boiled vigorously for a few moments and the remainder of the methanol removed (vacuum) whereupon the product crystallized. The latter was recrystallized from 50% ethanol; yield 4.3 g., m.p. 155–156°. A sample was sublimed at 150°/0.1 mm., m.p. 156–157°.

Anal. Calcd. for $C_{18}H_{21}NO_4$: C, 68.6; H, 6.71. Found: C, 68.5; H, 6.64. $[\alpha]_D^{20}$ −81.1° (c = 1, 10% HOAc).

14-Hydroxydihydrocodeine-B. A solution of 1 g. of *true* 14-hydroxycodeine in 30 ml. of 95% ethanol was shaken under hydrogen with 75 mg. of PtO_2. After the uptake of 1.2 moles of hydrogen (35 mins.), the usual manipulation yielded 0.84 g. of colorless crystals, m.p. 145–146°; the melting point was not depressed when mixed with 14-hydroxydihydrocodeine-B[13] (of m.p. 145–145.5°).

A small sample of the above product was acetylated (acetic anhydride–pyridine) and the product worked up as usual. Recrystallized from ethanol, the substance had the m.p. 181° alone or admixed with diacetyldihydrocodeine-B[13] (of m.p. 181–181.5°).

6,14-Diacetoxycodeine. A solution of 1.4 g. of *true* 14-hydroxycodeine in a mixture of 3 ml. of acetic anhydride and 1.5 ml. of dry pyridine was kept at 25° for 24 hr. The resulting crystalline magma was poured onto ice and treated slowly with 6*N* NH_4OH producing a colorless, crystalline precipitate which was collected and recrystallized from 95% ethanol; yield 1.2 g. colorless prisms. After a second recrystallization, m.p. 199° (evac. tube).

Anal. Calcd. for $C_{22}H_{25}NO_6$: C, 66.2; H, 6.31; CH_3CO, 21.5. Found: C, 66.1; H, 6.38; CH_3CO, 21.3. $[\alpha]_D^{20}$ −46.2° (c = 1.02, 10% HOAc).

BETHESDA, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents. V. Some Sulfur-Substituted Derivatives of Cysteine[1]

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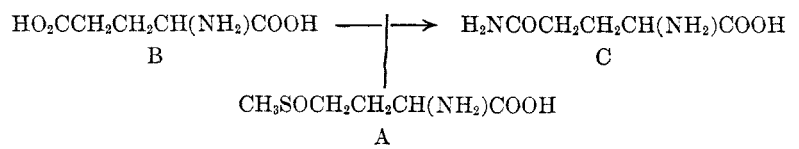
A number of *S*-alkyl and *S*-aryl derivatives of DL- and L-cystine, as well as some of their sulfoxides and sulfones, have been prepared for testing as possible anticancer agents.

In a search for compounds with anticancer activity, a number of derivatives of DL- and L-cysteine have been prepared. These compounds can be considered as potential amino acid antimetabolites; they could affect certain metabolic systems in a way similar to that in which methionine sulfoxide (A) acts as a glutamic acid (B) antagonist in the conversion of glutamic acid (B) to glutamine (C).[2]



The *S*-alkyl- and *S*-arylcysteines in Table I were prepared by a variety of methods. Direct alkylation of L-cysteine with reactive halogen compounds in the presence of dilute aqueous alkali and at room temperature gave good yields of compounds I–IV. The preparation of *S*-methyl-L-cysteine (V) was carried out with dimethyl sulfate and required a long reaction time in order to achieve a good yield. The *N*-benzoyl and *N*-acetyl derivatives of *S*-methyl-L-cysteine (V) were prepared by conventional procedures. The preparation of *S*-isopropyl-L-cysteine (VI) by direct alkylation of L-cysteine with isopropyl bromide or isopropyl iodide gave low yields of VI. The procedure of Gawron and Lieb[3] utilizing the alkylation with isopropyl bromide of the sodium salt of L-cysteine prepared from L-cystine in liquid ammonia gave a high yield of *S*-isopropyl-L-cysteine (VI).

The preparation of *S*-trimethylsilylmethyl-L-cysteine (VII) from L-cysteine and (chloromethyl)-trimethylsilane required a long reaction time in refluxing aqueous dioxane, an indication of the low order of activity of the halogen of the silane. An effort was made to prepare a phosphorylated compound by reaction of L-cysteine in alkali with (chloromethyl)phosphonic acid or dialkyl (chloromethyl)phosphonates. New ninhydrin-positive material was formed in these reactions as shown by paper chromatography, but efforts to purify the products were unsuccessful. Although many of the compounds in Table I are designated as derivative of L-cysteine, it is recognized that the conditions used in methods A, B, and D might lead to various degrees of racemization.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. For the preceding paper of this series, *cf.* Elmer J. Reist, Leon Goodman, Roland R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 3962 (1958).

(2) E. Borek, P. Sheiness, and H. Waelsch, *Federation Proc.*, **5**, 123 (1946).

(3) O. Gawron and J. A. Lieb, *J. Am. Chem. Soc.*, **74**, 834 (1952).

