TABLE II
SOLUBILITIES IN KREBS-RINGER PHOSPHATE BUFFER AT 37°

Compd	λ_{max}, mμ	$\epsilon \times 10^{-4}$	Soly, M
XII	216, 276, 384	5.0, 2.1, 1.3	1.4×10^{-3}
XX	252, 291	1.7, 1.7	3.5×10^{-5}
XXI	237	1.9	4.2×10^{-3}
XXII	240	1.9	2.4×10^{-3}
XXIV	210, 252, 320, 332	6.6, 5.1, 1.3, 1.6	3.2×10^{-6}

Within the group of compounds studied, type a effect is given by aliphatic or heterocyclic disulfides containing amino groups. If the basicity of the amino nitrogens is decreased, as for instance by N-acetylation of cystamine (to give XV), or by introduction of appropriate ring substituents in heterocyclic bases (to give XIX or XXIII), the compounds acquire type b effect. A similar change is obtained when the amino groups of cystamine are replaced by carboxyls (to give XVI). It is interesting to note that formation of the N-oxide (to give XVIII) does not alter the type a effect of 2,2′-dithiodipyridine.

The solubility of XX and XXIV was very low; a concentration comparable to that of the other disulfides studied could not be attained. At the maximum possible concentration these two compounds had no significant effect on the properties studied.

The accumulation of lactate from glucose, caused by action of type b compounds on Ehrlich ascites cells in air, indicates that the formation of pyruvate through the glycolytic pathway is not prevented by these compounds. However, the further oxidation of pyruvate through the Krebs cycle is undoubtedly inhibited. There appears thus to be a selective inhibition of the Krebs cycle by type b compounds, while the glycolytic pathway is relatively undisturbed. Compounds of type a, on the other hand, are strong inhibitors of the glycolytic pathway. The study of these compounds at the enzyme and molecular level is being continued.

Skrede, *et al.*,[6] have studied the effect of several disulfides on citrate oxidation by rat liver mitochondria. It is interesting to note that compounds which we classify as type a were inhibitory, whereas compounds of type b were not. Thus, cystamine at 2×10^{-3} M inhibited respiration to the extent of 70% in the first hour; on the other hand, at the same concentration, N,N′-diacetylcystamine caused no inhibition of mitochondrial oxidation of citrate.

Experimental Section

Materials and Methods. Manometry.—These experiments were carried out as reported previously,[2] except that, for the aerobic glycolysis, instead of lactate determination, the CO_2 evolution in a atmosphere of O_2-CO_2 (95:5) was determined in Krebs-Ringer bicarbonate buffer, pH 7.4. The amount of heparin added was 50 U.S.P. units/ml of ascitic fluid.

Solubility.—These experiments were carried out as described previously[2] and are reported in Table II.

Melting points were determined on the Fisher-Johns block.

6,6′-Dithiodinicotinic Acid (XIX).—6-Mercaptonicotinic acid was oxidized with iodine and KI at pH 7, according to the procedure described by Fox and Gibas.[7] The disulfide was purified by repeated extraction with hot acetone; mp 265°, quantitative yield.

Anal. Calcd for $C_{12}H_8N_2O_4S_2$: C, 46.75; H, 2.62. Found: C, 47.11; H, 2.82.

6,6′-Dithiodinicotinamide (XX).—6-Mercaptonicotinamide was oxidized with iodine and KI in alkaline medium (KOH), according to the procedure described by Miller, *et al.*[8] The disulfide was recrystallized from 2-propanol; mp 263–265°, yield 60%.

Anal. Calcd for $C_{12}H_{10}N_4O_2S_2$: C, 47.04; H, 3.29. Found: C, 46.34; H, 3.57.

2,2′-Dithiodipyrimidine (XXI).—2-Mercaptopyrimidine was oxidized with iodine and KI in alkaline medium.[8] The product was recrystallized from ethyl acetate–petroleum ether (bp 60–110°); mp 139–140°, yield 53%.

Anal. Calcd for $C_8H_6N_4S_2$: C, 43.22; H, 2.72. Found: C, 43.57; H, 2.95.

6,6′-Dimethyl-2,2′-dithiodipyrimidine (XXII).[9]—2-Mercapto-6-methylpyrimidine was oxidized in the same manner.[8] The product was recrystallized from acetone–petroleum ether (bp 30–60°); mp 108–109°, yield 90%.

Anal. Calcd for $C_{10}H_{10}N_4S_2$: C, 47.96; H, 4.02. Found: C, 48.36; H, 3.90.

