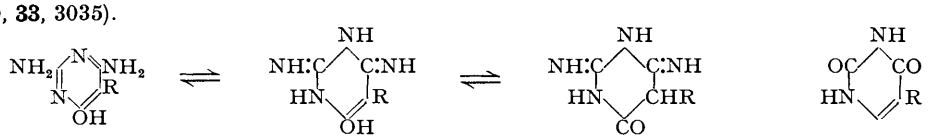
229. *Some 2 : 4-Diamino-5-acylamido-6-hydroxypyrimidines.*

By WALTER WILSON.

Several 5-acylamides have been made from 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2) either by using an acid chloride or anhydride, or by direct condensation with a carboxylic acid. For example, acetic anhydride gave 2 : 4-*diamino-5-acetamido-6-hydroxypyrimidine* (I; R = NHAc). The structure of this was established by an independent synthesis from ethyl acetamidocyanoacetate. Direct proof of the preferential acylation of the 5 amino-group in (I; R = NH_2) was thus obtained. The structure of xanthopterin (IV) is confirmed by these results. The reactions of pyrimidines of the types studied are best represented by the formula (I*A*) or (I*B*), which also agree with the light-absorption data. 2 : 4-*Diamino-5-p-aminobenzenesulphonamido-6-hydroxypyrimidine* was less active than sulphanilamide against a number of bacteria.

IN the course of work on compounds related to folic acid, several 2 : 4-diamino-5-acylamido-6-hydroxypyrimidines have been prepared. A few acyl derivatives of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2), which were believed to be the 5-acylamides, had been obtained previously by heating the pyrimidine with carboxylic acids at temperatures from 80° to 140° (Bayer, G.PP. 206,454, 213,711; B.P. 15,573; Traube and Dudley, *Ber.*, 1913, **46**, 3839; Purrmann, *Annalen*, 1940, **546**, 98; Wellcome, B.P. 579,138). Amino-groups in positions 2 and 4 of highly substituted amino-hydroxy-pyrimidines are often unreactive, whereas those in the 5 position acylate normally and react with cyanic acid (*e.g.*, Isay, *Ber.*, 1906, **39**, 257; Gabriel and Coleman, *Ber.*, 1901, **34**, 1236; Levene and Senior, *J. Biol. Chem.*, 1916, **25**, 607; Traube, *Ber.*, 1900, **33**, 3035).



(I.) (I*A*.) (I*B*.) (II.)

Treatment of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2) with acetic anhydride and aqueous alkali gave 2 : 4-*diamino-5-acetamido-6-hydroxypyrimidine* (I; R = NHAc) which formed a stable *picrate*. The remaining amides described here did not form picrates, although with the exception of the oxamic acid they were soluble in dilute mineral acids.

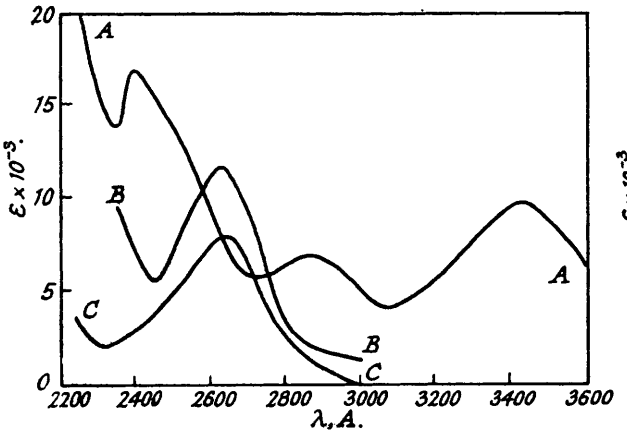
The 5-acetamido-compound has also been made by condensing guanidine with ethyl

acetamidocyanoacetate, a method which established unequivocally the preferential acetylation of the 5-amino-group.

The pyrimidine (I; R = NH_2) gave 2 : 4-*diamino*-5-*benzamido*-6-*hydroxypyrimidine* (I; R = NHBz) with benzoyl chloride and aqueous alkali. Two other amides were prepared from the pyrimidine by interaction with carboxylic acids under suitable conditions (cf. Bayer *et al.*, *loc. cit.*). Thus the pyrimidine (as the sodium hydrogen sulphite compound) with lactic acid at 115—125° yielded 2 : 4-*diamino*-5-α-*hydroxypropionamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}CO{\cdot}CH(OH){\cdot}CH_3$). Amide formation occurred even more readily with oxalic acid, and 2 : 4-*diamino*-6-*hydroxypyrimidyl*-5-*oxamic acid* (I; R = $NH{\cdot}CO{\cdot}CO_2H$) separated rapidly in high yield from hot aqueous solutions of the reactants. This oxamic acid was soluble in hot sodium hydrogen carbonate solution, and on cooling the sparingly soluble *sodium* salt was obtained. The oxamic acid is undoubtedly an intermediate in the usual preparation of leucopterin (III) by fusing the pyrimidine (I; R = NH_2) with oxalic acid dihydrate at 160—260° (Purrmann, *Annalen*, 1940, **544**, 188; Totter, *J. Biol. Chem.*, 1944, **154**, 105).

