Phenothiazine-1-carboxylic Acids

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Metalation of 2-trifluoromethylphenothiazine followed by carbonation of the metallo intermediates leads to the formation of a mixture of 2-trifluoromethyl- and 8-trifluoromethylphenothiazine-1-carboxylic acids, with the first material predominating. Both compounds exhibit antiinflammatory properties in experimental animals, with the 8 isomer being the more potent.

Phenothiazine and its 10-substituted derivatives exhibit a variety of biological effects that have found utility in medical practice. The parent compound has been a useful anthelmintic, while substituted compounds, such as phenergan and chlorpromazine, exhibit antihistaminic or ataractic activities. This area has been reviewed extensively by Schenker and Herbst.[2]

The purpose of this paper is to report on the chemistry and biological properties of certain ring-substituted phenothiazine-1-carboxylic acids. Phenothiazines of this type have not been described in any great number. Baltzly, *et al.*,[3] obtained a product by metalation and carbonation of 3-methoxyphenothiazine which they suggested had the carboxyl function located in either the 1 or 4 position. These suggestions were based on the earlier observations of Gilman and co-workers[4] that phenothiazine itself was metalated exclusively in the 1 position and that 10-ethylphenothiazine was metalated in the 4 position. The actual position of the carboxyl function from the reaction of 3-methoxyphenothiazine was not confirmed.

Sen and Sharma[5] prepared 1-nitrophenothiazine-3-carboxylic acid and 3-nitrophenothiazine-1-carboxylic acid by the Smiles rearrangement[6,7] of the appropriately substituted diphenyl thioethers. The corresponding acid compounds were obtained by reduction in acetic amino with Adams catalyst.

Driscoll and Nealey[8] recently prepared 3,7-dinitrophenothiazine-1-carboxylic acid by nitration of the unsubstituted acid and obtained a dichlorophenothiazine-1-carboxylic acid chloride when phenothiazine-1-carboxylic acid was treated with thionyl chloride.

We undertook to prepare phenothiazine-1-carboxylic acids containing substituents in the 8 position and first studied metalation of 2-trifluoromethylphenothiazine with butyllithium followed by carbonation of the metallo derivative. This procedure proved to be more temperature dependent than other reported phenothiazine metalations. Prolonged reaction at room temperature or in refluxing ether resulted in polymer formation. However, a reaction temperature of 5–10° and a reaction time of about 6 hr gave a 50–60% yield of an acidic product which was shown by thin layer chromatography to be a mixture of two components.

The major acidic component was isolated and purified in two steps: first by careful pH-controlled precipitation of the acids from an aqueous alkaline solution, followed by fractional crystallization of the more acidic material from a mixture of acetic–trifluoroacetic acid. It comprised about 80% of the total crude. A small amount of the isomeric acid was obtained in the pure state by chromatographic separation of residues from the purification of the major component.

The first material was identified as 2-trifluoromethylphenothiazine-1-carboxylic acid by its elemental analysis and spectral characteristics. The infrared spectrum of this material showed in addition to the expected patterns a carboxyl stretching band at 1655 cm^{-1}, characteristic of an intramolecularly hydrogen-bonded carbonyl, typical of one adjacent to an NH grouping. Aromatic substitution pattern indicated a 1,2-substituted phenyl ring (750 cm^{-1}) and a 1,2,3,4-substituted phenyl ring (833 cm^{-1}).

Elemental analysis of the minor component showed it to be isomeric with the major product. Its infrared spectrum with carbonyl absorption at 1660 cm^{-1} and bands for 1,2,3-substituted phenyl (750, 705 cm^{-1}) and 1,2,4-substituted phenyl (875, 812 cm^{-1}) suggested that the compound was 8-trifluoromethylphenothiazine-1-carboxylic acid. Conclusive evidence for this structure was obtained by an independent synthesis of the compound.

When a Smiles rearrangement was carried out on methyl 2-(2-formamido-4-trifluoromethylphenylmercapto)-3-nitrobenzoate and the product saponified, 8-trifluoromethylphenothiazine-1-carboxylic acid, identical with the minor product from the metalation reaction, was obtained. Raney nickel desulfurization of the material gave a compound that was identical with an authentic sample of N-(3-trifluoromethylphenyl)-anthranilic acid in all respects.

Nmr spectra of **2** and **3** are in accord with their structural assignments. In the spectrum of **2** the 2H proton is a quartet shifted downfield to δ = 7.76 ppm with J_{23} = 7.5 cps and J_{24} = 1.8 cps. The spectrum of **3** shows no X component of an AB*X* quartet consistent with the absence of a proton *ortho* to the carboxyl group.

