10^{-8}): 0.1 N HCl, 277 (shoulder) (8.8), 305 (12.3); 0.1 N NaOH, 264.5 (9.4), 290 (9.66).

Anal. Calcd. for $C_9H_{13}ClN_4$: C, 50.80; H, 6.12; N, 26.30. Found: C, 50.98; H, 6.48; N, 26.00.

5-Amino-6-chloro-4-cyclohexylaminopyrimidine (IIc).—A solution of 5-amino-4,6-dichloropyrimidine (10 g., 61 mmoles) and cyclohexylamine (30 g., 305 mmoles) in ethanol (70 ml.) was heated in a stainless steel bomb[10] at 130° for six hours. The solution was evaporated to dryness, the residue triturated with ether (500 ml.), and the insoluble solid (cyclohexylamine hydrochloride) removed by filtration. The ether was removed and the residue extracted by decantation with boiling water (6 × 200 ml.). The combined, cooled extracts yielded 2.15 g. of a light yellow solid, m.p. 138°.

Anal. Calcd. for $C_{10}H_{15}ClN_4 \cdot H_2O$: C, 49.00; H, 6.95; N, 22.85. Found: C, 49.27; H, 6.89; N, 22.57.

The sample (2.0 g.) was dried *in vacuo* over P_2O_5 at 100° for four hours; yield 1.87 g., m.p. 139°; λ_{max} in $m\mu$ (ϵ × 10^{-8}): 0.1 N HCl, 275 (shoulder) (8.78), 304 (12.3); 0.1 N NaOH, 262.5 (9.65), 288 (9.45).

Anal. Calcd. for $C_{10}H_{15}ClN_4$: C, 52.90; H, 6.62; N, 24.70. Found: C, 53.26; H, 6.71; N, 24.62.

The light brown residue from the above aqueous extraction was dried *in vacuo* over P_2O_5 at 100° for four hours; yield 7.68 g., m.p. 138°. The ultraviolet spectrum of this material was practically identical with those of the analytical sample above. The total yield of 5-amino-6-chloro-4-cyclohexylaminopyrimidine was 9.83 g. (71%).

9-*n*-Butyl-6-chloropurine (IIIa).—A solution of 5-amino-4-*n*-butylamino-6-chloropyrimidine (8.1 g.) in diethoxymethyl acetate[11] (75 ml.) was heated at 100° for 1.5 hours and then evaporated to a small volume *in vacuo*. Distillation of the residue under reduced pressure gave a light yellow liquid; yield 7.42 g., b.p. 142° (0.2 mm.).

9-Cyclohexyl-6-hydroxypurine (IVc).—A solution of 5-amino-6-chloro-4-cyclohexylaminopyrimidine (1.0 g.) in anhydrous formic acid (10 ml.) was refluxed for 5 hours, evaporated to a small volume, diluted with water (10 ml.) and neutralized to pH 8 with concd. ammonium hydroxide. The mixture was cooled, and the solid which deposited was collected by filtration and dried over P_2O_5; yield 800 mg., m.p. 268–270°. A carbon and hydrogen analysis indicated

that this material was 98% pure. Recrystallization of a small sample from water raised the melting point to 273–275°; λ_{max} in $m\mu$ (ϵ × 10^{-8}): 0.1 N HCl, 249 (11.7); 0.1 N NaOH, 254 (13.1).

9-*n*-Butylpurine (Va).—A solution of 9-*n*-butyl-6-chloropurine (3.47 g., 16.5 mmoles) in a 1:1 mixture of ethanol-water (100 ml.) was hydrogenated over a 5% Pd/C catalyst (2 g.) in the presence of magnesium oxide (2 g.). After the mixture had absorbed 420 ml. of hydrogen (theoretical 411 ml.), the catalyst and excess magnesium oxide were removed by filtration and washed with hot ethanol (50 ml.). To the combined filtrates was added 50 ml. of a 5% sodium carbonate solution and the whole evaporated to dryness *in vacuo*. The residue was extracted with ether (3 × 75 ml.) and the ether removed at reduced pressure. Distillation of the residue *in vacuo* gave a colorless liquid; yield 2.45 g. (84.5%). A carbon and hydrogen analysis indicated that this material was 98% pure. A second distillation was necessary to obtain the analytical sample; yield 1.84 g.; λ_{max} in $m\mu$ (ϵ × 10^{-8}): 0.1 N HCl, 263 (5.6); 0.1 N NaOH, 264 (7.6).

