Growth toxicity of this amino acid analog is only slightly affected by higher concentrations of DL-valine (*i.e.*, in the presence of 20 γ/ml. of DL-valine, the minimal concentration of antagonist required for complete inhibition is increased from 6 to 20 γ/ml.) and is not affected to any appreciable extent by DL-leucine. Equal concentrations of a mixture of isoleucine and valine are no more effective than the isoleucine concentrations alone in preventing the inhibitory effects of 2-amino-3-methylthiobutyric acid. However, threonine does prevent the inhibitory effects of the antagonist appreciably.

Threonine is known to serve as a precursor of isoleucine in *E. coli*,[9,10] and the reversal by threonine of an isoleucine antagonist has been previously studied in detail.[11]

The inhibitory effects of 2-amino-3-methylthiobutyric acid and its reversal by isoleucine have been studied most extensively with *E. coli*, as indicated in Table III. The growth inhibitions produced by this analog were competitively reversed by increasing concentrations of isoleucine over approximately a 100-fold range with an inhibition index (ratio of inhibitor to substrate necessary for complete inhibition of growth) of about 30. From these results, it is apparent that 2-amino-3-methylthiobutyric acid is a specific and effective antagonist of isoleucine in the microorganisms studied.

(9) H. E. Umbarger and E. A. Adelberg, *J. Biol. Chem.*, **192**, 883 (1951).
(10) H. E. Umbarger, *J. Bacteriol.*, **65**, 203 (1953).
(11) W. M. Harding and W. Shive, *J. Biol. Chem.*, **206**, 401 (1954).

Synthesis of Some Pyrimidine Amino Acids by the Rhodanine Method and Tests *vs.* the Ehrlich Ascites Carcinoma[1]

VILHJALMUR G. SKULASON, CLAUDE PIANTADOSI,[2] BENJAMIN F. ZAMBRANA,[3] AND J. LOGAN IRVIN

Departments of Medicinal Chemistry and Biochemistry, University of North Carolina, Chapel Hill, North Carolina

Received August 10, 1964

Some pyrimidine amino acids and intermediates (α-keto acids, α-thioketo acids, and α-oximino acids) have been synthesized by the rhodanine method from 2-mercapto-6-oxo-pyrimidine-4-carboxaldehyde and various 1- and 5-methyl and ethyl derivatives thereof. The reduction of the oximino acids to the amino acids was performed with sodium amalgam in alkaline solution followed by neutralization with a weakly acidic ion-exchange resin. Representative examples of these compounds were tested as inhibitors of growth and protein synthesis in Ehrlich ascites carcinoma in mice.

Granacher[4] has shown the applicability of rhodanine to organic syntheses. Its active methylene group permits reactions with aldehydes yielding stable condensation products.[5] These are easily cleaved with alkali to thioketo acids which are believed to exist in equilibrium with the tautomeric sulfhydryl forms since they give a deep green color with ferric chloride.[4] Oximino acids, produced by the action of hydroxylamine on the thioketo acids, can be converted with sodium amalgam to amino acids.[4] The oximino acids also can be converted with hydrochloric acid in the presence of formaldehyde to the corresponding keto acid.[6] This synthetic sequence, if applicable to the pyrimidinealdehydes, was considered to be of unusual interest since the intermediates as well as the amino acids might be expected to show some antitumor activity in view of their structural relationship to various metabolites.

The pyrimidine nucleosides and nucleotides are important as coenzymes and as metabolic precursors of the nucleic acids. Consequently, it is not surprising that a number of bacteriostatic and carcinostatic drugs are derivatives of pyrimidines. We have reported[7] recently that various derivatives of pyrimidine-4-carboxaldehydes inhibit growth of the Ehrlich ascites carcinoma in mice. It seemed to be of interest to prepare various α-amino acids, α-keto acids, and α-thioketo acids with pyrimidine substituents in the β-position for testing as possible inhibitors of tumor growth. It was found that these compounds could be prepared from the rhodanine derivative as outlined previously. The rhodanine derivative itself was of some interest since it can be considered to be a thio analog of a γ-lactone, and certain lactones[8,9] have been reported[10,11] to inhibit tumor growth. Substitution of pyrimidines in the β-position of the amino acid alanine might yield a potential inhibitor of protein synthesis as well as nucleic acid synthesis, and the corresponding α-oximino acid would be of interest in view of a previous report[12] of antitumor activity in this series. Substitution of pyrimidines in the β-position of pyruvic acid and thiopyruvic acid might yield an inhibitor of lactic dehydrogenase as well as a pyrimidine antimetabolite. This might be of special interest in the inhibition of tumors in which there is particular dependence upon glycolysis[13] for which lactic dehydrogenase is an essential enzyme.

