Experimental

Barbituric acid, uracil, 6-methyluracil, and 4,6-dihydroxypyrimidine were commercial pyrimidines used as received. The 5-carboxaldehydes of 2,4,6-trihydroxy-,[2,4] 2,4-dihydroxy-[2] and 6-methyl-2,4-dihydroxypyrimidine[2] were prepared as previously described. 4,6-Dihydroxypyrimidine 5-carboxaldehyde was prepared by the Reimer–Tiemann reaction but no attempt was made to isolate the aldehyde. The procedures used in preparation of the derivatives are given in footnotes to the tables. The compounds were dried at 150° (1 mm.) for 4 hr. prior to analysis.[5]

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(5) Analyses by Micro Tech. Laboratories.

Orotylamino Acids

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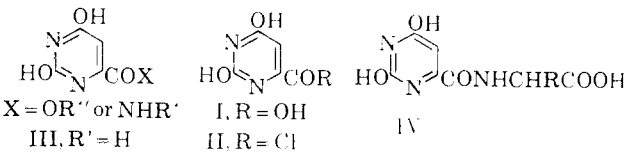
Orotic acid (uracil-6-carboxylic acid, I) has received an increasing amount of attention because of its important function in nucleic acid biosynthesis. In this connection, several of its carboxyl derivatives have been prepared as possible cancer chemotherapeutic agents.[2]

It also has been claimed to promote growth in a number of animal species[3] and, in fact, frequently has been suggested to be a vitamin. However, extensive nutritional examination in this and other laboratories indicates that the effect is sporadic in occurrence. A growth factor thought to be I or a closely related compound has been found in a variety of natural feedstuffs,[4] but only fragmentary evidence of the structure of the biologically active principal has been reported.

Review of the chemical and physical information available indicated that this "whey factor" indeed might be a carboxyl derivative of I, perhaps a substituted amide or peptide conjugate. Although orotamide (III) has been known for many years,[5] only a few N-substituted amides have been reported. All of these compounds have been prepared by aminolysis of simple orotic esters.[2] Attempts to prepare the orotyl derivatives of amino acids by the use of dicyclohexyl carbodiimide failed because of the insoluble nature of I. Likewise, syntheses of these substances from ethyl orotate were unsuccessful. Although fruitless efforts to prepare orotyl chloride (II) have been reported,[2] the compound was formed smoothly and in high yield in the present investigation.[6] Reaction of I and thionyl chloride in benzene in the presence of a catalytic quantity of *N*,*N*-dimethylformamide[7] gave the desired product, even though both I and II appeared to be insoluble in the reaction mixture.

As expected, II proved to be somewhat unstable, and the general insolubility and non-volatility of both I and II precluded purification. Although satisfactory analytical data proved difficult to obtain, the infrared spectrum of the isolated product exhibited a strong band ascribable to the carbonyl part of the COCl function (5.52 μ), a C–Cl band (12.25 μ), and a sharp amide band (5.90 μ). Conversion to the acid chloride was essentially quantitative as shown by the absence of carboxyl absorption in the infrared and failure to observe any insoluble I following the reaction of crude II with excess concentrated ammonium hydroxide or methanol. In both instances, removal of the solvent reactant provided high yields of III or methyl orotate.



Reaction of II with a variety of α-amino acids by the Schotten–Baumann technique provided the corresponding orotylamino acids (IV) (Table I). Attempts to conduct the preparation in a nonpolar solvent with pyridine as acid acceptor were unsuccessful. The amino acid derivatives were found to be remarkably soluble in polar solvents; although crude yields were high, considerable loss of material was suffered upon purification.

The preparation of orotic esters from II and the appropriate alcohol proceeded smoothly as indicated above (Table II). 2-Ethylhexyl orotate was found to be of particular interest; it melted at 109–110° (orotic acid m.p. 345°) and was found to be very soluble in nonpolar solvents such as ethyl ether and benzene. The biological properties of these "fat-soluble" derivatives are now under investigation.

