Synthesis of Some Derivatives of 4-Phenoxathiincarboxylic Acid

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The preparation of a series of amino acid derivatives of 4-phenoxathiincarboxylic acid is described.

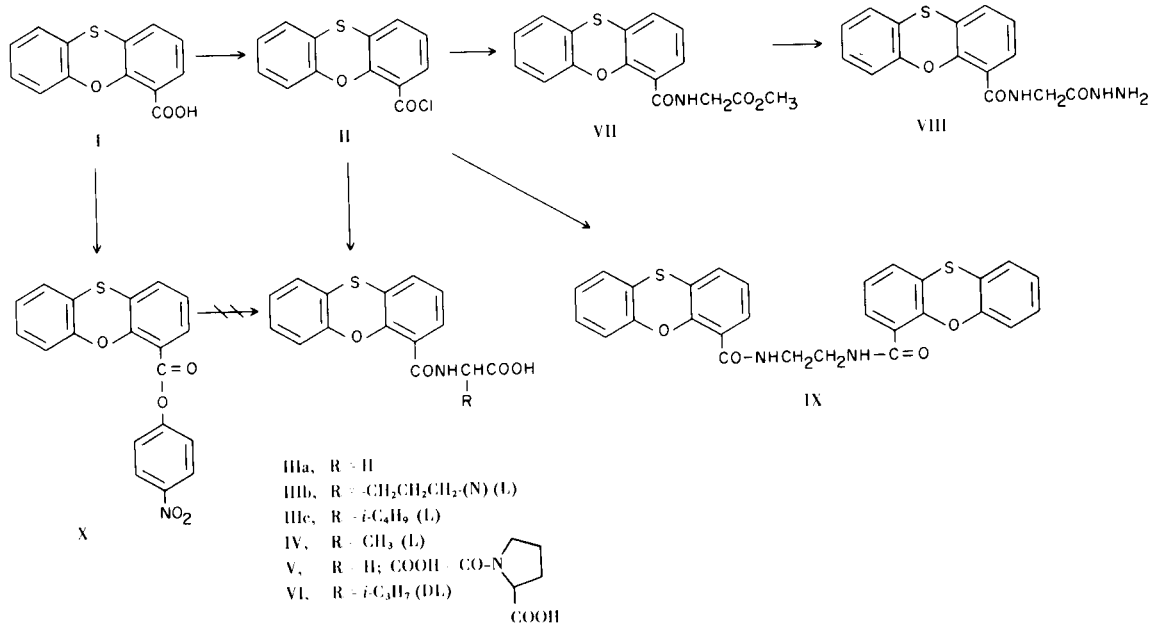
Actinomycin is a bright red, heteroaroyl peptide reported by Waksman and Tishler in 1942 as isolated from cultures of *Streptomyces antibioticus* (2). The structure of actinomycin was the subject of extensive investigations by Brockmann and Johnson during the past twenty years (3). Although a powerful antibiotic, actinomycin is highly toxic to humans and therefore the antibiotic activity was of little value. Several investigators (4a-c) recently have described a cytostatic activity. This has revived the chemotherapeutic interest in the compound and considerable attention has been given to the synthesis of actinomycin analogs in an attempt to reduce the toxicity while retaining the other activity (5-11).

The interesting pharmacological properties of the complex heteroaroyl polypeptide, actinomycin, suggested the possibility of potential activity of simple heteroaroylamino acids (12,13). The potent pharmacological activity of phenoxathiin led to the choice of this heteroaroyl function for initial investigations.

This paper describes the synthesis of some derivatives of 4-phenoxathiincarboxylic acid.

4-Phenoxathiincarboxylic acid (I), prepared by the carbonation of the *n*-butyllithium metalation product of phenoxathiin (14), was chlorinated with thionyl chloride to yield 4-phenoxathiincarboxylic acid chloride (II). The reaction of II with glycine, L-proline, L-leucine, L-alanine, glycyl-L-proline, and *dl*-valine by the Schotten-Baumann technique yielded 4-phenoxathiincarbonylglycine (IIIa), 4-phenoxathiincarbonyl-L-proline (IIIb), 4-phenoxathiincarbonyl-L-leucine (IIIc), 4-phenoxathiincarbonyl-L-alanine (IV), 4-phenoxathiincarbonyl-glycyl-L-proline (V), and 4-phenoxathiincarbonyl-*dl*-valine (VI), respectively. Each of the amino acid amides gave the infrared spectrum anticipated for these compounds: 3400 cm^{-1} (-NH), 1720 cm^{-1} (-carboxyl), and 1630 cm^{-1} (-amide). The optically active amino acids were not racemized in this reaction.

