Anal. Calcd. for $C_7H_9N_3O$: C, 55.62; H, 6.00; N, 27.80. Found: C, 55.82; H, 6.15; N, 27.74.

2,4-Dimethyl-5-pyrimidinecarbonitrile (V).—A mixture of 15.3 g. (0.101 mole) of VI, 50 ml. of $POCl_3$, and 200 ml. of xylene was stirred and refluxed for 8 hr. After excess $POCl_3$ and xylene were removed *in vacuo*, the residue was taken up in 400 g. of ice water, and the acidic solution was neutralized with dilute NaOH. Extraction with four 500-ml. portions of chloroform gave, after removal of the chloroform *in vacuo*, 6.5 g. (49%) of the crude nitrile. An analytical sample was obtained by vacuum sublimation at 35° (0.1 mm.), m.p. 50.5–53.0°.

Anal. Calcd. for $C_7H_7N_3$: C, 63.14; H, 5.30; N, 31.56. Found: C, 62.99; H, 5.33; N, 31.17, 31.31.

The infrared spectrum of a Nujol mull exhibited absorption at 4.49 (C≡N) and 6.34 μ (C=N).

2,4-Dimethyl-5-acetamidomethylpyrimidine (VII).—A mixture of 8.3 g. (0.067 mole) of V, 200 ml. of acetic anhydride, 3 g. of anhydrous sodium acetate, and 5 g. of Raney nickel was hydrogenated at 3–4 atm. for 24 hr. at room temperature on a Parr apparatus. The mixture was heated to boiling and filtered. After the catalyst was boiled with 100 ml. of additional acetic anhydride and the combined filtrates were concentrated to dryness *in vacuo*, the residue was extracted with two 400-ml. portions of boiling CCl_4. Removal of the solvent from the combined extracts gave 4.8 g. (40%) of the crude product. An analytical sample was obtained by recrystallization from heptanes; m.p. 88.5–90.0°.

Anal. Calcd. for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45. Found: C, 59.98; H, 6.98; N, 23.60.

The infrared spectrum of a Nujol mull exhibited absorption at 3.05 (NH), 6.13 (amide C=O), and 6.31 μ (C=N).

2,4-Dimethyl-5-hydroxymethylpyrimidine (I) from VII.—A solution of 4.8 g. (0.026 mole) of VII and 50 ml. of 2 *N* NaOH was refluxed for 5 hr. The solution was neutralized with concentrated HCl and 2.5 ml. of the concentrated acid was added in excess. A solution of 5 g. (0.07 mole) of sodium nitrite was added. The mixture was stirred at 60° for 15 hr., neutralized with dilute NaOH, and continuously extracted with ethyl acetate for 19 hr. After removal of the ethyl acetate, the residue was extracted with two 400-ml. portions of boiling CCl_4. Removal of the solvent from the combined extracts *in vacuo* gave 1.4 g. (39%) of the crude carbinol. The product was recrystallized from ligroin to give a white crystalline solid, m.p. 55–57°.

The infrared spectra (mineral oil mulls) of various samples of I showed small but discernible differences in the 9–10-μ region and indicated that two crystalline habits were formed because of variations in the crystallization temperature. Solution spectra of the two samples in CCl_4 were identical. The infrared spectrum of the 55–57° melting material reported in this experiment exhibited absorption at 3.15 (OH), 6.31 (C=N), and 9.66 μ (C–OH).

Methanolic solutions of the two samples were analyzed by vapor phase chromatography using the conditions described above. Each of these solutions contained only one component, the retention time of which was identical in both cases. When the components from the two chromatograms were collected, they exhibited identical infrared spectra. Upon analysis of a mixture of the two solutions, only peak enhancement was observed.

The Effect of Some Sulfur-Containing Pyridine Derivatives on the Carbohydrate Metabolism of Ehrlich Ascites Tumor[1]

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The effect of 22 pyridine derivatives on the carbohydrate metabolism of Ehrlich ascites tumor was studied. Most of the compounds are pyridinethiols, sulfides, and disulfides; several of them are new compounds, of which the synthesis is described. 2,2′-Dithiodipyridine was found to inhibit respiration and glycolysis; pyridines containing the grouping $-(CH_2)_xS-$ (with $x = 1$ or 2) in the 4 position inhibited oxygen uptake and increased lactate accumulation; 5-nitro-2-pyridinethiol and the corresponding disulfide and thioether had the common property of stimulating oxygen uptake in the presence of added glucose.

