Fasted mixed white mice were used to screen the tosylthiocarbamates for hypoglycemic activity. The compounds were suspended in 5% acacia and administered intraperitoneally in a dose of 0.1 ml. After 1 hr., a sample of blood was obtained by decapitation and after immediate centrifuging, a serum sample was collected.

No hypoglycemic activity was obtained in the tosylbiuret series signifying that these molecules do not accommodate well with the receptor sites necessary to initiate the release of endogenous insulin.

n-Propyl *N*-tosylthiocarbamate was shown to be approximately as active as tolbutamide when tested in mice according to the enzymatic method described in this communication.

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Keyphrases

Tosylbiurets, thiocarbamates—synthesis

Propyl tosylthiocarbamate—hypoglycemic activity

Colorimetric analysis—spectrophotometer

Antiradiation Compounds XII. Dithiocarbamates of Strongly Basic Acridines and Quinaldines

By WILLIAM O. FOYE, DOUGLAS H. KAY, and PRAMOD R. AMIN*

Dithiocarbamates of strong heterocyclic bases (pKa 8–12) in the acridine and quinaldine series have been obtained, with further examples of the formation of imino-*N*-carbodithioates. Significant radiation protection of mice was found for the dithiocarbamate ester of 9-aminoacridine, whereas dithiocarbamates of both stronger and weaker bases were nonprotective.

Significant radiation protection in mice irradiated with 1000 r (γ-rays) has been found for the dithiocarbamate of a strongly basic heterocyclic imine (1). The base was 1,2-dihydro-1-methyl-2-iminopyridine, obtained by ring-*N*-alkylation of 2-aminopyridine, and has a pKa of 12.2. Dithiocarbamates of the corresponding 4-iminopyridine and 2-iminopyrimidine gave no radiation protection at this radiation level, however. In order to determine structural requirements for radiation protection by dithiocarbamates of strongly basic heterocycles, preparation of dithiocarbamates of this type of compound has been extended to include acridines and quinaldines.

Dithiocarbamates were generally prepared from these bases, for which pKa values ranged from 8–12 (2) in alcohol with or without the presence of additional base such as pyridine or triethylamine. The dithiocarbamates were obtained as esters, salts of the heterocyclic base, or salts of the added base; the nature of the product was unpredictable. Characteristic absorption for dithiocarbamates was observed in the UV at 220, 240–250, 260–275, and 290–310 mμ (3) and in the IR near 1,000 cm.$^{-1}$ (1) for these products, providing further evidence for dithiocarbamate formation of imines. In contrast to dithiocarbamate formation of aliphatic amines which generally takes place quite rapidly (4), some of the products reported here required a number of days to form.

Radiation protection in mice was found for the dithiocarbamate of 9-aminoacridine (pKa = 10.0) *versus* 825 r (X-rays). No protection was reported for several other members of these series made from heterocycles having either greater or lesser basicities.

DISCUSSION

The method of Albert (5) was used to prepare 3-aminoacridine (pKa = 8.0). Treatment of this compound with carbon disulfide in ethanol gave an undefinable product, but in the presence of a large excess of triethylamine, the triethylammonium salt of 3-acridyldithiocarbamate was obtained. In contrast to this behavior, 9-aminoacridine (pKa = 10.0) was converted to ethyl 9-acridyldithiocarbamate when treated with carbon disulfide in ethanol in either the presence or absence of triethylamine. The yield was larger in the former case, however. The UV absorption spectrum in ethanol showed peaks at 243 and 270 mμ for the 3-isomer and 261 and 304 mμ for the 9-isomer.

The procedure of Albert (6) was used to prepare 9-imino-10-methylacridinium iodide (pKa = 11.1). This compound was treated with sodium hydroxide to yield 9-amino-9-hydroxy-10-methylacridine which was dehydrated to yield 9,10-dihydro-9-imino-10-methylacridine. Both the hydroxy and the de-

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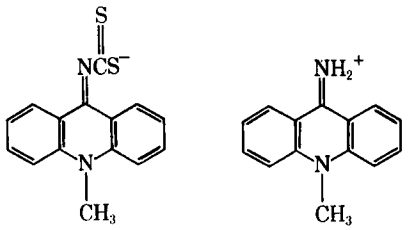
* In part.

