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v-Triazolo(d)pyrimidines. I. 2-Aryl-5-amino-7-hydroxy Derivatives[1]

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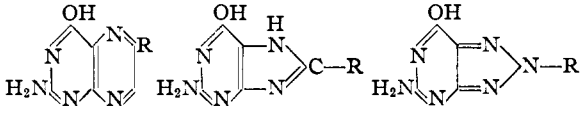
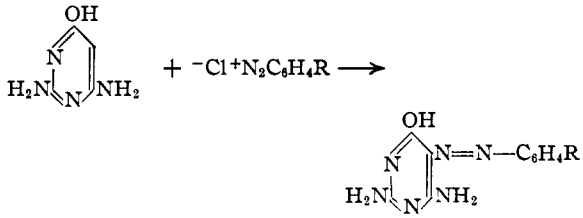
The concept of hindering the transformation of normal constituents of living cells by means of different yet architecturally similar substances has been of considerable utility in the design of chemotherapeutic agents. Recently there have been reported several investigations of metabolite antagonists for the biologically important purines[2] and pteridines.[3] Among the variations of these two classes which have been studied is the replacement of the imidazole or pyrazine groups in these heterocycles with a vicinal triazole group, forming the v-triazole (d) pyrimidine ring system. Thus 5-amino-7-hydroxy-v-triazolo(d)pyrimidine interferes with the biological action of its structural analog guanine.[4]

The v-triazolo(d)pyrimidine nucleus is not new, members of this class having been prepared first in 1901 by Gabriel and Coleman.[5] Hitherto, however, no derivatives have been described containing substituents on any of the nitrogen atoms of the v-triazole portion of this heterocycle. The synthesis of the v-triazolo(d)pyrimidines substituted in this manner was undertaken with the thought that such compounds might possess the requisite chemical and geometrical characteristics for either anti-folic acid or anti-purine behavior. The first sub-division investigated has been the 2-aryl-5-amino-7-hydroxy derivatives, the loading here being similar to the 2-amino-4-hydroxy-6-substituted pteridines or the 8-substituted guanines.

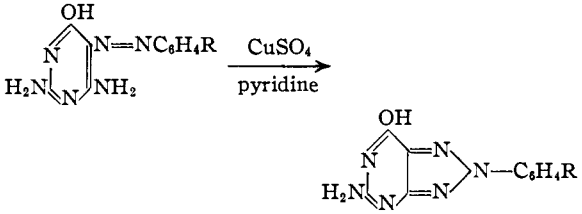


2-Amino-4-hydroxy-6-pteridine derivative | 8-Guanine derivative | 5-Amino-7-hydroxy-2-v-triazolo(d)-pyrimidine derivative

The starting material for the synthesis of these compounds is 2,4-diamino-6-hydroxypyrimidine. Previously it had been shown that diazotized *p*-nitroaniline can couple with this compound, presumably in the 5 position.[6] Accordingly a series of aromatic amines were diazotized and coupled with this pyrimidine, a yellow, orange or red dye being obtained in each case. That the azo link in these products was in fact at the 5 position of the pyrimidine ring was established by reduction of the compound derived from aniline to the known 2,4,-5-triamino-6-hydroxypyrimidine. The 2,4-diamino-5-arylazo-6-hydroxypyrimidines thus formed (Table I) all contained water of crystallization, usually being sesquihydrates. Quantitative removal of this water was found to be impractical and the hydrated azo compounds were used as such for transformation to the triazoles.



Conversion of *o*-amino azo compounds to 2-substituted v-triazole derivatives has been effected by such procedures as heating,[7] chromic acid oxidation,[8] and oxidation with alkaline copper sulfate.[9] In the present study copper sulfate in a pyridine–water mixture[10] was utilized to effect the oxidative ring closure. The various 2-substituted v-triazolo(d)pyrimidines were formed in yields of 50 to 80% by use of this reagent (Table I). The particular compounds chosen for the present initial study were those containing a variety of para substituted aryl groups in the 2 position. The 2-aryl-5-amino-7-hydroxy-v-triazolo(d)pyrimidines were found to be light yellow or white crystalline substances having very little solubility in water or dilute acids. The compounds of this series are, however, soluble in alkali due, in the case of the phenyl and *p*-carbethoxyphenyl derivatives, to the phenolic character of the 7-hydroxyl group. Although the phenolic group complicated the titration of 2-*p*-carboxyphenyl-5-amino-7-hydroxy-v-triazolo-(d)pyrimidine when strong alkali was used, by means of a base of intermediate strength, piperidine, a fairly accurate estimation of its equivalent weight could be made. The value found, 287, is reasonably close to the calculated value, 272.2.