9-Cyclopentyl-6-mercaptopurine (VIb).—A solution of 6-chloro-9-cyclopentylpurine (500 mg., 2.25 mmoles) and thiourea (170 mg., 2.25 mmoles) in *n*-propyl alcohol (15 ml.) was refluxed for 15 minutes. The solid that deposited was collected by filtration, dissolved in 1 N NaOH (10 ml.), and the solution acidified with acetic acid. The white precipitate was removed by filtration, washed with water, and dried *in vacuo* over P_2O_5 at 80°; yield 400 mg.

A small sample was recrystallized from methyl isobutyl ketone; m.p. >300°; λ_{max} in $m\mu$ (ϵ × 10^{-8}): 0.1 N HCl, 224 (9.35), 325 (19.1); 0.1 N NaOH, 232 (14.4), 311 (22.4).

6-Amino-9-cyclohexylpurine (VIIc).—A solution of 6-chloro-9-cyclohexylpurine (1.5 g.) in ethanolic ammonia (50 ml. saturated at 0°) was heated in a bomb for 15 hours at 105–110°. The solution was evaporated to dryness *in vacuo*, and the residue boiled in benzene (50 ml.). A brown, insoluble material was removed from the benzene solution by filtration, and the filtrate allowed to cool. The solid which deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 700 mg., m.p. 199–200°. Recrystallization from Skellysolve C did not raise the melting point; λ_{max} in $m\mu$ (ϵ × 10^{-8}): 0.1 N HCl, 260 (14.5); 0.1 N NaOH, 262 (14.9).

(11) H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 260 (1937).

Birmingham 5, Alabama

The *in vivo* Hydroxylation of 1-Ethynylcyclohexyl Carbamate[1,2]

By Robert E. McMahon

Received September 3, 1957

1-Ethynylcyclohexyl carbamate-C^{14} (ethinamate-C^{14}) has been prepared and its metabolism studied in rats. Hydrolytic cleavage of the carbamate grouping was found to be a minor catabolic route, the major route being hydroxylation to hydroxyethynylcyclohexyl carbamate (hydroxyethinamate). Hydroxyethinamate is eliminated in urine both in the unbound form and as the β-glucuronide. Hydroxyethinamate was isolated from human urine and its identity conclusively shown by physical properties and by derivative formation. No evidence was found that the acetylene group was altered by the body. The significance of these findings is discussed.

Introduction

The metabolic conversion of the acetylenic grouping in animal organisms has been little studied. Hoppe-Seyler[3] early reported the conversion of *o*-nitrophenylpropiolic acid to indoxyl. Bohm[4] later reported that *o*-nitrophenylacetylene is likewise metabolized to indoxyl. These reports together with the observation by El Masry, Smith and

Williams[5] of the slow conversion of phenylacetylene to phenylacetic acid by rabbits appear to represent the major investigations in which metabolic products of acetylenic compounds have been clearly identified.

The recently introduced hypnotic drug ethinamate (1-ethynylcyclohexyl carbamate, I) has been reported by Langecker, Schumann and Junkmann[6] and by Swanson, Anderson and Gibson[7] to be

(1) Eli Lilly and Company Trade-mark, "Valmid" (Ethinamate, Lilly).

(2) Preliminary Report, Medicinal Chemistry Division, A.C.S. 130th Meeting, Atlantic City, New Jersey, September, 1956.

(3) G. Hoppe-Seyler, *Z. physiol. Chem.*, **7**, 178 (1882).

