2,2′-Anhydro-1-β-D-arabinofuranosylthymine[9b] (III).—The hydrochloride of III was prepared by detritylation of II with ethereal HCl in the manner described.[9b] The free nucleoside III was obtained from its hydrochloride salt by a procedure which is simpler and affords higher yields than the Dowex 1 (acetate) method previously described.[9b]

A suspension of the hydrochloride of III (1.0 g) in 15 ml of ethanol was stirred and 1 equiv of triethylamine was added dropwise. The reaction mixture was allowed to stand at room temperature 1 hr. The solid (III) was filtered off and washed with ethanol followed by ether. The product had a mp 225–228° and was obtained in 92% yield.[14] Ultraviolet spectra and chromatographic data obtained for III were the same as for an authentic sample.[9b]

1-β-D-Arabinofuranosylthymine (IV).[9b]—Twenty milliliters of 1 *N* H_2SO_4 was added to the 2,2′-anhydro nucleoside (III, 1.0 g, 4.0 mmoles) and the reaction mixture was refluxed for 45 min. On cooling the reaction mixture, 0.84 g of 1-β-D-arabinosylthymine (IV), mp 245–248°, precipitated. The mother liquor was neutralized ($BaCO_3$) and the salts were removed by filtration. On evaporation of the filtrate, an additional 0.11 g (mp 248–250°, over-all yield 88%) was obtained. Crystallization of the combined solids from 25% ethanol gave a 93% recovery. Chromatographic data, infrared, and ultraviolet spectra of this sample were identical with those reported for authentic IV.[9b]

1-(Tri-*O*-acetyl-β-D-arabinofuranosyl)thymine (V).—To a stirred suspension of 1.3 g (5.2 mmoles) of 1-β-D-arabinofuranosylthymine in 20 ml of pyridine was added 1.6 ml (17 mmoles) of acetic anhydride. The suspension was heated at 45° for a few minutes until solution occurred. The reaction mixture was allowed to stand at room temperature for 18 hr. Ethanol (0.5 ml) was added to quench the reaction and the pyridine evaporated off. The syrup was evaporated (twice with 50% ethanol and then azeotroped with absolute ethanol. The crystalline residue was triturated with ether and filtered, 1.9 g (94%), mp 138–140°. Crystallization from ethanol gave short colorless needles, mp mp 140–142°, $[\alpha]^{26}D$ +65° (*c* 0.3, ethanol).

Anal. Calcd for $C_{16}H_{20}N_2O_8$: C, 50.00; H, 5.25; N, 7.29. Found: C, 50.00; H, 5.22; N, 7.28.

1-(Tri-*O*-acetyl-β-D-arabinofuranosyl)-4-thiothymine (VI).—To a stirred pyridine (50 ml) solution containing 1.9 g (4.9 mmoles) of acetylated nucleoside V was added 2.4 g (10.9 mmoles) of P_2S_5. The mixture was refluxed and when solution was complete (within the first 30 min) 0.12 ml of water was added and the reaction mixture was refluxed for 3 hr. After the reaction mixture had cooled, the pyridine solution containing the product was decanted from a yellow hygroscopic precipitate. The solid was extracted twice with pyridine, and the combined pyridine solutions were evaporated *in vacuo* to an amber-colored syrup. The syrup was treated with 50% ethanol and the ethanol–water mixture was concentrated. This procedure was repeated. The yellow residue was taken up in chloroform and filtered from a small residue. The $CHCl_3$ solution was evaporated *in vacuo*, and ethanol was added to the yellow glass. Precipitation of a light yellow solid occurred, 2.0 g, mp 124–126°. Crystallization of the solid from ethanol gave light yellow needles, mp 126–127°, $[\alpha]^{26}D$ +148° (*c* 0.25, acetone). Spectral properties in 50% ethanol showed maxima at 332 and 243 mμ, minima at 275 and 222 mμ; spectral ratio in 50% ethanol 332/243 mμ = 5.3.

Anal. Calcd for $C_{16}H_{20}N_2O_7S$: C, 48.00; H, 5.04; N, 6.99; S, 7.99. Found: C, 47.87; H, 4.86; N, 7.08; S, 7.92.