The proportion of 2-trifluoromethylphenothiazine-1-carboxylic acid obtained by method A is further evidence of the inductive influence of the trifluoromethyl group on aromatic metalation reactions and agrees with the observations of Roberts and Curtin[9]

(1) American Foundation for Pharmaceutical Education Fellow, 1947–1950.

(2) V. E. Schenker and H. Herbst, *Progr. Drug Res.*, **5**, 269 (1963).

(3) R. Baltzly, M. Harfenist, and F. J. Webb, *J. Am. Chem. Soc.*, **68**, 2673 (1946).

(4) H. Gilman, D. A. Shirley, and P. R. VanEss, *ibid.*, **66**, 625, 1214 (1944).

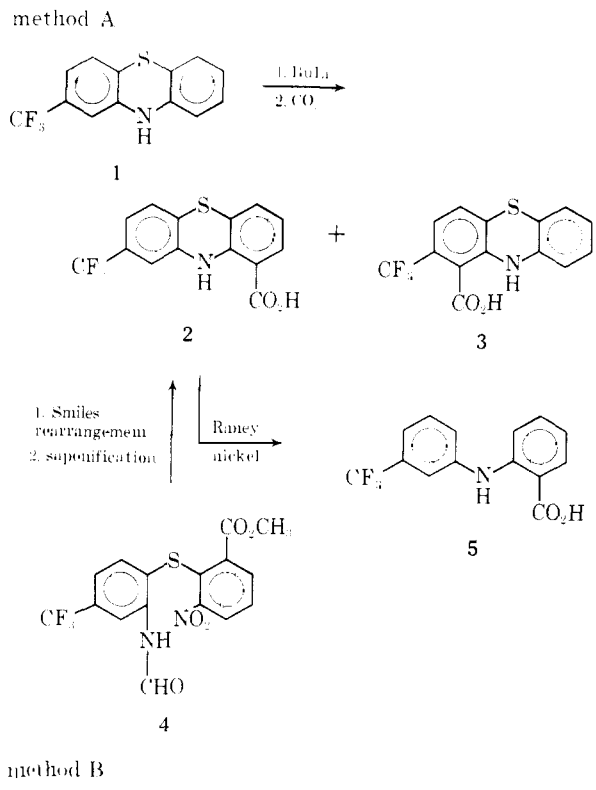
(5) A. B. Sen and R. C. Sharma, *J. Indian Chem. Soc.*, **35**, 202 (1958).

(6) W. J. Evans and S. Smiles, *J. Chem. Soc.*, 181 (1935).

(7) J. F. Bunnett and R. E. Zahler, *Chem. Rev.*, **49**, 362 (1951).

(8) J. S. Driscoll and R. H. Nealey, *J. Heterocyclic Chem.*, **2**, 272 (1965).

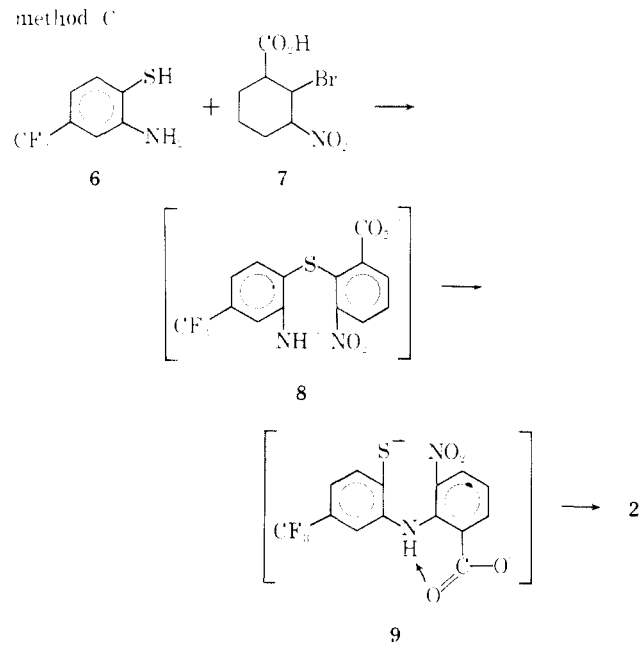
(9) J. D. Roberts and D. Y. Curtin, *J. Am. Chem. Soc.*, **68**, 1658 (1946).