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Amides of N-Acylcysteines as Mucolytic Agents

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The synthesis of N-acylated cysteines[1-3] as mucolytic agents was extended to include some new carboxamides,[4,5] typified by L-2-acetamido-3-mercaptopropionamide (**2**), the amide of N-acetyl-L-cysteine (NAC). Only two 2-amino-3-mercaptopropionamides have been reported[6,7] previously. 2-Amino-N-β-naphthyl-3-mercaptopropionamide[6] was prepared in connection with oxytocin studies, and 2-amino-3-mercapto-N-*n*-octadecylpropionamide[7] was obtained in crude form for use as an emulsifying agent.

Chemistry.—Despite unsuccessful attempts by earlier investigators[8,9] to obtain L-cystine diamide dihydrochloride (**19**)[9] by ammonolysis of L-cystine dimethyl ester dihydrochloride,[10] we have found that **19** can be isolated in good yield, provided complete conversion to the dihydrochloride is assured by acidification with

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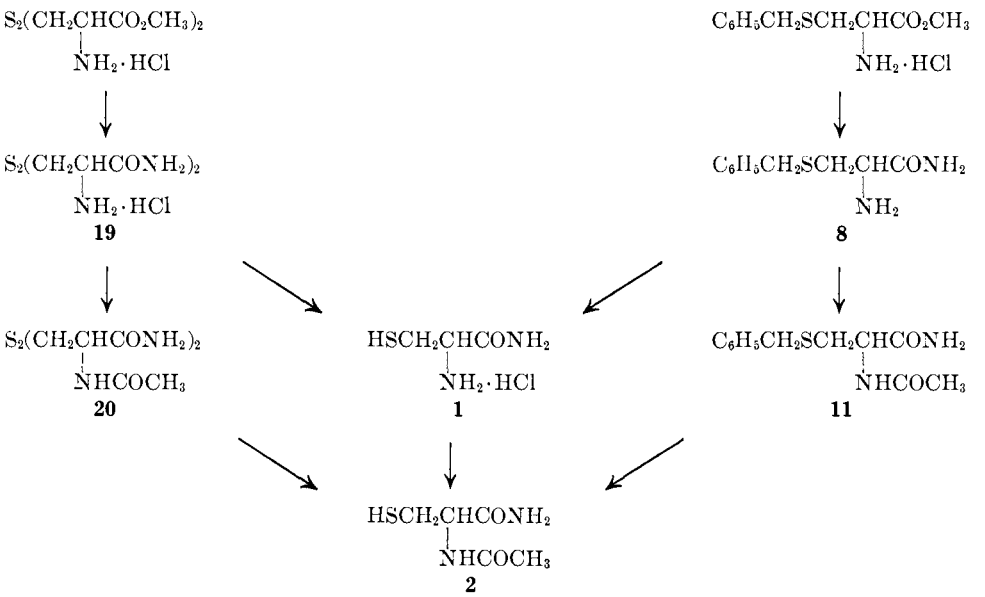
SCHEME I



alcoholic hydrogen chloride. Reduction of **19** with sodium in liquid ammonia gave a desired intermediate, L-2-amino-3-mercaptopropionamide hydrochloride (**1**), in excellent yield. Selective N-acetylation[1-3] of **1** gave **2** (Scheme I).

Intermediate **1** was also prepared by sodium–liquid ammonia debenzylation of L-2-amino-3-benzylthiopropionamide (**8**) and purified through its insoluble mercuric mercaptide. Earlier workers[9] had obtained **1** in solution by reduction of L-N,N′-bis(benzyloxycarbonyl)cystine diamide or L-S-benzyl-N-(benzyloxycarbonyl)cysteine amide, but isolated it only in the form of its oxidation product, L-cystine diamide.

Two other routes to **2** involved acetylation of intermediates **19** and **8**, followed by reduction of the resulting acetylated compounds (**20** and **11**, respectively).

Four analogs of **2** were synthesized (Table I). Compounds **4** and **5** were prepared by reduction of the corresponding acylated cystine diamides (**21** and **23**, respectively). L-2-Formamido-3-mercapto-N-phenylpropionamide (**6**) was readily prepared by removal[10] of the S-diphenylmethyl blocking group from L-3-diphenylmethylthio-2-formamido-N-phenylpropionamide (**17**). Compound **17** was obtained in excellent yield from L-S-diphenylmethyl-N-formylcysteine[10] and aniline by the N,N′-dicyclohexylcarbodiimide method. Applying the carbodiimide method to the condensation of NAC with aniline produced **7**. These findings are similar to the results reported by Sheehan and Hess[11] where N-carbobenzoxyserine was found to react in a like manner.