(1) Presented before the Division of Organic Chemistry, American Chemical Society, Atlantic City, September 19, 1949.

(2) Kidder, Dewey, Parks and Woodside, *Science*, **109**, 511–513 1949); Vaughan, Krapcho and English, THIS JOURNAL, **71**, 1885 (1949).

(3) Cosulich and Smith, *ibid.*, **70**, 1922 (1948); Seeger, Cosulich, Smith and Hultquist, *ibid.*, **71**, 1753 (1949).

(4) Roblin, Lampen, English, Cole and Vaughan, *ibid.*, **67**, 290 (1945).

(5) Gabriel and Coleman, *Ber.*, **34**, 1249 (1901).

(6) Lythgoe, Todd and Topham, *J. Chem. Soc.*, 315 (1944).

(7) Charrier, *Gazz. chim. ital.*, **52**, I, 261 (1922).

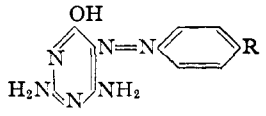
(8) Zincke, *Ber.*, **18**, 3136 (1885).

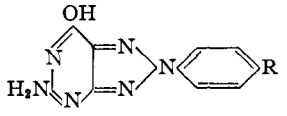
(9) Neri, *Gazz. chim. ital.*, **67**, 610 (1931); Charrier and Beretta, *ibid.*, **55**, 745 (1925); Charrier and Jorio, *ibid.*, **68**, 640 (1938).

(10) Schmidt and Hagenbocker, *Ber.*, **54B**, 2191, 2201 (1921).

TABLE I

2,4-DIAMINO-5-ARYLAZO-6-HYDROXYPYRIMIDINES 2-ARYL-5-AMINO-7-HYDROXY-V-TRIAZOLO(D)PYRIMIDINES[c]





		Carbon, %		Hydrogen, %		Nitrogen, %		Carbon/nitrogen			Carbon, %		Hydrogen, %		Nitrogen, %	
R[a]	Formula	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Yield, %	Calcd.	Found	Calcd.	Found	Calcd.	Found
H	$C_{10}H_{10}ON_6 \cdot 1.5H_2O$	46.68	46.62	5.09	4.91	32.67	32.71	1.429	1.425	72	52.63	52.79	3.53	3.66	36.83	36.95
p-COOH	$C_{11}H_{10}O_3N_6 \cdot 1.5H_2O$	43.85	44.22	4.35	4.30	27.90	27.66	1.572	1.599	64	48.53	48.28	2.96	3.28	30.88	30.95
p-$COOC_2H_5$	$C_{13}H_{14}O_3N_6 \cdot 1.5H_2O$	47.41	47.27	5.17	4.91	25.52	25.89	1.858	1.826	80	51.99	51.87	4.03	4.11	27.99	27.71
p-CONHCHCOOH $(CH_2)_2COOH$[b]	$C_{16}H_{17}O_6N_7 \cdot H_2O$	45.60	46.04	4.54	4.60	23.27	23.44	1.960	1.964	50	47.88	47.61	3.77	4.02	24.43	24.22

[a] All the compounds decomposed above 300° except the azoglutamic acid compound which decomposed at 284° and the triazolo(d)pyrimidineglutamic acid compound which decomposed at 240°. [b] *p*-Aminobenzoyl-l(+)glutamic acid was used as starting material. [c] The empirical formulas of the triazolo(d)pyrimidines were the same as the corresponding azo compounds except that they are anhydrous and contain two less hydrogen atoms.

