963. *Pyrimidines. Part V.* The Synthesis of 5-Amino-4-hydroxypyrimidine, a New Isomer of Cytosine.*

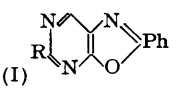
By (Miss) M. P. V. Boarland and J. F. W. McOmie.

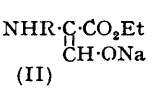
5-Amino-4-hydroxypyrimidine has been prepared by the Raney nickel desulphurisation of the corresponding 2-ethylthio-compound. The so-called *trans*-α-carbethoxyamino-β-*S*-ethylthiureidoacrylic acid has been shown to be a mixture of the 5-carbethoxyamino- and 5-formamido-derivatives of 2-ethylthio-4-hydroxypyrimidine by means of ultra-violet absorption spectra studies and by paper chromatography.

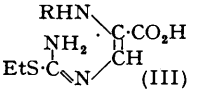
Of the seven possible aminohydroxypyrimidines only three are known, *viz.*, 4-amino-2-hydroxy- (cytosine), 2-amino-4-hydroxy- (*iso*cytosine) (cf. Johnson and Hahn, *Chem. Reviews*, 1933, **13**, 193), and 4-amino-6-hydroxy-pyrimidine (Brown, *J. Soc. Chem. Ind.*, 1950, **69**, 353; Cavalieri and Bendich, *J. Amer. Chem. Soc.*, 1950, **72**, 2587). In view of the biological importance of cytosine, we now describe the synthesis and some properties of 5-amino-4-hydroxypyrimidine. Although it is known (Marshall and Walker, *J.*, 1951, 1004; Boarland and McOmie, *J.*, 1952, 3716) that simple hydroxypyrimidines exist in the pyrimidone form, this may not be true for pyrimidines containing more than one potentially tautomeric group. For convenience, the compounds are referred to below as hydroxypyrimidines.

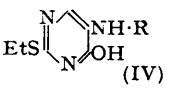
5-Amino-4-hydroxypyrimidine should be obtainable by reduction of the 5-nitro-compound. Since attempts at nitration of 4-hydroxypyrimidine failed (A. Albert and R. Royer, personal communication), its nitrosation was investigated: this failed, however, with amyl nitrite–sodium ethoxide (cf. Henrich, *Monatsh.*, 1897, **18**, 142) or –hydrogen chloride (Barltrop, Johnson, and Meakins, *J.*, 1951, 181), or ethyl nitrate–sodium ethoxide or –aluminium chloride. An attempt to nitrosate 2-thiouracil with sodium nitrite in dilute acetic or dilute hydrochloric acid (Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383) was also unsuccessful.

A second possible route was *via* 5-benzamido-4-hydroxypyrimidine. Johnson (*Amer. Chem. J.*, 1905, **34**, 191) failed to effect simple deacylation of 5-benzamido-2-ethylthio-4-hydroxypyrimidine with acid or alkali. 5-Benzamido-4-hydroxy-2-methylthiopyrimidine was readily obtained by us from methylthiuronium sulphate and the sodium salt of ethyl


(I)


(II)


(III)


(IV)

benzamidoformylacetate in the presence of aqueous potassium hydroxide, and was desulphurised with Raney nickel to 5-benzamido-4-hydroxypyrimidine. Although this gave almost the theoretical quantity of benzoic acid on acid hydrolysis, 5-amino-4-hydroxypyrimidine could not be isolated. Two further reactions of 5-benzamido-4-hydroxy-2-

* Part IV, *J.*, 1952, 4691.

methylthiopyrimidine are recorded: treatment with phosphorus oxychloride gave a good yield of 2′-methylthio-2-phenylpyrimidino(5′ : 4′-4 : 5)oxazole (I; R = MeS), which was desulphurised with Raney nickel to give (I; R = H). The ultra-violet absorption spectra of these oxazoles in ethanol are shown in Fig. 1. Use of acetic anhydride instead of phosphorus oxychloride gave 5-acetamido-4-hydroxy-2-methylthiopyrimidine in low yield, but no oxazole.