1-β-D-Arabinofuranosyl-5-methylcytosine (VII).—The thionucleoside (VI, 1.3 g, 3.25 mmoles) within a glass container was treated with liquid NH_3 (20 ml) and heated in a steel bomb for 29 hr at 55–60°. The liquid ammonia was driven off by a stream of dry nitrogen. The resulting pale yellow syrup was dissolved in water (30 ml), neutralized with 2 *N* acetic acid, and applied to a Dowex 50 (H^+) 100–200 mesh column (1.4 × 14 cm). The column was washed with water until the effluent was free from ultraviolet-absorbing material and then eluted with 2 *N* NH_4OH. The ultraviolet absorbing fractions were evaporated to dryness. The white residue was dissolved in 50% ethanol, treated with charcoal, and filtered using a filter aid of diatomaceous earth. Colorless cubic crystals precipitated, 0.72 g (80%), which sintered at ~150°, and effervesced at 160–165°; $[\alpha]^{26}D$ +105° (*c* 0.39, water); ultraviolet absorption properties at pH 7.3 (neutral species), maximum at 277 mμ (ε 8600), minimum at 253 mμ (ε 4600); in 0.1 *N* HCl (cationic species), maximum at 287 mμ (ε 12,700), minimum at 244 mμ (ε 1400).

Anal. Calcd for $C_{10}H_{13}N_3O_5 \cdot H_2O$: C, 43.63; H, 6.22; N, 15.14. Found: C, 44.00; H, 6.26; N, 15.14.

Acknowledgments.—The authors are indebted to Dr. J. H. Burchenal for the screening data of MCA in leukemia P815 and to Drs. G. S. Tarnowski and F. Schmid for data on Sarcoma 180. The authors are grateful to Dr. G. B. Brown for his warm and continued interest.

(14) The hydrochloride of 2,2′-anhydro-1-β-D-arabinofuranosyluracil was also converted to its free base in 97% yield by this treatment.

Synthesis of Some Orotic Acid Analogs as Potential Antimetabolites[1]

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The elucidation and biosynthesis of pyrimidine nucleotides has established orotic acid (2,6-dihydroxy-4-carboxypyrimidine) as an important metabolite. The significance of inhibition of this metabolic route is generally accepted as being reflected in antiviral, antineoplastic, and antibiotic effects on the organism.[3] Many isosteres and analogs of orotic acid as well as other pyrimidine derivatives have been shown to interfere with orotic acid metabolism and subsequently with nucleic acid formation.[4] We wish to report on the synthesis and biologic evaluation of a series of 5-substituted orotic acid analogs designed as potential antimetabolites of orotic acid. Inhibition by pyrimidines at this level of nucleic acid biosynthesis has the advantage of the use of molecules which can be more easily prepared than their corresponding nucleotides and also have the opportunity of being more specific in action since earlier metabolites (*e.g.*, aspartic acid, DPN, etc.) have more diverse metabolic applications.

In view of the antimetabolic activity of some 2,4-diaminopyrimidines[4f,5] on orotic acid a series of 2-amino-, 2-hydroxy-, and 2-thio-6-amino-4-carboxypyrimidines substituted at the 5 position was synthesized. Although the 5 position of orotic acid is not involved in the biosynthesis of the pyrimidine nucleotides, it has been mentioned by Stone and Potter[6] as the choice position for substitution in the preparation of orotic acid antagonists. A limited number of 5-substituted orotic acids have been synthesized and shown to possess anti-

(1) (a) Abstracted from a portion of the thesis submitted by S. Borodkin to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1962. (b) Supported by a research grant from Smith Kline and French Laboratories, Philadelphia, Pa.

(2) Deceased.

