel-methanol).

Anal. Calcd. for $C_{19}H_{26}N_4O_8S \cdot {}^1/_2H_2O$: C, 57.11; H, 6.81; N, 14.02; S, 8.02. Found: C, 57.72; H, 6.42; N, 13.84; S, 8.09.

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