ized, and sterilized by free flowing steam for 10 min. Cells for the inoculum were grown in Henderson and Snell[6] basal medium and harvested after 6–8 hr at 35°.

E. coli.—The methods used were similar to those described[5] with the exceptions that total volumes of 10 ml/50-ml erlenmeyer flask and incubation on a reciprocating shaker at 28° were employed.

Testing Procedures.—Initially, all compounds were tested at final concentrations of 100, 200, 400, and 800 μg/ml, both in the absence and presence of exogenous L-cysteine hydrochloride (1.5 μg/ml, a level required for about half-maximal growth) with *L. mesenteroides*. With *E. coli* similar levels of compound were evaluated but only in the absence of cysteine.

(6) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

N-Acylcysteines

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As a continuation of work on N-acylcysteines,[1,2] a number of cysteine and cystine derivatives, including several N-acylcysteine salts, were synthesized. The mucolytic effects of representative compounds have been reported.[3a] The biological activities of many of these derivatives as amino acid antagonists in bacteria are presented elsewhere.[3b]

The selective N-acylation method,[1,2] involving the reaction of a mercaptoamino acid with an equivalent of acetic anhydride in the presence of an acid acceptor or buffering agent, *e.g.*, sodium acetate, was employed for the preparation of N-acetyl-DL-cysteine (**9**) and N-acetyl-D-penicillamine (**10**). The previously unreported **9** was also obtained from inactive cystine[4] and from S-benzyl-DL-cysteine.[5]

N-Formyl-L-cysteine (**1**), prepared[6] originally by the reduction of N,N′-diformyl-L-cystine, was more readily obtained in crystalline form by heating[7] S-diphenylmethyl-N-formyl-L-cysteine with trifluoroacetic acid and phenol.

N-Acetyl-S-guanyl-DL-cysteine (**18**) was prepared by the addition of thiourea to 2-acetamidoacrylic acid.

The compounds not described in the Experimental Section are listed in Table I.

Experimental Section[8]

Examples of Preparative Methods. A. N-Acetyl-S-benzyl-DL-cysteine (**15**) was prepared in 37% yield according to the procedure of Eiger and Greenstein[9] and in 95% yield by the following method.

To a cold (0–3°) solution of 21 g (0.1 mole) of S-benzyl-DL-cysteine[5] and 10 g of NaOH in 100 ml of H_2O was added slowly 15 ml (16.2 g, 0.16 mole) of Ac_2O. The resulting suspension was warmed to 45–50° at which point a dark solution resulted. After 0.5 hr, the reaction mixture was cooled to 0–10° and treated with 75 ml of 4 *N* HCl to precipitate 24 g (95%) of white solid of mp 155–157°. *Anal.* ($C_{12}H_{15}NO_3S$) N, S.

B. N-Acetyl-DL-cysteine (**9**). (**a**) Debenzylation of **15** (21 g, 0.083 mole) was achieved in liquid NH_3 with metallic Na (*ca.* 2.3 equiv). The white sodio derivative was suspended in THF and treated slowly with 17 ml of concentrated HCl to give 8.5 g (63%) of crude product in two crops. Recrystallization from 2-PrOH gave the purified product, mp 127–129°. *Anal.* ($C_5H_9NO_3S$) N, SH.

(**b**) Debenzylation of 42.3 g of S-benzyl-DL-cysteine[5] to the sodio derivative and then selective acetylation[1,2] with 1 equiv of Ac_2O, in 6 ml of HOAc and 100 ml of 80% THF, gave the product in an over-all yield of 60%.

(**c**) DL-Cystine[4] (0.1 mole) in 135 ml of 2 *N* NaOH was acetylated in the cold with 40 ml of Ac_2O to N,N′-diacetylcystine, which, without isolation, was reduced with excess Zn dust. After filtering to remove excess Zn, the filtrate was passed through a Dowex 50W-X8 (200–400 mesh, H^+ form) column and concentrated to give the product in an over-all yield of 23%.

C. N-Acetyl-D-penicillamine (**10**).[10]—Selective acetylation of D-penicillamine·HCl[11] gave this derivative in 77% crude yield. Recrystallization from H_2O gave the purified product of mp 178–179° dec, $[\alpha]^{25}D$ +22° (*c* 1, 50% EtOH), lit.[10] $[\alpha]^{25}D$ +18°. *Anal.* ($C_7H_{13}NO_3S$) N, SH.