To further characterize the new compounds, their ultraviolet absorption spectra were determined (Figs. 1–2). These 2-aryl-v-triazolo(d)pyrimidines showed maxima between 225 and 230 mμ and minima between 290 and 300 mμ. Comparison of the ultraviolet spectra for the phenylazo compound (Fig. 1) with that of the corresponding v-triazolo(d)pyrimidine clearly shows a distinction between these substances.

Certain biological properties of these compounds are being investigated elsewhere.

Experimental

All of the 2,4-diamino-5-arylazo-6-hydroxypyrimidines were prepared by the same general method which is described for the preparation of 2,4-diamino-5-phenylazo-6-hydroxypyrimidine. No significant difficulty was encountered in the diazotization or coupling operations with any of the compounds described. The azo compounds may be purified further by dissolving in dilute alkali and reprecipitating with acetic acid. The preparation of the various 2-aryl-5-amino-7-hydroxy-v-triazolo(d)pyrimidines

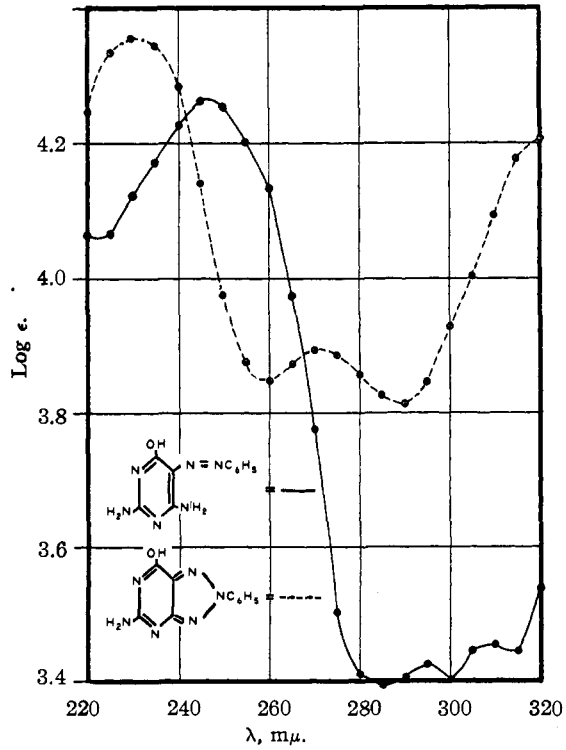


Fig. 1.—Absorption spectra of 2-phenyl-5-amino-7-hydroxy-v-triazolo(d)pyrimidine and 2,4-diamino-5-phenylazo-6-hydroxypyrimidine in 0.1 *N* NaOH.

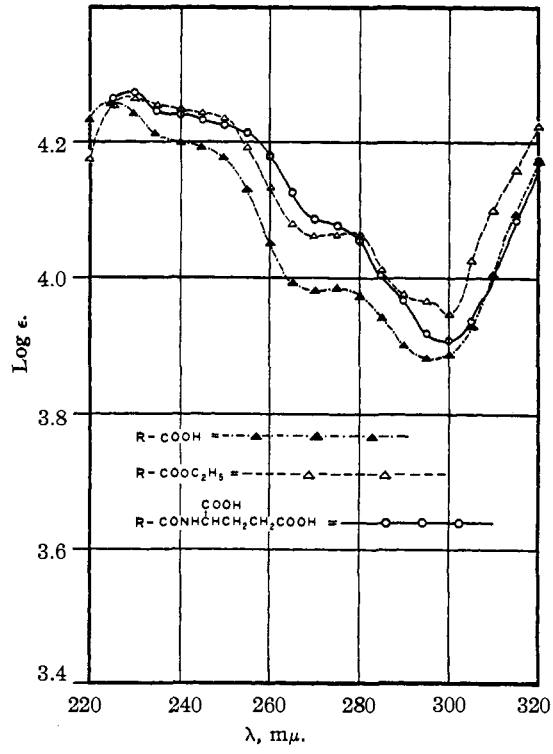


Fig. 2.—Absorption spectra of 2-*p*-carboxy and 2-*p*-carbethoxyphenyl-5-amino-7-hydroxy-v-triazolo(d)-pyrimidine and N [4-(5-amino-7-hydroxy-2-v-triazolo(d)-pyrimidyl)-benzoyl]-l-(+)-glutamic acid in 0.1 *N* NaOH: R = 5-amino-7-hydroxy-2-v-triazolo(d)pyrimidyl-*p*-phenyl.

differ only in the ease of oxidation and the manner of isolation. Three typical oxidation and isolation procedures are described.