TABLE I

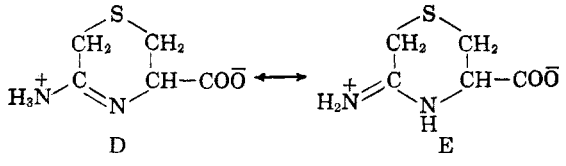
S-ALKYLATED AND *S*-ARYLATED L- AND DL-CYSTEINES

$RSCH_2CH(\overset{+}{N}H_3)\overset{-}{C}OO$

Compound No.[c]	R	Method[c]	Yield, %	M.P., °C. (Dec.)	$[\alpha]_D$ (°C.)[t]	Analyses Calcd. C	H	N	Found C	H	N
I	HO_2CCH_2[a]	A	72	197–203[h]	0.0 (25)[u]						
II	$C_6H_5COCH_2$[a,e]	A	68	90–95	−0.6 (28)	53.2	5.68	5.64	53.6	5.54	5.62
III	$NCCH_2$[a,f]	A	49	>300	−14.6 (25)	37.5	5.03	17.5	37.1	4.83	16.9
IV	NH_2COCH_2[a]	A	77	188–190[i]	−6.6 (26)[v]						
V	CH_3[a]	B[g]	88	230[j]	0.0 (27)[w]						
VI	$(CH_3)_2CH$[a]	C[g]	33	237–239[k]	+3.0 (27)[x]						
VII	$(CH_3)_3SiCH_2$[a]	D	99	165–198	0.0 (28)[y]	40.5	8.27	6.76	40.5	8.28	6.45
VIII	C_6H_5[b]	E	72	190[l]							
IX	2-$CH_3C_6H_4$[b]	E	84	193[m]		56.8	6.21	6.63	57.2	6.30	6.64
X	3-$CH_3C_6H_4$[b]	E	84	190–191[n]		56.8	6.21	6.63	56.7	6.32	6.57
XI	4-$CH_3C_6H_4$[b]	E	54	195–197[o]		56.8	6.21	6.63	56.4	6.06	6.61
XII	4-ClC_6H_4[b]	E	77	195–196[p]							
XIII	4-$NO_2C_6H_4$[b]	E	94	175[q]		44.6	4.17	11.6	44.6	4.17	11.4
XIV	2-$NH_2C_6H_4$[b]	E	94	240		50.9	5.70	13.2	50.9	5.95	13.4
XV	3-$CF_3C_6H_4$[a]	F	17	183–185	+68.2 (28)	45.3	3.77	5.28	45.0	4.00	5.30
XVI	4-FC_6H_4[a]	F	12	185–186[r]	+82.7 (28)[z]	50.2	4.68	6.51	50.4	4.83	6.50

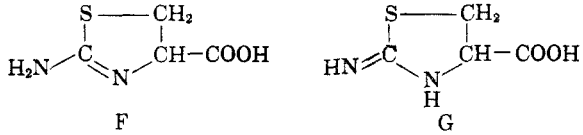
[a] Derivative of L-cysteine. [b] Derivative of DL-cysteine. [c] See Experimental. [d] All compounds were determined to be homogeneous by paper chromatography. [e] Calculated analytical figures are for the hemihydrate. Compound II could be recrystallized from water but only with very poor recovery; the analytical figures are for the crude, washed product. [f] The compound actually exists in the cyclic form (see Discussion). The analytical figures are for the crude, washed product. [g] Method of Gawron and Lieb.[3] [h] Lit. m.p. 204–207°,[4] 175–176°,[5] 193–194°.[6] [i] Lit. m.p. 188–190°.[4] [j] Lit. m.p. 247–248°,[4] 238°.[7a] [k] Lit. m.p. 237–239°,[8] 223–224°.[3] [l] Decomposed without melting; lit. reports compound to decompose without melting above 160°.[9] [m] Lit. m.p. for L-isomer, 166–167°.[10] [n] Lit. m.p. for L-isomer, 175–176°.[10] [o] Lit. m.p. for L-isomer, 200–202°.[10] [p] Lit. m.p. 202°.[11] [q] Lit. m.p. 151°.[11] [r] Lit. m.p. 180–183°.[12] [s] Method of du Vigneaud, Loring, and Craft.[7b] [t] 1% solutions in 1*N* HCl unless otherwise noted. [u] Lit.[4] $[\alpha]_D^{24}$ +0.5° (1% in 1*N* HCl); this compound is not racemic since compounds XXIV and XXX were prepared from I and are optically active. [v] Lit.[4] $[\alpha]_D^{24}$ −6.0° (1% in 1*N* HCl). [w] Lit.[4] $[\alpha]_D^{24}$ −9.6° (1% in 1*N* HCl); product V is evidently racemic. [x] Lit.[8] $[\alpha]_D^{21}$ −19.0° (1% in H_2O). [y] Probably racemic. [z] 0.56% in 1*N* HCl.

Compound III is designated as *S*-cyanomethyl-L-cysteine in Table I. It seems clear, however, that the actual form of the compound is the cyclic structure (D) [or possibly its tautomer (E)]. The infrared spectrum of III showed a typical amino



D E

acid zwitterion spectrum but showed no nitrile absorption near 4.5μ. The ninhydrin color of III was a faint yellow, in contrast with the distinct blue color noted with I, IV, V, VI, and VIII, another indication that the compound probably has the cyclic structure. Alkylation of *N*-benzoyl-L-cysteine with chloroacetonitrile[13] led to *N*-benzoyl-*S*-cyanomethyl-L-cysteine, whose infrared spectrum showed strong nitrile absorption at 4.44μ, a strong suggestion that III, lacking this infrared band, does not possess the open-chain structure but has the cyclic amidine structure (D or E). These results find analogy in the work of Schöberl,[14] who showed that the compound thought to be *S*-cyano-L-cysteine is in reality the cyclic thiazoline (F or G, presumably as the zwitterion).



F G

Compound II was prepared in good yield from either phenacyl chloride or phenacyl bromide. The product gave a yellow color with ninhydrin and it was suspected that the true structure was the cyclized formula (H). The infrared spectrum of

(4) M. D. Armstrong and J. D. Lewis, *J. Org. Chem.*, **16**, 749 (1951).

(5) L. Michaelis and M. P. Schubert, *J. Biol. Chem.*, **106**, 331 (1934).

(6) S. A. Harris, N. R. Easton, D. Heyl, A. N. Wilson, and K. Folkers, *J. Am. Chem. Soc.*, **66**, 1757 (1944).

(7) (a) S. Yurugai, *J. Pharm. Soc. Japan*, **74**, 519 (1954). (b) V. du Vigneaud, H. S. Loring, and H. A. Craft, *J. Biol. Chem.*, **105**, 481 (1934).

(8) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **32**, 866 (1949).

(9) E. Baumann and C. Preusse, *Z. Physiol. Chem.*, **5**, 336 (1881).