In connection with a cancer chemotherapy research project, we have synthesized a series of pyridinethiols, sulfides, and disulfides, several of which are new. Although they were prepared primarily as model compounds, we have tested their effect on certain aspects of the metabolism of Ehrlich ascites tumor.

As is known, some key enzymes of the glycolytic pathway contain sulfhydryl groups in their active centers and are thus susceptible to interaction with other thiols or disulfides. Furthermore, nicotinamide adenine dinucleotide (NAD) acts as coenzyme for the dehydrogenases involved in glycolysis. It has been established[2] that certain carcinostatic alkylating agents exert their action by decreasing the availability of NAD to the cell, thus causing an inhibition of glycolysis. In addition, there are indications that NAD in ascites tumor cells may be synthesized by a route different from that of normal cells.[3] This may provide a basis for selective inhibition of the energy-yielding metabolism of tumor cells.

The properties studied are: oxygen uptake in the absence and presence of added glucose, and aerobic and anaerobic glycolysis. Among the 22 compounds reported here, we have found different types of activities, which can to some extent be correlated to structural features.

Materials and Methods

Manometric Experiments.—Swiss mice, bearing 5- to 10-day-old ascites tumors, were sacrificed by cervical fracture. The fluid was collected, heparinized, and used immediately. Manometric determinations were carried out in a conventional Warburg apparatus at 37°. Readings were taken every 5 min. for 1 hr. after introduction of the compound under study. Lactic acid was determined by the method of Barker and Summerson.[4]

When oxygen uptake was measured, the gas phase was air. The main compartment of the flask contained 2.5 ml. of heparin-

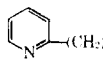
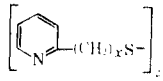
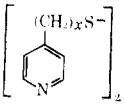
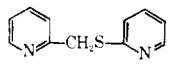
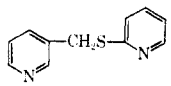
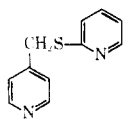
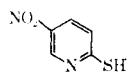
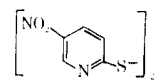
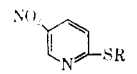
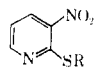
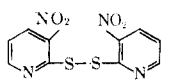
(1) This investigation was supported by Public Health Service Research Grant CA 07296, from the National Cancer Institute.

(2) (a) H. Holzer, P. Glogner, and G. Sedlmayr, *Biochem. Z.*, **330**, 59 (1958); (b) H. Holzer and H. J. Boltze, *Z. Krebsforsch.*, **64**, 113 (1961).

(3) (a) H. Holzer, G. Friedrich, and H. Grisebach, *Biochim. Biophys. Acta*, **51**, 600 (1961); (b) H. Holzer and H. Kroeger, *Biochem. Z.*, **330**, 579 (1958).

(4) S. B. Barker and W. H. Summerson, *J. Biol. Chem.*, **138**, 535 (1941).

