Nucleosides. XVII. Pyrimidinyl Amino Acids[1]

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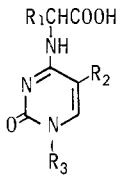
N-(2-Oxo-4-pyrimidinyl) amino acids were prepared by reaction of 4-methylthio-2-pyrimidinones with amino acids. N-(2-Oxo-4-pyrimidinyl)-glycine, -L-alanine, -L-phenylalanine (IVc), -L-tryptophan (IVd), -β-alanine, -*o*- and *p*-aminobenzoic acid (Va), and -glycylglycine were obtained. N-(2-Thio-4-pyrimidinyl)-L-tryptophan was also prepared as well as the 5-methyl, 5-fluoro (IVf), 5-chloro, and 5-bromo analogs of N-(2-oxo-4-pyrimidinyl)-D,L-alanine. The ribonucleosides of IVc, d, and Va were synthesized by treatment of 1-β-D-ribofuranosyl-4-methylthio-2-pyrimidinone with the appropriate amino acid. The 1-(2′-deoxy-β-D-ribofuranosyl) derivative of IVf was synthesized by similar methods. Preliminary results with some of these compounds in experimental tumors showed no significant antitumor activity. None of the pyrimidinyl amino acids tested supported the growth of certain pyrimidine- or amino acid-requiring mutants of *Escherichia coli*.

It was demonstrated in another series[2] that certain exocyclic N-alkylated derivatives of 5-fluoro-2′-deoxycytidine exhibited significant antitumor activity against transplanted mouse leukemia B82, though the parent compound, 5-fluoro-2′-deoxycytidine, had a better chemotherapeutic index in this system. It was also demonstrated that the exocyclic N-methyl derivatives of the naturally-occurring cytosine nucleosides (*e.g.*, cytidine, 5-methylcytidine, 2′-deoxycytidine, and 5-methyl-2′-deoxycytidine) are essentially resistant to conversion to their corresponding uridine analogs by nucleoside deaminase(s) derived from *Escherichia coli* B. The N-methyl derivative of 5-fluoro-2′-deoxycytidine however *is* converted appreciably to 5-fluoro-2′-deoxyuridine in this enzymatic system though to a lower extent than 5-fluoro-2′-deoxycytidine.

In addition, several purinyl and pyrimidinyl amino acids are known to occur in nature as antibiotics or as intermediates in the biosynthesis of nucleotides, such as adenylosuccinate,[3] amicetin,[4] and guanine propionate[5a] and its nucleoside.[5b] A number of N-(6-purinyl)[6a–d] and N-(6-purinoyl)[6e] amino acids have also been synthesized. These studies suggest that other exocyclic-N-substituted derivatives of cytosines might be useful as compounds of potential biochemical interest.

This paper deals with the synthesis of certain N-(4-pyrimidinyl) amino acids of structure I including some 1-β-D-ribofuranosyl and 1-(2-deoxy-β-D-ribofuranosyl) derivatives thereof.

There are several approaches to the synthesis of pyrimidinyl amino acids. Some have been prepared



I

by reaction of halogenopyrimidines with amino acids.[7] Unfortunately, this method is not easily applicable to the synthesis of I since 4-halogeno-2-pyrimidinones are not readily available. Prokofév, *et al.*,[8] have reported the synthesis of N-(2-oxo-6-methyl-4-pyrimidinyl)-glycine by treatment of 4-amino-2-chloro-6-methylpyrimidine with chloroacetic acid. This method is ambiguous since later reports in the literature[9] indicate that, in general, alkylation occurs on a ring nitrogen rather than on the exocyclic amino group in certain aminopyrimidines. Feldman, *et al.*,[10] have utilized the ready replacement of the 2-alkylmercapto group[11] by amines to prepare N-(4-oxo-pyrimidinyl) amino acids by reaction of 2-methylthio-4-pyrimidinone with amino acids.

The facile synthesis of 4-thio-2-pyrimidinones by direct thiation of uracils[12] and the availability of 4-thio nucleosides[2,13] made this approach the method of choice. The thiouracils (II) used herein were prepared by the method of Mizuno, *et al.*,[12] and then converted to the 4-methylthio derivatives III, by modification of the procedure of Wheeler and Johnson.[14]