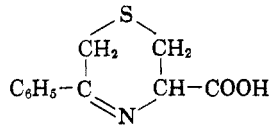
(10) H. D. West and G. R. Mathura, *J. Biol. Chem.*, **208**, 315 (1954).

(11) H. Behringer and E. Fackler, *Ann.*, **564**, 73 (1949).

(12) S. J. Zbarsky and L. Young, *J. Biol. Chem.*, **152**, 599 (1944).

(13) Leon Goodman, Leonard O. Ross, and B. R. Baker, Paper XII of this series, *J. Org. Chem.*, in press.

(14) A. Schöberl, M. Kawohl, and R. Hamm, *Ber.*, **84**, 571 (1951).

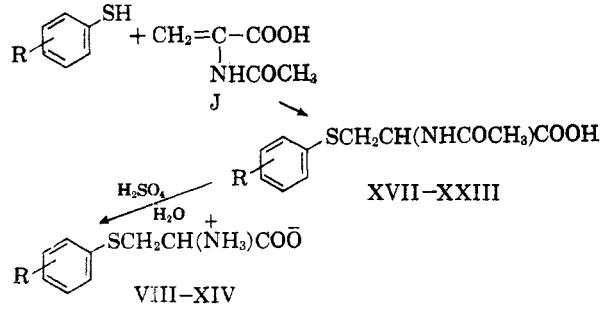


H

compound II, however, showed a strong aromatic ketone carbonyl band at 6.01μ and ammonium ion absorption at 3.30 and 6.12μ, which is typical of α-amino acids. Further, the ultraviolet spectrum of II was very similar to that of acetophenone in water and was essentially the same in 0.1N hydrochloric acid, a strong indication that II exists as the open-chain, α-amino acid. Compound II decomposed upon warming in water and it was necessary to crystallize the compound rapidly from hot water in order to obtain even low recoveries. It is interesting that Dittmer[15] prepared a related compound, aspartophenone [$C_6H_5COCH_2$-$CH(NH_2)COOH$] and reported it to be more effective biologically after heating, possibly an indication of cyclization upon heating.

Attempts to *S*-alkylate L-cysteine with chloroacetone were completely unsuccessful; the products were highly colored, nonhomogeneous solids.

Two methods were used to prepare the *S*-aryl cysteines (VIII–XVI). The method of Behringer and Fackler,[11] involving the base-catalyzed addition of a thiophenol to α-acetamidoacrylic acid (J), gave the corresponding mercapturic acids (XVII–XXIII) (*cf.* Table II) which, in turn, were subjected to acidic hydrolysis, to give the *S*-aryl-DL-cysteines

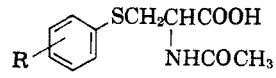


(VIII–XIV). Both the preparation of the mercapturic acid and the acid hydrolysis gave generally good yields, although the use of *ortho*-substituted thiophenols seemed to result in somewhat lower yields.

The second method used to prepare *S*-arylcysteines involved the reaction between a diazotized substituted aniline and the cuprous salt of L-cysteine. This method has been described by du Vigneaud[16] and by West and Mathura[10] and, although it gives generally low yields, it is convenient when the appropriate substituted aniline is available, and has the additional advantage of giving the L-form of the substituted cysteine. It was found, in the present work, that the use of a catalytic quantity of the cuprous salt of L-cysteine with the remainder of the L-cysteine present as the free amino acid represented a distinct inprovement over the procedure which utilized a stoichiometric amount of the preformed cuprous salt of L-cysteine. A disadvantage of the diazonium salt procedure for the preparation of *S*-aryl-L-cysteines resulted from the contamination of the products with appreciable amounts of L-cystine, which was difficult to remove by recrystallization from water. However, it was found that the contaminating L-cystine could be readily removed by recrystallizing the product from water containing sodium sulfite; the L-cysteine was thereby converted to soluble amino acids without affecting the desired *S*-aryl-L-cysteines.[17] Compounds XV and XVI were prepared using both of the described modifications of the diazonium salt synthesis.

TABLE II

DL-MERCAPTURIC ACIDS



Compound No.	R	Yield, %	M.P., °C.	Lit. M.P., °C.
XVII	H	61	150–151	151[11]
XVIII[a]	2-CH_3	30	134–136	For L-isomer, 144.5–145[10]
XIX[b]	3-CH_3	61	128–130	For L-isomer, 141[10]
XX	4-CH_3	51	155–156	154[11]
XXI	4-Cl	65	148–150	148[11]
XXII	4-NO_2	56	168–170	167–168[11]
XXIII[c]	2-NH_2	34	145	

[a] Calcd. for $C_{12}H_{15}NO_3S$: C, 56.9; H, 5.96; N, 5.53. Found: C, 56.8; H, 5.71; N, 5.66, 5.77. [b] Calcd. for $C_{12}H_{15}NO_3S$: C, 56.9; H, 5.96; N, 5.53. Found: C, 56.7; H, 5.97; N, 5.62. [c] Calcd. for $C_{11}H_{14}N_2O_3S$: C, 52.0; H, 5.55; N, 11.0. Found: C, 52.0, 52.2; H, 5.69, 5.77; N, 10.6.

Most of the compounds listed in Table I were subjected to oxidation with 30% hydrogen peroxide with the use of a variety of conditions. The alkylated L-cysteines (I, III, and IV) were converted to their sulfoxides (XXIV, XXV, and XXVI, respectively) in excellent yield by heating the compounds in aqueous solution for a short time with a large excess of 30% hydrogen peroxide. Such a procedure gave complete degradation of *S*-methyl- and *S*-isopropyl-L-cysteine. Only a low yield of the sulfoxide XXVII of *S*-isopropyl-L-cysteine (VI) could be obtained by the reaction of VI with a stoichiometric amount of hydrogen peroxide in acetic acid as solvent, the procedure employed by Stoll and Seebeck.[8] The material yielded poor analytical figures (comparable to those obtained by Stoll and Seebeck[8]) but was homogeneous ac-

(15) K. Dittmer, *Ann. N. Y. Acad. Sci.*, **52**, 1292 (1950).