(4) F. Bohm, *ibid.*, **261**, 35 (1939).

(5) A. M. El Masry, J. N. Smith and R. T. Williams, *Biochem. J.*, **65**, No. 2, 10P (1957).

(6) H. Langecker, H. J. Schumann and K. Junkmann, *Arch. exptl. Pathol. Pharmakol.*, **219**, 130 (1953).

(7) E. E. Swanson, R. C. Anderson and W. R. Gibson, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 40 (1956)

characterized by rapid onset of action and by short duration of sedation. The latter workers[7] reported that ethinamate was rapidly metabolized and that no metabolites containing the acetylenic group could be detected in urine. These results led to the speculation that the short duration of action of ethinamate is due to its rapid metabolism in the body by alteration of the ethynyl grouping. This speculation was reinforced by the previous report by Perlmann and Johnson[8] that methylethyl-ethynylcarbinol, also a short acting hypnotic, is rapidly metabolized and that no metabolites containing the ethynyl group are detectable in urine.

It seemed desirable, therefore, to carry out a quantitative study of the metabolism of ethinamate and to isolate and identify chemically the primary metabolites. Radiocarbon labeling was employed to facilitate the study.

Results and Discussion

In choosing the site for the radiocarbon label in the ethinamate molecule, we were guided by the report of Bryan, Skipper and White[9] that carbonyl-labeled ethyl carbamate-C^{14} underwent hydrolytic cleavage to the extent of 90% when administered to mice. Since ethinamate is also an alkyl ester of carbamic acid, it was thought best to check first the possibility that the primary metabolic step might be simply the cleavage of the carbamate function. The carbonyl carbon was thus chosen as the site for the label.

Labeling was carried out by the following reaction sequence. The intermediate chlorocarbonate was not isolated. The over-all radiochemical yield was 23%.
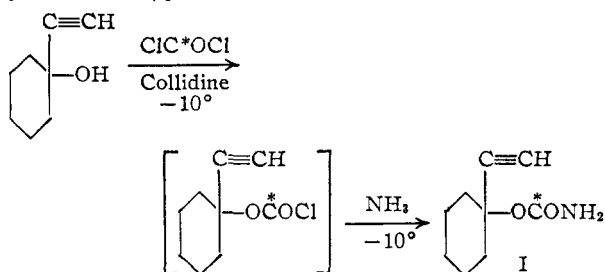
In order to determine the metabolic pattern the labeled ethinamate was administered to the rats and the rate and mode of elimination determined. The results are summarized in Table I. During the 8 hr. period of collection only 10% of the radioactivity appeared as respiratory carbon dioxide, showing that cleavage of the carbamate link is a route of minor importance. Urinary excretion proved to be the favored route of elimination, 63% of the radioactivity appearing in the urine in 8 hr.

Paper chromatography of the urine in a n-butanol–ammonia system proved the most satisfactory method for the estimation of the distribution of radioactive metabolites. In one experiment (see Table II) an 0–8 hr. urine sample showed the following distribution of labeled metabolites: un-

(8) P. L. Perlmann and C. Johnson, J. Am. Pharm. Assoc., Sci. Ed., 41, 13 (1952).

(9) C. E. Bryan, H. E. Skipper and L. White, J. Biol. Chem., 177, 941 (1949).

TABLE I

RATE OF ELIMINATION OF RADIOACTIVE METABOLITES[a]

Time hr. (accumulated)	% R.A. dose eliminated in Respiratory CO_2	Urine
0-1	1.8	8
0-2	5.0	25
0-4	8.4	46
0-8	10.0	63

[a] Based on four rats receiving 50 mg./kg.

changed ethinamate (R_f = 0.86), 2%, metabolite A (R_f = 0.02), 48%, metabolite B (R_f = 0.73), 41%, and other metabolites, 9%. There were then two major metabolites, one (A) apparently considerably more polar than the other. Since both metabolites were radioactive, it seemed likely that the carbamate group was intact. There appeared to be at least three minor metabolites present. These have not been investigated further and in Table II are combined under "other" metabolites.