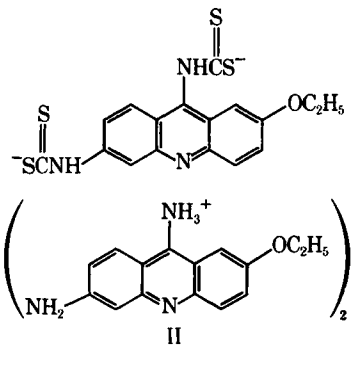
hydrated compounds were found to have identical IR and UV absorption spectra in ethanol (peaks at 220 and 265 mμ).

Treatment of either of these compounds with carbon disulfide in absolute ethanol yielded 9,10-dihydro - 10 - methylacridyl - 9 - immonium 9,10-dihydro - 10 - methylacridyl - 9 - imino - *N* - carbodithioate (I), which showed UV absorption at 221, 243, 265, and 288 (shoulder) mμ.

6,9-Diamino-2-ethoxyacridine monohydrate (pKa 11.6) was prepared from the lactate salt by treatment with aqueous sodium hydroxide followed by oven drying until the correct weight was lost to give the monohydrate. Treatment of this compound with carbon disulfide in acetone gave bis-(2-ethoxy-6-aminoacridyl-9-ammonium) 2-ethoxyacridyl-6,9-bis(dithiocarbamate)(II).



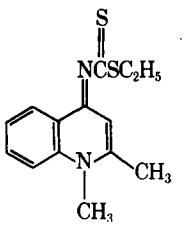
I



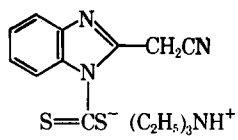
II

Pyridinyl 4-quinaldinyldithiocarbamate was obtained from the reaction at 25° of 4-aminoquinaldine (pKa = 9.4), pyridine, carbon disulfide, and triethylamine, and required 16 days. By analogy to the aminoacridine reactions with carbon disulfide and triethylamine in ethanol, similar treatment of 4-aminoquinaldine gave a product for which the analytical data was reasonable for the ethyl ester of the dithiocarbamate. However, an analytically pure sample was not obtainable.

The preparation of 1,4-dihydro-4-imino-1-methylquinaldine hydriodide (m.p. 280–283°) has not previously been described. However, Ochiai (7) has described an isomer, 4-methylaminoquinaldine (m.p. 239°), its hydriodide (m.p. 285° dec.), and the picrate (m.p. 264° dec.). The free base, 1,4-dihydro-4-imino-1-methylquinaldine (pKa ~ 12.0) was obtained by treating the hydriodide with potassium hydroxide followed by a toluene extraction. Proof that these conditions had not caused rearrangement to 4-methylaminoquinaldine (which was found to occur with certain pyrimidines and pyridines) (1) was shown by the formation of the picrate (m.p. 243–245°) of the free base in toluene. The toluene was removed, and the free base in absolute ethanol was treated with carbon disulfide to yield ethyl 1,4 - dihydro - 1 - methylquinaldine - 4 - imino - *N*-carbodithioate (III).



III



IV

Treatment of 2-benzimidazolylacetonitrile (pKa = 10.7) in absolute ethanol with carbon disulfide and triethylamine readily yielded the triethylammonium salt of the dithiocarbamate (IV).

Antiradiation Properties—Anitradiation screening results of several of the compounds at the Walter Reed Army Institute of Research have been reported through the courtesy of Dr. D. P. Jacobus. Tests were carried out in mice *versus* 825 r (X-rays) with an observation period of 30 days. The dithiocarbamates of 3-aminoacridine, 4-imino-1-methylquinaldine and 2-benzimidazolylacetonitrile provided no protection at this level of radiation dosage. The dithiocarbamate ester of 9-aminoacridine, however, showed fair protection (25–44% survival) at this radiation dosage but no protection at 1000 r (γ-rays) at compound dosage levels of less than 50 mg./kg.

EXPERIMENTAL

Analyses for carbon, hydrogen, and nitrogen were done by Weiler and Strauss, Oxford, England. Sulfur analyses were done by Parr bomb peroxide fusion. Melting points were taken on a Mel-Temp apparatus and are corrected. IR absorption spectra were obtained with a Perkin-Elmer model 137B spectrometer. The authors are indebted to Mr. J. R. Lo for determination of pKa values, obtained with a Beckman research pH meter with glass and calomel electrodes.