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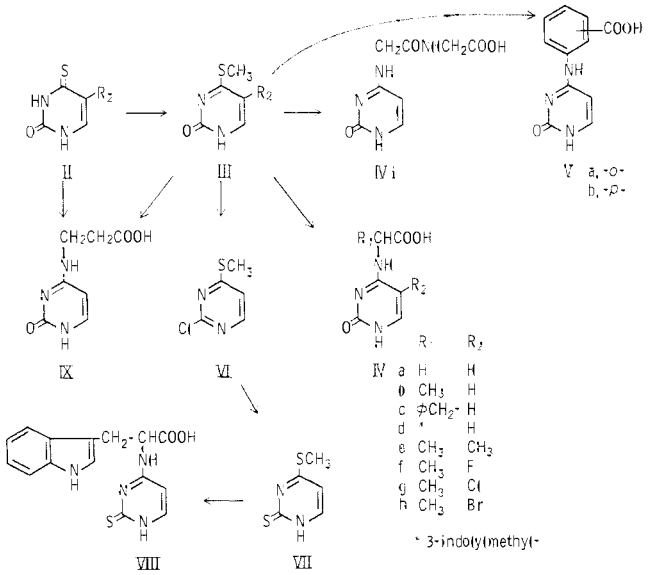
TABLE I
ULTRAVIOLET ABSORPTION PROPERTIES OF 4-PYRIMIDINYL AMINO ACIDS[a]

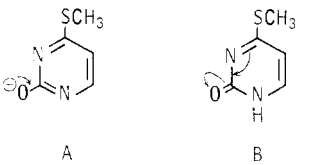
Compounds	N HCl λ_{max} mμ	N HCl ϵ_{max}	N HCl λ_{min} mμ	N HCl ϵ_{min}	pH 6.50 λ_{max} mμ	pH 6.50 ϵ_{max}	pH 6.50 λ_{min} mμ	pH 6.50 ϵ_{min}	N NaOH λ_{max} mμ	N NaOH ϵ_{max}	N NaOH λ_{min} mμ	N NaOH ϵ_{min}
IVa	281	11,500	242	1,820	267	8,200	218	6,260	285	9,160	253	1,930
	212.5	8,200			sh231	7,270						
b	282	12,450	243	1,990	268	9,130	248	6,830	284	9,780	253	2,400
	213	8,700			sh232	7,800						
c	284	11,350	244	2,260	267.5	8,850	247.5	6,600	285	9,760	251	2,260
					sh235	7,150						
d	280	16,500	244	4,180	271	65,450	247	9,580	283	16,700	252	4,920
									288	16,500		
e	287	12,300	245	4,470	272	8,840	252	6,340	287	9,260	255	2,420
	215	12,620										
f	287	9,500	248	2,720	275	8,220	257	6,940	292.5	9,580	255	1,690
	214	9,580			242.5	7,550	229	7,400				
g	293	8,600	252	4,790	280	6,820	256	4,750	294	8,150	259	1,400
	220	11,000										
h	295	8,680	255	1,750	282	6,800	259	4,760	295	8,230	262	4,870
	219	11,400										
i	282	11,700	241	2,100	267.5	7,780	249	5,960	283	8,880	252	1,770
	210	8,870			sh230	7,850						
Va	296	17,100	259	6,600	302	22,500	252	6,100	314	27,100	280	7,600
					245	6,300	240	6,270	271	8,000	258	6,600
b	291	12,240	254	4,000	295	64,500	256	5,800	304	15,500	275	5,100
	sh220	13,900			306	64,000			sh260	6,300		
VIII	276	23,000	245	10,200	267	30,800	235	64,500	sh260	17,750	sh280	60,300
	sh315	5,300										
IX	279	9,380	241	1,580	267	6,960	248	5,180	285	7,530	254	1,660
	214	6,870			231	6,500	228	6,450				
XIIa	288	15,700	247	2,700	274	13,500	230	8,400	275	63,500	231	8,600
					sh245	8,800			sh245	8,800		
XIIb	282	17,400	246	4,000	275	17,700	245	10,300	278	18,100	246	60,250
	287.5	17,100										
XIIc	292.5	11,500	252	2,920	280	10,840	228	6,570	281	10,520	229	6,720
	218	9,000			sh252	8,470			sh250	7,740		
XIII	301	18,100	260	5,750	305	24,500	256	5,850	312	67,500	260	8,150
					sh245	6,150						

[a] Values determined with a Cary Model 15 recording spectrophotometer. Sh, shoulder.



Reaction of III with amino acids in the presence of one equivalent of aqueous sodium carbonate at pH 8–9 under reflux for 3–18 hr. yielded (after acidification) the pyrimidinyl amino acids IV and V in satisfactory yield.

The pH of the reaction is important. If the reaction is carried out at pH 7 or below (no addition of sodium carbonate), extensive hydrolysis of the methylthio group occurs and uracils are the predominant products. In the pH region above 10 the reaction proceeded very slowly. This latter phenomenon may be due to the dissociation of the –NHCO– grouping on the 1,2 positions of III to give enolate A which could serve as



A B

an electron donor, thereby decreasing the susceptibility of C-4 to nucleophilic attack.