Treatment of II with methyl glycinate hydrochloride



(15) affords 4-phenoxathiincarbonylglycine methyl ester (VII) as an oil, which on treatment with hydrazine hydrate gave 4-phenoxathiincarbonylglycyl hydrazide (VIII) in good yield. Interaction of two equivalents of II with one equivalent of ethylenediamine in dioxane, in the presence of triethylamine, readily yielded *N,N'*-ethylenebis(4-phenoxathiincarboxamide) (IX).

p-Nitrophenyl 4-phenoxathiincarboxylate (X) was prepared by coupling I and *p*-nitrophenol in ethyl acetate with *N,N'*-dicyclohexylcarbodiimide (DCC). Attempts to prepare the amino acid amides, using X as an intermediate were unsuccessful.

EXPERIMENTAL

All melting points are uncorrected. Analyses are obtained from Schwarzkopf Microanalytical Laboratory, Woodside, New York. Ultraviolet spectra were obtained with a Perkin-Elmer Spectracord 4000 in absolute ethanol solution. Infrared spectra were obtained on a Perkin-Elmer infracord determined as mulls in series 11-41 Halocarbon oil from 4000 to 1300 cm^{-1} and in Nujol from 650 to 1300 cm^{-1}.

4-Phenoxathiincarboxylic Acid (I).

This compound was prepared in yields of approximately 70% by a metalation reaction of phenoxathiin using one equivalent of *n*-butyllithium according to the procedure of Gilman (14). The crude product was recrystallized from glacial acetic acid to yield I, m.p. 171-173° (lit. (14), m.p. 171-172°).

4-Phenoxathiincarboxylic Acid Chloride (II).

Heating 2.44 g. (0.01 mole) of 4-phenoxathiincarboxylic acid (I) under reflux for 0.5 hour with an excess of thionyl chloride (5 ml.) completed the reaction. The resulting solution was concentrated under reduced pressure to remove the excess of thionyl chloride. The residual acid chloride solidified as a golden yellow solid on cooling, m.p. 65-75°; ir ν max 1800 cm^{-1} (-COCl). The product (II) was obtained in quantitative yield.

4-Phenoxathiincarbonylglycine (IIIa).

A solution of 0.92 g. (12.3 mmoles) of glycine 9.80 g. (24.6 mmoles) of a 10% sodium hydroxide solution, and 2.80 g. (10.6 mmoles) of 4-phenoxathiincarboxylic acid chloride (II) was stirred for 0.25 hour. The mixture was filtered and the filtrate was acidified with 5% hydrochloric acid to give 2.4 g. of a precipitate, m.p. 140-180°. After washing the solid several times with 100 ml. portions of ether the solid was recrystallized from methyl alcohol with Norit treatment and gave IIIa, m.p. 220.5-222.5°; ir ν max 3400, 1720, and 1620 cm^{-1}.

Anal. Calcd. for $C_{15}H_{11}NO_4S$: C, 59.78; H, 3.68. Found: C, 59.58; H, 3.63.

4-Phenoxathiincarbonyl-L-proline (IIIb).

A solution of 3.00 g. (11.4 mmoles) of 4-phenoxathiincarboxylic acid chloride (II), 1.31 g. (11.4 mmoles) of L(-)-proline, and 23.0 g. (22.3 mmoles) of a 4% sodium hydroxide solution was stirred for 3 hours and the product was precipitated by the addition of 1*N* hydrochloric acid. The product was triturated with 95% ethyl alcohol and ligroin (66-75°) to give 0.31 g. (7.8%) of IIIb, m.p. 110-114° dec.; ir ν max 3400, 1720 and 1600 cm^{-1}.

Anal. Calcd. for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43. Found: C, 63.45; H, 4.33.

4-Phenoxathiincarbonyl-L-leucine (IIIc).

A solution of 2.63 g. (0.01 mole) of II in 5 ml. of dry benzene was added to a mechanically stirred solution of L-leucine (1.31 g., 0.01 mole) in 10 ml. of 2*N* sodium hydroxide. During the reaction period, the solution was kept alkaline (Thymol blue) with 2*N* sodium hydroxide. After the addition was completed, the stirring was continued for 2 hours and the solution was acidified (Congo red) with 2*N* hydrochloric acid while cooling in an ice bath. The product was collected by filtration, washed with water, and recrystallized from ethanol. The product weighed 1.46 g. and another 0.92 g. was obtained from the mother liquor representing a total yield of 67%. Further recrystallization of the solid from aqueous ethanol gave pale yellow crystals, m.p. 185-186°; ir ν max 3400, 1720, and 1630 cm^{-1}; $[\alpha]_D^{25}$ -104° (c, 0.25 absolute ethanol).

Anal. Calcd. for $C_{19}H_{19}NO_4S$: C, 63.84; H, 5.37. Found: C, 63.64; H, 5.39.

4-Phenoxathiincarbonyl-L-alanine (IV).