D. Piperazinium N-Acetyl-L-cysteine Hydrate (**4**).—To a solution of 16.3 g (0.1 mole) of N-acetyl-L-cysteine (NAC) in 50 ml of MeOH was added a solution of 9.7 g (0.05 mole) of piperazine hexahydrate in *ca.* 50 ml of MeOH. The reaction temperature increased from 20 to 31°. After cooling, the precipitated solid was collected, washed with MeOH, and dried; yield 14.3 g (66%). Recrystallization of 5 g from 150 ml of MeOH–EtOH (1:2) gave 3.1 g of white solid, mp 152.5–154.5° dec. *Anal.* [$(C_5H_9NO_3S)_2 \cdot C_4H_{10}N_2 \cdot H_2O$] C, H, SH.

E. N,N′-Dichloroacetyl-L-cystine (**23**).—A solution of 32.4 g (0.22 mole) of $CHCl_2COCl$ in 100 ml of anhydrous Et_2O and 110 ml of 2 *N* NaOH were added simultaneously over 1 hr to a cold solution (4–8°) of 24 g (0.1 mole) of L-cystine in 103 ml of 2 *N* NaOH. The reaction mixture was stirred for 4 hr, allowed to stand overnight, and acidified with 6 *N* HCl. Extraction with EtOAc yielded 13 g (30%) of product. It was recrystallized from H_2O in 67% yield, mp 166.5–168.5° dec, $[\alpha]^{25}D$ −53.6° (*c* 2.5, 1 *N* NaOH). *Anal.* ($C_{10}H_{12}Cl_4N_2O_6S_2$) N, S, Cl.

F. L-Cysteine Hydantoin (**11**).[12]—Fifty grams of dried Dowex 50W-X8 (200–400 mesh, H^+ form) was added slowly to a warm (50–60°) mixture of 20 g (0.0688 mole) of L-cystine hydantoin (**25**),[13] 10 g of Zn dust, and 125 ml of H_2O. After holding 2.5 hr at 55–60°, the mixture was filtered to remove the resin (Zn^{2+} form) and excess Zn dust. The filter cake was washed with hot H_2O and the total filtrate was cooled to give 15.6 g (77%) of product in three crops. Recrystallization from H_2O gave 10.5 g (53%) of pure product of mp 147.5–149°, $[\alpha]^{25}D$ −97.8° (*c* 1, DMSO). *Anal.* ($C_4H_6N_2O_2S$) C, H, N.

G. N-Acetyl-S-guanyl-DL-cysteine (**18**).—A stirred suspension of 8.1 g (0.106 mole) of thiourea, 20 ml of 5.4 *N* HCl, 40 ml of CH_3OH, and 12.9 g (0.1 mole) of 2-acetamidoacrylic acid[14] was warmed at 38–40° for 5 hr. At the end of this time the slightly turbid solution was filtered. The filtrate was concentrated to a semisolid which was dissolved in 15 ml of H_2O and treated with 8.4 g of $NaHCO_3$ to bring the pH of the solution to *ca.* 6. Addition of 20 ml of MeOH precipitated the crude product which was recrystallized from 75% MeOH to give 4 g (19%) of pure product, mp 195.5–196.5° dec. The nmr spectrum was consistent with the structure. *Anal.* ($C_6H_{11}N_3O_3S$) C, H, N.

H. L-Cysteinium N-Acetyl-L-cysteinate (**2**).—To a solution of 48.4 g (0.4 mole) of L-cysteine and 500 ml of H_2O was added

(1) T. A. Martin and C. W. Waller, U. S. Patent, 3,184,505 (1965).

(2) T. A. Martin, J. R. Corrigan, and C. W. Waller, *J. Org. Chem.*, **30**, 2839 (1965).

(3) (a) A. L. Sheffner, *Ann. N. Y. Acad. Sci.*, **106**, 298 (1963); U. S. Patent 3,091,569 (1963); *Pharmacotherapeutica*, **1**, 46 (1965); (b) W. A. Zygmunt and T. A. Martin, *J. Med. Chem.*, **11**, 623 (1968).

(4) H. L. Loring and V. du Vigneaud, *J. Biol. Chem.*, **102**, 287 (1933).

(5) O. Gawron and A. J. Glaid III, *J. Am. Chem. Soc.*, **71**, 3232 (1949).

(6) W. O. Foye and M. Verderame, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 273 (1957).

(7) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).

(8) We are grateful to Messrs. John G. Schmidt, Clarence Kennedy, and Charles M. Combs of our Control Laboratories for the analytical and instrumental data. The infrared spectra of all the described compounds were consistent with the assigned structures. The melting points are corrected (Thomas–Hoover capillary apparatus). In general, all preparative operations involving sulfhydryl compounds were carried out in an atmosphere of nitrogen, using deionized water. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

(9) I. Z. Eiger and J. P. Greenstein, *Arch. Biochem.*, **19**, 467 (1948).