In the pH region 8–9 in which approximately more than half of III ($pK \sim 9.5$) is in the undissociated form, contributions as shown in B may facilitate nucleophilic attack on C-4. It is also to be noted that, in the 8–9 pH region, the amino acids exist partially at least in the noncationic form available for nucleophilic substitution.

With the exception of the aromatic amino acid derivatives, all the pyrimidinyl amino acids gave ultraviolet absorption spectra generally similar to that for cytosine[15] (see Table I). The alanyl and glycyl deriva-

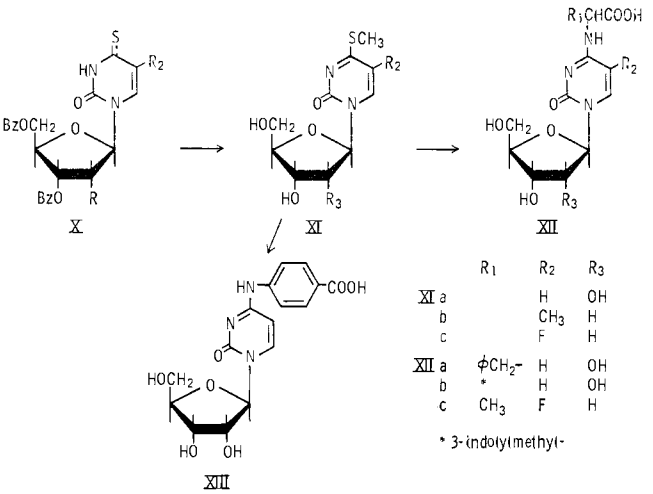
[15] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

tives of IV were stable to *N* hydrochloric acid or *N* sodium hydroxide (100° for 18 hr.). This stability is to be contrasted with that for N-(6-purinyl)-α-alanine[6d] which was reported to be unstable to acid or to boiling water and with that for N-(6-hydroxy-2-purinyl)-α-alanine[5a] which is unstable in aqueous solution as well as in acid or alkaline media.

The synthesis of VIII was accomplished by reacting III (R = H) with phosphorus oxychloride in diethylaniline and treating the crystalline monochloro derivative VI thus obtained with thiourea to afford VII. Refluxing of VII with L-tryptophan in water (pH 8–9) gave N-α-(2-thio-4-pyrimidinyl)-L-tryptophan (VIII) with the evolution of methyl mercaptan. VIII was negative to ninhydrin spray test which shows that the α-amino group of tryptophan is substituted.

The reaction of II (R_2 = H) with β-alanine at reflux temperature in water containing one equivalent of sodium carbonate for 30 hr. yielded the β-amino acid derivative IX in 60% yield. On the other hand, when the methylthio derivative III was used in place of II in this reaction, the reaction time was reduced to 3 hr. and IX was obtained in 90% yield. These data attest to the greater susceptibility of III *vs.* II to nucleophilic displacement on C-4.

The 5-chloro and -bromo analogs of IVb were synthesized by reaction of IVb with N-chloro- and N-bromosuccinimide, respectively.



For the synthesis of amino acid nucleosides of structure I, the readily available acylated 4-thionucleosides (X, R = OBz, R_2 = H and R = H, R_2 = CH_3,) were employed as starting materials. These were deacylated in alkali and methylated *in situ* to their 4-methylthio analogs XI. Of these, only the thymidine analog XIb was obtained in crystalline form. Unfortunately, this crystalline substance XIb when treated with an amino acid (*i.e.*, α-alanine, phenylalanine, or tryptophan) for 20 hr. gave only trace amounts of product. The bulk of the reaction mixture was thymidine and starting material.

4-Methylthiouridine (XIa) was more reactive than 4-methylthiothymidine XIb with amino acids (*i.e.*, phenylalanine, tryptophan, and *p*-aminobenzoic acid) and after ion-exchange chromatography, gave low yields (20–30%) of product XIIa,b, and XIII. Again, the main side reaction was the hydrolysis of the thioether of XIa to uridine. 4-Thio-5-fluoro-2′-deoxyuridine[2] was converted to the 4-methylthio derivative XIc and reacted with α-alanine. After ion-exchange chromatography on Dowex I (formate), a 40% yield of crystalline XIIc was obtained.

Screening Studies.[16]—As in a previous study,[17] some of these compounds were tested against transplanted mouse leukemia B82. Preliminary screening results in this system[16a] were obtained with the following compounds: II and III (R_2 = F), IVa–d, XIb, and XIIc. None of these compounds was active against this tumor except the phenylalanyl derivative IVc and the tryptophanyl derivative IVd. With IVc at 250 mg./kg./day × 7, the inhibition was slight and without significant toxicity. With IVd (at the same dose level) significant tumor inhibition (~ 73%) was observed but this activity was accompanied by severe weight loss.