(1) This investigation was supported by Public Health Service Grants CA-06364-02, CA-06364-03, and CA-02756-08 from the National Cancer Institute, and in a small part from the American Cancer Society Institutional Grant to the University of North Carolina.
(2) To whom inquiries should be sent.
(3) Predoctoral Trainee supported by Public Health Service Training Grant 5 TI-GM-404-02 from the National Institute of General Medical Sciences.
(4) C. Granacher, *Helv. Chim. Acta*, **5**, 610 (1922).
(5) P. L. Julian and B. M. Sturgis, *J. Am. Chem. Soc.*, **57**, 1126 (1935).
(6) W. H. Perkin, Jr., W. M. Roberts, and R. Robinson, *J. Chem. Soc.*, **101**, 232 (1912).
(7) C. Piantadosi, V. G. Skulason, J. L. Irvin, J. M. Powell, and L. Hall, *J. Med. Chem.*, **7**, 337 (1964).
(8) O. Doebner, *Ber.*, **27**, 344 (1894).
(9) R. Kuhn and D. Jerchel, *ibid.*, **76**, 413 (1943).
(10) P. B. Medawar, G. M. Robinson, and R. Robinson, *Nature*, **151**, 195 (1943).
(11) R. Kuhn, D. Jerchel, F. Moewas, E. F. Moller, and H. Letzre, *Naturwissenschaften*, **31**, 468 (1943).
(12) J. E. Wilson, J. L. Irvin, J. E. Suggs, and K. Liu, *Cancer Res.*, **19**, 272 (1959).
(13) O. Warburg and F. Kubowitz, *Biochem. Z.*, **189**, 242 (1927).

TABLE I

RESULTS OF SCREENING TESTS *vs.* THE EHRLICH ASCITES CARCINOMA[a]

Compd.	Dosage, mg./kg./day	Mortality treated group	Av. wt. change *T*/*C*, g.	Av. TPCV *T*/*C*, ml.	Av. TPCV % of controls
IV-4	188	1/8	3.8/5.2	2.4/2.8	85
	313	3/8	2.1/4.9	1.9/3.1	61
V-4	110	1/8	3.2/4.1	2.6/3.5	74
	217	1/8	3.0/4.5	1.7/2.7	63
	326	2/8	3.4/5.1	1.6/3.2	50
VI-2	216	1/8	2.1/4.0	2.0/2.9	69
	326	2/8	2.9/4.6	1.6/3.3	49
VII-3	204	2/8	4.7/5.1	2.7/2.8	97
	306	1/8	4.0/4.4	2.2/2.6	85
VIII-2	146	1/8	4.2/5.0	3.2/3.1	>100
	292	2/8	3.8/4.8	2.2/2.8	79
	486	3/8	3.2/4.8	1.8/3.0	60
VIII-3	154	2/8	3.7/4.6	2.9/2.9	100
	308	1/8	4.1/5.0	2.9/3.4	85
	514	3/8	3.0/4.1	2.1/3.0	70
IX[b]	110	0/8	2.8/4.4	1.9/3.2	59
	220	0/8	2.7/4.8	0.7/2.8	25

[a] T = treated group, C = control group, TPCV = total packed-cell volume of tumor cells; average mortality of control groups to day of assay = 36%. [b] Compound IX is 2-mercapto-6-oxo-1-ethyl-5-methylpyrimidine-4-carboxaldehyde, the parent aldehyde from which IV-4, V-4, VI-2, VII-3, and VIII-3 were synthesized.