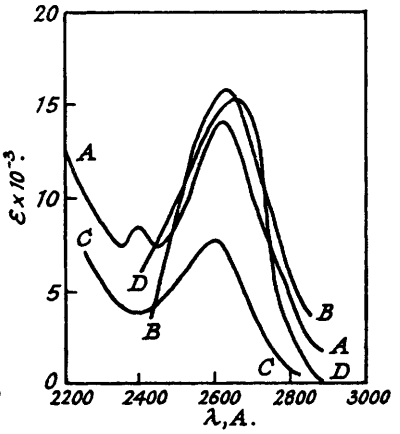
The facile condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine with aqueous oxalic acid suggested that the participating 5-amino-group might be abnormally reactive. Further work is necessary to establish this, since the insolubility of the product would tend to favour the reaction.

FIG. 1.



A. *Leucopterin in* 0·1N-*sodium hydroxide.*
B. 2 : 4-*Diamino*-6-*hydroxypyrimidyl*-5-*oxamic ocid in* 0·1N-*sodium hydroxide.*
C. *Thymine in water.*

FIG. 2.



2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine;* A, *in* 0·1N-*sodium hydroxide;* B, *in* 0·1N-*hydrochloric acid.*
2 : 4-*Diamino*-5-α-*hydroxypropionamido*-6-*hydroxypyrimidine;* C, *in* 0·1N-*sodium hydroxide;* D, *in* 0·1N-*hydrochloric acid.*

The lack of reactivity of amino-groups in positions 2 and 4 was illustrated by the recovery of 2 : 4-diamino-6-hydroxypyrimidine on attempted benzoylation by the Schotten–Baumann method.

The ultra-violet absorption characteristics of the above pyrimidines are summarised in the table. For these measurements the author is indebted to Dr. W. F. Elvidge and Mr. R. C. Voss of the Analytical Department, Boots Pure Drug Co. Ltd. Most of the acylamido-compounds have similar spectra, although prepared by different methods. This confirms the view that they are all 5-acylamides, but spectrographic data alone would be inconclusive without the examination of some 2- and 4-acylamides.

Heyroth and Loofbourow (*J. Amer. Chem. Soc.*, 1934, **56**, 1728) correlated the absorption intensities with the degree of unsaturation in the pyrimidine ring, which is changed by tautomerism when hydroxy- or amino-groups are in positions 2, 4, or 6. The intensity maxima (ϵ = 10,000—20,000) of the 2 : 4 : 5-triamino-6-hydroxypyrimidine derivatives are intermediate between that of barbituric acid and those of uracil and thymine. The structures (I*A*) or (I*B*) for the compounds are a convenient representation of the relative reactivities of the amino-groups and indicate a relationship to barbituric acid and uracil. Resonance probably plays an important part in such molecules, but this factor cannot be usefully discussed here.

Measurements have been carried out on some of the compounds in both acid and alkaline

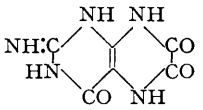
solutions (Fig. 2). The maxima are usually accentuated by acids, but, although this may be due to the tautomers (I*B*) being favoured, caution is essential in interpreting these results, since some of the compounds are unstable in alkaline solutions.

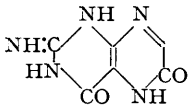
The absorption curve of thymine alters with the pH, but in neutral or acid solutions a curve is obtained, with $\lambda_{max.}$ 2640 A. ($\epsilon_{max.}$ 7800) (Fig. 1), resembling those obtained with the 5-acylamidopyrimidines. Under these conditions, thymine probably exists in the form (II; R = Me), which recalls structure (I*A*) for the 5-acylamidopyrimidines.

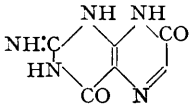
The 5-benzylideneamino- and 5-benzamido-compounds gave curves of a different type, attributed to the presence of the powerful phenyl chromophore. Although the sulphonamide has a maximum absorption at 2590 A., this is not believed to be significant since sulphanilamide also absorbs at this wave-length (Elvidge, *Q. J. Pharm.*, 1941, **14**, 141).