TABLE II

DISTRIBUTION OF RADIOACTIVE METABOLITES IN URINE BY PAPER CHROMATOGRAPHY

Time of collection (hr.)	% R.A. present at R_f 0.02	0.73	0.86	Other
0-0.25	8	41	43	8
0.25-0.50	12	71	13	4
0.50-1	19	64	6	11
1-2	41	48	2	9
2-4	66	27	..	7
4-8	70	23	..	7
0-8	48	41	2	9
0-8[a]	91	..	..	9
0-8[b]	..	90	10	..
0-8[c]	5	81	5	9
0-8[d]	5	90	..	5

[a] After ether extraction (pH 7). [b] Ether extract. [c] After β-glucuronidase incubation. [d] After ether extraction followed by β-glucuronidase incubation.

A study of the relative rates of elimination of urinary metabolites proved of interest. Periodic urine samples were collected and the ratio of labeled metabolites estimated by paper chromatography. It was observed that elimination of unchanged ethinamate was important only in the first 15 minutes, becoming negligible after 1 hr. The interesting observation was made (Table II) that in early collections metabolite B was the important component but that with increased time the proportion of A increased at the expense of B. After 2 hr. the ratio of A to B approached a constant value. A likely explanation from these observations was that metabolite B was an intermediate in the formation of metabolite A from ethinamate. That A was the more polar compound was indicated by its slow movement on paper tape and by the fact that it could not be extracted with ether as could B (see Table II). This led to the postulate that A was in reality a conjugated form of B. This guess was reinforced by the results of β-glucuronidase incubation. Treatment of metabolite A with β-glucuronidase (pH 6.8) led to its conversion to a radioactive material having the same mobility on paper as did metabolite B (Table II).

An attempt was made to isolate metabolite B in pure form. Extraction of urine with methylene chloride followed by chromatographic purification procedures yielded an oil which could not be purified further. The infrared spectrum in chloroform was, however, very instructive. The presence of

$$\overset{O}{\overset{\|}{}}$$

both the $-C\!\equiv\!CH$ and $OCNH_2$ groupings were indicated with certainty. Furthermore, although the 2.8–3.0 μ region was partially obscured by absorption due to the $-NH_2$ grouping, the presence of a hydroxyl group was strongly suggested. This led us to postulate that metabolite B was indeed hydroxyethinamate (III).
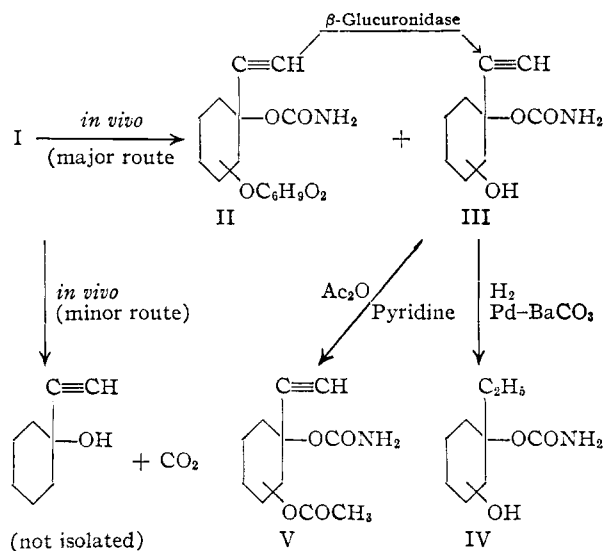
As the amount of material available from rats was quite limited, we turned our attention to the isolation of hydroxyethinamate (III) from the urine of humans receiving "cold" ethinamate. Using the same procedures as employed previously, it was possible to isolate from 25 liters of urine from humans who had received 44 g. of cold ethinamate, 625 mg. of a semi-crystalline material having an infrared spectrum ($CHCl_3$) identical with that of the rat metabolite. Further purification gave a nicely crystalline material, m.p. 135–136°, the elemental analysis and molecular weight (calculated from X-ray data) of which confirmed its identity as hydroxyethinamate (III).