Mucolytic Data.—The activity of three compounds in reducing the viscosity of a mucoprotein[12] solution is shown in Table II. NAC is included as reference material. Substantially greater mucolytic activity is demonstrated by the 2-acylamino-3-mercaptopropionamides (**1**–**5**) at each time period. Two less soluble compounds (**6** and **7**) do not show good activity.

Oxidative Stability.—In addition to the good mucolytic properties exhibited by several of these products, a greater resistance to autoxidation was demonstrated. The results in Table III show that thiol group oxidation in **2** is minor in comparison to that in NAC and L-cysteine.

Acute Toxicities.—The acute intravenous toxicities for L-2-acetamido-3-mercaptopropionamide (**2**) and NAC were conducted with groups of ten male albino mice[13] weighing 18–28 g and with groups of ten male albino rats[14] weighing 120–168 g. Solutions of **2** in distilled water at pH 5 and in N-saline, adjusted to pH 6.8–7, were administered to mice and rats, respectively. Sodium salt solutions of NAC in distilled water at pH 7 were used in both species. Injections in mice were made at a rate of 0.3 ml/min with volumes of solution that ranged from 10 to 30 ml/kg and in rats at 0.1 ml/min with a volume of 10 ml/kg. Toxic side effects of each compound indicated a generalized depression of the central nervous system. Deaths occurred within 24 hr after treatment and apparently resulted from respiratory failure. The median lethal doses (LD_{50})[15] in mice were determined to be 2820 (2611–3046) and 3800 mg/kg (3420–4220) for **2** and NAC, respectively. In rats the LD_{50} doses were 1870 mg/kg (1655–2113) for **2** and 2550 mg/kg (2473–2625) for NAC.

Experimental Section[16]

Examples of Preparative Methods. A. L-3,3′-Dithiobis(2-aminopropionamide) Dihydrochloride or L-Cystine Diamide Dihydrochloride (19).—L-Cystine dimethyl ester dihydrochloride[10] (34.1 g, 0.1 mole) was added with stirring to 300 ml of liquid NH_3. The NH_3 was allowed to evaporate and the residue was warmed (50°) *in vacuo*. The residual crude solid (27.5 g) was slurried with warm MeOH. A trace of insoluble material was collected (discarded) and the filtrate was acidified with alcoholic HCl to give 24 g (77 %) of **19**.

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(13) Swiss Webster strain of mice obtained from Laboratory Supply Company, Indianapolis, Ind.
(14) Wistar strain of rats obtained from Harlan Industries, Cumberland, Ind.
(15) Median lethal doses were calculated according to the method of T. J. Litchfield and F. Wilcoxon, *J. Pharmacol. Expll. Therap.*, **96**, 99 (1949).
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TABLE I

L-$R_1SCH_2CHCONHR_3$ with NHR_2

No.	R_1	R_2	R_3	Prep[a] method	Yield, %	Mp, °C[b]	Recrystn solvent[c]
1	H	H	H	C, D	84, 53	191–192 dec	A
2	H	$COCH_3$	H	H–K	88–50	148–150	B
3[e]	H	$COCH_3$	H	L	6	161–162.5	C
4	H	$COCH_2CH_3$	H	L	21	160–162.5 dec	D
5	H	$COCH_3$	CH_3	L	36	192–194.5	E
6	H	CHO	C_6H_5	N	69	166.5–167.5	E
7	H	$COCH_3$	C_6H_5	O	11	195–196	F
8	$C_6H_5CH_2$	H	H	B	58	75.5–77.5	G
9	$C_6H_5CH_2$	H	H	B	43	210–212 dec	E
10	$C_6H_5CH_2$	CHO	H	R	8	145–146	H
11	$C_6H_5CH_2$	$COCH_3$	H	E, F	83, 54	150.5–151.5	I
12[e]	$C_6H_5CH_2$	$COCH_3$	H	F	4	176–178	E
13	$C_6H_5CH_2$	COC_6H_5	H	Fv	60	168–168.5	E
14	$C_6H_5CH_2$	$COCH_3$	CH_3	F	75	155.5–156.5	D
15[e]	$C_6H_5CH_2$	$COCH_3$	$(CH_2)_2OH$	P	55, 73	108–110	D
16	$(C_6H_5)_2CH$	H	H	B[d]	50	110.5–112	J
17	$(C_6H_5)_2CH$	CHO	C_6H_5	O	92	140.5–141.5	K
18	CH_3CO	$COCH_3$	H	M	27	141.5–144.5	J
19	$NH_2COCHCH_2S$	H	H	A	77	225.5–227.5 dec	L
20	$NH_2COCHCH_2S$	$COCH_3$	H	G	70	240.5–241.5 dec	M
21	$NH_2COCHCH_2S$	$COCH_2CH_3$	H	G	47	195–197	E
22	$NH_2COCHCH_2S$	COC_6H_5	H	Q	76	239.5–240.5 dec	N
23	$NHCOCHCH_2S$	$COCH_3$	CH_3	G, Kv	88, 69	263.5–264.5 dec	O