(10) H. M. Crooks, Jr., in "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p 470.

(11) B. E. Leach and J. H. Hunter, *Biochem. Prepn.*, **3**, 111 (1953).

(12) J. V. Karabinos and J. L. Szabo, *J. Am. Chem. Soc.*, **66**, 649 (1944).

(13) W. C. Hess, *ibid.*, **56**, 1421 (1934).

(14) H. W. Coover, Jr., and J. B. Dickey, U. S. Patent 2,622,074 (1952

TABLE I

L-$R_1SCH_2CHCO_2R_3$
|
NHR_2

No.[a]	R_1	R_2	R_3 (salt)[b]	Prep method (variation)[c]	Yield, %	Mp, °C	Recrystn solvent[d]	Formula	Analyses	$[\alpha]^{25}D$, deg (solvent)[e]
1	H	CHO	H	Ref 7	58	139.5–140.5 dec	A	$C_4H_7NO_3S$	N, SH	+15.9 (1)
3	H	$COCH_3$	(1)	D (1)	85	108.5–110.5	B	$C_5H_9NO_3S \cdot C_5H_{12}NO_2$	N, SH	
5	H	$COCH_3$	(2)	D (2)	84	*Ca.* 48–61[f]		$C_5H_9NO_3S \cdot C_{18}N_{30}N_2S$	C, H, N, SH	
6	H	$COCH_3$	(3)	D (3)	98	109.5–112.5 dec		$C_5H_9NO_3S \cdot C_2H_7NO$	C, H, N	+18.2 (1)
8	H	$COCH_3$	Na	I (4)	...	*Ca.* 120–122[f]		$C_5H_8NO_3SNa$	C, H, N, SH	+21.52 (2)
12	C_2H_5	$COCH_3$	Na	(5)	48	*Ca.* 78–138[f]		$C_7H_{12}NO_3SNa$	N, Na[g]	−15.45 (3)
13	C_2H_5	$COCH_3$	H	(6)	78	122–123.5	B	$C_7H_{13}NO_3S$	N, S	0.0 (4)
14	$C_6H_5CH_2$	$COCH_3$	H	(7)	75	141.5–143.5	C	$C_{12}H_{15}NO_3S$	N, S	−35.4 (5)
16	$C_6H_5CH_2$	$CO(CH_2)_8CH{=}CH_2$	H	(7)	54	78–79.5	D	$C_{21}H_{31}NO_3S$	C, H, S	+1.6 (6)
17	$(C_6H_5)_2CH$	$COCH_3$	H	A	15	165.5–167.5	C	$C_{18}H_{19}NO_3S$	S	
19	CH_3CO	$COCH_3$	H	Ref 2	34	120.5–122.5	A	$C_7H_{11}NO_4S$	N, S	−42.0 (3)
20	CH_3CO	$COCH_3$	H	(8)	84	120–121	E	$C_7H_{11}NO_4S$	N, S	
21	HO_2CCHCH_2S (NHCHO)	CHO	H	Ref 6	56	184.5–185.5 dec	F	$C_8H_{12}N_2O_5S_2$	C, H, N	−104.4 (7)
22	HO_2CCHCH_2S (NHCO$(CH_2)_8CH{=}CH_2$)	$CO(CH_2)_8CH{=}CH_2$	(4)	E, D	36	176.5–177.5 dec	G	$C_{28}H_{48}N_2O_6S_2 \cdot C_4H_{10}N_2$	C, H, N, S	
24	HO_2CCHCH_2S (NHCOCCl$_3$)	$COCCl_3$	H	(9)	38	100–102 dec	D	$C_6H_6Cl_3N_2O_5S_2$	N	−37.37 (8)
25	L-Cystine hydantoin			Ref 12	84	>300	H	$C_8H_{10}N_4O_4S_2$	C, H, N, S	−242.33 (9)