Johnson (*loc. cit.*) converted ethyl carbethoxyaminoacetate by sodium and ethyl formate in hot benzene into the sodium salt of ethyl carbethoxyaminoformylacetate (II; R = CO_2Et). This with ethylthiuronium bromide gave a product which he formulated as *trans*-α-carbethoxyamino-β-*S*-ethylthiureidoacrylic acid (III; R = CO_2Et) and with boiling aqueous sodium hydroxide yielded 5-amino-2-ethylthio-4-hydroxypyrimidine (IV; R = H). In the same paper, he reported that sodium hydroxide opened the pyrimidine ring of 5-benzamido-2-ethylthio-4-hydroxypyrimidine (IV; R = Bz), to give the corresponding acrylic acid (III; R = Bz). In view of the conflicting behaviour of these two acrylic acids and the improbability that hot sodium hydroxide would effect ring

FIG. 1. FIG. 2. FIG. 3.

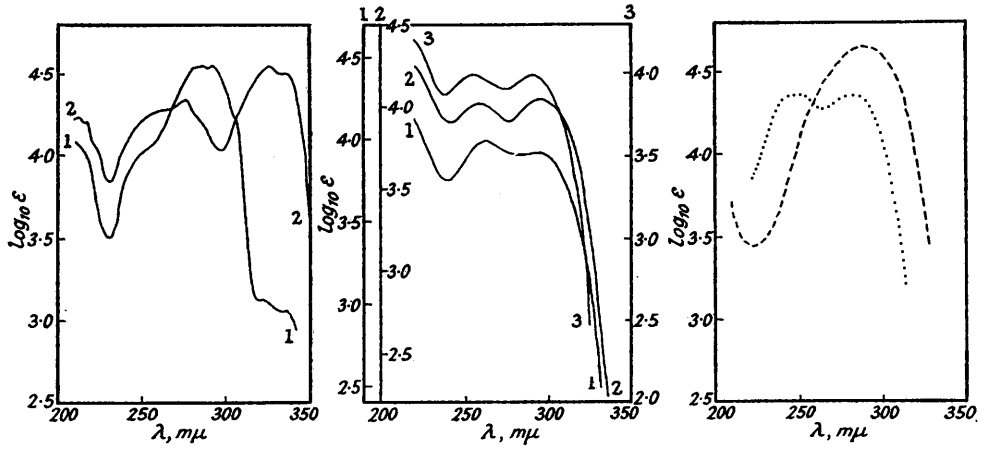


FIG. 1. 1, 2-*Phenylpyrimidino*(5′ : 4′-4 : 5)*oxazole in ethanol.* 2, 2′-*Methylthio-2-phenylpyrimidino*-(5′ : 4′-4 : 5)*oxazole in ethanol.*

FIG. 2. 1, 5-*Amino-2-ethylthio-4-hydroxypyrimidine.* 2, 2-*Ethylthio-5-formamido-4-hydroxypyrimidine.* 3, 5-*Carbethoxyamino-2-ethylthio-4-hydroxypyrimidine.* (*All in* 0·1N-NaOH.)

FIG. 3. 5-*Amino-4-hydroxypyrimidine* (. . . . *in* 0·1N-NaOH; – – – – *in* N-HCl).

closure to a pyrimidine derivative, the acid (III; R = CO_2Et) was investigated more fully. The compound was readily obtained by Johnson's method and like his material showed no sharp melting point, but decomposed at about 256°. Its ultra-violet absorption spectrum was very similar to those of 5-amino-2-ethylthio-4-hydroxypyrimidine (IV; R = H) and its 5-carbethoxyamino-derivative (IV; R = CO_2Et). Since it seemed unlikely that the absorption of an acrylic acid would resemble that of a highly conjugated pyrimidine system, the possibility that the compound was a pyrimidine was considered.