This compound was prepared in a manner similar to the synthesis of III. From 2.63 g. (0.01 mole) of II and 0.89 g. (0.01 mole) of L-alanine, 2.16 g. (69%) of 4-phenoxathiincarbonyl-L-alanine (IV) m.p. 198-199° was obtained; ir ν max 3400, 1720, and 1630 cm^{-1}; $[\alpha]_D^{25}$ -96° (c, 0.25 absolute ethanol).

Anal. Calcd. for $C_{16}H_{13}NO_4S$: C, 60.94; H, 4.15. Found: C, 60.69; H, 4.14.

4-Phenoxathiincarbonylglycyl-L-proline (V).

Following the procedure for the synthesis of III 0.53 g. (0.002 mole) of II and glycyl-L-proline (16) (0.35 g., 0.002 mole) gave 0.52 g. (65%) of 4-phenoxathiincarbonylglycyl-L-proline (V), m.p. 110-112°; ir ν max 3400, 1720, and 1640 cm^{-1}; $[\alpha]_D^{25}$ -76° (c, 0.25, absolute ethanol).

Anal. Calcd. for $C_{20}H_{18}N_2O_5S$: C, 60.29; H, 4.55. Found: C, 60.16; H, 4.86.

4-Phenoxathiincarbonyl-*dl*-valine (VI).

From 1.17 g. (0.01 mole) of *dl*-valine and 2.63 g. (0.01 mole) of II gave 2.93 g. (85%) of 4-phenoxathiincarbonyl-*dl*-valine (VI), m.p. 190-191°; ir ν max 3400, 1720, and 1630 cm^{-1}.

Anal. Calcd. for $C_{18}H_{17}NO_4S$: C, 62.95; H, 4.99. Found: C, 62.78; H, 5.12.

4-Phenoxathiincarbonylglycine Hydrazide (VIII).

Finely powdered glycine methyl ester hydrochloride (1.26 g., 0.01 mole) was added to a solution of 2.63 g. (0.01 mole) of II in 25 ml. of dry benzene, and the mixture was heated under reflux for 16 hours. Any undissolved material was separated from the hot solution. The filtrate was cooled, and no precipitate was formed. The solvent was then removed by distillation under reduced pressure. The oily product (VII) was redissolved in 40 ml. of methanol and heated on the steam-bath for 1.5 hours with 85% hydrazine hydrate (1 ml.). The pale yellow crystals which separated on cooling were recrystallized from methanol to give 1.42 g. of 4-phenoxathiincarbonylglycine hydrazide (VIII), m.p. 235-236°; ir ν max 3350, 2900, 1700, and 1640 cm^{-1}. By gradual concentration of the mother-liquors a further quantity of the product (0.84 g.) was obtained. The total weight represented 72% yield.

Anal. Calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.32. Found: C, 56.63; H, 4.19; N, 13.00.

N,N'-Ethylenebis(4-phenoxathiincarboxamide) (X).

A solution of 2.63 g. (0.01 mole) of II in 40 ml. dioxane was

added dropwise to a stirring solution of 1.5 ml. (0.01 mole) of triethylamine and 0.4 ml. (0.005 mole) of ethylenediamine. A precipitate appeared almost immediately. After the addition was completed the mixture was heated under reflux for 20 minutes, cooled, and filtered. The crude product was washed several times with water and recrystallized from dimethylformamide-water to give 2.51 g. (99%) of pale yellow powder, m.p. 247-249°; ir ν max 3350, 1630, 1600 and 1530 cm^{-1}.

Anal. Calcd. for $C_{28}H_{20}N_2O_4S_2$: C, 65.61; H, 3.93. Found: C, 65.84; H, 4.01.

p-Nitrophenyl-4-phenoxathiincarboxylate (X).

A solution of 2.44 g. (0.01 mole) of I and 1.67 g. (0.012 mole) of *p*-nitrophenol in 60 ml. of ethyl acetate was stirred and cooled in an ice-bath while 2.06 g. (0.01 mole) of dicyclohexylcarbodiimide in 5 ml. ethyl acetate was added at 0°. The mixture was stirred at 0° for 0.5 hour and for an additional 4 hours at room temperature. After the addition of 0.5 ml. of glacial acetic acid, the dicyclohexylurea was removed by filtration and washed with ethyl acetate. The combined filtrates were removed by distillation under reduced pressure, and the residue was crystallized from hot ethanol to yield 2.41 g. (66%) of *p*-nitrophenyl 4-phenoxathiincarboxylate (X) as yellow needles, m.p. 158-159.5°; ir ν max 1740 cm^{-1} (ester CO), 1600, 1510 and 1350 cm^{-1} (NO_2), 750 and 735 cm^{-1} (substituted phenyls).

Anal. Calcd. for $C_{19}H_{11}NO_5S$: C, 62.46; H, 3.03. Found: C, 62.59; H, 3.29.

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Anal. Calcd. for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.03. Found: C, 48.59; H, 7.16.