TABLE I: RELATIONSHIP BETWEEN STRUCTURE AND ACTIVITY OF SOME PYRIDINE DERIVATIVES

Group	Formula	x or R	Compd.	Ref. prepn.	Concn., M	Metabolic characteristics,[a] % change Q_{O_2}	$Q_{O_2}(G)$	Q_L^{air}	$Q_L^{N_2}$
A		0	I	...	10^{-3}	−10	0	0	0
		1	II	...	10^{-3}	−32	−12	0	−6
		2	III	*b*	10^{-3}	−20	0	0	0
B		0	IV	*c*	10^{-3}	−90	−41	−58	−100
		1	V	*d*	10^{-3}	−49	−17	−35	−12
C_1		2	VI	*b*	10^{-3}	−85	−70	+76	+6
C_2		1	VII	New	10^{-3}	−90	−43	+52	−41
		2	VIII	*b*	10^{-3}	−91	−67	+68	−10
D_1			IX	New	10^{-3}	−7	−8	+7	0
D_2			X	New	10^{-3}	−35	−35	+41	0
D_3			XI	New	10^{-3}	−63	−46	+45	0
E		2-Pyridyl	XII	*e*	10^{-3}	−15	0	−14	−34
		Phenyl	XIII	*f*	10^{-3}	0	0	+8	0
F			XIV	*g*, *h*	8×10^{-4}	+37	+92	+92	+44
G			XV	*h*	2.1×10^{-5}	−10	+79	−7	0
H		5-Nitro-2-pyridyl	XVI	*i*	4.4×10^{-5}	−9	+31	+7	0
		2-Pyridyl	XVII	*j*	10^{-3}	−9	0	+15	0
		Phenyl	XVIII	*k*	2.6×10^{-5}	0	+7	+12	+11
		2-Pyridylethyl	XIX	New	10^{-3}	−12	0	+6	0
I		2-Pyridyl	XX	*l*	5.4×10^{-4}	0	0	+6	0
		Phenyl	XXI	New	2.3×10^{-5}	0	0	0	0
J			XXII	*m*	8.7×10^{-5}	−9	0	−9	0

[a] Experiments were carried out at 37°. Q values were determined as described in text and are expressed in μl./mg. dry weight of ascitic fluid/hr.: Q_{O_2} = rate of oxygen uptake in air; $Q_{O_2}(G)$ = rate of oxygen uptake in air in the presence of added glucose (0.05 M); Q_L^{air} = rate of formation of lactic acid in air in the presence of added glucose (0.05 M); $Q_L^{N_2}$ = rate of formation of lactic acid in N_2–CO_2 (95:5) in the presence of added glucose (0.01 M). Aerobic and anaerobic rates of lactic acid formation in most cases were determined (a) by direct lactic acid determination, and (b) by measurement of CO_2 evolved from bicarbonate buffer. These two sets of determinations gave closely agreeing results. For details see text. Compounds were used as the hydrochlorides whenever possible. I and II were commercial products. Dry weights were determined by drying a known volume of ascitic fluid in an oven at 105° for 1 hr. [b] L. Bauer and L. A. Gardella, *J. Org. Chem.*, **26,** 82 (1961). [c] W. Marckwald, W. Klemm, and H. Trabert, *Ber.*, **33,** 1556 (1900). The dihydrochloride of IV was prepared from the base in ether using dry HCl and was recrystallized from ethanol; m.p. 112–115°. *Anal.* Calcd. for $C_{10}H_{10}Cl_2N_2S_2 \cdot 0.5H_2O$: C, 39.74; H, 3.64; Cl, 23.51; N, 9.27. Found: C, 39.92; H, 3.96; Cl, 23.14; N, 9.14. [d] L. Nutting, R. Silverstein, and C. Himel, U. S. Patent 2,951,848 (Sept. 6, 1960); *Chem. Abstr.*, **55,** 4542*b* (1961). The dihydrochloride of V was prepared from the base in ether, using dry HCl, and was recrystallized twice from 2-propanol; m.p. 170–173°. *Anal.* Calcd. for $C_{12}H_{14}Cl_2N_2S_2$: C, 44.86; H, 4.39. Found: C, 44.82; H, 4.54. [e] J. Renault, *Ann. Chim.* (Paris), **10,** 135 (1955), reported melting point is 215°; we found 219–221°. [f] L. G. S. Brooker, G. H. Keyes, R. H. Sprague, R. H. Van Dyke, E. Van Lare, G. Van Zandt, and F. L. White, *J. Am. Chem. Soc.*, **73,** 5326 (1951). [g] W. T. Caldwell and E. C. Kornfeld, *ibid.*, **64,** 1695 (1942). [h] C. Räth, *Ann.*, **487,** 95 (1931). [i] A. R. Surrey and H. G. Lindwall, *J. Am. Chem. Soc.*, **62,** 1697 (1940). [j] T. Takahashi, T. Yatsuka, and Y. Onuma, *J. Pharm. Soc. Japan*, **64,** 235 (1944); *Chem. Abstr.*, **45,** 4717*d* (1951). [k] B. Pützer and F. Schönhöfer, German Patent 550,325 (Oct. 24, 1930); *Chem. Abstr.*, **26,** 4062 (1932). [l] The synthesis of XX was reported while this work was in progress: O. R. Rodig, R. E. Collier, and R. K. Schlatzer, *J. Org. Chem.*, **29,** 2652 (1964). We had prepared it by essentially the same method. [m] XXII has been prepared by H. Saikachi, *J. Pharm. Soc. Japan*, **64,** 201 (1944); *Chem. Abstr.*, **45,** 4717*b* (1951), from 2-chloro-3-nitropyridine and KSH. The reported melting point is 249–250°. S. G. Fridman, *Zh. Obshch. Khim.*, **26,** 864 (1956); *Chem. Abstr.*, **50,** 14753*a* (1956), prepared the same compound from 2-chloro-3-nitropyridine and Na_2S_2; the reported melting point is 203–205°. We have essentially repeated the preparation of Fridman but obtained a product with the same m.p. as that reported by Saikachi (see Experimental).