Reaction of II with 2-aminoethanol in benzene resulted in the isolation of *N*-(2-hydroxyethyl)orotamide rather than the related aminoester. The product was identical in its properties with that prepared by aminolysis of either ethyl or butyl orotate,[2] and its structure was confirmed by spectral data.

Contrary to several reports,[3] dietary administration of orotic acid and its esters was without appreciable effect on the growth rate or feeding efficiency of chickens grown on practical type rations. However, as shown in Table III, orotylmethionineamide, orotylglycine, and *N*-hydroxyethylorotamide produced statistically significant increases in weight compared to untreated controls in five-week trials.

Despite these responses, the results of other series of experiments were not always consistent. Although the present work supports the view that the "whey factor" indeed may be an orotic acid conjugate, it is apparent that the mechanism by which this type of compound exerts its physiological effect remains to be explained.

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TABLE I
OROTAMIDES

Compound	M.p., °C.	Yield, %[a]	Molecular composition	Nitrogen, % Calcd.	Nitrogen, % Found
Orotamide[b]	373	95	$C_5H_5N_3O_3 \cdot H_2O$	24.3	24.5
N-(2-Hydroxyethyl)orotamide	245	48	$C_7H_9N_3O_4$	21.0	21.0
Orotylglycine[b]	269–270	43	$C_7H_7N_3O_5 \cdot H_2O$	18.2	17.9
Orotylglycine ethyl ester	247–249	52	$C_9H_{11}N_3O_5$	17.4	17.6
Orotylglycylglycine	280–281 dec.	46	$C_9H_{10}N_4O_6$	20.7	20.6
Orotyl-DL-methioninamide[c]	249–250	36	$C_{10}H_{14}N_4O_4S \cdot 2H_2O$	17.4	17.6
Orotyl-DL-methionine	222	15	$C_{10}H_{13}N_3O_5S$	14.6	14.6
Orotyl-DL-phenylalanine	237–238	19	$C_{14}H_{13}N_3O_5$	13.9	13.8
Orotyl-DL-tryptophan	233–235 dec.	30	$C_{16}H_{14}N_4O_5$	16.4	16.1
Orotyl-DL-valine	253–254	14	$C_{10}H_{13}N_3O_5$	16.5	16.3

[a] After purification. [b] Monohydrate. [c] Dihydrate.

TABLE II
OROTYL ESTERS

Compound	M.p., °C.	Yield, %[a]	Molecular composition	Nitrogen, % Calcd.	Nitrogen, % Found
Methyl orotate	241–242[b]	92	$C_6H_6N_2O_4$	16.5	16.5
Ethyl orotate	208–209[c]	87	$C_7H_8N_2O_4$	15.2	15.3
2-Ethylhexyl orotate	109–110	51	$C_{13}H_{20}N_2O_4$	10.4	10.4
Octadecyl orotate	142–143	22	$C_{23}H_{40}N_2O_4$	6.9	7.1

[a] After purification. [b] Lit.[9] m.p. 240–242°. [c] Lit.[9] m.p. 207–208°.

TABLE III
EFFECT OF OROTIC ACID AND ITS DERIVATIVES ON CHICKENS[a]

Compound	Dietary level, %	Period, weeks	Growth % of control
Orotic acid	.010	6	103
Octadecyl orotate	.023	6	100
Orotamide	.010	5	101
Ortotylglycine	.013	5	105
Orotylmethioninamide	.017	5	110
N-Hydroxyethyl orotamide	.010	5	116

[a] These experiments were conducted by the Wisconsin Alumni Research Foundation, Madison, Wisconsin.

Experimental[8]

Orotyl Chloride (II).—Anhydrous orotic acid (I) was prepared by heating the commercial monohydrate in an oven at 125° until constant weight was obtained. I (15.6 g., 0.10 mole), 40 g. (0.33 mole) of thionyl chloride, 150 ml. of dry, thiophene-free benzene, and 0.5 ml. of N,N-dimethylformamide were boiled under reflux for 1 hr. with efficient stirring and the exclusion of moisture. The mixture was allowed to cool, and the product was isolated on a sintered-glass filter, washed first with thionyl chloride and then with benzene, and dried in a vacuum desiccator. The yield of acid chloride containing a trace of unreacted orotic acid was 16.8 g. (96% as the pure product).