Screening Results.—The various pyrimidine derivatives were tested *vs.* the Ehrlich ascites carcinoma in Swiss–Webster white mice by procedures described previously.[12,14] The results of tests of representative compounds are recorded in Table I in which compounds are designated by the table number (IV–VIII) and compound number (Arabic numerals). The rapid increase in body weight of control mice (C) is a measure of the accumulation of ascitic fluid and tumor cells, and the effectiveness of treatment of the mice in treated groups (T) is shown in part by smaller increases in body weight of mice in these groups (column 4). However, the total packed-cell volume of tumor cells (TPCV) (columns 5 and 6) determined on the 6th day after intraperitoneal transplantation of the tumor is the most reliable index of the multiplication of the tumor cells. The dosages recorded in column 2 were divided into two intraperitoneal injections/day commencing 24 hr. after transplantation of the tumor and continuing for 5 days.

The data of Table I indicate that *vs.* the Ehrlich tumor none of the derivatives tested are as effective as the parent pyrimidinealdehyde. Of the derivatives, the effectiveness *vs.* the tumor is approximately in the following order of decreasing activity: V-4 = VI-2 > IV-4 > VIII-2, VII-3, and VIII-3. Compounds V-4, VI-2, and IV-4 (and probably other members of these series) seem sufficiently active to warrant testing against other tumors.

Inhibition of Protein Synthesis.—The effects of the pyrimidine derivatives upon protein synthesis were studied by determining the inhibition of incorporation of L-phenylalanine-1-C^{14} into the proteins of Ehrlich ascites carcinoma cells which were incubated aerobically for 1 hr. with the labeled amino acid and the pyrimidine *in vitro* in Krebs–Ringer phosphate at 37° by a procedure described previously in detail.[12] Each incubation flask contained 5 ml. of a 40% suspension of tumor cells in Krebs–Ringer phosphate (pH 7.2), 1 ml. of Krebs–Ringer phosphate containing 0.1 μmole of L-phenylalanine-1-C^{14} (0.2 μc.), and 1 ml. of the pyrimidine in Krebs–Ringer phosphate (or 1 ml. of Krebs–Ringer phosphate, alone, in the controls). After the incubation of the experimental and control flasks, the total proteins were isolated and freed of lipids and nucleic acids as described previously.[12] A portion, usually 10 mg., of the total protein sample from each incubation flask was plated on stainless steel planchets, and the radioactivity was determined in a windowless gas-flow counter and scaler. The remainder of the total protein sample from each flask was extracted with 0.2 *N* HCl by stirring for 3 hr. at 4°. The HCl-insoluble proteins were removed by centrifugation. The supernatant solution was brought to pH 11 by addition of ammonium hydroxide, and 2 vol. of 95% ethanol was added. The precipitated basic proteins (designated in the tables as acid-soluble proteins) were collected by centrifugation and were plated on planchets for determination of radioactivity. In Tables II and III radioactivities are expressed as counts per min. per mg. of protein (c.p.m./mg.) after correction for background and self-absorption.

TABLE II

THE EFFECT OF 2-MERCAPTO-6-OXO-1-ETHYL-5-METHYL-4-PYRIMIDYLMETHYLIDENERHODANINE (IV-4) ON THE INCORPORATION OF PHENYLALANINE-1-C^{14} (PA) INTO PROTEINS OF EHRLICH ASCITES CARCINOMA CELLS DURING INCUBATION *in Vitro* IN KREBS–RINGER PHOSPHATE[a]

Compd. IV-4, μmoles	Molar ratio IV-4/PA	Total proteins C.p.m./mg.	Total proteins % of controls	Acid-soluble proteins C.p.m./mg.	Acid-soluble proteins % of controls
..	Controls	2277 ± 52		852 ± 136	
1	10	2060 ± 235	91	190 ± 31	22
..	Controls	1870 ± 147		742 ± 75	
1	10	1331 ± 50	71	275 ± 62	37
5	50	968 ± 115	52	111 ± 44	15
10	100	150 ± 116	8		
..	Controls	2642 ± 38		981 ± 151	
10	100	19 ± 7	0.7	20 ± 3	2

[a] 0.1 μmole of phenylalanine-1-C^{14} added to each incubation flask.

