# Antiradiation Compounds XI. Sulfur-Containing Derivatives of Nitriles and Aminonitriles

By WILLIAM O. FOYE and JOEL M. KAUFFMAN

**Attempts to obtain iminothio esters and dithio esters from α-aminoacetonitrile gave stable products only with the amino group acylated by the carbobenzoxy function. An iminothio ester derived from β-aminopropionitrile was found to be relatively stable as the dihydrochloride, however. Examples of both α- and β-cyanoalkylthiosulfates were found to be stable as the sodium salts. Antiradiation testing of the β-amino iminothio ester dihydrochloride and the α-cyanothiosulfate showed no protective properties.**

INORGANIC CYANIDES have shown radiation-protective properties in animals (1), but relatively few organic nitriles have been tested for this property. Malononitrile (2), hydroxyacetonitrile (3), 2-hydroxybutyronitrile (4), and α-benzyloxyiminopropionitrile (5) have all shown some degree of radiation protection, and several examples of cyano-containing dithio acid dianions (6) are radiation protective. The *N*-cyano derivative of 2-mercaptoethylguanidine (MEG) has also been found protective in animals, but less so than MEG itself (7). An attempt has been made, therefore, to obtain sulfur-containing derivatives of organic nitriles which might have protective ability, and in particular those which might lead to sulfur-containing derivatives of amino acids *in vivo*. Glycine itself is radioprotective for some enzymes (8), and its thioamide has shown radiation-protective properties in plants (9).

In regard to the synthesis of amino dithio acids, no method of liberating an amine or amine salt from an N-blocked derivative in the presence of a dithiocarboxylic acid or ester is known. It appeared possible to obtain dithio esters or iminothio esters with free amino groups, however. Accordingly, benzyl cyanomethylcarbamate (I) was prepared

$$C_6H_5CH_2OCONHCH_2CN \xrightarrow[HCl]{C_2H_5SH}$$

I

$$C_6H_5CH_2OCONHCH_2\overset{\overset{NH_2^+Cl^-}{\|}}{C}SC_2H_5 \xrightarrow[Na_2CO_3]{H_2S}$$

II

$$C_6H_5CH_2OCONHCH_2CS_2C_2H_5$$

III

from carbobenzoxy chloride, aminoacetonitrile bisulfate, and sodium carbonate. Although the preparation of I from aminoacetonitrile hydrochloride with gradual addition of sodium hydroxide has been reported (10), the method described here gives a cleaner product with less trouble, since carbonate provides the optimum pH for the reaction. The product can also be recrystallized rather than reprecipitated. Reaction with ethanethiol and hydrogen chloride gave the iminothio ester (II), which was converted to the dithio ester (III) with hydrogen sulfide and sodium carbonate. The iminothio ester (II) remained stable only long enough for chlorine and nitrogen analyses, which could be run immediately after isolation of the product. Characteristic absorption for C=S in (III) was shown in the infrared at 1050 cm.$^{-1}$ and in the ultraviolet at 270 and 365 mμ.

Attempts to remove the carbobenzoxy group from the α-aminodithio ester were made with hydrogen bromide in acetic acid, hydrogen chloride in acetic acid, trifluoroacetic acid, and phosphonium iodide. All of these methods, as well as aqueous procedures, failed. Attempts to thiohydrolyze the dithio ester (III) to the dithio acid with hydrogen sulfide and triethylamine also failed.

Benzyl cyanoethylcarbamate (IV) was prepared from aminopropionitrile fumarate and sodium carbonate rather than from the free amine and hydroxide as previously reported (11). Reaction with methanethiol and hydrogen chloride gave the iminothio ester (V), which surprisingly had suffered loss of the carbobenzoxy group. Apparently, a distance of two carbons is required between an unblocked amino and iminothio ester functions for stability.

$$C_6H_5CH_2OCONHCH_2CH_2CN \xrightarrow[HCl]{CH_3SH}$$

IV

$$Cl^-{}^+NH_3CH_2CH_2\overset{\overset{NH_2^+Cl^-}{\|}}{C}SCH_3$$

V

To obtain compounds containing both cyano and reactive sulfur functions, the preparation of sodium cyanomethylthiosulfate was attempted using chloroacetonitrile and sodium thiosulfate. Analysis indicated the product (VI) to be a monohydrate, but the infrared spectrum showed only weak absorption for a cyano group and much stronger absorption at 1,640 cm.$^{-1}$. Suspecting that the product might be the amide rather than the nitrile, the amide (VII) was prepared from chloroacetamide and sodium thiosulfate. The amide differed in crystalline form, solubility, infrared absorption, melting behavior, and $R_f$ value on thin-layer chromatography, thus confirming that the nitrile had been obtained. The homologous cyanoethylthiosulfate was also prepared, and again obtained as the monohydrate.