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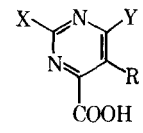
TABLE I
4-PYRIMIDINECARBOXYLIC ACIDS

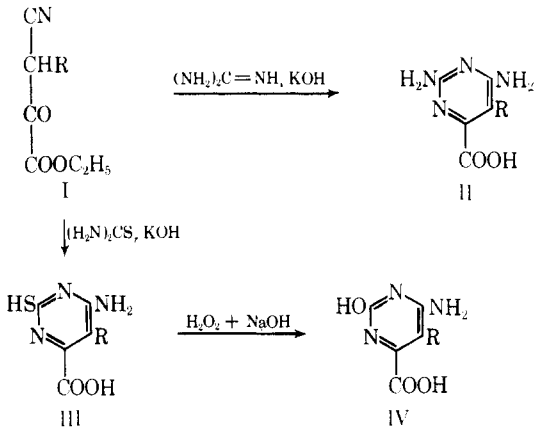


No.	X	Y	R	% yield	Mp, °C[a]	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Sulfur, % Calcd	Sulfur, % Found	Ultraviolet spectra[b] λ, mμ (ε) Max	Ultraviolet spectra[b] λ, mμ (ε) Min
1	NH_2	NH_2	CH_3	27	260–261	$C_6H_8N_4O_2$	42.85	43.03	4.79	4.43	33.32	33.11			284 (6000)	257 (3500)
2	NH_2	NH_2	C_2H_5	30	252–253	$C_7H_{10}N_4O_2 \cdot H_2O$	41.99	42.30	6.04	6.16	27.99	28.10			284 (6000)	258 (3500)
3	NH_2	NH_2	n-C_3H_7	24	243–244	$C_8H_{12}N_4O_2 \cdot 0.5H_2O$	46.81	46.78	6.38	6.14	27.31	27.10			284 (5500)	258 (3000)
4	NH_2	NH_2	n-C_4H_9	22	245–246	$C_9H_{14}N_4O_2 \cdot H_2O$	47.35	47.33	7.06	6.99	24.55	24.32			284 (6000)	258 (3500)
5	NH_2	NH_2	i-C_4H_9	29	238–239	$C_9H_{14}N_4O_2$	51.41	51.05	6.71	6.82	26.66	26.48			285 (5500)	258 (3000)
6	NH_2	NH_2	$C_6H_5CH_2$	31	248–249	$C_{12}H_{12}N_4O_2 \cdot H_2O$	54.95	54.93	5.38	5.44	21.37	21.28			284 (6000)	258 (4000)
7	NH_2	NH_2	C_6H_5	32	238–240	$C_{11}H_{10}N_4O_2 \cdot 0.5H_2O$	56.35	56.39	4.52	4.74	23.91	23.82			284 (6500)	268 (5500)
8	NH_2	NH_2	p-$CH_3OC_6H_4$	29	248–249	$C_{12}H_{12}N_4O_3 \cdot H_2O$	55.38	55.13	4.65	4.92	21.53	21.39			None	None
9	SH	NH_2	CH_3	37	241–243	$C_6H_7N_3O_2S \cdot H_2O$	35.46	35.76	4.46	4.04	20.68	20.60	15.78	15.74	273 (19,000)	None
10	SH	NH_2	C_2H_5	35	238–239	$C_7H_9N_3O_2S$	42.21	42.03	4.55	4.62	21.10	21.40	16.07	16.32	273 (20,000)	None
11	SH	NH_2	n-C_3H_7	30	233–234	$C_8H_{11}N_3O_2S$	45.05	44.95	5.20	5.62	19.77	19.91	15.03	15.06	273 (20,000)	None
12	SH	NH_2	n-C_4H_9	29	216–217	$C_9H_{13}N_3O_2S$	47.56	47.73	5.76	6.01	18.49	18.41	14.11	14.11	273 (21,000)	None
13	SH	NH_2	i-C_4H_9	28	231–232	$C_9H_{13}N_3O_2S$	47.56	47.67	5.76	6.07	18.49	18.54	14.11	14.26	274 (22,000)	None
14	SH	NH_2	$C_6H_5CH_2$	35	277–279	$C_{12}H_{11}N_3O_2S$	55.15	54.98	4.24	4.38	16.09	16.05	12.26	12.26	273 (24,000)	None
15	SH	NH_2	C_6H_5	25	279–281	$C_{11}H_9N_3O_2S \cdot H_2O$	49.82	49.74	4.17	4.28	15.85	16.05	12.06	12.02	279 (24,000)	None
16	SH	NH_2	p-$CH_3OC_6H_4$	29	283–286	$C_{12}H_{11}N_3O_3S \cdot H_2O$	51.97	51.68	4.00	4.29	15.16	14.93	11.55	11.72	280 (24,000)	None
17	SH	NH_2	i-$C_3H_7OCH_2$	13	[c]	$C_9H_{13}N_3O_3S$	44.43	44.04	5.38	5.35	17.28	17.02	13.11	13.05	245 (13,500), 277 (21,000)	252 (12,500)
18	SH	NH_2	$C_2H_5OCH_2$	8	[d]	$C_8H_{11}N_3O_3S$	41.90	42.01	4.84	4.95	18.33	18.22	13.98	13.89	245 (14,000), 276 (21,000)	252 (13,000)
19	OH	NH_2	CH_3	96	258–260	$C_6H_7N_3O_3 \cdot 0.5H_2O$	40.45	40.60	4.52	4.61	23.59	23.70			288 (8500)	250 (2000)
20	OH	NH_2	C_2H_5	78	247–248	$C_7H_9N_3O_3$	45.90	46.00	4.95	4.97	22.94	22.98			289 (8500)	250 (1500)
21	OH	NH_2	n-C_3H_7	72	219–221	$C_8H_{11}N_3O_3 \cdot 0.5H_2O$	46.82	46.75	5.85	5.80	20.49	20.81			290 (9000)	251 (2000)
22	OH	NH_2	n-C_4H_9	74	217–218	$C_9H_{13}N_3O_3 \cdot H_2O$	47.15	47.92	6.60	6.56	18.33	18.35			290 (8500)	250 (2000)
23	OH	NH_2	i-C_4H_9	72	249–250	$C_9H_{13}N_3O_3$	51.41	51.05	6.71	6.82	19.80	19.86			291 (8500)	251 (2000)
24	OH	NH_2	$C_6H_5CH_2$	81	258–260	$C_{12}H_{11}N_3O_3$	58.77	59.29	4.52	4.57	17.14	17.10			289 (8500)	250 (3000)
25	OH	NH_2	C_6H_5	73	356–359	$C_{11}H_9N_3O_3 \cdot 0.5H_2O$	55.00	55.17	4.20	4.31	17.49	17.76			288 (7500)	260 (4000)
26	OH	NH_2	p-$CH_3OC_6H_4$	97	356–357	$C_{12}H_{11}N_3O_4$	55.16	55.19	4.24	4.08	16.09	15.90			284 (8000)	265 (7000)
27	OH	NH_2	i-$C_3H_7OCH_2$	83	[d]	$C_9H_{13}N_3O_4$	47.57	47.37	5.76	5.68	18.50	18.46			283 (7500)	248 (2500)
28	OH	NH_2	$C_2H_5OCH_2$	78	[d]	$C_8H_{11}N_3O_4$	45.07	45.46	5.20	5.54	19.71	19.41			283 (7500)	248 (2500)