(16) V. du Vigneaud, J. L. Wood, and F. Binkley, *J. Biol. Chem.*, **138**, 369 (1941).

(17) This method of purification of compounds XV and XVI was suggested by O. P. Crews, Jr.

TABLE III

ALKYLATED AND ARYLATED L- AND DL-CYSTEINE SULFOXIDES

$RSOCH_2CH(\overset{+}{N}H_3)C\overset{-}{O}O$

Compound No.	R	Method[f]	Yield, %	M.P., °C. (Dec.)	$[\alpha]_D$ (°C.)	Analyses					
						Calcd.			Found		
						C	H	N	C	H	N
XXIV	HO_2CCH_2[a]	G	73	190–191	+27.3 (29)[h]	30.8	4.65	7.18	31.0	4.87	7.07
XXV	$NCCH_2$[a,g]	G	80	>300	−30.3 (28)[i]	34.1	4.54	15.9	34.2	4.67	14.9, 14.1
XXVI	NH_2COCH_2[a]	G	84	157–160	+14.1 (29)[j]	30.9	5.19	14.4	30.8	5.33	14.44
XXVII	$(CH_3)_2CH$[a,d]	H[e]	18	154–156[c]		38.3	7.50		38.0	6.98	
XXVIII	C_6H_5[b]	J	46	186–187		50.7	5.20	6.57	50.4	5.47	6.46
XXIX	3-$CH_3C_6H_4$[b]	K	37	137–138		52.8	5.76	6.16	53.0	5.75	6.01

[a] Derivative of L-cysteine. [b] Derivative of DL-cysteine. [c] Lit. m.p. 155–156°.[8] [d] The calculated analytical values are for the hemihydrate; Stoll and Seebeck[8] reported similar analytical results. [e] Method of Stoll and Seebeck.[8] [f] See Experimental. [g] Compound XXV exists in the cyclic form as shown by the absence of nitrile infrared absorption near 4.5μ. [h] 1% in 1*N* HCl. [i] 0.86% in 1*N* HCl. [j] 0.55% in 1*N* HCl.

cording to paper chromatography and possessed an infrared spectrum in accord with structure XXVII. A number of attempts were made to convert *S*-methyl-L-cysteine (V) to its sulfoxide in a worthwhile yield, but these were uniformly unsuccessful. It is evident that the simple *S*-alkyl-L-cysteines are rapidly degraded under mild oxidizing conditions. Conditions were found that permitted the isolation of *S*-aryl-L-cysteine sulfoxides XXVIII and XXIX by hydrogen peroxide oxidation of VIII and X, respectively. Generally, the oxidation of the *S*-arylcysteines listed in Table I could not be stopped at the sulfoxide stage, so that separation of the mixture of parent amino acid, sulfoxide, and sulfone was impractical. Even carrying out the oxidations in strong acid as suggested by Bordwell and Bouton[18] did not favor the formation of sulfoxides. The data concerning the sulfoxides are summarized in Table III.

The use of infrared spectra and of paper chromatography was especially helpful in determining the composition of the products of the oxidations. The sulfoxide absorpton band near 9.75μ and the two sulfone bands near 7.70 and 8.80μ were completely reliable for the identification of both the pure oxidation products and mixtures of them. The solvent system *n*-butyl alcohol/methyl ethyl ketone/17*N* ammonia/water (5/3/1/1)[19] was generally capable of resolving mixtures of the parent amino acids, sulfoxides, and sulfones, all of which could be detected with ninhydrin. In the above solvent system, the parent acid moved faster than the sulfone, which, in turn, moved faster than the sulfoxide.[20] In the acidic solvent system *t*-butyl alcohol/formic acid/water (6.95/0.10/2.95)[21] the order of movement was also parent acid>sulfone>sulfoxide. An exception was the case of compounds III and XXV, where the sulfoxide XXV moved faster than the parent acid III, a further indication that both III and XXV exist in the cyclic form.

Only one of the alkylated cysteines, namely, *S*-carboxymethyl-L-cysteine (I), could be converted to its sulfone (XXX) by direct oxidation. The conversion was effected by use of a large excess of 30% hydrogen peroxide and a long reaction time.[22] The yield of sulfone (XXX) was low and was accompanied by large amounts of inorganic solids formed by degradation reactions. The oxidation of the *S*-arylcysteines (VIII–XIII and XV–XVI) to the corresponding sulfones (XXI–XXX–VIII) was carried out in aqueous acetic acid or aqueous hydrochloric acid with excess hydrogen peroxide and proceeded in generally good yield (*cf.* Table IV). It was not possible to convert *S*-2-aminophenyl-DL-cysteine (XIV) to a sulfoxide or sulfone with either 30% hydrogen peroxide or alkaline potassium permanganate; only tars resulted. Possibly the (aminophenyl)cysteine sulfones are inherently unstable, since the addition of 4-acetamidobenzenesulfinic acid to α-acetamidoacrylic acid followed by acid hydrolysis yielded a solid which appeared to be the desired sulfone as determined by infrared spectrum and paper chromatography but which decomposed during recrystallization.

In the isolation of water-soluble sulfoxides and sulfones prepared with a large excess of hydrogen peroxide, it was found convenient to destroy the excess peroxide before isolation of the product by addition of small amounts of a commercial 5% platinum-on-charcoal catalyst.

(18) F. G. Bordwell and P. J. Bouton, *J. Am. Chem. Soc.*, **79**, 717 (1957).

(19) M. Wolfe, *Biochim. et Biophys. Acta*, **23**, 186 (1957).

(20) In the present work circular paper chromatograms were used to establish homogeneity. These were rapid and reliable with proper controls but did not give accurate R_f values.

(21) L. B. Rockland and J. C. Underwood, *Anal. Chem.*, **26**, 1557 (1954).