[a] See Experimental Section for examples of the preparative methods. [b] All melting points are corrected (Thomas–Hoover capillary apparatus). [c] A = 90% aqueous MeOH, B = 90% aqueous EtOH, C = anhydrous EtOH, D = EtOAc–EtOH, E = EtOH, F = EtOAc–MeOH, G = EtOAc, H = 50% aqueous EtOH, I = 85% aqueous EtOH, J = 2-PrOH, K = 80% aqueous EtOH, L = 66% aqueous MeOH, M = H_2O, N = slurried with DMF–EtOH, O = MeOH. [d] (1) *c* 5, H_2O; (2) 1, MeOH; (3) 1, H_2O; (4) 1, EtOH

TABLE II

COMPARISON OF THE RATE AND EXTENT OF REDUCTION OF VISCOSITY OF MUCOPROTEIN SOLUTION[a]

Compd	% decrease in viscosity		
	3 min	30 min	60 min
L-2-Acetamido-3-mercapto-propionamide (**2**)	27	33	34
L-2-Acetamido-3-mercapto-N-methylpropionamide (**5**)	20	27	28
L-2-Propionamido-3-mercapto-propionamide (**4**)	20	26	29
N-Acetyl-L-cysteine (NAC)	9	16	21

[a] In each test the reaction mixture consisted of the mucoprotein (porcine gastric mucin), 1.5%; NaCl, 0.9%; and test compound, 0.05 *M*, in a total volume of 1 ml. The solutions were adjusted to pH 8.0 and held at 37° for the period of time specified.

TABLE III

COMPARISON OF OXIDATIVE STABILITY[a]

Compd	% reduction in thiol concn		
	15 min	30 min	60 min
L-2-Acetamido-3-mercapto-propionamide (**2**)	6	7	12
N-Acetyl-L-cysteine (NAC)	18	52	93
L-Cysteine	65	100	100

[a] Solutions of the three compounds having concentrations of 0.4 μmole/ml and containing 10^{-10} mole/ml of $CuSO_4$ were prepared in pH 8 0.1 *M* Tris buffer. After O_2 had bubbled through similar aliquots of each compound for specified periods of time, the residual thiol concentrations were then determined by the *p*-chloromercuribenzoate method, described by P. D. Boyer, *J. Am. Chem. Soc.*, **76,** 4331 (1954), and modified by A. L. Sheffner, E. M. Medler, K. R. Bailey, D. G. Gallo, A. J. Mueller, and H. P. Sarett, *Biochem. Pharmacol.*, **15,** 1523 (1966).

B. L-2-Amino-3-benzylthiopropionamide (8) and HCl (9).—After 133 g (0.51 mole) of S-benzyl-L-cysteine methyl ester hydrochloride was added to 2.1 l. of MeOH saturated at 10–15° with NH_3, a slow stream of NH_3 was passed into the mixture for 1 additional hr. The flask was stoppered securely and allowed to stand for 3 days. The reaction mixture was concentrated to a slurry and diluted with 500 ml of dry Et_2O. Compound **9** was collected, washed with Et_2O, and dried; yield 54 g (43%). The free base **8** was obtained by concentrating the filtrate to a slurry, adding 250 ml of dry Et_2O, and filtering; yield 62 g (58%) of white solid.

L-2-Amino-3-mercaptopropionamide Hydrochloride (1). C.—Compound **19** (15.5 g, 0.05 mole) in 300 ml of liquid NH_3 was treated with Na, in small pieces, until the blue color persisted for a few minutes. After the NH_3 had evaporated, the residual white solid was dried under reduced pressure, slurried with 85 ml of 80% MeOH, and acidified with MeOH–HCl. The NaCl was removed by filtration and the filtrate was concentrated to a slurry. Dilution with MeOH and cooling gave 13.2 g (84%) of product in two crops.