[a] The compounds described in the Experimental Section are omitted from the table. [b] (1) Carboxymethyltrimethylammonium (prepared from betaine), (2) 10-(1-methyl-3-pyrrolidylmethyl)-phenothiazinium, (3) 2-hydroxyethylammonium, (4) piperazinium. [c] See Experimental Section for the letters; (1) product was isolated by concentrating the reaction mixture (solvent, 2-PrOH) and diluting with dry Et_2O; (2) the product was obtained from the methanolic reaction solution by evaporation *in vacuo*; (3) the salt precipitated from the reaction solvent, THF; (4) a 5% aqueous solution was used; (5) the procedure was similar to that of A. D. Brown and A. R. Matzuk, U. S. Patent 2,888,380 (1959); (6) the DL form was obtained from a method similar to that used for N-acetyl-DL-methionine: V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932); (7) the method of G. L. Shiple and C. P. Sherwin, *ibid.*, **55**, 671 (1923), was used; (8) the DL form was prepared by the procedure of M. W. Farlow, U. S. Patent 2,406,362 (1946), except for omitting the catalyst (ascaridole) and effecting the reaction at a lower temperature (60°); (9) the method of J. S. Fruton and H. T. Clarke, *J. Biol. Chem.*, **106**, 667 (1934), was followed. [d] A = H_2O, B = 2-PrOH, C = EtOH, D = EtOAc–Skellysolve B (bp 63–69°), E = EtOAc, F = 50% aqueous MeOH, G = 95% aqueous MeOH, H = DMSO. [e] (1) *c* 1, H_2O; (2) 5, H_2O; (3) 2, H_2O; (4) 1, 1 *N* HCl; (5) 1, EtOH; (6) 2, EtOH; (7) 2, 1 *N* NaOH; (8) 2.5, 1 *N* NaOH; (9) 1, DMSO. [f] Hygroscopic solid. [g] Calculated on the basis of the found 4.88% H_2O content.

65.3 g (0.4 mole) of N-acetyl-L-cysteine (NAC). After stirring the solution for 2 hr and diluting with 500 ml of H_2O, the product was isolated by lyophilization. A 91% yield (102 g) of white solid was obtained; mp *ca.* 162–168° dec, $[\alpha]^{25}D$ +1.13° (*c* 3, H_2O). *Anal.* ($C_5H_9NO_3S \cdot C_3H_7NO_2S$) C, H, N; SH: calcd, 23.2; found, 22.7.

It is noted that the melting point of **2** is intermediate between those of the two reactants: NAC, 109–110°, and L-cysteine, 221.5–223° dec. In contrast, a mechanical mixture of equimolar quantities of the two reactants showed a depressed melting point, *ca.* 101–150° dec, and an ir spectrum significantly different from that of **2**. The components of this salt are, however, loosely bound to one another. L-Cysteine was precipitated in 97% recovery from a 50% aqueous solution of **2** by diluting with EtOH. A 50% recovery of recrystallized NAC was obtained from the filtrate.

I. Calcium N-Acetyl-L-cysteinate Acetate (7). (**a**) To a stirred mixture of 163.2 g (1 mole) of NAC, 60 g (1 mole) of HOAc, and 600 ml of H_2O, was added, in portions, 110 g (1.1 moles) of $CaCO_3$. A rapid evolution of CO_2 followed each addition. After stirring overnight, the reaction mixture was freed of dissolved CO_2, at reduced pressure (aspirator), and filtered (Celite bed). The filter bed was washed with 1 l. of H_2O. Evaporation of the filtrate by lyophilization gave 243 g (93%) of product. Further drying in a vacuum oven at 90–95° lowered the weight to 234 g (89.5%), $[\alpha]^{25}D$ +12.4° (*c* 5, H_2O). *Anal.* [$C_5H_8NO_3(C_2H_3O_2)Ca$] C, H, N, SH.

(**b**) To a stirred solution of 16.3 g (0.1 mole) of NAC in 105 ml of 85% aqueous MeOH was added 17.6 g (0.11 mole) of $Ca(OAc)_2 \cdot H_2O$. The suspension was stirred at room temperature for 3 hr and at 40° for 2 hr. After standing overnight, the suspension was diluted with 15 ml of H_2O and warmed to 40°. The resulting solution was cooled and filtered. Evaporating the filtrate under reduced pressure (*ca.* 2–3 mm) gave 25.5 g (97%) of a white spongy solid. Purification was achieved by reprecipitating the product from a solution in 50% MeOH (1 g/ml) by the addition of a double volume each of acetone and 2-PrOH. The resulting sticky mass was converted to a finely divided, white suspension by slight warming. The pure product was collected on a filter, washed with warm 2-PrOH, and dried in a vacuum oven at 70°; yield, 16 g (61%) of white powder of mp *ca.* 244° dec. The nmr spectrum was consistent with the structure. *Anal.* N, SH, Ca.

The Anticancer and Antimalarial Properties and Preparation of Arylazopyrimidines[1a]

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Since Lythgoe and coworkers[2] reported that azo coupling occurs in the 5 position of the pyrimidine ring, various substituted 5-arylazopyrimidines have been synthesized and their mode of action in various biological systems has been studied. However, very few data were available on the activity of these compounds against tumors in animals or man.