(22) While this paper was being refereed the preparation, by another method, of XXX, m.p. 185.5–186° dec., was reported by B. J. Finkle and E. L. Smith, *J. Biol. Chem.*, **230**, 679 (1958).

TABLE IV

ALKYLATED AND ARYLATED L- AND DL-CYSTEINE SULFONES

$RSO_2CH_2CH(\overset{+}{N}H_3)C\overset{-}{O}O$

Compound No.	R	Method[d]	Yield, %	M.P., °C. (Dec.)	$[\alpha]_D^{28}$	Analyses					
						Calcd.			Found		
						C	H	N	C	H	N
XXX	HO_2CCH_2[a]	L	25	193–194[22]	+10.2[e]	28.4	4.30	6.63	28.8	4.67	6.63
XXXI	C_6H_5[b]	M	61	155–156		47.2	4.83	6.11	47.3	5.21	6.08
XXXII	2-$CH_3C_6H_4$[b]	N	27	160–162		49.4	5.39	5.76	49.4	5.60	5.83
XXXIII	3-$CH_3C_6H_4$[b]	N	17	135–137		49.4	5.39	5.76	49.7	5.44	5.84
XXXIV	4-$CH_3C_6H_4$[b]	N	44	155–157		49.4	5.39	5.76	49.1	5.30	5.39
XXXV	4-ClC_6H_4[b]	M	97	171–172		41.1	3.80	5.31	41.2	3.96	5.36
XXXVI	4-$NO_2C_6H_4$[b]	M	70	156–157		39.4	3.68	10.2	39.7	3.54	10.2
XXXVII	3-$CF_3C_6H_4$[a,c]	N	68	148–150	+12.7[f]	39.2	3.62	4.57	39.3	3.76	4.41
XXXVIII	4-FC_6H_4[a]	N	52	172–174	+18.6[g]	43.8	4.07	5.67	43.6	4.28	5.74

[a] Derivative of L-cysteine. [b] Derivative of DL-cysteine. [c] The calculated values are for the hemihydrate. [d] See Experimental. [e] 1% in 1*N* HCl. [f] 0.57% in 1*N* HCl. [g] 0.84% in 1*N* HCl.

Although a number of the compounds listed in the tables showed considerable toxicity, none of them showed selective activity against the mouse tumors, Sarcoma 180, Carcinoma 755, or Leukemia 1210.[23]

EXPERIMENTAL[24]

S-Phenacyl-L-cysteine (II). *Method A.* A suspension of 1.0 g. (6.35 mmoles) of L-cysteine hydrochloride and 0.97 g. (6.35 mmoles) of α-chloroacetophenone in a mixture of 4 ml. of water and 2 ml. of 95% ethanol was prepared. To the well-stirred mixture was added, dropwise, a solution of 0.84 g. (12.7 mmoles) of potassium hydroxide in 5 ml. of water. Upon complete addition of the base, 1.07 g. (68% yield) of compound II precipitated as a crystalline, slightly yellow compound. It could be recrystallized in poor yield from water on a small scale but decomposed rapidly on prolonged heating in water. Purification of the material was carried out by washing thoroughly with cold water and acetone. The material melted at 90–95° (dec.) and was homogeneous on paper using the *t*-butyl alcohol/formic acid/water system.[21] Compound II gave a faint yellow color with ninhydrin.

Anal. Calcd. for $C_{11}H_{13}NO_3S.{}^1/_2H_2O$: C, 53.2; H, 5.68; N, 5.64. Found (washed material): C, 53.6; H, 5.54; N, 5.62. Found (recrystallized material): C, 52.90; H, 5.79; N, 5.74.

Compound II had the following ultraviolet absorption: $\lambda_{max}^{H_2O}$ 247 mμ (ε 14,000), $\lambda_{max}^{0.1NHCl}$ 244 mμ (ε 13,200).

Infrared spectrum: λ_{max}^{KBr} 3.30, 6.12, and 6.55 ($\overset{+}{N}H_3$); 6.01 (aromatic ketone C = O); 6.33 and 7.17 (CO_2^-); 13.75 and 14.59 μ (mono-substituted phenyl).

Attempts to oxidize compound II with hydrogen peroxide in either water or ethanol led to rapid darkening of the solution and isolation and dark-colored, nonhomogeneous solids.

N-Benzoyl-S-methyl-L-cysteine. Method B. Benzoylation of 0.50 g. (3.7 mmoles) of *S*-methyl-L-cysteine (V) under conventional Schotten-Baumann conditions gave 0.77 g. (87%) of yellow solid, m.p. 40–50°. A portion of it was crystallized from water (1 g./10 ml.) and melted at 50–59°. (Izumiya *et al.*[27] reported m.p. 59–61° for the compound, prepared by an unstated method. It seems probable that the material described above is partially racemized.)

Anal. Calcd. for $C_{11}H_{13}NO_3S$: C, 55.2; H, 5.48. Found: C, 55.3; H, 5.64.

Infrared spectrum: λ_{max}^{KBr} 2.98 (NH); 5.77 (carboxyl C = O); 6.09 (amide C = O); 6.53 (amide NH); 13.95μ (mono-substituted phenyl).

N-Acetyl-S-methyl-L-cysteine. Method C. A solution of 1.0 g. (7.4 mmoles) of *S*-methyl-L-cysteine (V) in 10 ml. of acetic anhydride was allowed to stand at room temperature for 18 hr. The excess acetic anhydride was evaporated *in vacuo*, leaving a sirupy residue which solidified after standing a few days at 0°. The residue was recrystallized from 5 ml. of 95% ethanol to yield 0.40 g. (33%), m.p. 147–149°.

Anal. Calcd. for $C_6H_{11}NO_3S$: C, 40.7; H, 6.26. Found: C, 40.7; H, 6.05.

Infrared spectrum: λ_{max}^{KBr} 2.99 (NH); 3.85 (carboxyl OH); 5.88 (carboxyl C = O); 6.21 (amide C = O); 6.45μ (amide NH).

