Hydroxypyrimidine-5-carboxaldehyde Derivatives in Cancer Chemotherapy

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Interest in the tumor growth retardation characteristics of aldehyde hydrazone derivatives[1] and pyrimidines in general has prompted an extension of previous investigations[2] concerning derivatives of pyrimidine-5-carboxaldehydes.

Substituted hydrazone derivatives of the 5-carboxaldehydes of 2,4,6-trihydroxy-, 2,4-dihydroxy-, 6-methyl-2,4-dihydroxy- and 4,6-dihydroxypyrimidine have been prepared and characterized along with a variety of anil derivatives of 2,4,6-trihydroxypyrimidine-5-carboxaldehyde (Tables I and II).

TABLE I

DERIVATIVES OF 2,4,6-TRIHYDROXYPYRIMIDINE-5-CARBOXALDEHYDE

Reagent used[a]	Procedure[b]	M.p., °C.	Recryst.[c] from	N Analyses, % Calcd.	Found
Isonicotinic acid hydrazide	I	330	A	25.45	25.56
p-Nitrophenylhydrazine	I	305	DMF	24.05	23.91
p-Hydrazinobenzoic acid	I	339	DMF/W	19.30	19.01
2-Hydrazinoquinoline	II	275–276	DMS/W	23.56	23.42
p-Nitroaniline[d]	I	>360	DMF	20.29	20.47
3,4-Dichloroaniline	I	>360	DMF	14.00	14.01
p-Fluoroaniline	I	345	DMF/W	16.86	16.96
2,5-Difluoroaniline	I	325	DMF/W	15.73	15.77
p-Hydroxyaniline	I	>360	DMF/W	16.96	17.06
p-Anisidine	I	>360	DMF/W	16.09	16.35
m-Anisidine	I	324	DMF/W	16.09	16.01
Dimethylaminoaniline	I	357/dec	DMF/W	20.42	20.43
p-Diethylaminoaniline	I	200/dec	DMF/W	18.53	18.76
p-Aminobenzoic acid	I	>360	DMF/W	15.27	15.30
3-Aminopyridine	I	>360	DMS/W	24.13	23.99
Pyridoxamine	I	307	DMF/W	18.29	18.05
Adenine[e]	I	330	DMF/W	35.89	35.65
Sulfathiazole	I	351/dec	DMA/W	17.80	17.92
Sulfamerazine	I	320/dec	DMF/W	20.89	20.83
Sulfapyridine	I	326/dec	DMF/W	18.08	17.99
Sulfaguanidine	I	323/dec	DMF/W	23.85	23.70
Sulfamethazine	I	276/dec	DMF/W	20.18	20.14
Sulfadiazine	I	344/dec	DMS/W	21.64	21.59

[a] The first four reagents yield hydrazone derivatives. The remainder yield anil derivatives. [b] Procedures I and II were used in preparing derivatives: I. The aldehyde was prepared by the Reimer–Tiemann reaction.[2] The reaction mixture was cooled and filtered to remove precipitated salts, including the potassium salt of the aldehyde. This salt mixture was suspended in enough 6 *N* sulfuric acid to form a thick slurry which was heated for a few min. at 60°. The suspension was cooled, filtered and the precipitate washed with cold 0.1 *N* sulfuric acid and with cold water until free of potassium. The dried aldehyde was suspended in boiling dimethylformamide. To this solution was added a slight excess of the amine or hydrazine in dilute acetic acid. The resulting mixture was boiled for a few min., cooled and water added to assure complete precipitation of the product. The product was collected on a filter and recrystallized. II. The aldehyde was prepared by refluxing barbituric acid with dimethylformamide.[3] The reaction mixture was cooled overnight and filtered to yield a solid intermediate. To this solid in hot water was added the hydrazine in dilute acetic acid. The reaction mixture was heated for a few min., cooled and the product collected on a filter, dried and recrystallized. [c] A, not recrystallized; DMF, dimethylformamide; W, water; DMS, dimethylsulfoxide; DMA, dimethylacetamide. [d] Calcd. for $C_{11}H_8N_4O_5$: C, 47.83; H, 2.92. Found: C, 47.63; H, 2.86. [e] Calcd. for $C_{10}H_7N_7O_3$: C, 43.40; H, 2.58. Found: C, 43.17; H, 2.88.