Modest and coworkers[3] found that at least one amino group adjacent to the arylazo link is necessary for optimum activity. They concluded that the aryl group should be unsubstituted or contain electron-releasing substituents for maximum microbiological activity. The work of Tanaka and co-workers,[4] and of Roy-Burman and Sen[5] substantiates these findings. Tanaka and co-workers tested a number of 5-arylazo-2,4,6-triaminopyrimidines containing electron-withdrawing substituents such as sulfonic acid, sulfonamide, carboxylic acid, phosphonic acid, or arsonic acid on the aryl group and they found these compounds to be less active in microbiological systems. However, compounds containing electron-withdrawing substituents on the phenylazo group were effective against Yoshida ascites sarcoma and Ehrlich ascites carcinoma. They concluded that these compounds interfere with nucleic acid synthesis. The inhibitory actions by two of the most active compounds, 5-phenylazo-2,4,6-triaminopyrimidine, and 5-phenylazo-2,4-diamino-6-hydroxypyrimidine were not reversed by most bases and nucleotides involved in nucleic acid synthesis. Roy-Burman and Sen[5] found that the inhibitory effects of arylazopyrimidines in the *Streptococcus faecalis* (ATCC 8043) system could be more efficiently reversed by 5-formyltetrahydrofolic acid than by folic acid. These findings would seem to indicate that arylazopyrimidines may act as antagonists of folic acid and that they may interfere with the enzymatic conversion of folic acid to 5-formyltetrahydrofolic acid. Recent studies by Hampshire and coworkers[6] on the inhibitory effects of 5-arylazo-2,4,6-triaminopyrimidines on folic acid reductase from rat liver indicate that these compounds have a wide range of activities depending on the substituent present on the aryl group. The low activity of 2,4,5,6-tetraaminopyrimidine indicates that reductive cleavage of the 5-azo linkage does not occur in this system. There is no direct correlation between enzyme-inhibitory activity and toxicity or antitumor activity of the 5-arylazopyrimidines studied.

Chadwick and coworkers[7] used spectrophotometric methods with a rat liver homogenate fortified with a NADPH-generating system to determine the ease of reduction of the azo linkage in azopyrimidines and azobenzenes. They found that the azobenzenes were rapidly reduced under these conditions while the arylazopyrimidines were recovered unchanged. They postulated that 5-arylazopyrimidines are inhibitors rather than substrates of liver azo reductase.

Hibino[8] reported that the remission rates in cases of acute leukemia initially treated with a combination of three of the four agents, 6-purinethiol, steroid hormones, mitomycin C, and 4-(2,4,6-triamino-5-pyrimidinylazo)-benzenesulfonic acid, were as high as 85.7%. The remission rates achieved by using steroidal hormones, mitomycin C, and 6-purinethiol either alone or in combination were much lower. This indicated that 4-(2,4,-6-triamino-5-pyrimidinylazo)benzenesulfonic acid has a high synergistic effect when used in combination therapy.

(1) (a) This work was supported in part by a grant from the Michigan Cancer Foundation and Grant CA-06140-06 from the National Cancer Institute. (b) To whom inquiries should be addressed. (c) Abstracted from the thesis of Frederick E. Dutton, submitted as partial fulfillment of Master of Arts Degree.

(2) B. Lythgoe, A. R. Todd, and A. Topham, *J. Chem. Soc.*, 315 (1944).

(3) E. J. Modest, H. N. Schlein, and G. E. Foley, *J. Pharm. Pharmacol.*, **9**, 68 (1957).

(4) (a) K. Tanaka, K. Kaziwara, Y. Aramaki, and M. Kawashina, *Gann*, **47**, 401 (1956); (b) K. Tanaka, E. Omura, T. Sugawa, Y. Sanno, Y. Ando, K. Imai, and K. Kavashina, *Chem. Pharm. Bull.* (Tokyo), **7**, 1 (1959); (c) K. Tanaka, E. Omura, M. Kawashina, J. Watanabe, H. Yokotani, H. Ito, Y. Suguro, M. Ishidata, T. Araki, Y. Aramaki, and K. Kaziwara, *ibid.*, **7**, 7 (1959).

(5) P. Roy-Burman and D. Sen, *Biochem. Pharmacol.*, **13**, 1437 (1964).

(6) J. Hampshire, P. Hebborn, A. M. Triggle, D. J. Triggle, and S. Vickers, *J. Med. Chem.*, **8**, 745 (1965).

(7) M. Chadwick, J. Hampshire, P. Hebborn, A. M. Triggle, and D. J. Triggle, *ibid.*, **9**, 874 (1966).

(8) S. Hibino, *Cancer Chemotherapy Rept.*, **No. 13**, 141 (1961).