S-Trimethylsilylmethyl-L-cysteine (VII). *Method D.* A mixture of 0.79 g. (5.0 mmoles) of L-cysteine hydrochloride, 0.61 g. (5.0 mmoles) of (chloromethyl)trimethylsilane (Peninsular ChemResearch, Inc.), 10 ml. of dioxane, and 3 ml. of water was cooled to 0° and to it was added a cold solution of 0.66 g. (10.0 mmoles) of potassium hydroxide in 3 ml. of water. The two-phase mixture was heated, under nitrogen and with stirring, at 80–90° for 24 hr., at the end of which time the *p*H of the mixture was 8. The mixture was adjusted to *p*H 6. with 6 drops of 6*N* hydrochloric acid and was filtered from inorganic salts. The filtrate was evaporated to dryness *in vacuo* and the residue was extracted with 60 ml. of boiling absolute ethanol. The extract was filtered hot and the alcoholic filtrate was evaporated to dryness *in vacuo*, leaving 1.15 g. of residue. Paper chromatography[24] in system D showed the presence of a trace of cystine along with the desired product. Partition chromatography[28] of

(23) The anticancer assays were performed by the Biology Department, Stanford Research Institute, under a contract with the Cancer Chemotherapy National Service Center.

(24) Microanalyses were by the Microanalytical Laboratory of Stanford Research Institute, Menlo Park, Calif., and Berkeley Analytical Laboratory, Albany, Calif. Melting points were taken on the Fisher-Johns apparatus and are uncorrected. The paper chromatograms were run by the circular technique on Whatman No. 1 paper, using ninhydrin spray to detect the spots and using the following solvent systems:

A[21] *t*-butyl alcohol/formic acid/water (6.95/0.10/2.95)

B[25] *n*-butanol/acetic acid/water (5/2/3)

C[19] *n*-butanol/methyl ethyl ketone/water/17*N* ammonia (5/3/1/1)

D[26] water-saturated *n*-butanol.

Optical rotations were obtained using a Standard Polarimeter Model D attachment to the Beckman DU spectrophotometer, calibrated with standard sucrose solutions [A. S. Keston, Abstracts of the 127th meeting, American Chemical Society, 18C (1955)].

(25) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

572 mg. of the residue on Celite using *n*-butanol/water removed cystine and inorganic salts and gave an 89% recovery of VII. On this basis the yield was 98.7%. The purified material decomposed gradually over the range 165–198°, the decomposition being vigorous at 195–198°. The compound could also be recrystallized from water (about 1 g./10 ml.) but the recovery was poor. The analytical data are given in Table I.

Infrared spectrum: λ_{max}^{KBr} 3.25–3.40, 6.14, and 6.65 (NH_3^+); 6.25 (shoulder) and 7.17 (CO_2^-); 8.01 and 11.75μ ($SiCH_3$).

A picrate was prepared from 0.20 g. of VII and 0.42 g. of picric acid in a total volume of 50 ml. of water. The picrate, 0.25 g., m.p. 145.5–147.5°, was recrystallized from 25 ml. of hot water and had m.p. 146–148.5°.

Anal. Calcd. for $C_7H_{17}NO_2Si.{}^1/_2C_6H_3N_3O_7.{}^1/_2H_2O$: C, 36.3; H, 5.94. Found: C, 36.6; H, 6.14.

Infrared spectrum: λ_{max}^{KBr} 3.25–3.40 and 6.10 (NH_3^+); 5.77–5.90 (carboxyl C = O); 6.43 and 7.55 (NO_2); 7.85 (phenolate ion); 8.01 and 11.75 ($SiCH_3$); 12.66 μ (picrate ion).

*N-Acetyl-S-2-aminophenyl-*DL*-cysteine* (XXIII). *Method E.* A solution of 1.50 g. (10 mmoles) of α-acetamidoacrylic acid[29] and 1.62 g. (13 mmoles) of 2-aminobenzenethiol in 10 ml. of dioxane (Eastman White Label) and 15 drops of piperidine was heated under reflux for 1.5 hr. Dioxane was removed by evaporation *in vacuo* to leave a brown, sirupy residue. The residue was extracted with 5 ml. of methylene chloride and the nonextractable material was dissolved in 5 ml. of water and brought to *p*H 8 with concentrated ammonium hydroxide. The *p*H was adjusted to 4 with 6*N* hydrochloric acid and the product slowly crystallized to yield 1.0 g. (34%) of XXIII, m.p. 145°. The analytical data are given in Table II.

Infrared spectrum: λ_{max}^{KBr} 3.00 and 3.07 (NH and NH_2^+); 5.83 (carboxyl C = O); 6.20 (amide C = O); 6.55 (amide NH); 13.20 μ (*o*-disubstituted phenyl).

Absolute ethanol was used as the solvent in many of the mercapturic acid preparations summarized in Table II.

*S-2-Aminophenyl-*DL*-cysteine* (XIV). *Method F.* A mixture of 1.0 g. (39 mmoles) of *N*-acetyl-*S*-2-aminophenyl-DL-cysteine (XXIII), 4 ml. of concentrated sulfuric acid, and 16 ml. of water was heated under reflux for 30 min. The hot solution was brought to *p*H 7 with concentrated ammonium hydroxide. The product (0.78 g., 94%) precipitated and was crystallized from hot water, m.p. 240° (dec.). On paper chromatography in system B, it gave a single red-brown spot. The analytical data are given in Table I.

Infrared spectrum: λ_{max}^{KBr} 2.91, 2.99, and 6.1–6.2 (NH_2); 3.25, 6.1–6.2, and 6.75 (NH_3^+); 6.30 and 7.15 (CO_2^-); 13.32 μ (*o*-disubstituted phenyl).