2,4-Diamino-5-phenylazo-6-hydroxypyrimidine.—Aniline (4.7 g., 0.05 mole) was dissolved in 25 ml. of water and 20 ml. of concentrated hydrochloric acid. The mixture was cooled with stirring to 10°, then 25 g. of ice added. To the stirred mixture was added a solution of 3.5 g. (0.05 mole) of sodium nitrite in 25 ml. of water, the temperature being held below 3° during addition. The excess nitrous acid was destroyed by the addition of a saturated aqueous solution of urea. The cold solution was then added slowly to a stirred solution of 2,4-diamino-6-hydroxypyrimidine [11.29 g. (0.05 mole) of 2,4-diamino-6-hydroxypyrimidine sulfate dissolved with 52 g. of sodium acetate in 450 ml. of water, the mixture being filtered before use]. An orange precipitate soon formed which was stirred for ten minutes, then filtered, washed twice with water, twice with alcohol and twice with ether. After air drying overnight, the product was ground and dried eighteen hours at 50° under vacuum. The orange solid weighed 12.5 g. (practically quantitative yield) and melted over 300°.

An aqueous suspension of the orange solid was boiled in a test-tube with sodium hydrosulfite until all of the solid dissolved and the orange color had disappeared. The mixture was cooled, diluted with an equal volume of water and acidified with 10% sulfuric acid. After collecting the precipitate on a filter it was recrystallized from 2% sulfuric acid. The white solid obtained was dried over potassium hydroxide in a desiccator. Neutralization equivalent calculated for 2,4,5-triamino-6-hydroxypyrimidine sulfate, $C_4H_7N_5O \cdot H_2SO_4 \cdot H_2O$: 257; found, 260. The sample, after drying to remove water of crystallization, was analyzed. *Anal.* Calcd. for $C_4H_7N_5O \cdot H_2SO_4$: C, 20.08; H, 3.79; N, 29.28. Found: C, 19.90; H, 4.12; N, 29.53.

N-[4-(2,4-Diamino-5-pyrimidylazo-6-hydroxy)-benzoyl]-glutamic Acid.—This material was prepared by the same general procedure described for the previous compound. The compound separates first as an orange gel during coupling but with vigorous stirring and dilution with additional quantities of water becomes granular in nature and may be collected on a filter. The material after washing with water, redissolving in ammonium hydroxide, and reprecipitating with acetic acid was obtained as a light yellow solid. A consistent specific rotation could not be obtained in alkaline solution with the NaD line or at 650 mμ, presumably because of the color of the compound.

2-Phenyl-5-amino-7-hydroxy-v-triazolo(d)pyrimidine.—To a mixture of 26.4 g. of copper sulfate pentahydrate, 54 ml. of water and 54 ml. of pyridine at reflux temperature was added 9.2 g. (0.036 mole) of 2,4-diamino-5-phenylazo-6-hydroxypyrimidine sesquihydrate and 34 ml. of pyridine. The mixture was stirred vigorously under reflux for three hours, then poured into 600 ml. of water, filtered, washed with water and finally with dilute acetic acid. The precipitate was slurried with hot 50% acetic acid and filtered; this process was repeated with two additional portions of 50% acetic acid. The precipitate was washed once with water, then dissolved in 2 *N* sodium hydroxide solution, treated with charcoal and filtered. The filtrate was diluted to about 1200 ml. and acidified with acetic acid. The precipitate which separated soon coagulated and was filtered. It was treated with hot 50% acetic acid as before, dissolved in 1 *N* sodium hydroxide, diluted with water and again precipitated with acetic acid. The light yellow product was filtered and washed twice with water, twice with alcohol and twice with ether. After air drying overnight, it was dried for eighteen hours at 50° under vacuum; yield 5.8 g. (70.5%), m. p. over 300°.