ized ascitic fluid[5] diluted with Krebs–Ringer phosphate buffer[6] (4 ml. to 25 ml.). The compound studied was dissolved or suspended in either 0.3 ml. of isotonic saline or 0.3 ml. of 0.5 *M* glucose and was tipped in from the side arm after 10 min. of equilibration. The center well contained 0.2 ml. of 10% KOH and a strip of filter paper. The same procedure was used for determination of aerobic CO_2 evolution[7]; in this case the ascitic fluid was diluted with Krebs–Ringer bicarbonate buffer[8] and the center well contained 0.2 ml. of distilled water. The number of ascites cells was on the average 20 million/flask.

For anaerobic experiments, the gas phase was N_2–CO_2 (95:5). The main compartment contained 2.7 ml. of heparinized ascitic fluid diluted with Krebs–Ringer bicarbonate buffer[6] (1 ml. to 25 ml.). The compound studied was dissolved or suspended in either 0.3 ml. of isotonic saline or 0.3 ml. of 0.1 *M* glucose and was tipped in from the side arm after 10 min. of gassing and equilibration. The number of ascites cells was on the average 5 million/flask.

Manometric determinations were carried out at least three times when the compound studied showed activity; if the compound was inactive, they were carried out twice. In Table I, the figures are presented as the average of at least two determinations.

For the purpose of comparison, the effect of compound IV was studied using (a) the whole ascitic fluid diluted with Krebs–Ringer buffer, as indicated above, and (b) ascitic cells washed three times with isotonic saline and resuspended in the same Krebs–Ringer buffer. It was found that the effect of IV on Q_{O_2}, $Q_{O_2}(G)$, and $Q^{N_2}_{CO_2}$ was essentially the same in both cases.

Thin layer chromatography and autoradiography were used to detect whether accumulation of any phosphorylated glycolytic intermediates occurred. The following mixture was incubated for 1 hr. at 37°, with shaking in air, in a small test tube: 0.10 ml. of ascitic fluid diluted with Krebs–Ringer phosphate buffer (4 ml. to 10 ml.); 0.03 ml. of an aqueous solution of uniformly labeled C^{14}-glucose (1 μmole of glucose containing 3 μc.); 0.15 ml. of a solution of the compound tested in Krebs–Ringer phosphate buffer.

After incubation, 0.02 ml. of trichloroacetic acid (1 g./ml. of solution) was added, proteins were centrifuged off, and 0.002-ml. spots of the clear supernatant were applied to the chromatography plate. The plates were coated with a layer of cellulose powder (0.3-mm. thickness, Camag Type D, no binder) and developed with a mixture of acetone–acetonitrile–1 *N* HCl (64:26:10), as reported previously.[8] After drying, autoradiograms of these plates were made by exposing them for 4–8 days to No-Screen X-ray film (Du Pont or Kodak).

Determination of Solubilities.—The solubilities were determined for the compounds which did not readily give 10^{-2} *M* solutions in the buffers. They were determined only in Krebs–Ringer phosphate buffer, since approximate values were sufficient for our purpose.