$Na^+{}^-O_3SSCH_2CN \cdot H_2O$ VI

$Na^+{}^-O_3SSCH_2CONH_2$ VII

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**Antiradiation Screening**—Results obtained from the Walter Reed Army Institute of Research, through the courtesy of Drs. D. P. Jacobus and T. R. Sweeney, showed that Compounds V and VI provided no protection in mice. Compound V was tested *versus* 1,000r ($\gamma$-rays) and Compound VI was tested *versus* 825r (X-rays), both compounds administered at dose levels of less than 50 mg./kg.

## EXPERIMENTAL

Melting points were determined in capillaries on a Mel-Temp block (Laboratory Devices Co.) and are corrected. Elemental analyses were determined by Dr. Carol K. Fitz, Needham, Mass. Infrared absorption spectra were obtained with a Perkin-Elmer model 137B spectrometer with sodium chloride optics. Evaporations were done using a rotary evaporator at 40 mm.

**Benzyl Cyanomethylcarbamate (I)**—Aminoacetonitrile bisulfate (89 g., 0.58 mole) (Aldrich Chemicals), water (500 ml.) and ice (500 g.) were stirred while sodium carbonate monohydrate (220 g., 1.77 moles) was added. Additional ice (1 kg.) and ether (1 l.) were added, the temperature of the ether layer dropped to −2°, and carbobenzoxy chloride (94 g., 0.55 mole) (Nutritional Biochemicals) was poured in at once. Stirring was continued overnight as the mixture warmed to room temperature, and the ether layer was dried ($MgSO_4$) and evaporated. The residue was recrystallized from carbon tetrachloride–methanol, giving 73.7 g. (70%) of white needles, m.p. 61–63° [lit. (10) m.p. 62°]; $\nu_{min.}^{KBr}$ 3320 (NH), 2260 (very weak) (C≡N), 1700 (C=0) cm.$^{-1}$.

**Benzyl 2-Ethylthio-2-iminoethylcarbamate Hydrochloride (II)**—Benzyl cyanomethylcarbamate (1.9 g., 0.01 mole), ethanethiol (1.5 ml., 0.02 mole) (Eastman Organic Chemicals), and dry ether (20 ml.) were treated with hydrogen chloride at 4° for 30 min. The solution became cloudy and deposited 3.2 g. (100%) of white product after 3 days storage at 4°; m.p. 138–142° (dec.). The analytical sample was obtained by recrystallization from chloroform–ligroin (1:1); m.p. 135–141° (dec.); $\nu_{min.}^{CHCl_3}$ 2860 ($NH^+$), 1720 (C=O) cm.$^{-1}$.

*Anal.*—Calcd. for $C_{12}H_{17}ClN_2O_2S$: Cl, 12.28; N, 9.69. Found: Cl, 12.71; N, 9.88.

**Ethyl N-Carbobenzoxydithioglycinate (III)**—The iminothio ester (II) (1.9 g., 0.007 mole) and sodium carbonate (3.2 g., 0.03 mole) were covered with 30 ml. of dry ether and treated with dry hydrogen sulfide ($CaCl_2$) at a rate sufficient to stir the mixture for 4.5 hr. Filtration and removal of solvent from the filtrate in a dry nitrogen stream left 0.4 g. (22%) of bright yellow crystals, m.p. 60–64°, 150° (dec.). The compound was insoluble in cold alkaline solution, but soluble in sodium hydroxide solution after warming for 5 min. The analytical sample was obtained by recrystallization from ether–ligroin, m.p. 64–65°; $\nu_{min.}^{CCl_4}$ 1730 (C=O), 1050 (C=S) cm.$^{-1}$; $\lambda_{max.}$ ($CH_3OH$) 270 ($\epsilon$ 14), 365 ($\epsilon$ 16) m$\mu$.