In Sarcoma 180,[16b] IVd showed no activity. In Ehrlich ascites in the mouse, at dose levels of 500 and 250 mg./kg./day × 7, IVd showed slight inhibition of tumor growth.[16b]

Escherichia coli auxotrophs were employed to test for the ability of the pyrimidinyl amino acids (IVa–d) to support growth in these microbial systems.[16c] Four auxotrophs[17b] were used, *viz.*, B_u- (uracil requiring), W_c- (cytosine requiring), M83-5 (phenylalanine requiring), and M165A-52 (tryptophan requiring). None of the compounds supported the growth of the pyrimidine-requiring organisms, indicating that cleavage at the aliphatic side of the exocyclic amino function did not occur. Nor did IVc or IVd support the growth of M83-5 or M165A-52, respectively, indicating that the C–N bond on the heterocyclic side of the exocyclic amino function also remained intact in this system. Similar results were obtained with a uracil-requiring mutant of *Bacillus subtilis*.[16c]

Experimental[18]

4-Thiothymine (II, R_2 = CH_3).[19]—A mixture of thymine (63 g., 0.5 mole) and phosphorus pentasulfide (58 g., 0.26 mole) in pyridine (1200 ml.) was refluxed for 3 hr. with stirring. After cooling, the upper layer was decanted (lower layer discarded) and concentrated to dryness *in vacuo*. The residue was recrystallized from boiling water, yield, 59 g. (83%). This product II is sufficiently pure for use in the next step.

4-Methylthio-5-methyl-2-pyrimidinone (III, R_2 = CH_3).—4-Thiothymine (12 g.) was dissolved in *N* sodium hydroxide (84.5 ml.), methyl iodide (15 g.) was added, and the mixture stirred for 1 hr. at room temperature. The separated crystals were collected by filtration and recrystallized from water; 10 g., needles, m.p. 214–215° were obtained (lit.[19] m.p. 205–211°).

Anal. Calcd. for $C_6H_8N_2OS$: C, 46.14; H, 5.16; N, 17.94; S, 20.53. Found: C, 46.25; H, 5.14; N, 17.98; S, 20.66.

5-Fluoro-4-thiouracil (II, R_2 = F).—5-Fluorouracil (19.5 g.)[20] and phosphorus pentasulfide (17.5 g.) in reagent grade pyridine (400 ml.) were refluxed for 2 hr. At the beginning of the

(16) The authors are indebted to the following investigators from this Institute for kindly providing their preliminary results: (a) J. H. Burchenal, J. R. Purple, and E. Bucholz, (b) P. C. Merker and F. Schmidt, and (c) L. Kaplan and L. Provenzano.

(17) (a) N. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4060 (1961); (b) The authors are indebted to Dr. B. D. Davis of Harvard University for the phenylalanine- and tryptophan-requiring mutants and to Dr. S. S. Cohen of the University of Pennsylvania for the uracil- and cytosine-requiring mutants.

(18) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by the Galbraith Laboratories, Inc., Knoxville, Tennessee, and by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

(19) H. L. Wheeler and D. F. McFarland, *Am. Chem. J.*, **43**, 19 (1910).

(20) R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).

reaction, water was added dropwise (~ 0.4 ml.) to maintain an orange-turbid color.[21] The pyridine layer was evaporated and the residue was taken up in water and stirred. The precipitated product was collected by filtration (16.7 g., 76%). Pure compound was obtained after recrystallization from water; m.p. 277–278° dec.

Anal. Calcd. for $C_4H_3FN_2OS$: C, 32.86; H, 2.07; N, 19.17; F, 13.00; S, 21.93. Found: C, 32.87; H, 2.07; N, 19.35; F, 13.22; S, 22.06.

5-Fluoro-4-methylthio-2-pyrimidinone (III, R_2 = F).—5-Fluoro-4-thiouracil (8 g.) in *N* sodium hydroxide (54.8 ml.) was treated with methyl iodide (20 g.) at room temperature. The separated crystals were recrystallized from water, yielding needles (5.6 g., 64%), m.p. 222–224°.

Anal. Calcd. for $C_5H_5FN_2OS$: C, 37.49; H, 3.15; N, 17.49; F, 11.86; S, 20.02. Found: C, 37.65; H, 3.23; N, 17.36; F, 12.06; S, 20.16.