The amount of compound necessary to make a 10^{-2} *M* solution in 5 ml. of Krebs–Ringer phosphate buffer was weighed out and shaken at 37° for 1–2 hr. with 5 ml. of Krebs–Ringer phosphate buffer. It was then filtered or centrifuged, and the clear solution was diluted with 9 vol. of methanol. The optical density at the maximum wave length was measured with a Beckman DU spectrophotometer, and the solubility was calculated from this value. Molar extinction values were determined by dissolving a known amount of compound in absolute methanol and diluting it with Krebs–Ringer phosphate buffer to obtain a 9:1 mixture (see Table II).

TABLE II
SOLUBILITIES OF SOME PYRIDINE DERIVATIVES IN KREBS–RINGER PHOSPHATE BUFFER AT 37°

Compd.	λ_{max}, mμ[a]	ϵ[b]	Soly., *M*[c]
XIII	243	1×10^4	1.5×10^{-3}
XIV	400	1.5×10^4	8.0×10^{-4}
XV	311	2.2×10^4	2.1×10^{-5}
XVI	337	1.7×10^4	4.4×10^{-5}
XVII	325	1.5×10^4	1.1×10^{-3}
XVIII	330	1.4×10^4	2.6×10^{-5}
XX	351	4.3×10^3	5.4×10^{-4}
XXI	360	4.7×10^3	2.3×10^{-5}
XXII	348	5.7×10^3	8.7×10^{-5}

[a] λ_{max} is the wave length of maximum absorption (A) determined in methanol–Krebs–Ringer phosphate buffer (90:10). [b] ϵ is the molar extinction = $[A/(c \times 1)]MW$, where c is the concentration in g./l., 1 is the light path in cm., and MW is the molecular weight. [c] Solubilities given are in Krebs–Ringer phosphate buffer at 37° (see text).

Results and Discussion

Table I reports the results of the manometric experiments; an attempt is made to relate structural characteristics to the observed activity. Three main types of activities are observed among the compounds studied. (1) Inhibition of both respiration and glycolysis is caused by disulfide IV and, to a lesser extent, by V (group B). The fact that IV causes no accumulation of phosphorylated glycolytic intermediates, as was shown by thin layer chromatography and autoradiography, suggests that this compound may be a "disulfide poison" affecting glycolysis at the hexokinase level. Cystamine, another disulfide, has been shown to have a similar effect on glycolysis.[9] The thiols corresponding to disulfides IV and V (*i.e.*, I and II, group A) and XII, the thioether corresponding to disulfide IV, have little or no activity. If in this thioether XII one of the pyridine rings is replaced by a phenyl group, an inactive compound (XIII) is obtained. A study of the action of IV at the enzyme level is in progress and the results will be presented elewhere.

(2) Pyridines containing the grouping $-(CH_2)_xS-$ (where x = 1 or 2) in the 4 position have the common property of inhibiting oxygen uptake and increasing aerobic lactate accumulation (groups C_1, C_2, and D_3). We have studied only one compound (X, group D_2) where the above grouping is in the 3 position of a pyridine ring and found that it has the same activity as the 4-substituted compounds. If the substitution is in the 2-position of the pyridine ring, the compound has little or no activity (II, III, V, IX, and XIX). The fact that the grouping $-(CH_2)_xS-$ may be part of a thiol, disulfide, and thioether and still cause the same activity if it occupies the position 4 of the pyridine ring seems to indicate that the action of these compounds is not due to a thiol–disulfide interaction. The compounds of groups C and D do not affect anaerobic glycolysis, with the exception of VII, which inhibits it.

(3) Compounds XIV, XV, and XVI[10] (groups F, G, and H), which are, respectively, a 2-thiol, 2-disulfide, and 2-thioether of 5-nitropyridine, have the common property of stimulating oxygen uptake in the presence

(5) **Liquaemin®** Sodium 200 (Organon) was added, 0.5 ml./10 ml. of ascitic fluid, giving a final level of 1000 U.S.P. heparin units/ml. of fluid.

(6) W. W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," Burgess Publishing Co., Minneapolis, Minn., 1957, p. 149.

(7) J. Hunter, M. Woods, and D. Burk, *Acta Biol. Med. Ger.*, **11**, 681 (1963).

(8) D. R. Grassetti, J. F. Murray, Jr., and J. L. Wellings, *J. Chromatog.*, **18**, 612 (1965).