*Anal.*—Calcd. for $C_{12}H_{15}NO_2S_2$: C, 53.60; H, 5.62; N, 5.21. Found: C, 53.64; H, 5.56; N, 5.22.

**Benzyl 2-Cyanoethylcarbamate (IV)**—2-Aminopropionitrile fumarate (15.4 g., 0.06 mole) (Aldrich Chemicals) was carbobenzoxylated by the method above. In the purification of the product, propanol was added to the ether solution until all organic solid was dissolved. Dilution of the dried solution with ligroin and cooling gave glistening white plates in two crops totaling 17.6 g. (72%); m.p. 69–71° [lit. (11) m.p. 74°]; $\nu_{min.}^{KBr}$ 3310 (NH), 2250° (weak) (C≡N), 1695 (C=O) cm.$^{-1}$.

**3-Imino-3-methylthiopropylamine Dihydrochloride (V)**—This reaction, and all handling of the product, was carried out under dry nitrogen. The previous product (10.2 g., 0.05 mole) was dissolved in 300 ml. of dry 1,2-dimethoxyethane (distilled over sodium hydride), cooled to 5°, treated with 6 ml. of methanethiol (Eastman Organic Chemicals), and saturated with hydrogen chloride. Crystal formation appeared complete in 1 day. Solvent was decanted after 1 week, and the white, crystalline residue was washed with 70 ml. of dry 1,2-dimethoxyethane and vacuum dried, giving 9.1 g. (95%), m.p. 203–208° (dec.). It was hygroscopic and soluble in cold water. Analyses were obtained for chlorine and nitrogen immediately after isolation, but the product suffered partial decomposition before carbon-hydrogen analyses could be obtained.

*Anal.*—Calcd. for $C_4H_{12}Cl_2N_2S$: Cl, 37.1; N, 14.7. Found: Cl, 37.0; N, 14.6.

**Sodium Cyanomethylthiosulfate, Monohydrate (VI)**—Sodium thiosulfate pentahydrate (59.6 g., 0.2 mole) was dissolved in 50 ml. of distilled water and treated with 50 ml. of 95% ethanol and then chloroacetonitrile (15.1 g., 0.2 mole) (Aldrich Chemicals). The mixture was boiled under reflux for 20 min., cooled, and freed of solvent. The residue was leached with 100 ml. of boiling 95% ethanol, then with another 20-ml. portion of the same, and the extracts were cooled at −5° to give 32.0 g. (86%) of white prisms. Recrystallization from 95% ethanol gave the analytical sample; m.p. 150° (dec.); $\nu_{min.}^{KBr}$ 3490 (OH), 2250 (C≡N), 1640 (CH), 1380 ($SO_2$), 1220 ($SO_2$), 1040 cm.$^{-1}$.

*Anal.*—Calcd. for $C_2H_4NNaO_4S_2$: C, 12.43; H, 2.10; N, 7.25; Na, 11.9. Found: C, 12.5; H, 1.9; N, 7.35; Na, 11.9.

The Feigl spot test (12) for an aliphatic nitrile was strongly positive. On Eastman Chromagram sheets, using propanol–water, and iodine vapor as developer, this nitrile had an $R_f$ = 0.44, whereas the amide (VII) had an $R_f$ = 0.25.

**Sodium Carboxamidomethylthiosulfate (VII)**—Chloroacetamide (13) (2.4 g., 0.026 mole) in 12 ml. of warm ethanol solution and sodium thiosulfate pentahydrate (6.4 g., 0.026 mole) in 12 ml. of warm water were mixed and refluxed for 1 hr. The solvent was evaporated, and the residue was taken up in 15 ml. of ethanol with additional water for complete solution, and white needles precipitated at 5° (2.6 g., 55%); m.p. 180–203° (dec.); $\nu_{min.}^{KBr}$ 1670 (C=O), 1260 ($SO_2$), 1200 ($SO_2$), 1040 cm.$^{-1}$.