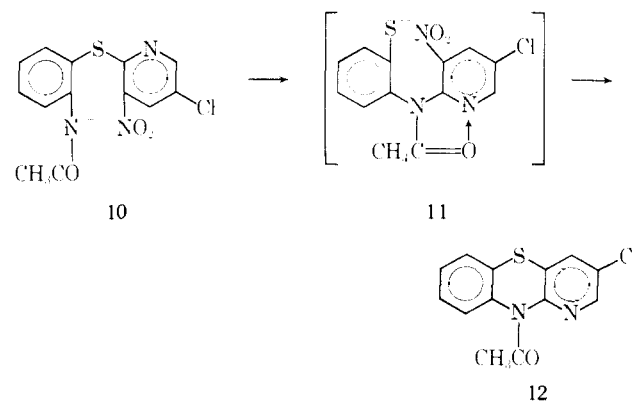
that this group activates the benzene ring to metalation. When 2-trifluoromethylphenothiazine is subjected to metalation with butyllithium, that position responsive to the inductive influence of the trifluoromethyl substituent is the major point of ring metalation and when carbonated leads primarily to formation of 2-trifluoromethylphenothiazine-1-carboxylic acid. Metalation in the unsubstituted ring is much less favored, as indicated by the small amounts of 8-trifluoromethylphenothiazine-1-carboxylic acid obtained.

Further work on improving the synthesis of the 8-substituted acid illustrated rather dramatically the effect of substituent groups on the course of the Smiles reaction. Our initial stepwise synthesis of 8-trifluoromethylphenothiazine-1-carboxylic acid followed the classical Smiles procedure. That is, 2-amino-4-trifluoromethylthiophenol and 2-bromo-3-nitrobenzoic acid were coupled under alkaline conditions to give 2-(2-amino-4-trifluoromethylphenylmercapto)-3-nitrobenzoic acid. After esterification, the intermediate was converted to the N-formyl derivative, which when heated with sodium hydroxide in ethanol–acetone formed 8-trifluoromethylphenothiazine-1-carboxylate ester.

Surprisingly, when 2-amino-4-trifluoromethylthiophenol and 2-bromo-3-nitrobenzoic acid were heated together in dimethylformamide in the presence of inorganic alkali at 120° or more, the phenothiazine acid formed without isolation of any intermediate or the need of amide activation for the Smiles rearrangement. We attribute the surprising ease of this sequence of reactions to the multiple aromatic substituents in the intermediate thioether. The strongly electronegative trifluoromethyl group in the amino thioether ring sufficiently decreases the basic nature of the primary amine so that it can become anionic under the reaction conditions and promote rearrangement to the diphenylamine. Presence of the bulky carboxyl group in the other ring now adjacent to the amino function stabilizes the conformation of the diphenylamine so that ring closure *via* elimination of the nitro group is facilitated.



A similar condition was described by Maki, *et al.*,[10] in preparing 3-chloro-10H-pyrido[3,2-*b*][1,4]benzothiazine from 2-(2-acetamidophenylmercapto)-5-chloro-3-nitropyridine. They suggested that when the bulky N-acetyl group in the intermediate diphenylamine coordinates with the pyrido nitrogen, the resulting conformation favorably locates the nitro group for elimination and ring closure.



Both 2-trifluoromethyl- and 8-trifluoromethylphenothiazine-1-carboxylic acid exhibit antiinflammatory activity in experimental animals, the 8 isomer (**2**) being more potent. In the guinea pig ultraviolet erythema assay[11] **2** has an oral ED_{50} of 4.3 mg/kg, while the 2-trifluoromethyl compound (**3**) does not afford protection below an oral dose of 40 mg/kg. The ED_{50} of phenylbutazone in this assay was 7.4 mg/kg.

8-Trifluoromethylphenothiazine-1-carboxylic acid (**2**) inhibited granuloma formation in the carrageenin-

(10) Y. Maki, M. Sato, and K. Yamane, *J. Pharm. Soc. Japan*, **85**, 429 (1965).

(11) C. V. Winder, J. Wax, V. Burr, M. Benn, and C. E. Rosiere, *Arch. Intern. Pharmacodyn.*, **116**, 261 (1958).

filter paper granuloma assay[12] in adrenalectomized rats when administered orally at levels as low as 5 mg/kg/bid or subcutaneously at 10 mg/kg/bid. The 2 isomer (**3**) was equally effective by subcutaneous administration but did not show oral activity when tested at levels as high as 80 mg/kg/bid. Phenylbutazone at a dose of 20 mg/kg/bid sc was used as a positive control in these procedures.