TABLE III

THE EFFECT OF α-OXIMINO-β-(2-MERCAPTO-6-OXO-1-ETHYL-5-METHYL)-4-PYRIMIDYLPROPIONIC ACID (VI-2) AND α-AMINO-β-(2-MERCAPTO-6-OXO-1-ETHYL 5-METHYL)-4-PYRIMIDYLPROPIONIC ACID (VIII-3) ON THE INCORPORATION OF PHENYLALANINE-1-C^{14} (PA) INTO PROTEINS OF ERHLICH ASCITES CARCINOMA CELLS DURING INCUBATION *in Vitro* IN KREBS–RINGER PHOSPHATE[a]

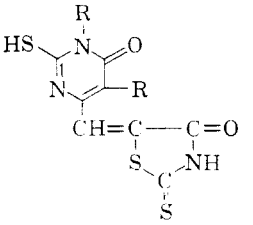
Compd., μmoles VI-2	Compd., μmoles VIII-3	Molar ratio Inhib./PA	Total proteins C.p.m./mg.	Total proteins % of controls	Acid-soluble proteins C.p.m./mg.	Acid-soluble proteins % of controls
...	...	Controls	2277 ± 52		852 ± 136	
1	...	10	2200 ± 140	97	810 ± 50	95
5	...	50	1898 ± 25	83	785 ± 70	92
...	1	10	1570 ± 79	69	640 ± 64	75
...	5	50	888 ± 94	39	375 ± 59	44
...	...	Controls	2940 ± 125		921 ± 85	
	1	10	1791 ± 81	61	532 ± 41	58
	5	50	820 ± 42	28	315 ± 54	34

[a] 0.1 μmole of phenylalanine-1-C^{14} added to each incubation flask.

(14) J. E. Wilson, J. E. Suggs, and J. L. Irvin, *Cancer Res. Suppl.*, **21**, 692 (1961).

TABLE IV

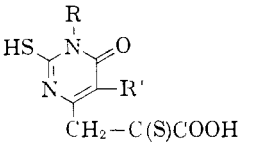
2-MERCAPTO-6-OXO-4-PYRIMIDYLMETHYLIDENERHODANINES



No.	R	R'	M.p., °C. dec.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Yield, %
1	CH_3	H	>300	$C_9H_7N_3O_2S_3$	37.88	38.02	2.47	2.66	14.73	14.68	33.71	33.35	100
2	H	C_2H_5	251–252	$C_{10}H_9N_3O_2S_3$	40.11	40.22	3.03	3.15	14.04	13.78	32.13	32.04	90
3	CH_3	CH_3	253–254	$C_{10}H_9N_3O_2S_3$	40.11	39.92	3.03	2.87	14.04	13.91	32.13	31.92	96
4	C_2H_5	CH_3	246–247	$C_{11}H_{11}N_3O_2S_3$	43.40	43.13	3.64	3.79	13.80	13.70	31.60	31.58	95

TABLE V

2-MERCAPTO-6-OXO-4-(2-CARBOXY-2-THIONOETHYL)PYRIMIDINES



No.	R	R'	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Yield, %
1	CH_3	H	202–203	$C_8H_8N_2O_3S_2$	39.33	38.83	3.30	3.62	11.47	11.29	26.25	26.28	100
2	H	C_2H_5	189–190 dec.	$C_9H_{10}N_2O_3S_2$	41.84	41.50	3.90	4.11	10.85	10.55	24.83	24.54	100
3	CH_3	CH_3	184–185 dec.	$C_9H_{10}N_2O_3S_2$	41.84	41.69	3.90	4.14	10.85	10.71	24.83	24.38	95
4	C_2H_5	CH_3	184–185 dec.	$C_{10}H_{12}N_2O_3S_2$	44.10	44.07	4.44	4.30	10.29	10.08	23.54	23.29	87

The data of Table II indicate that the pyrimidine rhodanine derivative (IV-4) inhibits phenylalanine incorporation into total proteins by only 10–30% at a ratio of 10:1 with reference to the labeled amino acid, but strong inhibition is shown at ratios of 50 and 100. It is of considerable interest that this compound inhibits incorporation of phenylalanine into the acid-soluble proteins more strongly than into total proteins of the tumor cells. By contrast, the pyrimidine amino acid and pyrimidine oximino acid (Table III) do not show this selective effect. On the other hand, the pyrimidine amino acid (VIII-3) is a much stronger inhibitor of phenylalanine incorporation into total proteins of the tumor cells than either the rhodanine derivative or the oximino acid. Compound VIII-3 can be considered as a structural analog of phenylalanine, and this probably accounts for its strong inhibitory effect. Further work will be necessary to determine the mechanism of inhibition by this compound, but it seems possible that compound VIII-3 could inhibit protein synthesis by inhibiting synthesis of essential ribonucleic acid (RNA) molecules, such as S-RNA, as well as by inhibiting the activation of phenylalanine by the amino acid activating enzyme system. This compound may prove useful in studies of protein biosynthesis.