Leucopterin (III) and the oxamic acid (I; R = $NH{\cdot}CO{\cdot}CO_2H$) are physically similar, and differ chemically only by one molecule of water. The absorption curves are, however, quite distinct (Fig. 1).

The assignment of structures (IV) and (V) to xanthopterin and *iso*xanthopterin was originally based on the facile conversion of haloacetyl derivatives (assumed to be the 5-acylamides) of 2 : 4 : 5-triamino-6-hydroxypyrimidine into xanthopterin (Purrmann, *Annalen*, 1940, **546**, 98; Wellcome, B.P. 579,138). Gates (*Chem. Reviews*, 1947, **41**, 81) preferred a proof based on a complex series of degradations which have recently been carried out. The original proof was simpler, and, in the light of the present work, is adequate.


(III.)


(IV.)


(V.)

2 : 4 : 5-Triamino-6-hydroxypyrimidine was converted by a standard method into 2 : 4-*diamino*-5-p-*acetamidobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHAc$), which was then deacetylated to 2 : 4-*diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NH_2$). The author is indebted to Mr. C. E. Coulthard of the Bacteriological Department, Boots Pure Drug Co. Ltd., for the results of a preliminary biological examination of this sulphanilamidopyrimidine. It was less active than sulphanilamide in inhibiting the growth of a number of bacteria, including *Streptococcus hæmolyticus*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Pseudomonas pyocyanea*.

Experimental.

(Temperatures are uncorrected. Compounds for which no m. p.s are given decomposed on heating.)

2 : 4 : 5-*Triamino*-6-*hydroxypyrimidine* (I; R = NH_2).—The intermediate 2 : 4-diamino-6-hydroxypyrimidine sulphate was most satisfactorily prepared by the method of Traube and Dudley (*Ber.*, 1913, **46**, 3843). The subsequent procedure for conversion into the sodium hydrogen sulphite compound of the triaminopyrimidine was similar to those described recently (Totter, Shukers, Kolson, Mims and Day, *J. Biol. Chem.*, 1944, **152**, 149; Cain, Mallette, and Taylor, *J. Amer. Chem. Soc.*, 1946, **68**, 1996).

2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine* (I; R = NHAc).—The sodium hydrogen sulphite compound of 2 : 4 : 5-triamino-6-hydroxypyrimidine (2·45 g.) in water (5 c.c.) was treated with 2N-sodium hydroxide (20 c.c.) and acetic anhydride (2·0 c.c.) at 5°. The product was precipitated by carbon dioxide and recrystallised from boiling water. 2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine* formed pale yellow crystals, m. p. 343—344° (decomp.) (Found : N, 38·2. $C_6H_9O_2N_5$ requires N, 38·2%). The compound was soluble in dilute acids and dilute alkalis.

Ethyl Acetyloximinocyanoacetate.—Ethyl oximinocyanoacetate (Conrad and Schulze, *Ber.*, 1909, **42**, 735) was acetylated, after Cerchez and Colesui (*Compt. rend.*, 1932, **194**, 1954). The acetyl compound had b. p. 120—128°/1 mm. (lit., b. p. 148°/14 mm.) and was eventually obtained solid. It formed white crystals, m. p. 44°, from light petroleum (Found : N, 15·4. Calc. for $C_7H_8O_4N_2$: N, 15·2%).

Ethyl Acetamidocyanoacetate.—Aluminium amalgam reduction of the above oxime acetate (Cerchez and Colesui, *loc. cit.*) was unsatisfactory and the following method was adopted. Ethyl oximinocyanoacetate (28 g.) was dissolved in glacial acetic acid (150 c.c.), and zinc dust (32 g.) added during 1 hour with vigorous stirring and cooling to 25—30°. Acetic anhydride (20 c.c.) and acetic acid (50 c.c.) were added, and the mixture stirred for 2 hours at 70—80°. The zinc acetate was filtered off and washed with glacial acetic acid (4 × 50 c.c.). The acetic acid was distilled off under reduced pressure and the residue triturated with ethyl acetate, giving the acetamido-ester (21·2 g., 64%), m. p. 103—106°. This material was pure enough for the following reaction, in spite of the low m. p. Recrystallisation from ethyl acetate gave the pure compound, m. p. 129°.