*Anal.*—Calcd. for $C_2H_4NNaO_4S_2$: C, 12.43; H, 2.10; N, 7.25. Found: C, 12.56; H, 2.44; N, 7.24.

**Sodium Cyanoethylthiosulfate Monohydrate**—The same procedure as used for VI was employed with 2-bromopropionitrile (26.8 g., 0.2 mole) and sodium thiosulfate pentahydrate (59.6 g., 0.2 mole). After 1 hr. of refluxing, 33.4 g. (81%) of white solid was extracted and washed with cold 95% ethanol; infrared peaks were similar to those of VI; m.p. 250° (dec.).

*Anal.*—Calcd. for $C_3H_6NNaO_4S_2$: C, 17.40; H, 2.92; N, 6.76. Found: C, 17.58; H, 3.60; N, 6.10.

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**Keyphrases**

Antiradiation compounds
Nitriles, aminonitriles—sulfur derivatives
Paper chromatography—identity
IR spectrophotometry—structure
UV spectrophotometry—structure

# Pyrrolo [2,3-*d*]Pyrimidines

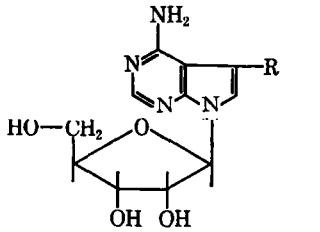
By RICHARD H. HAMMER

**The synthesis of a series of 4-alkylamino-7-methyl analogs of tubercidin from 4-chloro-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine is described.**

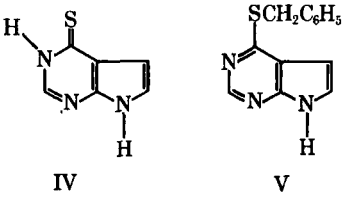
TUBERCIDIN (4-amino-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine) (I) and two other closely related naturally occurring nucleosides containing the pyrrolo[2,3-*d*]pyrimidine structure, toyocamycin (II), and sangivamycin (III), show pronounced cytotoxic activity (1–3). As a result of this activity and the unique stability of the base-sugar bond to enzymatic (4) and *in vitro* acid cleavage,[1] considerable interest in the synthesis and evaluation of tubercidin analogs has developed.

Recently, Gerster *et al.* (5) synthesized a series of 4-substituted derivatives of tubercidin with the ribofuranosyl group intact on the 7 position. Montgomery and Hewson (6) have observed a decrease in cytotoxic activity, compared to tubercidin, when substituting aliphatic and cycloaliphatic groups at the 7 position with a free amino group in the 4 position. Cell-culture cytotoxity studies on Compounds IV and V (Table III), synthesized in this laboratory (7), indicate an increase in activity when the bulky, lipophilic benzyl group is substituted on the mercapto group of IV. These observations prompted the synthesis of a series of 4-alkylamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines (VIII*a*-VIII*f*) where the series extends from the simpler alkyls, such as ethyl, to heterocyclic rings such as pyrrolidyl and piperidyl. In order to simulate the pKa of tubercidin, the described compounds all have a methyl group substituted on the 7-nitrogen.

Synthesis of these compounds has been accomplished through methylation of 4-chloro-7*H*-pyrrolo-[2,3-*d*]pyrimidine (VI) with methyl iodide (8) and reaction of this 7-methyl product (VII) with the appropriate amine in a Parr bomb at elevated temperatures to give the respective 4-alkylamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine compound (VIII*a*–VIII*f*).



I, R = H
II, R = C≡N
III, R = C(=O)—NH$_2$



IV V

## EXPERIMENTAL

Melting points were obtained on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Ultraviolet analysis was recorded on a Beckman model DB spectrophotometer. Titrimetric pKa values were obtained on a Sargent titrator, model D at 25°. NMR spectra were obtained on a Varian A-60 instrument at a field strength of 60 Mc./sec. Microanalysis was conducted by Galbraith Laboratories, Inc., Knoxville, Tenn.

**Method A**—To 0.175–0.20 g. of VII (8) was

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[1] Preliminary degradation studies in this laboratory show tubercidin to be completely stable for 66 hr. in 0.1 *N* HCl at 80° compared to a half-life of 72 min. for adenosine under identical conditions.