TABLE II

HYDRAZONE DERIVATIVES[a] OF HYDROXY SUBSTITUTED PYRIMIDINE-5-CARBOXALDEHYDES

Aldehyde of[b]	Derivative[c]	M.p., °C.	Recryst. from[d]	N Analyses, % Calcd.	Found
I	IN	334–335	A	27.02	26.86
I	NP	332	DMF	25.45	25.65
II	DP	336 dec.	DMF	25.15	25.17
II	NP	347	DMF	24.21	24.14
II	IN	323	DMF	25.63	25.36
II	P	316	DMF	22.94	23.06
III	NP[e]	>360	DMF	25.45	25.66
III	DP	285 dec.	DMF/W	26.25	26.38
III	BP	265–266	DMF	18.36	18.21

[a] All derivatives were prepared from solutions of the unisolated aldehyde prepared by the Reimer–Tiemann reaction.[2] The reaction mixture was cooled and filtered to remove any precipitated potassium chloride. The filtrate was acidified with acetic acid and refiltered if necessary. To the hot acidified filtrate was added an excess of the hydrazine in dilute acetic acid. The reaction mixture was boiled for a few min. and then cooled in an ice bath. The product was collected on a filter and recrystallized. [b] I, 2,4-Dihydroxypyrimidine; II, 2,4-dihydroxy-6-methylpyrimidine; III, 4,6-dihydroxypyrimidine. [c] IN, Isonicotinoylhydrazone, NP, *p*-nitrophenylhydrazone; P, phenylhydrazone; DP, 2,4-dinitrophenylhydrazone; BP, 1,1-diphenylhydrazone. [d] A, not recrystallized; DMF, dimethylformamide; W, water. [e] Calcd. for $C_{11}H_9N_5O_4$: C, 48.00; H, 3.30. Found: C, 48.13; H, 3.33.

Screening data[3] for these compounds have shown borderline response (±) in Sarcoma 180 tests in two or three determinations for the sulfamerazine, sulfapyridine, and sulfaguanidine anils and the *p*-carboxyphenylhydrazone (Table I) and for the isonicotinoylhydrazone derivative of the 2,4-dihydroxypyrimidine structure (Table II). The *p*-hydroxy and *p*-carboxy anils (Table I) have shown single ± Sarcoma 180 tests.

(1) (a) R. H. Wiley, H. K. White, and G. Irick, *J. Org. Chem.*, **24**, 1784 (1959); (b) R. H. Wiley and G. Irick, *ibid.*, **24**, 1925 (1959); **26**, 593 (1961); (c) R. H. Wiley, G. Irick, and H. K. White, *ibid.*, **26**, 589 (1961); (d) R. H. Wiley and G. Irick, *J. Med. Pharm. Chem.*, **5**, 49 (1962); (e) R. H. Wiley and R. L. Clevenger, *ibid.*, **5**, 1367 (1962).

(2) R. H. Wiley and Y. Yamamoto, *J. Org. Chem.*, **25**, 1906 (1960).

(3) The authors are indebted to Drs. C. C. Stock, R. K. Barclay, Christine Reilly, Elvira Falco, and Sophronia Myron, Sloan Kettering Institute for Cancer Research, for conducting these tests. The rating scales and procedures for the Sarcoma 180 test are given in *Cancer Res.*, Suppl. No. 1, 91 (1953), Suppl. No. 2, 179 (1955), and *Cancer Res.*, **18**, No. 8, 49 (1958).

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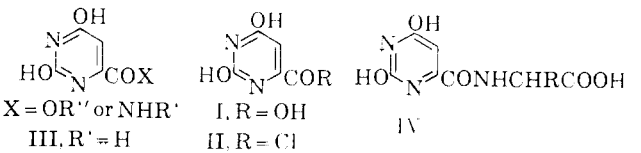
Orotic acid (uracil-6-carboxylic acid, I) has received an increasing amount of attention because of its important function in nucleic acid biosynthesis. In this connection, several of its carboxyl derivatives have been prepared as possible cancer chemotherapeutic agents.[2]

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, orotylmethioninamide, 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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