*S-3-(Trifluoromethyl)phenyl-*L*-cysteine* (XV). *Method G.* A solution of 5.50 g. (31.4 mmoles) of L-cysteine hydrochloride (monohydrate) in 100 ml. of 1.5*N* sulfuric acid was heated to 70–80° and, with stirring, 7.0 g. (49 mmoles) of cuprous oxide suspended in 100 ml. of water was added in 3 portions. After the solution had been stirred for 30 min., 5.50 g. (31.4 mmoles) of L-cysteine hydrochloride (monohydrate) was added, and the mixture was cooled to 0° with an ice-salt bath.

A solution of 9.0 g. (56 mmoles) of 3-(trifluoromethyl)-aniline, 100 ml. of water, and 10 ml. of concentrated sulfuric acid was cooled to −5° with an ice-salt bath and to it was added, with stirring, over a period of 2 hr., a solution of 5.0 g. (73 mmoles) of sodium nitrite in 50 ml. of water.

The cold diazonium salt solution was added dropwise, with stirring, to the cuprous cysteinate solution while the temperature was maintained at −5 to 0°. The stirred reaction mixture was allowed to rise to room temperature during a 12-hr. period and was then heated to 95°, at which temperature it was saturated with hydrogen sulfide. The resulting mixture was filtered, using Celite, and the filtrate was extracted with two 50-ml. portions of ether to remove phenols. The aqueous phase was adjusted to *p*H 6 with ammonium hydroxide, whereupon 3.5 g. (24%) of a crystalline product precipitated. This was recrystallized from water (1 g./60 ml.) to yield 2.55 g. (17%) of product, m.p. 183–185° (dec.). The product gave a single lavender-grey spot when chromatographed on paper using solvent system C. The analytical data are given in Table I.

Infrared spectrum: λ_{max}^{KBr} 3.25, 6.13, and 6.60 (NH_3^+); 6.33 and 7.00 (CO_2^-); 8.46 and 8.85 (CF_3); 12.65μ (*m*-disubstituted phenyl).

When the reaction was carried out with 5.0 g. (29 mmoles) of L-cysteine hydrochloride (monohydrate), 1.0 g. (7.0 mmoles) of cuprous oxide, and 6.2 g. (39 mmoles) of diazotized 3-(trifluoromethyl)aniline, the yield of XV was 33.1% (before recrystallization).

The product from several large-scale preparations of XV was seriously contaminated with L-cystine and it was very wasteful to remove the impurity by a number of recrystallizations from water. It was found that recrystallization of 0.50 g. of crude XV from 60 ml. of hot water which contained 0.50 g. of sodium sulfite gave a recovery of 0.40 g. of XV which was chromatographically homogeneous.

*S-Carboxymethyl-*L*-cysteine sulfoxide* (XXIV). *Method H.* A suspension of 1.0 g. (5.6 mmoles) of recrystallized *S*-carboxymethyl-L-cysteine (I) in a mixture of 20 ml. of water and 8 ml. (78 mmoles) of 30% hydrogen peroxide was heated on the steam bath for 15 min. To the hot solution was added 250 ml. of 95% ethanol and the resulting mixture was allowed to stand at room temperature for 2 days. During this period 0.80 g. (73%) of analytically pure product precipitated, m.p. 190–191° (dec.). The product gave a single red-brown spot when chromatographed on paper, using system A. It could be recrystallized from water but it appeared to decompose partially during the operation.

Anal. Calcd. for $C_5H_9NO_5S$: C, 30.8 H, 4.65. Found (for directly isolated product): C, 30.8; H, 4.72. Found (for the recrystallized product): C, 29.8; H, 4.74.

Infrared spectrum: λ_{max}^{KBr} 3.17 and 6.74 (NH_3^+); 5.90–6.00 (carboxyl C = O); 6.20 and 6.93 (CO_2^-); 9.70μ (S → O).

*S-Phenyl-*DL*-cysteine sulfoxide* (XXVIII). *Method J.* A mixture of 1.0 g. (5.1 mmoles) of *S*-phenyl-DL-cysteine (VIII), 20 ml. of water, and 10.0 ml. (98 mmoles) of 30% hydrogen peroxide was heated on the steam bath for 30 min., during which time complete solution was obtained. The solution was rapidly chilled to 15° with an ice bath, whereupon 0.50 g. (46%) of the sulfoxide XXVIII precipitated. The product gave a single gray-brown spot in solvent system C which moved slower than *S*-phenyl-DL-cysteine (VIII) or *S*-phenyl-DL-cysteine sulfone (XXXI). The analytical data are given in Table III.

Infrared spectrum: λ_{max}^{KBr} 3.4, 6.10, and 6.65–6.75 (NH_3^+); 6.31 and 7.25 (CO_2^-); 9.69 (S → O); 13.38 and 14.55μ (mono-substituted phenyl).

*S-m-Tolyl-*DL*-cysteine sulfoxide* (XXIX). *Method K.* To a solution of 1.0 g. (4.7 mmoles) of *S-m*-tolyl-DL-cysteine (X) in a mixture of 20 ml. of water and 5 ml. of glacial acetic acid was added 0.52 ml. (5.1 mmoles) of 30% hydrogen peroxide. The solution was heated on the steam bath for 1 hr. and was then evaporated *in vacuo*, yielding a sirup which solidified after standing at 0° for several days. The solid was recrystallized from 75% aqueous ethanol (1 g./25 ml.) to yield 0.4 g. (37%), m.p. 137–138° (dec.). The product gave a single gray-green spot in solvent system C which

(26) J. G. Buchanan, C. A. Dekkar, and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(27) N. Izumiya, A. Nagamatsu, and S. Ota, *Kyushu Mem. Med. Sci.*, **4**, 1 (1953).

(28) This separation was performed according to the procedure of H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955), and the elution pattern was followed by the relative intensity of an equal aliquot of each fraction spotted on paper and detected by ninhydrin.

(29) H. W. Coover and J. B. Dickey, U. S. Patent **2,622,-074** [*Chem. Abstr.*, **47**, 9998d (1953)].

moved slower than the parent compound (X). The analytical data are given in Table III.