Triethylammonium 3-Acridyldithiocarbamate—Six grams (0.031 mole) of 3-aminoacridine (5) was dissolved in 36 ml. of absolute ethanol containing 4.3 ml. (0.061 mole) of carbon disulfide and 50 g. (0.5 mole) of triethylamine. The reaction solution was stirred for 30 min. and then kept at 25° for 3 days. Precipitation occurred during this time. The solid was collected and washed with 10-ml. portions of absolute ethanol, acetone, and anhydrous ether. A yield of 3.2 g. (28%) was obtained, m.p. 123–125°, IR (KBr) 2,650(NH^+), 1,000(C=S) $cm.^{-1}$, UV max. (95% EtOH) 243 (ε84,610), 270 (ε121,540) mμ.

Anal.—Calcd. for $C_{20}H_{25}N_3S_2$: C, 64.96; H, 6.74; N, 11.31; S, 17.35. Found: C, 64.68; H, 6.61; N, 10.81; S, 17.53.

Ethyl 9-Acridyldithiocarbamate Monohydrate—9-Aminoacridine (10.0 g., 0.052 mole) was refluxed with 115 ml. of 95% ethanol and 25 ml. of triethylamine on a water bath. Then 14 ml. of carbon disulfide was added slowly during 20 min., and refluxing was continued for 1 hr., during which

time hydrogen sulfide was evolved and the reaction solution became orange in color.

The reaction mixture was stirred occasionally at room temperature for 2 days. During this time precipitation occurred and hydrogen sulfide was evolved. About 10 g. of a yellow solid was obtained which was recrystallized from 350 ml. of 95% ethanol. About 2.5 g. of solid was insoluble in the ethanol. On cooling and concentration of the filtrate, 3.5 g. (21%) of product was obtained, m.p. 188–190°, IR (mineral oil) 1,000 (C=S) cm.$^{-1}$, UV max. (95% EtOH) 261 (ϵ77,210), 304 (ϵ11,780) mμ.

Anal.—Calcd. for $C_{16}H_{14}N_2S_2 \cdot H_2O$: C, 60.73; H, 5.09; N, 8.85; S, 20.26. Found: C, 60.6; H, 5.3; N, 8.6; S, 20.1.

9,10-Dihydro-10-methylacridyl-9-immonium 9,10-Dihydro-10-methylacridyl-9-imino-N-carbodithioate—Three grams (0.014 mole) of 9,10-dihydro-9-imino-10-methylacridine (6) was dissolved in 12 ml. of absolute ethanol in a flask protected from moisture. Then 3.5 ml. (0.05 mole) of carbon disulfide was added and the reaction solution was gently refluxed above a water bath for 20 min. An orange solid was precipitated during this time.

The mixture was ice cooled and the orange solid was collected and washed with 15 ml. of absolute ethanol; 2.1 g. (63%) of product was obtained, m.p. 241–243°, IR (KBr) 2,050 (NH_2^+), 1,675 (C=N), 995 (C=S) cm.$^{-1}$, UV max. (95% EtOH) 221 (ϵ40,000), 243 (ϵ71,000), 265(ϵ86,270), 288 (shoulder)(ϵ24,000) mμ.

Anal.—Calcd. for $C_{29}H_{24}N_4S_2$: C, 70.70; H, 4.91; N, 11.37. Found: C, 70.93; H, 4.99; N, 11.19.

6,9-Diamino-2-ethoxyacridine Monohydrate—A mixture of 4 g. of sodium hydroxide dissolved in 100 ml. of distilled water, and 15 g. of 6,9-diamino-2-ethoxyacridine lactate monohydrate (0.042 mole) (Aldrich Chemical Co.) was stirred for 2 hr. The yellow solid was collected, washed with three 40-ml. portions of distilled water, and dried at 105–110° until it weighed 11.2 g., m.p. 116–118°, pKa(H_2O) 11.6.

Anal.—Calcd. for $C_{15}H_{16}N_3O \cdot H_2O$: C, 66.15; H, 6.62; N, 15.43. Found: C, 66,39; H, 6.64; N, 15.35.