D.—A slight excess of Na was added, during 20 min, to 38 g (0.18 mole) of **8** in 550 ml of liquid NH_3. Evaporation of NH_3 left a residual solid which was dissolved in 200 ml of H_2O. The solution was acidified with 130 ml of 6 *N* HCl, extracted with two 250-ml portions of Et_2O and concentrated slightly to remove dissolved Et_2O. A solution of 68 g (0.25 mole) of $HgCl_2$ in 160 ml of 2.25 *N* HCl was added and the resulting suspension was stirred for 3 hr. The solid was collected on a filter, washed with H_2O, and suspended in 600 ml of 3 *N* HCl. The mixture was stirred for 3 hr while a slow stream of H_2S was introduced. After the mixture was allowed to stand overnight, it was filtered to remove HgS. The clear filtrate was concentrated under reduced pressure at 40–50° to give the crude product. Recrystallization from 90% MeOH gave **1** as a white solid in 53% yield (15 g).

L-2-Acetamido-3-benzylthiopropionamide (11). E.—Seven grams (0.0685 mole) of Ac_2O was added to a stirred suspension of 10.5 g (0.05 mole) of **8** and 100 ml of EtOAc. The exothermic reaction raised the temperature to 40° and nearly all of the suspended solid was brought into solution. After warming at 65°

Formula	Calcd, % C	H	N	S(SH)	Found, % C	H	N	S(SH)	$[\alpha]^{25}D$, deg (solvent[d])
$C_3H_8N_2OS \cdot HCl$	23.00	5.79	17.89	(21.1)	23.23	5.65	17.53	(20.6)	+14.51 (1)
$C_5H_{10}N_2O_2S$	37.02	6.21	17.27	(20.3)	37.32	6.44	17.06	(20.4)	−12.28 (1)
$C_5H_{10}N_2O_2S$			17.27	(20.3)			17.09	(20.8)	0.0 (1)
$C_6H_{12}N_2O_2S$	40.89	6.86	15.90	18.20	41.10	6.96	15.78	18.29	−14.65 (2)
$C_6H_{12}N_2O_2S$	40.89	6.86	15.90	(18.76)	40.79	6.70	15.88	(18.0)	−28.23 (3)
$C_{10}H_{12}N_2O_2S$	53.55	5.40	12.49		53.50	5.46	12.38		+17.11 (4)
$C_{11}H_{14}N_2O_2S$	55.44	5.92	11.76		55.27	6.15	11.50		−45.82 (2)
$C_{10}H_{14}N_2OS$	57.11	6.71	13.32	15.25	57.03	6.70	13.32	14.85	
$C_{10}H_{14}N_2OS \cdot HCl$			11.36	12.99			11.30	12.73	+24.3 (5)
$C_{11}H_{14}N_2O_2S$	55.44	5.92	11.75	13.45	55.34	5.84	11.67	13.55	+1.15 (2)
$C_{12}H_{16}N_2O_2S$	57.12	6.39	11.10		57.24	6.48	10.90		−30.98 (6)
$C_{12}H_{16}N_2O_2S$	57.12	6.39	11.10	12.71	57.03	6.46	10.78	12.82	0.0 (2)
$C_{17}H_{18}N_2O_2S$	64.94	5.78	8.91	10.20	64.89	5.85	8.70	10.21	−73.4 (2)
$C_{13}H_{18}N_2O_2S$	58.62	6.81	10.52		58.66	6.97	10.37		−19.69 (6)
$C_{14}H_{20}N_2O_3S$	56.72	6.80	9.45	10.82	56.99	6.90	9.16	10.98	−1.2 (4)
$C_{16}H_{18}N_2OS$	67.10	6.33	9.78		66.92	6.37	9.69		0.0 (4)
$C_{23}H_{22}N_2O_2S$	70.74	5.68	7.18		70.72	5.73	7.13		+16.89 (4)
$C_7H_{12}N_2O_3S$	41.16	5.92	13.72		41.10	6.22	13.60		−21.8 (3)
$C_6H_{14}N_4O_2S_2 \cdot 2HCl$	23.15	5.18	18.01		23.43	5.14	17.74		−196.95 (7)
$C_{10}H_{18}N_4O_4S_2$	37.25	5.63	17.38		37.55	5.90	17.68		−124.49 (3)
$C_{12}H_{22}N_4O_4S_2$	41.12	6.34	15.99	18.30	40.87	6.29	15.71	18.01	−107.29 (4)
$C_{20}H_{22}N_4O_4S_2$	53.80	4.96	12.54	14.36	53.84	4.87	12.34	14.10	−243.3 (8)
$C_{12}H_{22}N_4O_4S_2$	41.12	6.33			41.14	6.40			−76.75 (3)