2-Chloro-4-methylthiopyrimidine (VI).—4-Methylthio-2-pyrimidinone (III, R_2 = H, 20.5 g.) was suspended in phosphorus oxychloride (80 ml.), stirred, and treated dropwise with diethylaniline (21.5 g.) at 55°. After 1.5 hr. at 55° most of the solvent was removed *in vacuo*. The residue was poured into ice–water (400 ml.) and extracted with ether. The ether layer was washed with water and dried over sodium sulfate, filtered from salts, and the filtrate evaporated to dryness. White crystals of 2-chloro-4-methylthiopyrimidine (VI)[22] were obtained, m.p. 66–68°.

Anal. Calcd. for $C_5H_5ClN_2S$: C, 37.38; H, 3.14; N, 17.44. Found: C, 37.39; H, 3.20; N, 17.40.

4-Methylthio-2-pyrimidinethione (VII).—Product VI was dissolved in absolute ethanol (450 ml.) containing thiourea (14 g.) and the solution refluxed for 1 hr. After concentrating to 100 ml. and cooling, crystals of the thiouronium salt separated, m.p. 166–167° dec.

Anal. Calcd. for $C_6H_9ClN_4S_2$: C, 30.43; H, 3.80; N, 23.67; S, 27.05. Found: C, 30.49; H, 4.09; N, 23.50; S, 26.52.

The thiouronium salt was dissolved in water (100 ml.) and 10% sodium hydroxide (*ca.* 50 ml.) was added. After acidification with acetic acid to pH 5, the precipitated crystals VII were collected and recrystallized from water. Yellow needles (16 g., 70% from III, R_2 = H), were obtained, m.p. 190–192°.

Anal. Calcd. for $C_5H_6N_2S_2$: C, 37.97; H, 3.80; N, 17.72; S, 40.51. Found: C, 38.11; H, 3.75; N, 17.81; S, 40.25.

N-(1H-2-Oxopyrimidinyl-4)-glycine (IVa).—A mixture containing 4-methylthio-2-pyrimidinone (4.0 g.), glycine (2.57 g., 1.2 equiv.), and sodium carbonate (1.82 g., 0.6 equiv.) in water (20 ml.) was refluxed for 3 hr. After cooling, the solution was acidified with formic acid (4 ml.), cooled, and the precipitate, 4.7 g., collected and recrystallized from water. Yield is *ca.* 80%, and the compound has no definite melting point.

Anal. Calcd. for $C_6H_7N_3O_3$: C, 42.61; H, 4.17; N, 24.83. Found: C, 42.40; H, 4.22; N, 24.63.

N-(1H-2-Oxo-4-pyrimidinyl)-L- and -DL-Alanine (IVb).—These products were obtained by the same method stated before, except the refluxing time was 5–8 hr. The yield was 75–85% for L-isomer, $[\alpha]^{22}D$ −104° (*c* 0.4, *N* HCl), m.p. > 280°, and DL-isomer, optically inactive, m.p. 264–265° dec.

Anal. Calcd. for $C_7H_9N_3O_3$: C, 45.90; H, 4.95; N, 22.94. Found: L-isomer: C, 45.87; H, 5.03; N, 23.21. DL-isomer: C, 45.96; H, 5.04; N, 22.92.

N-(1H-2-Oxo-4-pyrimidinyl)-L-phenylalanine (IVc).—A mixture of III (R_2 = H) (2 g.), L-phenylalanine (1.1 equiv.), and sodium carbonate (0.55 equiv.) in water (20 ml.) gave this product after 18 hr. at reflux temperature. Recrystallization from water gave 1.8 g., m.p. 136–138° dec., $[\alpha]^{20}D$ +49° (*c* 0.40, *N* HCl).

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.23; H, 5.05; N, 16.21. Found: C, 59.82; H, 5.54; N, 16.01.

N-(1H-2-Oxo-4-pyrimidinyl)-L-tryptophan (IVd).—A mixture of III (R_2 = H, 4.26 g.), L-tryptophan (1.1 equiv.), and sodium carbonate (0.55 equiv.) in water (50 ml.) gave crude product (7.5 g.) after 17 hr. reflux. This product was purified by dissolving it in dilute ammonium hydroxide and precipitation by addition of acetic acid, 6.1 g., m.p. 201–205° slow dec. This product showed a single spot in paper electrophoresis (0.1 *M* ammonium acetate, pH 7) and gave a negative ninhydrin reaction; $[\alpha]^{23}D$ −17° (*c* 0.538, 3 *N* HCl).

Anal. Calcd. for $C_{15}H_{14}N_4O_3 \cdot 1.5\ H_2O$: C, 55.38; H, 5.27; N, 17.22. Found: C, 55.74; H, 5.53; N, 17.24.