[a] All 4-pyrimidinecarboxylic acids melted with effervescence. [b] Determined on a Beckman Model DU spectrophotometer using 10^{-4} molar aqueous solutions and scanned from 220–400 mμ. The molar extinction coefficients were rounded to nearest 500 units. [c] Slow decomposition above 220°. [d] Slow decomposition above 200°.

tumor activity.[4b-d,7] The effect of substitution on this position seemed promising for structure–activity relationship studies. The series of 5-substituted 2,6-diamino-4-carboxypyrimidines (II) was prepared by treating 3-substituted ethyl cyanopyruvates (I) with guanidine (Scheme I). Attempts to prepare the 5-

SCHEME I



unsubstituted compound[8] (II, R = H) by this method failed as did attempts to make compounds in which the 5 substituent had electron-withdrawing characteristics (R = *p*-nitrophenyl, 3,4-dichlorophenyl, and isopropoxymethyl). The 3-substituted ethyl cyanopyruvates (I) were readily available by condensing ethyl oxalate with the appropriate nitrile.[9]

When the cyanopyruvates (I) were treated with thiourea, a series of 5-substituted 6-amino-4-carboxy-2-thiopyrimidines (III) was obtained. Although the reaction was more facile than the corresponding 2,6-diaminopyrimidine synthesis, the formation of pyrimidines with electron-withdrawing character on the 5 position was still not considered successful, even though small amounts were obtained.