Independent Synthesis of 2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine.*—Guanidine hydrochloride (4·8 g.) was dissolved in a solution of sodium ethoxide, prepared from sodium (2·3 g.) and ethanol (50 c.c.). After 5 minutes, ethyl acetamidocyanoacetate (8·5 g.) was added and the mixture was refluxed for 1 hour. The alcohol was removed in a vacuum, the residue dissolved in water (50 c.c.), and carbon

dioxide passed in to precipitate the pyrimidine [4·8 g.; m.p. 339—341° (decomp.), not depressed on mixing with a specimen prepared by direct acetylation of 2 : 4 : 5-triamino-6-hydroxypyrimidine]. With picric acid in water, the *picrate* was obtained in yellow needles, m. p. 254—255° (decomp.) (Found : N, 26·95. $C_6H_9O_2N_5,C_6H_3O_7N_3$ requires N, 27·15%). The same picrate (m. p. and mixed m. p.) was obtained from the acetamido-compound prepared by direct acetylation.

2 : 4-*Diamino-5-benzamido-6-hydroxypyrimidine* (I; R = NHBz).—2 : 4 : 5-Triamino-6-hydroxypyrimidine sulphate (2·4 g.) was dissolved in N-sodium hydroxide (40 c.c.), and benzoyl chloride (1·6 g.) added with shaking and cooling. Saturation with carbon dioxide precipitated a solid (2·6 g.; m. p. 313°), which on recrystallisation from dilute acetic acid gave the pure 5-*benzamido*-compound as pale yellow needles, m. p. 318—319° (Found : N, 28·6. $C_{11}H_{11}O_2N_5$ requires N, 28·6%).

2 : 4-*Diamino-5-α-hydroxypropionamido-6-hydroxypyrimidine* [I; R = NH·CO·CH(OH)·CH_3].—The pyrimidine sodium hydrogen sulphite compound (4·9 g.) and DL-lactic acid (3·6 g.) were heated at 115—125° (bath) for 1 hour. The product was washed with water and crystallised from the same solvent, giving pale yellow crystals of 2 : 4-*diamino-5-hydroxypropionamido-6-hydroxypyrimidine*, m. p. 299—301° (decomp.) (Found : N, 32·65. $C_7H_{11}O_3N_5$ requires N, 32·85%). The substance was soluble in dilute acids and dilute alkalis, but did not appear to form a picrate.

2 : 4-*Diamino-6-hydroxypyrimidyl-5-oxamic acid* (I; R = NH·CO·CO_2H).—The sodium hydrogen sulphite compound (109 g.) and oxalic acid dihydrate (124 g.) were dissolved in water (2220 c.c.) and boiled for 3 hours. The *oxamic acid* (73 g.) was filtered off and washed with hot water (Found : C, 33·9; H, 3·3; N, 32·4. $C_6H_7O_4N_5$ requires C, 33·8; H, 3·3; N, 32·8%). It resembled leucopterin in appearance, solubility in 2N-sodium hydroxide, and insolubility in all organic solvents. Unlike leucopterin, it dissolved in hot 5N-sulphuric acid and, with effervescence, in hot 6% sodium hydrogen carbonate solution. On cooling the latter solution, the sparingly soluble *sodium* salt separated (Found : N, 30·45. $C_6H_6O_4N_5Na$ requires N, 29·8%). The sodium salt (30 g.) with N-hydrochloric acid (200 c.c.) regenerated the oxamic acid (24·6 g.; calc. 27·2 g.), and sodium chloride (7·8 g.; calc. 7·5 g.) was obtained on evaporation of the filtrate.

Compound.	Solvent.	$\lambda_{max.}$ (A.) ($\epsilon_{max.}$).	$\lambda_{min.}$ (A.) ($\epsilon_{min.}$).
2 : 4-Diamino-6-hydroxypyrimidine	0·1N-NaOH	2620—2660 (13,220)	—
	0·1N-HCl	2630 (19,200)	—
2 : 4-Diamino-6-hydroxypyrimidine sulphate (1)	0·1N-NaOH	2620 (22,050); 2450 (10,140)	2470 (9100); 2410 (9100)
2 : 4 : 5-Triamino-6-hydroxypyrimidine sulphate (2)	0·1N-NaOH	2400 (5230); 2650 (5450); 2740 (5700)	2380 (4990); 2520 (4250); 2670 (5230)
2 : 4-Diamino-5-acetamido-6-hydroxypyrimidine (Fig. 2)	0·1N-NaOH	2400 (8400); 2610 (14,020)	2350 (7470); 2450 (7470)
	0·1N-HCl	2630 (15,900)	—
2 : 4-Diamino-5-(α-hydroxypropionamido)-6-hydroxypyrimidine (Fig. 2)	0·1N-NaOH (3)	2600 (7900)	2380 (3860)
	0·1N-HCl	2650 (15,300)	—
2 : 4-Diamino-6-hydroxypyrimidyl-5-oxamic acid (Fig. 1)	0·1N-NaOH	2630 (11,500)	2450 (5300)
2 : 4-Diamino-5-benzylideneamino-6-hydroxypyrimidine (4)	Water	2360 (4250); 2850 (3100)	2660 (800)
2 : 4-Diamino-5-benzamido-6-hydroxypyrimidine	0·1N-NaOH	2280—2300 (23,300)	—
2 : 4-Diamino-5-(*p*-aminobenzenesulphonamido)-6-hydroxypyrimidine	0·1N-NaOH	2590 (22,800)	2250 (8600)
Leucopterin (5) (Fig. 1)	0·1N-NaOH	2400 (16,800); 2850 (6880); 3420 (9940)	2350 (13,800); 2710 (5590); 3050 (3960)
Thymine (6) (Fig. 1)	0·1N-NaOH	2700 (5500); 2780 (5450); 2800—2860 (5250) (inflexion)	2520 (2520); 2730 (5050)
	Dilute ammonia	2640 (7650)	—
	Water or 0·1N-HCl (7)	2640 (7800)	2420 (2000)