Anal. Calcd. for $C_5H_3ClN_2O_3$: C, 34.4; H, 1.7; N, 16.1. Found: C, 35.1; H, 2.1; N, 16.5.

Substitution of the usual orotic acid monohydrate for the anhydrous starting material resulted in a product which was tan in color but from which satisfactory derivatives could be prepared.

Orotamide (III).—Crude II from 50 g. (0.29 mole) of I monohydrate was gradually added to 200 ml. of concd. ammonium hydroxide solution with vigorous stirring. After standing overnight, 1800 ml. of water was added and the mixture heated to boiling to dissolve the precipitated amide, decolorized with carbon, filtered, and allowed to cool. The collected solid weighed 37 g., m.p. 373° dec.; recovery of a second crop of crystals from the mother liquors provided a total yield of 47 g. (95%) as III monohydrate.

Anal. Calcd. for $C_5H_5N_3O_3 \cdot H_2O$: C, 34.7; H, 4.1; N, 24.3. Found: C, 35.0; H, 4.5; N, 24.5.

A sample was recrystallized from water and dried at 110° over phosphorus pentoxide.

Anal. Calcd. for $C_5H_5N_3O_3$: C, 38.7; H, 3.3. Found: C, 39.0; H, 3.4.

Orotylglycine.—A mechanically stirred solution of glycine (16 g., 0.21 mole) in 200 ml. of 4% aqueous sodium hydroxide solution was chilled to 5°. Solid II (35 g., 0.20 mole) was added in small portions while 75 ml. of 13% sodium hydroxide solution was added dropwise. After addition of II was complete, the solution was stirred briefly, acidified, and the pale yellow solid was collected by filtration, washed with a small volume of water, and recrystallized from 850 ml. of boiling water (decolorizing carbon). Yield, 21 g. (43%), m.p. 269–270°.

Anal. Calcd. for $C_7H_7N_3O_5 \cdot H_2O$: C, 36.4; H, 3.9; N, 18.2. Found: C, 36.2; H, 4.2; N, 17.9.

The infrared spectrum of the compound was consistent with the proposed structure. In addition, hydrolysis with hot 10% aqueous sodium hydroxide solution yielded orotic acid (shown by m.p. and infrared spectrum) and glycine (shown chromatographically by comparison with an authentic specimen).

2-Ethylhexyl Orotate.—The acid chloride (II) (from 20 g., 0.11 mole, of I) was added to 250 ml. of 2-ethylhexanol and the resulting mixture was warmed on the steam bath for 15 min., filtered hot, and the excess alcohol was removed *in vacuo*. The white solid residue was recrystallized twice from ethanol to provide 15 g. (51%) of crystalline ester, m.p. 109–110°.

Anal. Calcd. for $C_{13}H_{20}N_2O_4$: C, 58.2; H, 7.5; N, 10.4. Found: C, 58.5; H, 7.7; N, 10.4.

N-(2-Hydroxyethyl)orotamide.—The acid chloride (II) (35 g., 0.20 mole) was added in small portions to a rapidly stirred solution of 50 g. (0.82 mole) of 2-aminoethanol in 250 ml. of benzene. After stirring at room temperature for a few min., the mixture was boiled under reflux for 2 hr., cooled, and extracted repeatedly with water. The aqueous extract was evaporated to dryness and the residue was dissolved in hot water, ethanol added to turbulence, and the product allowed to crystallize. Two additional recrystallizations from boiling water provided 19.2 g. (48%) of white crystals, m.p. 275° (lit.[2] 278–280°). Spectral characteristics of the product were identical with those described in the literature[2] and with those of a reference sample prepared by reaction of 2-aminoethanol with ethyl orotate.

Anal. Calcd. for $C_7H_9N_3O_4$: C, 42.2; H, 4.6; N, 21.0. Found: C, 42.3; H, 4.8; N, 21.0.

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(8) All melting points were measured in a Vanderkamp block and are corrected. Infrared spectra were measured with a Perkin-Elmer Model 21 spectrophotometer.

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