Experimental[15]

(2-Mercapto-6-oxo-1-methyl-4-pyrimidylmethylidene)rhodanine.—This compound was prepared by a modification of the procedure described by Julian and Sturgis.[5] A mixture of 13.6 g. (0.08 mole) of 2-mercapto-6-oxo-1-methyl-pyrimidine-4-carboxaldehyde, 12.0 g. (0.09 mole) of rhodanine, 19.2 g. of freshly fused sodium acetate, and 160 ml. of glacial acetic acid was heated on the steam bath with stirring for 1 hr. After cooling, the mixture was poured, with stirring, into 300 ml. of water. The resulting yellow crystalline powder was filtered, washed well with water, alcohol, and ether, and air dried to give 22.5 g. of the compound. This was recrystallized from dimethylformamide and washed with water. The melting point was above 300° dec. Compounds made by a similar procedure are listed in Table IV.

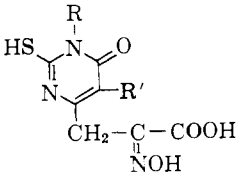
2-Mercapto-6-oxo-1-methyl-4-(2-carboxy-2-thionoethyl)pyrimidine.—This compound was prepared by a modification of the procedure described by Julian and Sturgis.[5] Twenty-two grams of (2-mercapto-6-oxo-1-methyl-4-pyrimidylmethylidene)-rhodanine was dissolved in 100 ml. of 15% NaOH solution in a 400-ml. beaker covered with a watch glass. The solution was heated vigorously on the steam bath with occasional shaking for 0.5 hr. It was diluted with 100 ml. of water, filtered, and cooled. The addition of 150 ml. of 10% aqueous HCl, with stirring, rapidly precipitated the compound as a yellow amorphous powder. This mixture was left standing in the refrigerator overnight. The acid was filtered, washed with water, and air dried to yield 18.5 g. of the compound. It was recrystallized from aqueous dimethylformamide by the addition of a few drops of HCl and finally washed with water; m.p. 202–203°. Compounds made by a similar procedure are listed in Table V.

α-Oximino-β-(2-mercapto-6-oxo-1,5-dimethyl)-4-pyrimidylpropionic Acid.—This compound was prepared by a modification of the procedure described by Julian and Sturgis.[5] To a solution of sodium ethylate prepared from 4.5 g. of sodium and 130 ml. of ethanol was added a warm solution of 13.5 g. of hydroxylamine hydrochloride in 12 ml. of water. The solution of hydroxylamine was filtered from the precipitated NaCl and poured onto 15 g. of the crude thioketo acid. The resulting solution was refluxed for 1 hr. and allowed to cool slowly to room temperature. The precipitate was filtered, washed with ethanol, and dissolved in a 4% solution of NaOH (42 ml.). The resulting solution was filtered, cooled, and cautiously acidified with stirring, with 30 ml. of 10% aqueous HCl. The acid precipitated immediately and the mixture was placed in the refrigerator for 0.5 hr. The filtrate was evaporated to dryness on the flash evapora-

(15) Analyses by Weiler and Strauss, Oxford, England, and by Spang Microanalytical Laboratories, Ann Arbor, Mich. All melting points were determined with the Mel-Temp apparatus and are corrected. Infrared spectra were measured with a Perkin-Elmer 137 spectrophotometer.

TABLE VI

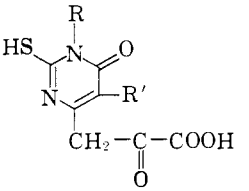
α-OXIMINO-β-(2-MERCAPTO-6-OXO)-4-PYRIMIDYLPROPIONIC ACIDS



No.	R	R′	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Yield, %
1	CH_3	CH_3	190–191 dec.	$C_9H_{11}N_3O_4S$	42.02	42.18	4.31	4.41	16.33	16.22	12.46	12.32	33
2	C_2H_5	CH_3	169–171	$C_{10}H_{13}N_3O_4S$	44.27	43.85	4.83	5.01	15.49	15.22	11.82	11.60	38
3	H	C_2H_5	208–210 dec.	$C_9H_{11}N_3O_4S$	42.02	42.01	4.31	4.22	16.33	16.45	12.46	12.80	72