N-(1H-2-Oxo-4-pyrimidinyl)-glycylglycine (IVi).—A mixture of III (R_2 = H, 1.3 g.), glycylglycine (1.1 equiv.), and sodium carbonate (0.55 equiv.) in water (10 ml.) treated in the same manner as before gave a product (1.8 g.) after 8 hr. reflux. Recrystallization from water afforded 1.3 g., m.p. 259–251° dec., which showed only a single spot in paper electrophoresis (0.1 *M* ammonium acetate, pH 7, 700 v., 90 min., +9.0 cm.). The glycyl derivative (IVa) shows a spot at +11.2 cm.

Anal. Calcd. for $C_8H_{10}N_4O_4 \cdot H_2O$: C, 39.34; H, 4.92; N, 22.95. Found: C, 38.72; H, 4.80; N, 22.81.

N-(1H-2-Oxo-4-pyrimidinyl)-*p*-aminobenzoic acid (Vb).—From the mixture of III (R_2 = H, 1.4 g.), *p*-aminobenzoic acid (1.0 equiv.), and sodium carbonate (0.5 equiv.) in water (10 ml.) after 2 hr. reflux, 1.9 g. of Va was obtained. The product was purified by dissolving in dilute ammonium hydroxide solution and precipitating by addition of formic acid, m.p. >270°.

Anal. Calcd. for $C_{11}H_9N_3O_4$: C, 57.14; H, 3.92; N, 18.17. Found: C, 56.96; H, 4.05; N, 18.01.

N-(1H-2-Oxo-4-pyrimidinyl)-anthranilic acid (Va).—The product (Vb) was obtained (7.5 g.) after refluxing III (R_2 = H, 6.0 g.), anthranilic acid (1.0 equiv.), and sodium carbonate (0.5 equiv.) in water (50 ml.) for 4 hr. in a manner described above. Va was purified in the same manner as Vb, m.p. 247–248° dec.

Anal. Calcd. for $C_{11}H_9N_3O_4$: C, 57.14; H, 3.92; N, 18.17. Found: C, 56.79; H, 4.26; N, 17.77.

N-(1H-2-Oxo-5-methyl-4-pyrimidinyl)-L-alanine (IVe).—A mixture of III (R_2 = CH_3, 1.56 g.) L-alanine (2 equiv.), and sodium carbonate (1 equiv.) in water (10 ml.) was refluxed for 12 hr. After acidification of the solution with formic acid to pH 5, the solution was evaporated to dryness and the residue taken up in ethanol, and the precipitate (L-alanine) was removed by filtration. The filtrate was evaporated to dryness, the residue taken up in water, and cooled in the refrigerator overnight. The separated crystals were recrystallized from water; yield 0.5 g., m.p. >290°, $[\alpha]^{25}D$ − 123° (*c* 0.94, *N* HCl).

Anal. Calcd. for $C_8H_{11}N_3O_3$: C, 18.37; H, 5.62; N, 21.30. Found: C, 48.67; H, 5.59; N, 21.34.

N-(1H-2-Oxo-5-fluoro-4-pyrimidinyl)-L-alanine (IVf).—The mixture of III (R_2 = F, 1.0 g.), L-alanine (1.1 equiv.), and sodium carbonate (0.55 equiv.) in water (10 ml.) gave the product after 8 hr. reflux. Recrystallization from aqueous alcohol gave 0.5 g., m.p. 239–240° dec., $[\alpha]^{25}D$ − 166° (*c* 0.67, *N* HCl).

Anal. Calcd. for $C_7H_8FN_3O_3$: C, 41.79; H, 3.98; F, 9.45; N, 20.90. Found: C, 42.01; H, 3.81; F, 9.29; N, 21.21.

N-(1H-2-Thio-4-pyrimidinyl)-L-tryptophan (VIII).—VII (1.55 g.), tryptophan (1.1 equiv.), and sodium carbonate (0.55 equiv.) in water (20 ml.) were refluxed for 5 hr. and worked up in the same manner as in the synthesis of IVd. The product (0.7 g.) had m.p. 178–195° dec.

Anal. Calcd. for $C_{15}H_{14}N_4O_2S \cdot 1.5H_2O$: C, 52.77; H, 5.02; N, 16.41; S, 9.39. Found: C, 52.74; H, 5.29; N, 15.89; S, 9.76.

N-(1H-2-Oxo-4-pyrimidinyl)-β-alanine (IX). (a) **From 4-Thiouracil.**—The mixture of II (R_2 = H, 19.2 g.), β-alanine (21 g.), and sodium carbonate (8 g.) in water (200 ml.) was refluxed for 34 hr. After concentration to a small volume, the precipitated sodium salt of IX (22 g.) was isolated and converted to the free acid (17 g.) by acidification of its aqueous solution with formic acid, m.p. 270–271° dec.

Anal. Calcd. for $C_7H_9N_3O_3$: C, 45.90; H, 4.95; N, 22.94. Found: C, 45.87; H, 4.83; N, 22.94.