Experimental Section[13]

2-Trifluoromethylphenothiazine-1-carboxylic Acid (3).—A solution of 16.0 g (0.06 mole) of 2-trifluoromethylphenothiazine in 400 ml of anhydrous ethyl ether under a nitrogen atmosphere was maintained at 5 to 10° as 382 ml (0.60 mole) of a hexane solution of butyllithium[14] was added rapidly dropwise. The stirred mixture was maintained at 0–10° for 6 hr and was then poured onto solid CO_2. After the ice had melted, the mixture was acidified with aqueous 10% HCl. The organic layer was separated and extracted exhaustively with aqueous 1% NaOH. Combined alkaline extracts were acidified to pH 2 with 10% HCl and a crude solid was collected. It was redissolved in aqueous 1% NaOH, the solution was decolorized with activated charcoal, and the filtrate was carefully adjusted to pH 4 with acetic acid. On standing, 2.0 g of a greenish brown solid separated and was removed. The filtrate was further acidified to pH 2 with 36% HCl causing a larger precipitation. This second precipitate was recrystallized from a 1:1 mixture of acetic–trifluoroacetic acid to give 8.0 g of **3**: mp 199–200° dec; ultraviolet spectrum, λ_{max}^{EtOH} 320 mμ (ε 3500), 258 mμ (ε 27,100), $\lambda_{max}^{EtOH.NH_3}$ 318 mμ (ε 4200), 258 mμ (ε 34,300); infrared spectrum, principal bands 3310 (NH), 2600 broad (OH), 1655 (CO), 1300, 1180, 1125 (CF_3), 908 (OH dimer), 833 (1,2,3,4-substituted phenyl), 750 (1,2-substituted phenyl) cm^{-1}.

Anal. Calcd for $C_{14}H_8F_3NO_2S$: C, 54.04; H, 2.59; N, 4.50. Found: C, 54.26; H, 2.69; N, 4.29.

8-Trifluoromethylphenothiazine-1-carboxylic Acid (2). Method A.—The greenish brown solid and the residues from recrystallization solvents from above were combined[15] and were chromatographed on alumina,[16] using methanol containing 1% triethylamine as the developer. Three colored bands separated on the column and were collected by continued elution. The collection containing the slowest moving band was concentrated and the residue was redissolved in aqueous 1% NaOH. On acidification of the solution to pH 5 a yellow solid separated, 0.55 g, mp 242–245° dec.

Method B. 2-(2-Amino-4-trifluoromethylphenylmercapto)-3-nitrobenzoic Acid.—A mixture of 2.19 g (0.01 mole) of 2-amino-4-trifluoromethylthiophenol hydrochloride, 2.46 g (0.01 mole) of 2-bromo-3-nitrobenzoic acid, and 2.8 g (0.02 mole) of anhydrous K_2CO_3 were mixed in 50 ml of DMF and stirred at room temperature for 24 hr. The yellow suspension was poured into 150 ml of distilled water and the alkaline solution was extracted once with ether. The aqueous phase was separated and acidified with 1:1 aqueous phosphoric acid. The yellow crude solid was separated (2.1 g), mp 160–163°. An analytical sample was recrystallized from toluene, mp 154–155°.[17]

Anal. Calcd for $C_{14}H_9F_3N_2O_4S$: C, 46.93; H, 2.53; N, 7.82. Found: C, 47.03; H, 2.79; N, 7.67.

Methyl 2-(2-Amino-4-trifluoromethylphenylmercapto)-3-nitrobenzoate.—A solution of 14.3 g (0.04 mole) of 2-(2-amino-4-trifluoromethylphenylmercapto)-3-nitrobenzoic acid in 300 ml of methanol was cooled to 0° and saturated with HCl gas. After standing at room temperature for 60 hr, the solution was concentrated under reduced pressure and the residue was recrystallized from methylcyclohexane to give 12.3 g of ester. An analytical sample was recrystallized from methylcyclohexane, mp 105–105.5°.

Anal. Calcd for $C_{15}H_{11}F_3N_2O_4S$: C, 48.39; H, 2.89; N, 7.52. Found: C, 48.54; H, 3.07; N, 7.40.

Compound 2.—Acetic anhydride (24 ml) was stirred into a solution of 10.6 g (0.028 mole) of methyl 2-(2-amino-4-trifluoromethylphenylmercapto)-3-nitrobenzoate in 75 ml of formic acid (95–100%), and the mixture was allowed to stand at room temperature for 4 hr. Solvent acids were removed under reduced pressure, the residue was stirred into a mixture of 120 ml of 1 *N* alcoholic NaOH solution and 120 ml of acetone, and the mixture was heated under reflux for 1 hr. The reaction mixture was concentrated *in vacuo* to a solid mass which was extracted with ethyl ether. The ether extracts were combined and concentrated to a semisolid that solidified on stirring in water. The solid was removed, washed, and dried to give 5.11 g of the ethyl ester of **2**, mp 85–89°. An analytical sample was obtained by recrystallizing from 2-propanol, mp 95–96°.