TABLE VII

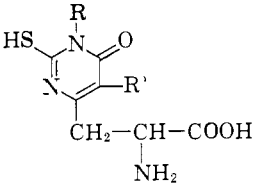
β-(2-MERCAPTO-6-OXO)-4-PYRIMIDYLPYRUVIC ACIDS



No.	R	R′	M.p., °C. dec.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Yield, %
1	CH_3	H	250–251	$C_8H_8N_2O_4S$	42.10	42.09	3.53	3.41	12.28	11.95	14.05	13.96	55
2	CH_3	CH_3	224–226	$C_9H_{10}N_2O_4S$	44.62	44.28	4.16	4.06	11.56	11.42	13.24	13.05	29
3	C_2H_5	CH_3	218–220	$C_{10}H_{12}N_2O_4S$	46.87	46.69	4.72	4.39	10.93	10.62	12.51	12.38	63
4	H	C_2H_5	181–183	$C_9H_{14}N_2O_4S$	44.62	44.43	4.16	4.28	11.56	11.48	13.24	13.09	60

TABLE VIII

α-AMINO-β-(2-MERCAPTO-6-OXO)-4-PYRIMIDYLPROPIONIC ACIDS



No.	R	R′	M.p., °C. dec.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Yield, %
1	H	C_2H_5	284–285	$C_9H_{13}N_3O_3S$	44.43	44.30	5.39	5.21	17.27	16.99	13.18	12.98	52
2	CH_3	CH_3	336–338	$C_9H_{13}N_3O_3S$	44.43	44.17	5.39	5.38	17.27	17.16	13.18	13.37	33
3	C_2H_5	CH_3	273–274	$C_{10}H_{15}N_3O_3S$	46.68	46.69	5.88	6.01	16.33	16.31	12.46	12.19	38

tor, and the residue was treated in the same way as described above. The precipitate was filtered, washed with water, and air dried. The yield after recrystallization from aqueous ethanol (1:1) was 5.0 g. (33%), m.p., 190–191° dec. Compounds prepared by a similar procedure are listed in Table VI.

β-(2-Mercapto-6-oxo-1,5-dimethyl)-4-pyrimidylpyruvic Acid.—This compound was prepared by a modification of the method described by Perkin, *et al.*[6] Two grams of the corresponding oximino acid was suspended in 8 ml. of 40% formaldehyde solution, and 10 ml. of concentrated HCl gradually was added. This mixture was stirred at room temperature for 3 hr. and placed in the refrigerator for 1.5 hr., and the resulting precipitate was filtered, washed thoroughly with water, and air dried. Recrystallization from ethanol gave 0.5 g. (29%) of the compound, m.p. 224–226° dec. Compounds made by a similar procedure are listed in Table VII.

α-Amino-β-(2-mercapto-6-oxo-1,5-dimethyl)-4-pyrimidylpropionic Acid.—Two grams of the corresponding crude oximino acid was dissolved in 80 ml. of ethanol. This solution was heated on the steam bath and 80 g. of 2% sodium amalgam was added in four 20-g. portions with shaking after each addition. When all the amalgam had been added, enough water (80 ml.) was added to effect a solution of the precipitate. The reaction mixture was heated gently on the steam bath for 0.5 hr. and filtered. The solution was passed through a column containing a weakly acidic ion-exchange resin, Amberlite IRC-50, and the filtrate was evaporated to dryness on a flash evaporator, treated with 20 ml. of ethanol, and placed in the refrigerator for 1 hr. The white precipitate which formed was filtered and washed thoroughly with ethanol. The yield was 630 mg. (33%), m.p. 336–338° dec. Recrystallization from aqueous ethanol did not change the melting point. This compound was soluble in both dilute NaOH and dilute HCl solutions. It gave a positive ninhydrin test. Its infrared spectrum showed absorption at 6.28 (COO^- asym. stretch), 6.50 (NH_3^+ sym. stretch), and 7.11 μ (COO^- sym. stretch).[16]

Other amino acids listed in Table VIII were prepared by an analogous manner.

(16) K. Nakanishi, "Infrared Absorption Spectroscopy—Practical," Holden-Day, Inc., San Francisco, Calif., 1962, p. 196.