(b) **From 4-Methylthio-2-pyrimidinone.**—The mixture of III (R_2 = H, 1.42 g.), β-alanine (1.07 g.), and sodium carbonate (0.63 g.) in water (15 ml.) was refluxed for 2 hr. The solution was acidified and the free acid IX was obtained; 1.65 g., (90%) as white needles, m.p. 271° dec. The ultraviolet spectral properties (see Table I) were identical with IX obtained from the previous method. The identity of both compounds was also shown by paper ionophoresis (+5.5 cm., single spot, 0.1 *M* ammonium acetate, 700 v., 1 hr.).

N-(1H-2-Oxo-5-chloro-4-pyrimidinyl)-DL-alanine (IVg).—The mixture of IVb (DL-form, 0.5 g.) and N-chlorosuccinimide (0.5 g.) in acetic acid (15 ml.) was heated for 30 min. at 100°. After removal of solvent *in vacuo*, the residue was triturated with water. The precipitate (0.45 g.) was recrystallized

(21) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958) (see ref. 35).

(22) A. D. Ainley, H. S. Curd, and S. Birtwell, *Chem. Abstr.*, **46**, 9619 (1952) [British Patent 658,202 (1951)], have reported this compound as an oil.

from water (with charcoal treatment); yield, 0.3 g., m.p. 239–240° dec.

Anal. Calcd. for $C_7H_8ClN_3O_3$: C, 38.63; H, 3.71; Cl, 16.29; N, 19.31. Found: C, 38.47; H, 3.67; Cl, 16.33; N, 19.13.

N-(1H-2-Oxo-5-bromo-4-pyrimidinyl)-D,L-alanine (IVh).—The mixture of IVb (D,L-form, 1.0 g.) and N-bromosuccinimide (1.1 g.) in acetic acid (30 ml.) was kept for 20 min. at 80–100° and treated as above. The product (1.0 g.) was recrystallized from water (0.85 g.), m.p. 225–227° dec.

Anal. Calcd. for $C_7H_8BrN_3O_3$: C, 32.08; H, 3.08; Br, 30.49; N, 16.03. Found: C, 32.00; H, 3.19; Br, 30.43; N, 15.85.

1-(β-D-Ribofuranosyl)-4-methylthio-2-pyrimidinone (XIa).—2',3',5'-Tri-O-benzoyl-4-thiouridine[13] (X, R = OBz, R_2 = H, 11.5 g.) was dissolved in *N* sodium hydroxide (60 ml.), water (40 ml.), and ethanol (100 ml.) and stirred for 1 hr. Methyl iodide (14.2 g.) and more *N* sodium hydroxide (20 ml.) were added, and the mixture stirred for 30 min. After neutralization with acetic acid, the solution was evaporated *in vacuo*, and the residue dissolved in ethanol. The insoluble material was removed by filtration and discarded, and the filtrate concentrated to a sirup. This sirup was treated with ethanol and the solids which formed were discarded. The filtrate was again concentrated to a sirup and the treatment with alcohol was repeated. The final filtrate was concentrated to a sirup and triturated repeatedly with acetone. The acetone-insoluble sirup (4.5 g., crude XIa) was used in the following reactions.

N-(1-β-D-Ribofuranosyl-2-oxo-4-pyrimidinyl)-L-phenylalanine (XIIa).—The solution of XIa (2.7 g.), L-phenylalanine (2.0 g.), and sodium carbonate (0.56 g.) in water (12.5 ml.) was refluxed for 15 hr. After acidification with acetic acid, the solution was concentrated *in vacuo* and the residue dissolved in ethanol. The insoluble material was removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in water, adjusted to pH 7.5, and applied to a column (Dowex I, formate, 2.5 × 16 cm. long). The column was washed with water which removed uridine and unchanged XIa. Formic acid (0.2 *M*) was used for elution. The eluate was collected in 100-ml. fractions. The ultraviolet absorbing fractions (measured at 290 mμ) containing product were combined and evaporated to dryness *in vacuo*, and the residue was dissolved in ethanol from which crystals were obtained (1.2 g.), m.p. 165–170° slow dec.

Anal. Calcd. for $C_{18}H_{21}N_3O_7 \cdot 1.5H_2O$: C, 51.67; H, 5.77; N, 10.04. Found: C, 51.90; H, 5.74; N, 10.26.