Bis(2-ethoxy-6-aminoacridyl-9-ammonium) 2-Ethoxyacridyl-6,9-bis(dithiocarbamate)Trihydrate—To 6.0 g. (0.022 mole) of 6,9-diamino-2-ethoxyacridine monohydrate in 200 ml. of acetone, 23 ml. (0.33 mole) of carbon disulfide was added with constant stirring. An orange solid precipitated immediately. The reaction mixture was stirred for 3 hr. and the solid was collected, washed with two 40-ml. portions of acetone, and air-dried, giving 5.0 g. (75%), m.p. 174–175°; IR(KBr) 3,000 (NH_3^+), 1,000 (C=S) cm.$^{-1}$.

Anal.—Calcd. for $C_{47}H_{45}N_9O_3S_4 \cdot 3H_2O$: C, 58.42; H, 5.32; N, 13.04. Found: C, 58.96; H, 4.95; N, 13.00.

Pyridinyl 4-Quinaldinyldithiocarbamate Monohydrate—Five grams (0.032 mole) of 4-aminoquinaldine (Aldrich Chemical Co.) was dissolved in 25 ml. of pyridine, 15 ml. of triethylamine, and 12 ml. (0.17 mole) of carbon disulfide in a stoppered flask and stirred occasionally during 16 days, the last 3 days with the stopper ajar. A yellow solid was slowly deposited and the reaction solution became dark red in color. The solid was collected, washed with three 20-ml. portions of acetone and one 20-ml. portion of anhydrous ether, yielding 3.0 g. (28%), m.p. 290–291° with decomposition beginning about 150°, IR (KBr) 2,750 (NH^+), 993 (C=S) cm.$^{-1}$.

Anal.—Calcd. for $C_{16}H_{15}N_3S_2 \cdot H_2O$: C, 57.97, H, 5.17; N, 12.67. Found: C, 57.78; H, 5.08; N, 13.42.

1,4-Dihydro-4-imino-1-methylquinaldine Hydriodide—Ten grams (0.063 mole) of 4-aminoquinaldine in 60 ml. of methanol was refluxed on a water bath, and 16.0 ml. (0.25 mole) of methyl iodide was added dropwise during 10 min. A white solid precipitated after about 20 min. The mixture was refluxed for 60 min. and then ice cooled. The solid was collected, washed with two 20-ml. portions of hot acetone, and recrystallized from methanol to yield 9.8 g. (49%), m.p. 280–283°.

Anal.—Calcd. for $C_{11}H_{13}IN_2 \cdot H_2O$: C, 41.81; H, 4.72; N, 8.81. Found: C, 42.11; H, 4.75; N, 8.92.

The picrate was crystallized from 95% ethanol, m.p. 243–245°.

Anal.—Calcd. for $C_{17}H_{15}N_5O_7$: C, 50.73; H, 3.76; N, 17.45. Found: C, 51.22, H, 4.03; N, 17.55.

Ethyl 1,4-Dihydro-1-methylquinaldine-4-imino-N-carbodithioate Monohydrate—Four grams (0.013 mole) of 1,4-dihydro-4-imino-1-methylquinaldine hydriodide monohydrate was mixed thoroughly with 3.0 g. of powdered potassium hydroxide in a mortar and extracted in a Soxhlet apparatus with toluene for 2.5 hr. Five milliliters of the toluene extract was treated with 5 ml. of a saturated solution of picric acid in toluene, giving a picrate melting at 243–245°, the value found for the picrate of 1,4-dihydro-4-imino-1-methylquinaldine.

The toluene solution of 1,4-dihydro-4-imino-1-methylquinaldine was evaporated at 25 mm. on a water bath. The tan hygroscopic solid obtained was dissolved in 15 ml. of hot absolute ethanol and the solution was filtered. The warm filtrate was treated with 1 ml. (0.014 mole) of carbon disulfide, and an orange solid slowly precipitated. The mixture was stirred for 1 hr., and the tan solid was collected and washed with 10 ml. of absolute ethanol and two 10-ml. portions of anhydrous ether, giving 1.0 g. (27%), m.p. 134–135°, IR(KBr) 1,650 (C=N), 1,005 (C=S) cm.$^{-1}$.

Anal.—Calcd. for $C_{14}H_{16}N_2S_2 \cdot H_2O$: C, 57.16; H, 6.66; N, 9.51; S, 21.78. Found: C, 56.97; H, 6.12; N, 9.75; S, 21.34.