Finally, a series of 5-substituted 6-amino-2-hydroxypyrimidines (IV) was prepared from the corresponding 2-thiols (III). Attempts to obtain the 6-amino-4-carboxy-2-hydroxypyrimidines (IV) directly by treating the cyanopyruvates (I) with urea were unfruitful. Urea, unlike guanidine and thiourea, is apparently too weak a nucleophile to give the desired pyrimidine.

An examination for antiviral effects was carried out against influenza virus (PR-8) and herpes simplex virus on compounds **1–3**, **6–11**, **14–16**, **19**, **20**, and **23–25** as listed in Table I. Activity against influenza virus (PR-8) was observed with **9** at a concentration of 32 mg/egg. Activity against herpes simplex virus was observed with **15** at concentrations of 8, 16, and 32 mg/egg and for **20** at 32 mg/egg. The toxic level for the three active drugs was 64 mg/egg. All other compounds did not exhibit activity. The data are summarized in Table II.

In vivo evaluation for antimalarial activity against *Plasmodium berghei* in rodents on compounds **2**, **4–14**, **16**, **20**, and **26** showed that **9** at 64 mg/kg of body weight caused an increase in survival times of 1.9 days. The results are summarized in Table II.

TABLE II
ANTIVIRAL AND ANTIMALARIAL SCREENING RESULTS

	Max nontoxic dose, mg/kg[b]		*Plasmodium berghei*	
No.[c]	PR-8-Flu	Herpes simplex	Dose, mg/kg	Mean survival time, days[d]
1	64	64	...	...
2	64	64	16	7.4
3	32	32	...	...
4	...	...	16	7.2
5	...	...	64	8.0
6	64	64	64	7.4
7	64	64	64	7.2
8	16	16	16	7.4
9	64	32	64	8.2
	Active, 32			
10	64	64	64	7.2
11	32	32	64	7.2
12	...	...	64	7.8
13	...	...	64	6.4
14	*d*	*d*	64	6.8
15	32	64	...	...
		Active, 8, 16, 32		
16	32	32	64	6.8
19	64	64	...	...
20	64	64	64	6.8
		Active, 32		
24	32	32	...	...
25	64	*d*	...	...
26	...	...	64	6.8

[a] Number corresponds to compounds in Table I. [b] Active chemicals designated with dosage levels. [c] Mean survival time of controls was 6.3 days. [d] Specific dosage levels used not available.

The results of *in vitro* antimicrobial testing against *Staphylococcus aureus* (resistant strain), *Klebsiella pneumoniae*, *Trichromonas foetus*, *Candida albicans*, and *Trichophyton mentagrophytes* of all the chemicals listed in Table I showed that **15** was active, causing *S. aureus* to fail to grow at 100–200 μg/ml and *C. albicans* at 5–14 μg/ml but was inactive in mice. Compound **10** was also active against *S. aureus* but had no *in vivo* activity.

Experimental Section[10]

5-Substituted 2,6-Diamino-4-carboxypyrimidine (II). Thirty-two grams (0.48 mole based on 85% purity) of KOH pellets was placed in a beaker and covered with 15 ml of water. When almost all of the KOH had dissolved, 285 ml of absolute ethanol was added. To this was added 34.4 g (0.36 mole) of guanidine hydrochloride, and the resulting solution came to a boil while stirring. After boiling for a few minutes the KCl was filtered from the guanidine solution while hot. The solution was cooled to room temperature after which 0.12 mole of the corresponding ethyl cyanopyruvate potassium salt (I), prepared by the method of Wislicenus and Silberstein,[9b] was added with stirring. The mixture was allowed to react for 24 hr at room temperature with stirring. The alcohol was then removed under reduced pressure and 300 ml of water was added to the residue. The resulting solution was made slightly acidic with concentrated HCl. After several hours the precipitate was filtered, washed