Notes.

(1) $(C_4H_6ON_4)_2,H_2SO_4$; *M*, 350.
(2) $C_4H_7ON_5,H_2SO_4$; *M*, 239. The compound decomposed quickly in alkaline solution. The measurements were made with a fresh solution. The curve obtained suggested that two similar compounds were present.
(3) This compound was unstable in alkaline solution; the measurements were therefore made on freshly prepared solutions.
(4) Prepared after Traube and Nithak (*Ber.*, 1906, **39**, 235).
(5) Prepared after Totter (*J. Biol. Chem.*, 1944, **154**, 105). The absorption figures agree with those recorded by Jacobsen and Simpson (*Biochem. J.*, 1946, **40**, 3).
(6) Prepared after Wheeler and McFarland (*Amer. Chem. J.*, 1910, **43**, 19).
(7) Deduced from the curve given by Stimson and Reuter (*J. Amer. Chem. Soc.*, 1945, **67**, 847).

Attempted Benzoylation of 2 : 4-*Diamino-6-hydroxypyridine.*—The diamine sulphate (3·7 g.) dissolved in 0·7N-sodium hydroxide (60 c.c.) was treated with benzoyl chloride (3·5 g.) and 2N-sodium hydroxide (30 c.c.) at 10°. After 2 hours, the solution was neutralised with 2N-hydrochloric acid, and benzoic acid (1·4 g.; m. p. 122°) was removed by filtration. Left at 0°, the mother liquors gave 2 : 4-diamino-6-hydroxypyrimidine (1·4 g.), bladed needles m. p. 277—279° (decomp.), raised to m. p. 286—288°

(decomp.) on crystallising from water (Found in material dried at 100°/1 mm.: N, 44·3. Calc. for $C_4H_6ON_4$: N, 44·4%) [Conrad, *Annalen*, 1905, **340**, 313, gives m. p. 286° (decomp.)].

2 : 4-*Diamino*-5-p-*acetamidobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHAc$). —The triamino-pyrimidine sulphate (4·8 g.) was dissolved in 0·3N-sodium hydroxide (120 c.c.) and stirred at 60—70° whilst *p*-acetamidobenzenesulphonyl chloride (5·2 g.) and 2N-sodium hydroxide (10 c.c.) were added. The mixture was stirred at 80° for 30 minutes, and was then neutral. The solid (4·7 g.) was washed with water and dried in a vacuum. Recrystallisation from boiling water gave the 5-p-*acetamidobenzenesulphonamide* in small, pale yellow needles, frothing at 280° (Found: N, 24·4. $C_{12}H_{14}O_4N_6S$ requires N, 24·85%).

2 : 4-*Diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NH_2$).— The above acetyl compound (1·8 g.) was boiled for 20 minutes with 5N-sodium hydroxide (20 c.c.). The solution was neutralised with hydrochloric acid, and the solid (1·1 g.) recrystallised from boiling water. 2 : 4-*Diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* formed small, pale brown crystals (Found: N, 28·15. $C_{10}H_{12}O_3N_6S$ requires N, 28·4%). The compound was much more soluble in water than the acetyl derivative. During the preparation, only a trace of ammonia was evolved. Hydrolysis of the acetyl compound with boiling 2N-hydrochloric acid gave a non-basic white compound, which was not the required sulphonamide.

The author thanks Drs. W. F. Short and D. A. Peak for their interest in this work. He is also indebted to Mr. C. A. Bartram for experimental assistance.

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