Triethylammonium 2-Benzimidazolylacetonitrile-1-carbodithioate—2-Benzimidazolylacetonitrile (5.5 g., 0.035 mole)[pKa(H_2O) 10.7] was warmed with 30 ml. of absolute ethanol, 42 ml. (0.6 mole) of carbon disulfide, and 70 ml. (0.56 mole) of triethylamine to effect solution. The solution was allowed to stand at 25° for 2 days with occasional stirring. The orange solid was collected and washed with two 30-ml. portions of anhydrous ether and 25 ml. of absolute ethanol, yielding 9.8 g. (84%), m.p. 132–134°, IR(KBr), 2,650 (NH^+), 2,200 (C≡N), 1,010 (C=S) cm.$^{-1}$.

Anal.—Calcd. for $C_{16}H_{22}N_4S_2$: C, 57.45; H. 6.62; N, 16.76; S, 19.17. Found: C, 57.46; H, 6.71; N, 16.75; S, 19.99.

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Keyphrases

Antiradiation compounds
Acridine dithiocarbamates—synthesis
Quinaldine dithiocarbamates—synthesis
Radioprotective activity—screening
IR spectrophotometry—identity
UV spectrophotometry—identity

Rheological Stability of a Procaine Penicillin G Suspension

By JAMES C. BOYLAN and ROBERT L. ROBISON

The rheology of a 58.6 percent procaine penicillin G suspension was followed for a 24-month period. Samples stored as recommended (5°) were unchanged after 2 years. Samples stored at 26 and 37° increased in viscosity, yield value, and thixotropy with time.

In formulating a product where rheological properties are important, it is not only necessary to ascertain that the freshly prepared product possesses the necessary rheological characteristics, but more importantly whether or not these characteristics change during the recommended shelf life of the product. A specific case where unchanging rheological properties are critical is an injectable suspension of procaine penicillin G (600,000 u./ml.). The rheological parameters of this type of suspension determine (*a*) ease of filling, (*b*) prevention of separation during shipping and storage, and (*c*) injectability. It is difficult to formulate a procaine penicillin G suspension that will be satisfactory under all these varied conditions.

In 1958, Ober and co-workers (1) published an outstanding rheological study of concentrated procaine penicillin G depot preparations. They reported that in aqueous suspensions of 40 to 70% (w/w) procaine penicillin G, the rheological structural breakdown point (the point of maximum torque at very low shearing rate) is the physical parameter that defines the structure of the suspension prior to any applied shearing force. Although the nature of this structure is not known, they reasoned that the existence of this structural breakdown point does allow highly concentrated procaine penicillin G suspensions to fluidize enough to allow passage through a hypodermic needle. Furthermore, it permits good depot formation by virtue of quickly regaining this structure. They also determined what effect particle-size distribution, specific surface of the powder, and percent solids had on these phenomena of structure formation, breakdown, and recovery. By using a variety of techniques, including rheology, a region of satisfactory formulation was ascertained.

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It is the purpose here to report the rheological changes obtained with a procaine penicillin G suspension under a variety of storage conditions and to illustrate how critically important storage conditions are to product performance.

EXPERIMENTAL

Materials—The suspension studied[1] had the following formula:

procaine penicillin G (USP)	58.6%
sodium citrate (USP)	4.0%
polysorbate 80 (USP)	0.4%
lecithin	1.5%
butyl parahydroxybenzoate	0.015%
water for injection (USP) *q.s.ad.*	100.0%

All figures are expressed as w/v.

The samples evaluated were disposable syringes taken from a production lot. Storage was under three conditions: 5, 26, and 37°. The marketed product bears a refrigeration storage statement and a 24-month expiration date.

Rheological Evaluation—The viscometer used in this study was a Ferranti-Shirley cone and plate viscometer[2] equipped with a 400 g. cm. spring, an automatic gap-setting device, an X-Y recorder,[3] and a constant-temperature water bath.[4] The use of this viscometer has been described elsewhere (2). Calibration of the instrument was carried out using N.B.S. standard viscosity oils.

Each sample was contained in a 1-ml. disposable syringe. Since the sample size required for this viscometer is approximately 1 ml., the sample was used in its entirety (except for some retention in the syringe). The sample to be evaluated was treated

[1] Duracillin A. S. (Lilly), 600,000 u./ml.
[2] Ferranti Electric Co., Plainview, Long Island, N. Y.
[3] Houston Instrument Co., Bellaire, Tex. (model HR-92).
[4] Brinkmann Instrument Co., Westbury, N. Y. (Haake model F).