Paper partition chromatography showed that our condensation product was a mixture of two compounds (cf. the melting point range of *ca.* 50°), and 5-carbethoxyamino-2-ethylthio- (IV; R = CO_2Et) and 2-ethylthio-5-formamido-4-hydroxypyrimidine (IV; R = CHO) seemed the two most probable components. The former is to be expected from ethylthiuronium bromide and ethyl carbethoxyaminoformylacetate, and it is probable that the crude sodium salt from the formylation of ethyl carbethoxyaminoacetate would be contaminated with ethyl formamidoformylacetate (II; R = CHO) formed by transacylation; the last compound with ethylthiuronium bromide would give the 5-formamidopyrimidine. These two acylaminopyrimidines were, therefore, synthesised by unambiguous routes. 5-Carbethoxyamino-2-ethylthio-4-hydroxypyrimidine was readily obtained by treatment of the 5-amino-compound (IV; R = H) (prepared by the action of hot alkali

on the condensation product) with ethyl chloroformate. 2-Ethylthio-5-formamido-4-hydroxypyrimidine was prepared in good yield by heating the 5-amino-compound with amyl formate (cf. Human and Mills, *J.*, 1948, 1457). The R_F values and absorption spectra established the presence of these compounds in the condensation product. The ultra-violet absorption spectra of the 5-amino-, 5-carbethoxyamino-, and 5-formamido-derivatives are recorded in the Table and Fig. 2.

Johnson (*loc. cit.*) reported that the action of acetic anhydride on the condensation product gave 5-carbethoxyamino-2-ethylthio-4-hydroxypyrimidine. This has been confirmed (mixed m. p., R_F, and ultra-violet absorption spectrum). Thus acetic anhydride merely purifies the condensation product (analytical figures indicated that the 5-carbethoxyamino-compound was the major component).

Raney nickel smoothly desulphurised 5-amino-2-ethylthio-4-hydroxypyrimidine in water (there was no reaction in ethanol), giving 5-amino-4-hydroxypyrimidine (for the absorption spectra see the Table and Fig. 3).

When tested by the agar-cup diffusion method, 1% solutions of 5-amino-4-hydroxypyrimidine and its 2-ethylthio-derivative showed no activity against *Staph. aureus*.

Substituents							
2	4	5	Medium	λ (mμ)	$\log_{10} \varepsilon_{max.}$	λ (mμ)	$\log_{10} \varepsilon_{max.}$
	OH	NH_2	0·1N-HCl	290	4·14	—	—
			0·1N-NaOH	248	3·88	282	3·87
EtS	OH	NH_2	0·1N-NaOH	263	3·99	295	3·92
EtS	OH	$NH{\cdot}CO_2Et$	0·1N-NaOH	255	3·99	291	3·99
EtS	OH	NH·CHO	0·1N-NaOH	259	4·02	296	4·05
MeS	OH	NHAc	0·1N-NaOH	256	4·09	291	4·10

Experimental

M. p.s are uncorrected. Microanalyses are by Messrs. W. M. Eno and B. S. Noyes, Bristol, and Drs. Weiler and Strauss, Oxford.

5-Benzamido-4-hydroxy-2-methylthiopyrimidine.—The sodium salt of ethyl benzamidoformylacetate (30 g.) [prep. (85% yield): "The Chemistry of Penicillin," Oxford Univ. Press, 1949, p. 515] in water (60 c.c.) was added to methylthiuronium sulphate (15·2 g.) and potassium hydroxide (6·2 g.) in water (80 c.c.). The solution was filtered and kept at 37° for 2 days. Acidification of the resulting gel with acetic acid precipitated a white solid, m. p. 230—240° (91%), which gave *5-benzamido-4-hydroxy-2-methylthiopyrimidine* as prismatic needles, m. p. 268°, from aqueous dioxan (Found: C, 55·5; H, 4·1; N, 16·3. $C_{12}H_{11}O_2N_3S$ requires C, 55·3; H, 4·2; N, 16·1%), insoluble in water, ethanol, or benzene; it showed a bright blue fluorescence in ultra-violet light.