Infrared spectrum: λ_{max}^{KBr} 3.3–3.4, 6.12, and 6.80 ($\overset{+}{N}H_3$); 6.27 (shoulder) and 7.25 (CO_2^-); 9.82 (S → 0); 13.25μ (*m*-disubstituted phenyl).

*S-Carboxymethyl-*L*-cysteine sulfone* (XXX). *Method L.* A mixture of 1.0 g. (5.6 mmoles) of recrystallized *S*-carboxymethyl-L-cysteine (I), 5 ml. of water, and 5 ml. (48 mmoles) of 30% hydrogen peroxide was heated at 45–60°, with occasional stirring, for 10 hr. A small amount of material remained in suspension at the end of this time and was removed by filtration. To the filtrate was added about 0.2 g. of 5% platinum-on-charcoal catalyst (Baker and Co.) in small portions; the vigorous reaction required cooling. A copious, white precipitate formed during this treatment. The precipitate was brought into solution with 100 ml. of hot (80°) water and the resulting mixture was filtered to remove the platinum catalyst. The filtrate was evaporated *in vacuo*, leaving a yellow solid. This was crystallized from 40 ml. of water, using decolorizing carbon, to give 0.3 g. of white material that crystallized very slowly. This amounted to a 25% yield but no effort was made to recover the product which remained in the mother liquors. The material was crystallized a second time from 25 ml. of hot water. It melted at 193–194° (dec.)[22] and gave a single red-brown spot on paper chromatography in solvent system A which moved faster than the sulfoxide XXIV and slower than the parent compound (I). The analytical data are given in Table IV.

Infrared spectrum: λ_{max}^{KBr} 3.35, 6.04, and 6.57 ($\overset{+}{N}H_3$); 5.79 (carboxyl C = O); 6.35 and 7.08 (CO_2^-), 7.70 and 8.83μ (SO_2).

*S-4-Nitrophenyl-*DL*-cysteine sulfone* (XXXVI). *Method M.* A solution of 0.50 g. (2.1 mmoles) of *S*-4-nitrophenyl-DL-cysteine (XIII) in 10 ml. of water, 2.5 ml. of 6*N* hydrochloric acid, and 1.50 ml. (14.7 mmoles) of 30% hydrogen peroxide was heated on the steam bath for 10 min. A small amount of an unidentified precipitate was filtered and the filtrate was heated for an additional 80 min. The solution was chilled and adjusted to *p*H 6–7 with concentrated ammonium hydroxide. A precipitate, 0.40 g. (70%) slowly formed and was recrystallized from water (1 g./20 ml.), m.p. 156–157° dec. The product gave a single light yellow spot on paper chromatography in solvent system C which moved slower than the parent compound (XIII). The analytical data are given in Table IV.

Infrared spectrum: $\lambda_{max\mu}^{KBr}$ 3.23–3.45, 6.08, and 6.76 (shoulder) ($\overset{+}{N}H_3$); 6.35 and 7.15 (shoulder) (CO_2^-); 6.52 and 7.40 (NO_2); 7.68 and 8.70 (SO_2); 12.05 (*p*-disubstituted phenyl).

*S-4-Fluorophenyl-*L*-cysteine sulfone* (XXXVIII). *Method N.* To a solution of 1.0 g. (4.64 mmoles) of S-4-fluorophenyl-L-cysteine (XVI) in 20 ml. of glacial acetic acid was added 5.0 ml. (49 mmoles) of 30% hydrogen peroxide. The resulting solution was allowed to stand at room temperature for 49 hr. after which the excess hydrogen peroxide was decomposed by the addition of about 0.2 g. of 5% platinum-on-charcoal catalyst, added in small portions. The charcoal was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml. of water and the resulting solution was neutralized to *p*H 7 with concentrated ammonium hydroxide yielding 0.80 g. (70%) of crystalline solid. This was recrystallized from hot water (0.2 g./25 ml.) to yield 0.60 g. (52%) of product, m.p. 172–174° (dec.). The product gave a single yellow spot on paper chromatography in solvent system C which moved slower than the parent amino acid (XVI). The analytical data are given in Table IV.

Infrared spectrum: $\lambda_{max\mu}^{KBr}$ 3.37–3.45, 6.05–6.10, and 6.60 ($\overset{+}{N}H_3$); 6.30 and 7.15 (CO_2^-); 7.60 and 8.72 (SO_2); 8.06 (C—F); 11.87 (*p*-disubstituted phenyl).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] VI. Synthesis of α-Amino-γ-sulfamoylbutyric Acids with Substituents on the Sulfonamide Nitrogen

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Chlorinolysis of L-cystine hydantoin and DL-homocystine hydantoin in 42% aqueous acetic acid gave 71% and 81% yields, respectively, of the corresponding 5-(chlorosulfonylalkyl)hydantoins. Procedures were devised for reaction of DL-5-(β-chlorosulfonylethyl)hydantoin with ammonia, alkylamines, arylamines, and glycinamide to form the respective sulfonamides, which, in turn, were base-hydrolyzed to the desired DL-α-amino-γ-sulfamoylbutyric acids. In contrast, L-5-(chlorosulfonylmethyl)hydantoin reacted satisfactorily only with the arylamines, and the resultant sulfonamides decomposed on attempted alkaline hydrolysis.

Interest in antagonists of L-glutamine as possible anticancer agents has been given considerable impetus by the observed anticancer activity of L-azaserine[2] and 6-diazo-5-oxo-L-norleucine.[3] These two compounds have been established to be antimetabolites of L-glutamine.[4] Reisner[5] has recently described the synthesis of α-amino-γ-sulfamoylbutyric acid (IVb, $R_1 = R_2 = H$) along with two

(1) This program is under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. For the preceding paper in this series, *cf.* L. Goodman, L. O. Ross, and B. R. Baker, *J. Org. Chem.*, **23**, 1251 (1958).

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(5) D. B. Reisner, *J. Am. Chem. Soc.*, **78**, 5102 (1956).