2-*p*-Carboxyphenyl-5-amino-7-hydroxy-v-triazolo(d)-pyrimidine.—Five and five-tenths grams (0.018 mole) of 2,4-diamino-5-*p*-carboxyphenylazo-6-hydroxypyrimidine sesquihydrate together with 17 ml. of pyridine was added to a mixture of 13.2 g. of copper sulfate pentahydrate, 27 ml. of water and 27 ml. of pyridine. The mixture was refluxed and purified as described above except that dilute ammonium hydroxide was used to dissolve the product instead of sodium hydroxide; yield 3.5 g. (71%) of light yellow solid, m. p. over 300°. The equivalent weight of this compound was determined by dissolving 30.097 mg. in 1 ml. of 0.959 *N* piperidine; back-titration to a phenolphthalein end-point required 7.00 ml. of 0.122 *N* hydrochloric acid. The equivalent weight from this experiment is 287. When titration was attempted with either 0.1 or 1 *N* sodium hydroxide, the observed end-point was not sufficiently sharp for analytical use.

N-[4-(5-Amino-7-hydroxy-2-v-triazolo(d)pyrimidyl)-benzoyl]1(+)-glutamic Acid.—Hydrous N-[4-(2,4-diamino-5-pyrimidylazo-6-hydroxy)-benzoyl]-glutamic acid (8.09 g., 0.019 mole) and 34 ml. of pyridine were added to a refluxing mixture of 26.4 g. of copper sulfate pentahydrate, 54 ml. of water and 34 ml. of pyridine. The mixture was stirred at reflux for two and one-half hours, then diluted with 130 ml. of pyridine and saturated with hydrogen sulfide on a steam-bath. The mixture was filtered and the filtrate evaporated to a low volume under vacuum on a steam-bath. An equal volume of water was added and the mixture again evaporated almost to dryness. The residue was dissolved in about 300 ml. of water, heated with neutral Norite and filtered. The filtrate was adjusted to *p*H 3.2 with acetic acid and centrifuged. The residue was dissolved in hot water and precipitated with acetic acid. The cooled mixture was again centrifuged and the residue recrystallized from large amounts of hot water. On drying 4.0 g. (52%) of white solid was obtained; m. p. 240° (d.); $\alpha^{20}D$ + 16.2 (in 4 moles of sodium hydroxide per mole compound in aqueous solution); $C = 2.0067$ g./100 ml.

The absorption spectra were obtained using a Model DU Beckmann Spectrophotometer. In all cases azopyrimidines and v-triazolo(d)pyrimidines were dissolved in 0.1 *N* sodium hydroxide, which was used also as comparison solvent. For 2,4-diamino-5-phenylazo-6-hydroxypyrimidine the concentrations used were 2.39×10^{-5} *M* for 220–270 mμ and 9.58×10^{-5} *M* for 265–320 mμ. For the v-triazolo(d)pyrimidines the following concentrations were employed for the wave lengths in question: phenyl, 2.38×10^{-5} *M*, 220–255 mμ and 300–320 mμ; 1.05×10^{-4} *M*, 250–300 mμ; *p*-carboxyphenyl, 2.78×10^{-5} *M*, 220–320 mμ; *p*-carbethoxyphenyl, 4.39×10^{-5} *M*, 220–320 mμ; benzoylglutamic acid, 1.91×10^{-5} *M*, 220–320 mμ. Measurement of the absorption spectra of the 2-*p*-carboxyphenylazo compound was attempted, but since the values obtained showed some deviation from Beer's law, the results are not included.

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Summary

A number of 2-aryl-5-amino-7-hydroxy-v-triazolo(d)pyrimidines have been prepared by oxidation of the corresponding 2,4-diamino-5-arylazo-6-hydroxypyrimidines. Ultraviolet absorption spectra and other physical properties of these compounds have been determined.

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