To further confirm the identity of III two derivatives were prepared. When III was reduced over palladium on barium carbonate, two moles of hydrogen was consumed rapidly to yield hydroxyethylcyclohexyl carbamate (IV), the saturated analog of III. To demonstrate the presence of the hydroxyl group, acetoxyethinamate (V) was prepared by treatment of hydroxyethinamate with acetic anhydride and pyridine.

Since metabolite B is hydroxyethinamate, metabolite A must be its β-glucuronide (II).

Hydroxyethinamate differs from related compounds in its reaction with Tollens reagent. Ethynylcyclohexanol, ethinamate and acetoxyethinamate all yield an insoluble silver complex when treated with Tollens reagent. Hydroxy-

ethinamate (III) does not. The failure of III to yield an insoluble silver complex is related to the presence of the hydroxyl group since acetylation produces a compound (V) which does yield an insoluble complex. The inability of the previous workers[7] to detect III in urine is readily explainable as the methods customarily used[6,7] to detect the ethynyl group all depend on the formation of an insoluble silver complex.

The question of the position of the hydroxyl group in hydroxyethinamate is still under investigation.

The known routes of metabolism of ethinamate does not then involve attack upon the acetylenic group. The speculation that the short duration of sedation produced by ethinamate is due to rapid alteration of the ethynyl side chain is thus not tenable. It would appear that the acetylene group is as stable to *in vivo* attack as is the saturated ethyl group.[10]

Experimental Part

Carboxyl-C^{14} Labeled Ethinamate (I).—In a 25-ml. 2-neck flask was placed 220 mg. (1.77 mmol.) of ethynylcyclohexanol, 6 ml. of toluene and a magnetic stirring bar. In one neck of the flask was placed a 10-ml. separatory funnel containing 220 mg. (1.82 mmol.) of collidine dissolved in 6 ml. of toluene. The reaction flask was connected to a vacuum line, and the contents were frozen in liquid nitrogen. The system was evacuated, and 178 mg. (1.8 mmol., 1.0 mc.) of phosgene-$C^{14,11}$ was transferred to the reaction flask. The reaction mixture was warmed to $-10°$ and the collidine solution added (0.5 hr.). Ammonia gas was added slowly from a gas buret until uptake ceased.

After the reaction mixture had warmed to room temperature, 8 ml. of 3 N HCl and 100 mg. of "cold" ethynylcyclohexyl carbamate were added. The organic layer was washed with water, dried and evaporated to dryness. The crude reaction product was purified by chromatography over neutral alumina (Woelm) in benzene–hexane mixtures. The yield was 126 mg., m.p. 93–95° (reported[6] 96–98°), specific activity 1.8 μc./mg. (23% radiochemical yield). A paper chromatogram (1:1 n-butanol–1.5 N ammonium hydroxide) showed only a single radioactive spot (R_f 0.86).

Animal Experiments.—The elimination rate studies in rats were carried out in glass metabolic cages similar to those described by Roth.[12] Male albino rats weighing 110–130 g. were used for all experiments. Labeled ethinamate was administered intravenously in a 1:1 normal saline–polyethylene glycol solution. Respired carbon dioxide was absorbed in 2.5 N sodium hydroxide. Urine samples were removed periodically for analysis.

Respiratory carbon dioxide was precipitated as barium carbonate with 0.5 M barium chloride and counted as an infinitely thick layer. The radioactivity content of urine samples was determined by liquid scintillation counting in a solution consisting of 0.1 ml. of urine, 4 ml. of isopropyl alcohol, 6 ml. of toluene and 30 mg. of diphenyloxazole.

Benzoic acid-C^{14} of known specific activity was utilized as an "internal standard." For each urine determination three counting samples were prepared containing "cold" benzoic acid and three containing a known amount of labeled benzoic acid. From the difference in the counting rate of the two sets of samples, the counting efficiency was calculated. A sample of the standard benzoic acid-C^{14} was also converted to barium carbonate to serve as a standard for counting of the solid samples.