5-Acetamido-4-hydroxy-2-methylthiopyrimidine.—The benzamido-compound (3·0 g.) was heated under reflux with acetic anhydride (30 c.c.) for 1 hour. After removal of excess of acetic anhydride under reduced pressure, the residue was poured into water. A dark oil separated, accompanied by some yellow crystals. After 3 days the crystals (1·2 g.) were collected and recrystallised from ethanol [pale yellow; m. p. 220—221° (decomp.); dull yellow fluorescence]. Sublimation at 100°/0·3 mm. removed benzoic acid (30 mg.; identified after recrystallisation from water by a mixed m. p. determination). The residue, recrystallised several times from ethanol (charcoal), gave *5-acetamido-4-hydroxy-2-methylthiopyrimidine* as rosettes of needles, m. p. 236° (Found: C, 42·6; H, 4·6; N, 21·2. $C_7H_9O_2N_3S$ requires C, 42·2; H, 4·5; N, 21·1%), readily soluble in dilute sodium hydroxide solution, sparingly soluble in cold water, and showing a bluish-white fluorescence.

5-Benzamido-4-hydroxypyrimidine.—A solution of 5-benzamido-4-hydroxy-2-methylthiopyrimidine (10·0 g.) in aqueous dioxan (300 c.c.) was heated under reflux with Raney nickel (40 g.) with vigorous stirring for 3½ hours. Filtration, evaporation (yield, 4·7 g.), and recrystallisation from aqueous ethanol gave *5-benzamido-4-hydroxypyrimidine* as very fine needles, m. p. 245—246° (Found: C, 61·3; H, 4·35; N, 19·3. $C_{11}H_9O_2N_3$ requires C, 61·5; H, 4·2; N, 19·5%), showing slight yellow fluorescence in ultra-violet light.

2'-Methylthio-2-phenylpyrimidino(5' : 4'-4 : 5)oxazole.—5-Benzamido-4-hydroxy-2-methylthiopyrimidine (9·0 g.) was heated under reflux with phosphorus oxychloride (50 c.c.) for 3 hours. After removal of excess phosphorus oxychloride under reduced pressure, the residue was poured on ice. The buff-coloured *compound* precipitated (77%) crystallised from ethanol as yellow needles, m. p. 155—156° (Found: C, 59·5; H, 3·7; N, 17·7. $C_{12}H_9ON_3S$ requires C, 59·3; H,

3·7; N, 17·3%), fluorescing intense yellow in ultra-violet light (bright blue in ethanol or benzene).

2-Phenylpyrimidino(5′ : 4′-4 : 5)oxazole.—The foregoing material (2·0 g.) in ethanol (150 c.c.) was heated under reflux with Raney nickel (8 g.) for 3 hours. After filtration, slight concentration, and cooling, 0·2 g. of the starting material was obtained. Further concentration yielded crystals (62%) (brilliant white fluorescence), which on recrystallisation from aqueous ethanol and sublimation at 110°/0·1 mm. gave the pure *oxazole*, needles, m. p. 114° (Found: C, 66·6; H, 3·8; N, 21·4. $C_{11}H_7ON_3$ requires C, 67·0; H, 3·6; N, 21·3%), soluble in ether, dioxan, or benzene, moderately soluble in methanol or ethanol, and sparingly soluble in water.

Ethyl Carbethoxyaminoformylacetate.—Ethyl formate (14 c.c.) and ethyl carbethoxyaminoacetate (20 g.) were added to finely powdered sodium (2·8 g.) under dry benzene (150 c.c.) and heated on a water-bath for ¾ hour. Next day dry ether was added to precipitate the yellow sodium salt which was collected and dried in a vacuum-desiccator (22·5 g.).

Condensation of Ethyl Carbethoxyaminoformylacetate and Ethylthiuronium Bromide.—Ethylthiuronium bromide (28 g.) and potassium hydroxide (8·5 g.) in water (50 c.c.) were added to the sodium salt of ethyl carbethoxyaminoformylacetate (37·3 g.) in water (50 c.c.), and the yellow solution was kept for 15 hours. Acidification with acetic acid gave a white product (16·8 g.), m. p. 200—256° (decomp.). Repeated crystallisation from a large volume of aqueous ethanol (50%) did not alter the m. p. range. Examination of a paper chromatogram (ethyl acetate–water–acetic acid; 3 : 2 : 1) in ultra-violet light revealed two spots (R_F 0·90 and 0·96), identical with those produced by 2-ethylthio-5-formamido- (R_F 0·90) and 5-carbethoxyamino-2-ethylthio-4-hydroxypyrimidine (R_F 0·96).