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(10) The melting points were taken on a Mel-Temp apparatus unless otherwise stated and are corrected. Elemental analyses were carried out by Dr. Weiler and Strauss, Oxford, England, and Alfred Bernhardt, Mulheim Germany.

with 300 ml of water, and then with 200 ml of acetone. The product was collected and recrystallized by dissolving in 500 ml of 1% NaOH solution, heating to boiling, and acidifying slightly with HCl while hot. A small portion was recrystallized from a large volume of water for analysis. Even though the compound was dried in a vacuum oven at 100°, the analysis indicated that water of crystallization was present. All water of crystallization was eliminated by heating 24 hr at 160° in a vacuum oven.[11]

5-Substituted 6-Amino-4-carboxy-2-thiopyrimidine (III).—To a solution of 16 g (0.24 mole based on 85% purity) of KOH in 600 ml of 95% ethyl alcohol, 60 g (0.72 mole) of thiourea was added with stirring, followed by 0.24 mole of the potassium ethyl cyanopyruvate[9a] (I) and the reaction mixture was stirred at room temperature for 24 hr. The alcohol was evaporated under reduced pressure and then 500 ml of water was added. The resulting solution was made strongly acidic with concentrated HCl. After several hours the yellowish crystals were separated and purified as described above. When the melting point was determined by the capillary tube method, melting did not occur until that of the decarboxylated derivative. The crystals however did give a characteristic decomposition point on a Fisher-Johns melting point apparatus. After drying the product 8 hr in a vacuum oven at 100°, the analysis of some pyrimidines in this series showed that water of crystallization was still retained.

5-Substituted 6-Amino-4-carboxy-2-hydroxypyrimidine (IV).—Ten grams of the corresponding 6-amino-4-carboxy-2-thiopyrimidine (III) was mixed with 120 ml of a 10% H_2O_2 solution and 120 ml of a 5% NaOH solution was added with stirring. The resulting solution effervesced and a sharp rise in temperature occurred. After the effervescence had subsided, the solution was boiled for several minutes and then allowed to cool. It was made strongly acidic with concentrated HCl. Precipitation occurred slowly. After 3 days enough NaOH solution was added to make the mixture just slightly acidic. The product was separated by filtration, washed with water and acetone, and dried. The product was purified in the same manner as described above. Some members of this series retained water of crystallization after being dried.

Biological Testing. *In Vitro* **Antiviral Tests.**—Ten-day embryonated eggs were inoculated with four twofold dilutions of the test compound starting with the maximum tolerated dose. Immediately afterwards, the eggs were inoculated with 100EID_{50} of virus. For the influenza virus (PR-8) system, the allantoic fluid was harvested at 24 hr, pooled, and titered for hemagglutinating virus. The HA reduction factor was calculated dividing the titer of the controls by that of the treated groups. An HA reduction factor of 10 or greater was considered significant. For the herpes simplex virus system, the eggs were candled each day for 10 days and survivors were recorded. A survival rate of 50% or greater over the control is considered significant.

Antimicrobial Screen.—The test compounds were dissolved or suspended in a nutrient agar at a final concentration of 7.5, 12.5, 50, and 200 mμ/ml. A Steers replicator apparatus simultaneously introduced the microorganisms (*Staphylococcus aureus* (resistant strain), *Klebsiella pneumonia*, *Trichomona foetus*, *Candida albicans*, and *Trichophyton mentagrophytes*) onto the surface of the agar. After appropriate incubation, the plates were observed for inhibition of growth.

Antimalarial Screen.—Mice were infected with a lethal dose of *Plasmodium berghei* 3 days prior to administration of the chemical. Administration of the chemical in oil was made subcutaneously at concentrations of 4, 16, and 64 mg/kg of body weight. The mean survival time of the mice was 6.3 ± 0.5 days. An extension in survival time of chemically treated mice was interpreted as evidence of antimalarial activity.

Acknowledgment.—The authors wish to thank Dr. John E. Hoover and the staff of the Microbiological Section at Smith Kline and French Laboratories, Philadelphia, Pa., for carrying out the antiviral and antimicrobial evaluation tests. The authors also wish to acknowledge the Walter Reed Army Institute of Research for carrying out the antimalarial tests.