The distribution of radioactive metabolites in urine was estimated by paper chromatography on No. 1 Whatman paper in a 1:1 n-butanol–1.5 N ammonium hydroxide sys-

(10) Weight is lent to this suggestion by work now in progress. A study of the metabolism of ethylcyclohexyl carbamate-C^{14} in rats shows it to be as rapidly eliminated as is ethinamate. The major metabolic route again appears to be ring hydroxylation.

(11) D. B. Melville, J. G. Pierce and C. W. H. Partridge, *J. Biol. Chem.*, **180**, 299 (1949).

(12) L. J. Roth, *Nucleonics*, **14**, No. 4, 104 (1956).

tem. The relative amounts of various metabolites present was determined by scanning in a gas-flow counter.

Cleavage of the glucuronide was carried out in the following fashion. One milliliter of urine was diluted to 4 ml. with pH 6.8 0.075 M phosphate buffer, and 40 units of Sigma β-glucuronidase was added. Incubation at 38° for 10 hr. gave nearly complete reaction.

Hydroxy Ethynylcyclohexyl Carbamate from Urine. (1) Rats.—A 24 hr. urine collection was made from a colony of rats which had received 800 mg. of "cold" ethinamate at an intravenous dose level of 50 mg./kg. To this urine was added that of two additional rats which had received labeled ethinamate. The crude metabolite was removed from the urine by extraction with methylene chloride at pH 10. Partial purification resulted from partition chromatography on silica gel employing a n-butanol–water system. Further purification was effected by alumina (Woelm) chromatography using benzene–methylene chloride mixtures for elution. The radioactive metabolite so isolated was an oil which could not be crystallized. The infrared spectra were obtained in acetonitrile and in chloroform solution.

Anal. Calcd. for $C_9H_{13}O_3N$: N, 7.65. Found: N, 5.55.

(2) Humans.—A total of 25 liters of urine was collected from humans who had received a combined total oral dose of 44 g. of "cold" ethinamate 8 hr. before collection. The extraction and chromatographic procedures were the same as those employed for the rat material. Those fractions eluted from alumina by benzene–methylene chloride mixtures which were shown by infrared analysis to contain both the acetylene group and the carbamate grouping were combined and evaporated to dryness. Trituration of the resulting oil gave a semi-crystalline mass which was filtered and washed with methylene chloride. This procedure yielded 625 mg. of semi-solid material, m.p. 128–132°. An infrared spectrum in chloroform solution was identical to that of the rat metabolite in the same solvent. Two recrystallizations from acetone–petroleum ether gave fine needles, m.p. 135–136°. A 2.9% solution in methanol in a one decimeter tube gave an observed optical rotation of +0.015°. The molecular weight calculated from X-ray crystallographic data was 183 (theory 183). The pure material was insoluble in chloroform but an infrared spectrum in acetonitrile was identical to that of the rate metabolite in the same solvent.

Anal. Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.65; H, 7.11; N, 7.22.

Hydroxyethylcyclohexyl Carbamate (IV).—Fifty milligrams (0.27 mmole) of hydroxyethinamate in 12 ml. of ethanol was reduced with hydrogen in 20 mg. of pre-reduced palladium on calcium carbonate catalyst. Uptake ceased in 13 minutes after 0.55 mmole of hydrogen had been consumed. The product was recovered by filtration, evaporation to dryness and two recrystallizations from benzene. The yield was 20 mg. (39%) of crystalline product, m.p. 128–129°. The infrared spectrum in acetonitrile was consistent with the structure.

Anal. Calcd. for $C_9H_{17}O_3N$: N, 7.48. Found: N, 7.29.