(5) 0.5, H_2O; (6) 2, EtOH; (7) 1, 1 *N* HCl; (8) 1, DMF. [e] Racemic form. [f] 1 *N* HCl was employed as the reaction solvent. [g] Benzoyl chloride was used as the acylating agent. [h] Carried out at atmospheric pressure. [i] The residual mass, remaining after the solvent (MeOH–NH_3) had evaporated, was suspended in MeOH and treated with an equivalent of anhydrous NaOAc. [j] The autoxidation product.

for 10 min and then cooling, the white product was collected, washed with EtOAc, and dried; yield 10.5 g (83%).

F.—After treating 200 g (0.765 mole) of S-benzyl-L-cysteine methyl ester hydrochloride with 2.1 l. of MeOH saturated with NH_3, the mixture was concentrated to dryness and treated with 500 ml of H_2O, 42 g (0.51 mole) of anhydrous NaOAc, and 86.5 g (0.846 mole) of Ac_2O. The reaction temperature rose to 50° and the product precipitated. After stirring the mixture with 400 ml of additional H_2O, the crude solid was collected, washed with H_2O, and dried; yield 170 g (87%). The DL form (**12**) was obtained as the first crop by solution of the crude solid in 1.2 l. of warm EtOH, followed by dilution with 150 ml of H_2O; yield 8 g (4%). Compound **11** was obtained on cooling the filtrate; yield 105 g (54%).

G. L-3,3′-Dithiobis(2-acetamidopropionamide) (20) was prepared in 70% yield from 6 g (0.018 mole) of **19** by using acylation conditions similar to those of method F.

2-Acetamido-3-mercaptopropionamide (2). H. Selective N-Acetylation of 1.—To a stirred mixture of 11 g (0.07 mole) of **1** and 97 ml of 82% aqueous tetrahydrofuran (THF) was added 10.9 g (0.08 mole) of $NaOAc \cdot 3H_2O$. The temperature of the reaction mixture dropped to 15° and stirring was continued until the temperature rose to 20°. The mixture was then kept at 0–5° while 6.7 ml (7.24 g, 0.071 mole) of Ac_2O was added over a period of 20 min. After stirring overnight at room temperature, 3 ml of 6 *N* HCl and 200 ml of THF were added. The NaCl was collected and the filtrate was concentrated to give a white solid which was recrystallized from EtOH; yield 7 g (61%).

I. Zinc Reduction of 20.—Concentrated H_2SO_4 (15 g, 0.153 mole) was added slowly to a stirred mixture of 49 g (0.152 mole) of **20**, 12 g of Zn dust, and 100 ml of 2 *N* HOAc. The exothermic reaction raised the temperature to 55°. After warming for 1 hr at 45–50°, the reaction mixture was concentrated. The residue was dissolved in 150 ml of warm EtOH. Crystallization afforded 43.5 g (88%) of crude **2** in four crops.

J. Sodium–Liquid Ammonia Reduction of 20.—Sodium was added in small pieces (until the blue color persisted for 1–2 min) to a mixture of 32.2 g (0.1 mole) of **20** and 400 ml of liquid NH_3. The solvent was removed. The white residual powder was slurried at 10–15° with 150 ml of 90% EtOH, and EtOH–HCl (80 ml, 4 *N*) was added slowly to bring the mixture to pH 7. After separating the inorganic salt by filtration, the filtrate was concentrated and cooled to give 19.5 g (57%) of crude **2**.

K. Debenzylation of 11.—To 500 ml of liquid NH_3 was added simultaneously (in portions) 25.2 g (0.1 mole) of **11** and Na. The NH_3 was allowed to evaporate under a stream of N_2 and the solid was dried *in vacuo*. The residual powder was stirred with 40 ml of ice–H_2O and the pH of the mixture was adjusted to 4–5 with concentrated HCl. The product **2** was collected and dried; yield 8.1 g (50%).