5-Amino-2-ethylthio-4-hydroxypyrimidine.—The above mixture of derivatives (6·3 g.) was heated under reflux with sodium hydroxide (3·1 g.) in water (30 c.c.) for 20 minutes. After a further 30 minutes at room temperature, the solution was cooled and acidified with acetic acid. The product, 5-amino-2-ethylthio-4-hydroxypyrimidine (4·1 g.), crystallised from water as fine needles, m. p. 159°. Johnson (*Amer. Chem. J.*, 1905, **34**, 191) reports m. p. 160°.

2-Ethylthio-5-formamido-4-hydroxypyrimidine.—5-Amino-2-ethylthio-4-hydroxypyrimidine (0·4 g.), suspended in amyl formate (10 c.c.; b. p. 121°) and gently warmed, gave a clear solution which rapidly deposited crystals. After ½ hour's heating under reflux and then cooling, the precipitated *formyl* derivative was collected (0·35 g.) and crystallised from a large volume of aqueous ethanol (50%) as prismatic needles, m. p. 269—270° (effervescence and slight decomp.) (Found: C, 42·6; H, 4·8; N, 21·3. $C_7H_9O_2N_3S$ requires C, 42·2; H, 4·5; N, 21·1%), slightly fluorescent in ultra-violet light.

5-Carbethoxyamino-2-ethylthio-4-hydroxypyrimidine.—(*a*) The mixed condensation product (1·0 g.) was dissolved in warm acetic anhydride (20 c.c.), cooled, and diluted with water. Next day the pale yellow crystals, m. p. 184° (0·2 g.), were collected. Recrystallisation from 15% acetic acid gave 5-carbethoxyamino-2-ethylthio-4-hydroxypyrimidine as prisms, m. p. 190—191° [Johnson (*loc. cit.*) reports m. p. 189—190°] (Found: C, 44·7; H, 5·35; N, 17·5; S, 13·45. Calc. for $C_9H_{13}O_3N_3S$: C, 44·5; H, 5·35; N, 17·3; S, 13·2%).

(*b*) A solution of 5-amino-2-ethylthio-4-hydroxypyrimidine (0·5 g.) and sodium carbonate (0·13 g.) in water (20 c.c.) was cooled in ice–salt, and ethyl chloroformate (0·25 c.c.) added slowly with shaking. A crystalline solid separated almost immediately. After ½ hour, the product, m. p. 188° (0·55 g., 90%), was collected. Recrystallisation from aqueous ethanol (50%) gave prismatic needles, m. p. and mixed m. p. with the product obtained as in (*a*), 190—191°; both products moved at the same rate on a paper strip chromatogram (R_F 0·95). The solid compound was slightly fluorescent in ultra-violet light, its ethanolic solution showing blue fluorescence.

5-Amino-4-hydroxypyrimidine.—5-Amino-2-ethylthio-4-hydroxypyrimidine (2·0 g.) was heated with Raney nickel (8 g.) in water (70 c.c.) under reflux with vigorous stirring for 3 hours. After filtration and evaporation, the residual *5-amino-4-hydroxypyrimidine* (0·8 g.) was crystallised from ethanol, giving prisms, m. p. 211—212° (Found: C, 43·3; H, 4·4; N, 38·3. $C_4H_5ON_3$ requires C, 43·2; H, 4·5; N, 37·9%), soluble in water, sparingly soluble in ethyl acetate, and slightly fluorescent in ultra-violet light.

Physical Measurements.—The methods have been described in Part II (*J.*, 1952, 3716).

The authors thank Professor K. E. Cooper and Dr. C. S. Shaw, of Canynge Hall, Bristol, for the bacteriological tests, and the Department of Scientific and Industrial Research for the award of a maintenance grant (to M. P. V. B.).

THE UNIVERSITY, BRISTOL. [*Received, July 21st*, 1952.]