N-(1-β-D-Ribofuranosyl-2-oxo-4-pyrimidinyl)-L-tryptophan (XIIb).—The solution of XIa (3.2 g.), L-tryptophan (2.05 g.), and sodium carbonate (0.56 g.) in water (15 ml.) was refluxed for 6 hr. The solution was diluted with water, adjusted to pH 9, and applied to a column (Dowex I, formate, 2.5 × 16 cm.). After washing the column with water, the product was eluted with *N* formic acid. The combined eluate was evaporated *in vacuo* to a sirup which was treated with ether to remove formic acid. The residue was taken up in a small amount of water from which the precipitate separated (yield 2.0 g.) and recrystallized from water, m.p. 176–178° dec.

Anal. Calcd. for $C_{20}H_{22}N_4O_7 \cdot H_2O$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.37; H, 5.22; N, 12.36.

N-(1-β-D-Ribofuranosyl-2-oxo-4-pyrimidinyl)-p-aminobenzoic Acid (XIII).—A mixture of XIa (5 g.), *p*-aminobenzoic acid (4.1 g.), and sodium carbonate (1.6 g.) in water (30 ml.) was refluxed for 3.5 hr. The solution was acidified with formic acid and the precipitate (*p*-aminobenzoic acid) was removed. Extensive hydrolysis of XIa to uridine was observed. The solution was adjusted to pH 9, applied to a column (Dowex I, formate 2.5 × 16 cm.), washed with water and 0.1 *N* formic acid, and eluted with *N* formic acid. The combined fractions containing the product were evaporated to a sirup and washed with ether. An amorphous solid was obtained from the residue which could not be crystallized; yield 1.4 g., m.p. 156–165° dec.

Anal. Calcd. for $C_{16}H_{17}N_3O_7$: C, 52.88; H, 4.72; N, 11.57. Found: C, 52,48; H, 4.75; N, 11.25.

1-(2-Deoxy-β-D-ribofuranosyl)-4-methylthio-5-methyl-2-pyrimidinone (XIb).—Compound X (R_2 = Me, R = H, 9.32 g.)[13] in *N* sodium hydroxide (40 ml.), ethanol (100 ml.), and water (70 ml.) was stirred for 1 hr. Methyl iodide (12 g.) and more *N* sodium hydroxide (20 ml.) were added and the mixture stirred for 30 min. After storage in the refrigerator overnight, needles separated and were collected. The filtrate was concentrated to a sirup and the sirup triturated with water from which additional needle crystals were obtained; total yield 4.9 g. (90%). One recrystallization from water afforded pure material, m.p. 176–178°.

Anal. Calcd. for $C_{11}H_{16}N_2O_4S$: C, 48.52; H, 5.92; N, 10.29; S, 11.78. Found: C, 48.49; H, 5.64; N, 10.22; S, 11.99.

N-[1-(2-Deoxy-β-D-ribofuranosyl)-5-fluoro-2-oxo-4-pyrimidinyl]-L-alanine (XIIc).—4-Thio-5-fluoro-2'-deoxyuridine (1.2 g.)[2] in *N* sodium hydroxide (6 ml.), water (20 ml.), and methyl iodide (1.5 g.) were stirred for 20 min. The solution was neutralized and evaporated to dryness. The residue was taken up in water (15 ml.), L-alanine (1 g.) and sodium carbonate (0.58 g.) were added, and the solution was refluxed for 2 hr. The reaction solution was applied to a column (Dowex I, formate, 2.5 × 16 cm.), washed with water, and eluted with 0.1 *N* formic acid. The fractions containing the product were combined and concentrated to a sirup, washed with ether, and the resulting crystalline residue was recrystallized from water; yield 0.7 g., m.p. 151–152° dec., $[\alpha]^{20}D$ −40° (*c* 0.54, *N* HCl).

Anal. Calcd. for $C_{12}H_{16}FN_3O_6$: C, 45.43; H, 5.08; F, 5.99; N, 13.24. Found: C, 45.14; H, 5.52; F, 6.16; N, 13.11.

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Imidazolidines. III.[1a] 2-Substituted 1,3-Bis-(*o*-hydroxybenzyl)-imidazolidines[1b]

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N,N'-Bis-(*o*-hydroxybenzyl)-ethylenediamine (I) undergoes an equimolecular condensation with aldehydes and aqueous formaldehyde in alcohol solution to form imidazolidine derivatives. In contrast, paraformaldehyde reacts with I in benzene to form 1,2-bis-[3-(3,4-dihydro-1,3,2H-benzoxazino)]-ethane. Acetone forms a stable imidazolidine derivative of I. Reactions of I with other ketones are discussed. None of the compounds showed appreciable antibacterial, antifungal, or antiviral activity.

Our interest to investigate the condensation reactions of N,N'-bis-(*o*-hydroxybenzyl)-ethylenediamine (I) was essentially twofold. Other similarly substituted ethylenediamines, N,N'-bis-(*p*-methoxybenzyl)-,[2] N,N'-bis-

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