L. DL-2-Acetamido-3-mercaptopropionamide (3).—The mother liquors from a larger (0.85 mole) run of the preceding example (K) were cooled to give 8.5 g (6%) of **3**.

M. L-2-Acetamido-3-acetylthiopropionamide (18).—After debenzylating 29 g (0.127 mole) of **8** in approximately 50% yield according to method D, the product without isolation was treated with about 2 equiv (15.1 g, 0.148 mole) of Ac_2O according to method H to give 6.5 g (27%) of the N,S-diacetyl derivative (**18**).

N. L-2-Formamido-3-mercapto-N-phenylpropionamide (6).—A mixture of 5.85 g (0.015 mole) of **17**, 2 g of phenol, and 50 ml of CF_3COOH was heated under reflux for 20 min. The resulting solution was concentrated to a semisolid which was slurried with a mixture of Et_2O and H_2O to give **6**; yield 2.3 g (69%).

O. L-2-Acetamido-3-mercapto-N-phenylpropionamide (7).—N,N′-Dicyclohexylcarbodiimide (10.3 g, 0.05 mole) was added at 10° to a stirred solution of 8.1 g (0.05 mole) of NAC, 4.65 g (0.05 mole) of aniline, and 90 ml of THF. The reaction temperature increased exothermically to 25°. After stirring the mixture overnight at room temperature, 10.5 g of 1,3-di(cyclohexyl)urea was collected. The filtrate was concentrated to a semisolid which was crystallized from EtOAc–Skellysolve B to give 1.3 g (11%) of **7** as white solid in two crops.

P. DL-2-Acetamido-3-benzylthio-N-(2-hydroxyethyl)propionamide (15).—A mixture of 7.5 g (0.02 mole) of 4-nitrophenyl N-acetyl-S-benzyl-DL-cysteinate,[17] 1.2 g (0.02 mole) of 2-amino-

(17) W. D. Cash, *J. Org. Chem.*, **27**, 3329 (1962).

ethanol, and 100 ml of THF was stirred overnight at room temperature and filtered. The filtrate was concentrated to a brown gum which was dissolved in 40 ml of 1:1 EtOH–Et_2O. The solution was decolorized with Nuchar, concentrated to a small volume, diluted with EtOAc–heptane, and stored at 0°. The separated solid was collected and triturated with acetone giving 3.3 g (55%) of **15**.

In a later preparation the gummy reaction product was triturated with acetone to give comparable product in 73% yield.

Q. L-3,3′-Dithiobis(2-benzamidopropionamide) (22).—Benzoyl chloride (15.6 g, 0.11 mole) was added slowly at 5–10° to a mixture of 15.6 g (0.05 mole) of **19**, 16.4 g (0.2 mole) of anhydrous NaOAc, 150 ml of H_2O, and 10 ml of toluene. After the mixture was stirred for 3 days at room temperature, it was filtered to separate **22**; yield 17 g (76%).

R. L-3-(Benzylthio)-2-formamidopropionamide (10).—A solution of 20.5 g (0.098 mole) of **8** in 200 ml of 97–100% HCO_2H was treated dropwise at 5–10° with 70 ml (0.74 mole) of Ac_2O. After being warmed slowly to room temperature, the mixture was diluted with 1 l. of EtOAc, and filtered. The filtrate was concentrated at reduced pressure to a small volume and diluted with 600 ml of H_2O. The white solid which separated was collected, washed with H_2O, and dried; yield 11.5 g of crude material. Recrystallization from 50% EtOH gave 2 g (8%) of **10**.

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Synthesis and Reactions of Some Pyrimidylethyl Isocyanates