Acetoxyethinamate (V).—Fifty milligrams of hydroxyethinamate was dissolved in 5 ml. of pyridine and 1 ml. of acetic anhydride added. The solution was stored at room temperature for 24 hr. Ether was added and the resulting ether solution washed with water. The product was recovered by evaporation to dryness. Recrystallization from benzene gave acetoxyethinamate, m.p. 157–158°. The infrared spectrum ($CHCl_3$) was consistent with this structure.

Anal. Calcd. for $C_{11}H_{15}O_4N$: C, 58.65; H, 6.71; N, 6.22. Found: C, 58.76; H, 6.86; N, 5.94.

Silver Complex Formation.—The substance to be tested (1 mg.) was dissolved in 1 ml. of 30% aqueous ethanol and a few drops of Tollens reagent added. Ethynylcyclohexanol gave a heavy white precipitate. Ethinamate gave a brown solution and a heavy precipitate. Hydroxyethinamate gave brown solution and no precipitate. Acetoxyethinamate gave a brown solution and a light precipitate.

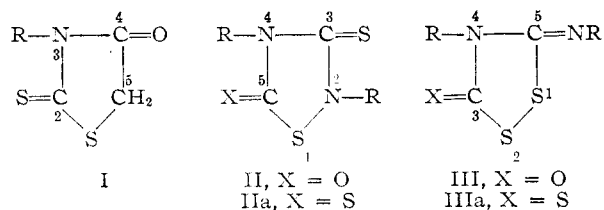# The Structure and Antimicrobial Activity of Some Isothiocyanate Oxides and Sulfides[1]

By C. K. Bradsher, F. C. Brown, E. F. Sinclair and S. T. Webster

Infrared absorption spectra of the isothiocyanate sulfides lend support to the 4-alkyl(aryl)-5-alkyl(aryl)-imino-1,2,4-dithiazolidine-3-thione structure (IIIa). On the basis of similar evidence, the simple alkyl isothiocyanate oxide bases are most probably 2,4-dialkyl-1,2,4-thiadiazolidine-3-thione-5-ones. The aryl and aralkyl isothiocyanate oxide bases, as well as all of the isothiocyanate oxide salts examined, appear to have the 1,2,4-dithiazolidine structure (III). Tests for antimicrobial activity have been carried out on several isothiocyanate sulfide and oxide derivatives.

It has been shown that 3-substituted rhodanine derivatives (I) possess pronounced antimicrobial activity.[2–4] More recently[5] a systematic study has indicated the advantage of having both the thione group at position 2 and the carbonyl at position 4. The present phase of our work involves

$$
\begin{array}{ccc}
\text{I} & \begin{array}{c}\text{II, X = O}\\\text{IIa, X = S}\end{array} & \begin{array}{c}\text{III, X = O}\\\text{IIIa, X = S}\end{array}
\end{array}
$$

the study of the effect of variations in the nature of the atoms or groups present at positions 1 and 5 of the rhodanine ring. Freund and co-workers[6–8] had

(1) This work was supported by a research grant (E-695(c)) from the National Microbiological Institute of the National Institutes of Health, Public Health Service. Taken in part from the theses submitted by E. Faye Sinclair and Sidney T. Webster in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Duke University, 1956 and 1957, respectively.

(2) G. J. M. van der Kerk, H. C. van Os, G. de Vries and A. K. Sijpesteijn, *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent*, **18**, 402 (1953); *C. A.*, **48**, 316 (1954).

(3) F. C. Brown, C. K. Bradsher, S. M. Bond and R. J. Grantham, *Ind. Eng. Chem.*, **46**, 1508 (1954).

(4) F. C. Brown, C. K. Bradsher, E. C. Morgan, M. Tetenbaum and P. Wilder, Jr., This Journal, **78**, 384 (1956).

(5) C. K. Bradsher, F. C. Brown and E. F. Sinclair, *ibid.*, **78**, 6189 (1956).

(6) M. Freund, *Ann.*, **285**, 154 (1895).

(7) M. Freund and E. Asbrand, *ibid.*, **285**, 166 (1895).

(8) M. Freund and G. Bachrach, *ibid.*, **285**, 184 (1895).