Anal. Calcd for $C_{16}H_{12}F_3NO_2S$: C, 56.63; H, 3.56; N, 4.18. Found: C, 56.63, 56.76; H, 3.56, 3.52; N, 4.18.

The crude ester from above was saponified with aqueous alcoholic NaOH and the basic solution was acidified with glacial acetic acid to give 3.94 g of **2**, mp 234–240° dec.

Method C.—A mixture of 2.19 g (0.010 mole) of 2-amino-4-trifluoromethylthiophenol hydrochloride, 3.7 g (0.015 mole) of 2-bromo-3-nitrobenzoic acid, and 5.5 g (0.4 mole) of K_2CO_3 in 30 ml of DMF was heated at 120–125° for 2 hr. The mixture was poured into 10 vol of 10% acetic acid, and the brown solid was removed and dried *in vacuo* (4.0 g), mp 218–230° dec. Reprecipitation from aqueous alkali and recrystallization from toluene gave 1.9 g (61%) of analytical material: mp 247–248° dec; ultraviolet spectrum, λ_{max}^{EtOH} 366 mμ (ε 4700), 259 mμ (ε 29,000), $\lambda_{max}^{EtOH.NH_3}$ 341 mμ (ε 5900), 263 mμ (ε 31,400), infrared spectrum, principal bands, 3820 (NH) 2600 broad, (OH), 1658 (CO), 1360, 1248, 1220 (CF_3), 910 (OH dimer) 875, 812 (1,2,4-substituted phenyl), 750, 705 (1,2,3-substituted phenyl) cm^{-1}.

Anal. Calcd for $C_{14}H_8F_3NO_2S$: C, 54.02; H, 2.59; N, 4.50. Found: C, 54.02; H, 2.66; N, 4.22.

Samples of **2** prepared by methods A, B, and C gave satisfactory elemental analyses and showed identical spectral and thin layer chromatographic characteristics.

Raney Nickel Desulfurization of 2.—A solution of 1.6 g (0.005 mole) of **2** and 0.4 g (0.003 mole) of K_2CO_3 in 50 ml of distilled water was mixed with 2 teaspoonfuls of activated Raney nickel and the mixture was heated under reflux for 2 hr. The resulting suspension was filtered and the filtrate was acidified with glacial acetic acid. The solid that separated (1.37 g) showed mp 131–133°.[18] The melting point was not depressed when mixed with an authentic sample of N-(3-trifluoromethylphenyl)anthranilic acid prepared by the condensation of *o*-chlorobenzoic acid and *m*-aminobenzotrifluoride. Infrared spectra of the material from desulfurization and authentic N-(3-trifluoromethylphenyl)-anthranilic acid were identical.

Acknowledgment.—The authors are indebted to Dr. Walter Thompson and Miss Margaret Carroll and their associates for the spectra and microanalyses.

(12) Modification of the methods reported by R. Meier, W. Schuler, and P. Desaulles, *Experientia*, **6**, 469 (1950), and A. Tanaka, F. Kobayaski, and T. Miyake, *Endocrinol. Japon.*, **7**, 357 (1960).

(13) All melting points were taken in a Thomas–Hoover melting point apparatus and are corrected.

(14) Ten grams of butyllithium/100 ml, Foote Mineral Co.

(15) Silica gel G thin layer chromatographic analysis ($CHCl_3$–EtOAc–acetic acid (80:20:0.5) used as a developer) of this mixture showed three major ultraviolet fluorescent materials, R_f 0.60, 0.67, and 0.90. The material at R_f 0.60 corresponded to previously isolated **3**, that at R_f 0.67 to isomeric **2**, and that at R_f 0.90 to unreacted **1**.

(16) Activity grade 4, aluminum oxide, Woelm neutral.

(17) When the melt was heated further, it solidified and remelted at 273–276° dec. The second melting point proved to be characteristic of the internal amide 4-nitro-8-trifluoromethyldibenzo[*b*,*f*][1,4]thiazepin-11-one.

(18) R. Moffett and B. Aspergen [*J. Am. Chem. Soc.*, **82**, 1600 (1960)] reported mp 134–136°.