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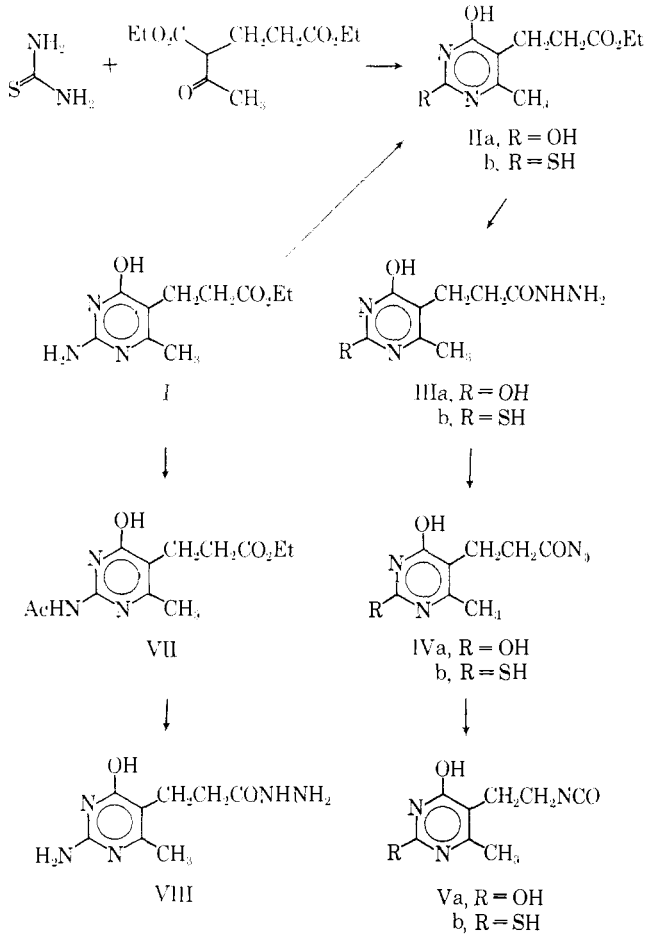
The synthesis of the *p*-aminobenzoyl-L-glutamic acid derivatives of pyrimidylethyl isocyanates was prompted by earlier work on nonclassical antimetabolites by Baker which demonstrated that drastic alterations in the tetrahydrofolic acid molecule brought forth related compounds with antimetabolite activity.[3]

The reactions outlined in Chart I illustrate the synthetic scheme followed for the acquisition of the intermediate isocyanates. Rearrangement of the azides was accompanied by a shift in infrared absorption from 2130–2140 (azide) to 2280 cm^{-1} (isocyanate).[4]

Acetylation of the 2-amino group of I to give ethyl 3-(2-acetamido-4-hydroxy-6-methyl-5-pyrimidyl)-propionate (VII) was undertaken to protect this active group in subsequent reactions. In spite of the fact that the acetyl group was lost on reaction of VII with hydrazine, giving the 2-amino hydrazide VIII, reaction of VIII with nitrous acid appeared to give 3-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl) propionyl azide which showed an infrared peak at 2160 cm^{-1}.

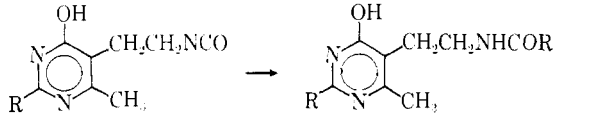
CHART I



However, rearrangement to the corresponding isocyanate was unsuccessful.

The presence of the isocyanate group in Va was demonstrated by its conversion to the isobutylcarbamate VIa. Also prepared were the *p*-aminobenzoic acid and *p*-aminobenzoyl-L-glutamic acid derivatives of Va and Vb, which are illustrated in Chart II.

CHART II



VIa, R = OH; R′ = $(CH_3)_2CHCH_2O$
b, R = OH; R′ = *p*-$HO_2CC_6H_4NH$
c, R = OH; R′ = $HO_2C(CH_2)_2CH(CO_2H)NHCOC_6H_4NH$
d, R = SH; R′ = *p*-$HO_2CC_6H_4NH$
e, R = SH; R′ = $HO_2C(CH_2)_2CH(CO_2H)NHCOC_6H_4NH$

Compounds VIb and VId were not inhibitory to the growth of *Streptococcus faecalis* (ATCC 8043) when tested at concentrations ranging from 10^{-3} to 10^{-8} *M*.[5] Compounds VIb, VIc, VId, and VIe also showed no inhibition when 10^{-3} *M* solutions were tested on the enzyme folic reductase.[6,7]

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(2) Taken from the thesis of K. Eskins, which was submitted as partial fulfillment of the requirements for the Ph.D. degree.

(3) (a) B. R. Baker and C. E. Morreal, *J. Pharm. Sci.*, **52**, 840 (1963); (b) B. R. Baker, C. E. Morreal, and B. T. Ho, *J. Med. Chem.*, **6**, 658 (1963); (c) B. R. Baker and H. S. Shapiro, *ibid.*, **6**, 664 (1963).

(4) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, p 263.

(5) S. F. Zakrzewski and C. A. Nichol, *J. Biol. Chem.*, **205**, 361 (1953).

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(7) W. C. Werkheiser, *J. Biol. Chem.*, **236**, 888 (1961).