(9) (a) P. Ciccarone and R. Milani, *Biochem. Pharmacol.*, **13**, 183 (1964); (b) R. Nesbakken and L. Eldjarn, *Biochem. J.*, **87**, 526 (1963).

(10) The effect of these three compounds has been screened on the growth of Walker carcinosarcoma 256 in tissue cultures by J. H. Gray and J. O. Ely, *Cancer Res.*, **18**, 391 (1958) (Cancer Chemotherapy Screening Data I), and by F. E. Reinhart, J. H. Gray, and W. G. Batt, *J. Franklin Inst.*, **261**, 669 (1956). XVI was found to cause complete inhibition at 1:200,000. The effect of XIV–XVI on yeast fermentation has been tested by G. E. Woodward and M. T. Hudson, *Cancer Res.*, **18**, 403 (1958) (Cancer Chemotherapy Screening Data I) and found to be negligible. S. J. de Courcy, Jr., *ibid.*, **18**, 397 (1958) (Cancer Chemotherapy Screening Data I), found that XIV inhibits the biosynthesis of nucleic acids in *L. casei*.

of added glucose [$Q_{O_2}(G)$]. In addition, the thiol XIV stimulates Q_{O_2}, Q_L^{air}, and $Q_L^{N_2}$, while XV and XVI do not affect these properties noticeably.

Some compounds similar to XV and XVI were studied and found to be inactive. They are XXII (group J), an isomer of XV containing the nitro groups in the 3 positions, and the compounds of group H. In Group H, variation of the structure of XVI by elimination of one nitro group or replacement of a 5-nitropyridine ring by a phenyl or by a 2-(2-pyridyl)-ethyl group gives inactive compounds.

Preliminary tracer experiments with compounds XIV–XVI indicate that XV and XVI stimulate the conversion of the C^{14} of glucose-1-C^{14} to CO_2. A more thorough study of the effect of these three compounds is in progress, aimed at establishing their mechanism of action and whether and to what extent the Crabtree effect is actually reversed by XV and XVI.[11]

Thin layer chromatography and autoradiography confirmed that the rate of glucose utilization of ascites tumor cells is increased by addition of these compounds, in the order (XIV > XV > XVI) of their apparent reversal of the Crabtree effect. The similarity of the structure of some of these compounds to known cofactors of carbohydrate metabolism may be related to their activity.

Among the compounds studied, there are some cases in which a thiol and the corresponding disulfide were tested. It is interesting to note that in certain cases the thiol and the disulfide exhibit little or no difference in their metabolic activities (*e.g.*, the pairs VI:VIII and II:V), whereas in other cases there are striking differences in their metabolic properties (*e.g.*, the pairs I:IV and XIV:XV). Such differences are probably due to the site of action of these compounds.

Experimental Section

Melting points were determined on the Fisher-Johns block. 2-Pyridinethiol (I) was obtained from L. Light and Co., Ltd., and recrystallized twice from 2-propanol; m.p. 123–125°. 2-(Mercaptomethyl)pyridine (II) was obtained from Aldrich Chemical Co., Inc., and used as received. Compounds III–VI, VIII, XII–XVIII, XX, and XXII were prepared according to the references given in Table I. Compounds VII, IX–XI, XIX, and XXI have not been reported before and the procedures used in their synthesis are given here in detail.

4,4′-(Dithiodimethylene)dipyridine Dihydrochloride (VII).—4-(Chloromethyl)pyridine hydrochloride (3.3 g., 0.02 mole) was treated with 2.0 g. of thiourea (0.026 mole) in 25 ml. of water. The resulting solution was heated under reflux for 1 hr. Concentrated NH_4OH (25 ml.) was added and reflux continued for 1 hr. The mixture was cooled and slowly treated with 2.5 ml. of 30% H_2O_2 in 10 ml. of water. After cooling, the product was filtered off and recrystallized from methanol; yield 1.0 g. (20.1%), m.p. 158–160°.

Anal. Calcd. for $C_{12}H_{12}N_2S_2$: C, 58.06; H, 4.84. Found: C, 58.30; H, 5.09.

The dihydrochloride was prepared in ether solution using anhydrous HCl; m.p. 198–200°.