(11) On several occasions attempts to remove all water of crystallization resulted in decarboxylation to the corresponding 5-substituted 2,4-diaminopyrimidine.

Synthesis of a Nonapeptide Sequence of Chymotrypsin[1]

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The nonapeptide glycyl-L-aspartyl-L-serylglycylglycyl-L-prolyl-L-leucyl-L-valyl-S-benzyl-L-cysteine, a sequence from chymotrypsin which contains the enzymically active serine residue, has been synthesized by the Merrifield solid phase method.[3] The modifications described by Stewart and Woolley[4] were used. All amino acids were introduced as their *t*-butyloxycarbonyl (Boc) derivatives. The synthesis of a related octapeptide by classical methods has been described.[5] The purified synthetic peptide was found, as expected, not to catalyze the hydrolysis of acetylphenylalanine ethyl ester. The histidine residue which is also necessary for catalytic activity resides in a different peptide chain of the chymotrypsin molecule. The plan of the present project was to synthesize separate peptides containing the essential serine and histidine and to link these peptides by means of a disulfide bridge.

Experimental Section

Synthesis of the Peptide on the Resin Support.—Boc-S-Benzyl-L-cysteine (2.54 g), 0.83 g of triethylamine, and 6.0 g of chloromethylated copolystyrene–divinylbenzene (2% cross-linked) (Biobeads, Bio-Rad Co., Richmond, Calif., 1.36 mequiv of Cl/g) were stirred and refluxed in 30 ml of absolute ethanol for 48 hr. The Boc-S-benzylcysteinyl resin was collected by filtration, washed thoroughly with ethanol, and dried, 6.8 g. The amount of Boc-S-benzylcysteine esterified to the resin was determined by hydrolysis of an aliquot of the product by refluxing for 24 hr with 1:1 dioxane–concentrated HCl. The liberated amino acids were measured with the Spinco amino acid analyzer; the sum of half-cystine and S-benzylcysteine was found to be 0.385 mmole/g of resin.

The coupling of the remaining eight amino acids to the S-benzylcysteinyl resin was carried out in the vessel previously described.[3a] The batch of resin (3.0 g, 1.16 mmoles of cysteine) was rocked with dioxane to swell the resin and was then rocked for 30 min with 4.0 *M* anhydrous HCl in dioxane to remove the Boc protecting group.[6] The resin was washed well (three times each) with dioxane and chloroform, and the hydrochloride of the cysteine was converted to the free base by rocking the resin for 10 min with 10% triethylamine in $CHCl_3$. After thorough washing with $CHCl_3$ and CH_2Cl_2, the resin was treated with a solution of 755 mg (3.48 mmoles) of Boc-L-valine in 10 ml of CH_2Cl_2 and rocked for 5 min to allow the amino acid to penetrate the resin. A solution of 720 mg (3.50 mmoles) of dicyclohexylcarbodiimide (Aldrich Chemical Co.) in CH_2Cl_2 (50% w/v, 1.44 ml) was then added and the vessel was rocked for 2 hr to allow the coupling reaction to go to completion. The resin was then washed thoroughly with CH_2Cl_2 and dioxane. In the same manner, the peptide resin was then acylated successively with the Boc derivatives[7] of leucine, proline, glycine, glycine, O-benzylserine, aspartic acid β-benzyl ester, and glycine. Following the

(1) Supported in part by Grant A1260 from the U. S. Public Health Service.

(2) Deceased July 1966. Address inquiries to J. M. Stewart, Rockefeller University.

(3) (a) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); (b) R. B. Merrifield, *Science*, **150**, 178 (1965).

(4) J. M. Stewart and D. W. Woolley, *Nature*, **206**, 614 (1965).

(5) H. T. Cheung, T. S. Murthy, and E. R. Blout, *J. Am. Chem. Soc.*, **86**, 4200 (1964).

(6) All dioxane was freed of peroxide before use by passing it through a short column of alumina (Merck No. 71707).

(7) Boc-L-amino acids were obtained from Cyclo Chemical Corp., Los Angeles, Calif.