Anal. Calcd. for $C_{12}H_{14}Cl_2N_2S_2$: C, 44.86; H, 4.39. Found: C, 44.40; H, 4.78.

2-(2-Pyridylthiomethyl)pyridine (IX.).—2-(Chloromethyl)-pyridine (8.25 g., 0.05 mole) and 2-pyridinethiol (5.5 g., 0.05 mole) were dissolved in 200 ml. of absolute ethanol. A solution of KOH (6.7 g., 0.12 mole) in 100 ml. of absolute ethanol was added slowly with stirring. The resulting mixture was stirred and refluxed for 2 hr. It was then filtered while hot, and the filtrate was evaporated under reduced pressure. The residual oil was distilled twice *in vacuo* through a short-path distillation apparatus. The product had b.p. 130° (1 mm.), yield 3.0 g. (29.6%).

Anal. Calcd. for $C_{11}H_{10}N_2S$: C, 65.31; H, 4.98. Found: C, 65.22; H, 5.22.

3-(2-Pyridylthiomethyl)pyridine (X) was prepared from 8.25 g. of 3-(chloromethyl)pyridine hydrochloride and 5.5 g. of 2-pyridinethiol in the same manner as IX above. The product had b.p. 152–155° (1 mm.), yield 3.0 g. (29.6%).

Anal. Calcd. for $C_{11}H_{10}N_2S$: C, 65.31; H, 4.98. Found: C, 65.35; H, 5.21.

4-(2-Pyridylthiomethyl)pyridine (XI) was prepared from 8.25 g. of 4-(chloromethyl)pyridine hydrochloride and 5.5 g. of 2-pyridinethiol in the same manner as IX. The product had b.p. 142° (1 mm.), yield 4.3 g. (42.5%).

Anal. Calcd. for $C_{11}H_{10}N_2S$: C, 65.31; H, 4.98. Found: C, 65.52; H, 4.94.

2-[2-(5-Nitro-2-pyridylthio)ethyl]pyridine (XIX).—2-Chloro-5-nitropyridine (4.0 g., 0.025 mole) and 2-(2-mercaptoethyl)pyridine (3.5 g., 0.025 mole) were heated under reflux for 2 hr. in 75 ml. of 2-propanol containing 1.5 g. of KOH. The mixture was filtered hot, and the product crystallized from the filtrate upon cooling. It was recrystallized from 2-propanol; m.p. 92°, yield 2.9 g. (44.4%).

Anal. Calcd. for $C_{12}H_{11}N_3O_2S$: C, 55.15; H, 4.24. Found: C, 55.08; H, 4.18.

2-Phenylthio-3-nitropyridine (XXI).—2-Chloro-3-nitropyridine (4.0 g., 0.025 mole), benzenethiol (2.5 g., 0.023 mole), and NaOH (0.9 g., 0.0225 mole) in 100 ml. of methanol were refluxed for 1.5 hr. The mixture was filtered hot, and the product crystallized from the filtrate on cooling. It was recrystallized from methanol; m.p. 101–104°, yield 4.5 g. (85.7%).

Anal. Calcd. for $C_{11}H_8N_2O_2S$: C, 56.89; H, 3.47. Found: C, 57.05; H, 3.50.

2,2′-Dithiobis(3-nitropyridine) (XXII).—Sodium sulfide (6.6 g., 60% Na_2S by weight, 0.05 mole) and 2.0 g. of sulfur were heated under reflux for 15 min. in 100 ml. of methanol. The mixture was filtered hot and the filtrate was added to 15.9 g. (0.10 mole) of 2-chloro-3-nitropyridine. This mixture was stirred and heated under reflux for 2 hr. The product was collected and washed with hot water and with 2-propanol; m.p. 250–252°, yield 13.0 g. (41.8%).

Anal. Calcd. for $C_{10}H_6N_4O_4S$: C, 38.70; H, 1.95; S, 20.66. Found: C, 38.63; H, 2.28; S, 19.23.

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(11) The reversal of the Crabtree effect by certain disulfides in Ehrlich ascites tumor has been studied by R. N. Etingoff and V. N. Gershanovich, *Biokhimiya*, **18**, 668 (1953).