194. *Pyrimidines. Part XIII.*[1] *Electrophilic Substitution at Position 6 and a Synthesis of Divicine (2,4-Diamino-5,6-dihydroxypyrimidine).*

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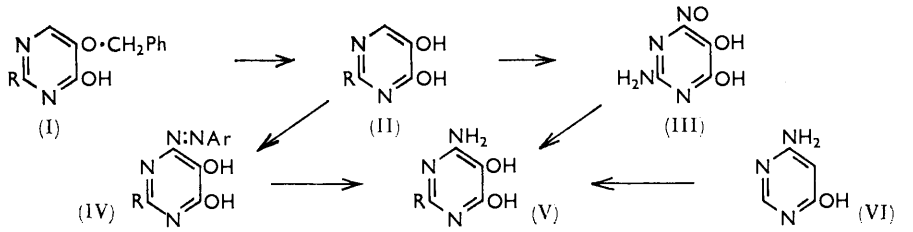
2-Amino-4,5-dihydroxypyrimidine undergoes nitrosation and coupling with diazonium salts at position 6; reduction of the resulting compounds then gives divicine. 4,5-Dihydroxypyrimidine couples with diazotised *p*-chloroaniline at position 6.

Electrophilic substitution in pyrimidines[2] occurs readily only when two electron-donating substituents, *e.g.*, NH_2 or OH, are present (though one such substituent is sufficient for halogenation) and the substituent enters at position 5. Electrophilic substitution at position 2 has not been recorded, but two examples are known at position 4(6). Bogert and Davidson found that 2,4,5-trihydroxypyrimidine would undergo nitrosation[3] and diazo-coupling[4] at position 6. We have applied these two reactions to 2-amino-4,5-dihydroxypyrimidine (II; R = NH_2) and thereby we have obtained compounds which on reduction gave divicine (2,4-diamino-5,6-dihydroxypyrimidine) (V; R = NH_2), as already briefly recorded.[5]

Further substitution in these 2,4,5-trisubstituted pyrimidines could only take place at position 6, and we have therefore examined the nitrosation and diazo-coupling reactions of 4,5-dihydroxypyrimidine, where substitution might occur at position 2 or at position 6.



Synthesis of Divicine.—2-Amino-5-benzyloxy-4-hydroxypyrimidine (I; R = NH_2) was made from benzyl benzyloxyacetate by formylation followed by condensation with guanidine carbonate. The benzyl group could be removed by catalytic reduction[6] or, more conveniently, by acid hydrolysis to give 2-amino-4,5-dihydroxypyrimidine (II; R = NH_2). When this was treated with nitrous acid it gave an almost quantitative yield of the sparingly soluble orange compound (III). Reduction of this compound by sodium dithionite or catalytically gave divicine (V; R = NH_2) in 94 and 49% yield, respectively. The dihydroxy-compound (II; R = NH_2) readily coupled with diazotised sulphanilic acid, but the product (IV; R = NH_2, Ar = *p*-$C_6H_4SO_3Na$) could not be obtained analytically pure; it was therefore reduced directly by sodium dithionite thereby giving divicine in low yield.

Divicine, the aglycone of the naturally occurring compound vicine,[7] has previously been synthesised[6] by the condensation of ethyl α-cyano-α-tetrahydropyran-2-yloxyacetate with guanidine followed by hydrolytic removal of the protective group. The synthetic divicine sulphate had an ultraviolet spectrum, and gave colour reactions almost identical with those of a sample made by acid hydrolysis of the glucoside vicine. However, the infrared spectra of the two materials were reported to show differences, attributed

[1] Part XII, McOmie and Turner, *J.*, 1963, 1070.
[2] Brown, "The Pyrimidines," Interscience Publishers, New York and London, 1962.
[3] Bogert and Davidson, *Proc. Nat. Acad. Sci. U.S.A.*, 1932, **18**, 215.
[4] Davidson and Bogert, *Proc. Nat. Acad. Sci. U.S.A.*, 1932, **18**, 490.
[5] McOmie and Chesterfield, *Chem. and Ind.*, 1956, 1453.
[6] Davoll and Laney, *J.*, 1956, 2124.
[7] Bendich and Clements, *Biochem. Biophys. Acta*, 1953, **12**, 462.

to impurities in the " natural " divicine. We have repeated the extraction [7] of vicine from *Vicia sativa* and have hydrolysed it to divicine which we characterised as the stable diacetate. The natural and synthetic diacetates gave identical infrared spectra.

During the course of this work we prepared 2,4,5-trihydroxypyrimidine (II; R = OH) and 4,5-dihydroxy-2-methylpyrimidine (II; R = Me) by the hydrolysis of the corresponding 5-benzyl ethers. After the completion of our work, Chang and Wu,[8] and Chang and Chiang,[9] reported the synthesis of several 5-hydroxypyrimidines *via* the corresponding benzyl ethers, the protective group being removed in each case by catalytic hydrogenolysis.

Electrophilic Substitution in 4,5-Dihydroxypyrimidine.—When a solution of *p*-chlorobenzenediazonium chloride was added to a solution of 4,5-dihydroxypyrimidine (II; R = H) in sodium hydroxide, a deep red precipitate was formed which gave the correct analysis for the expected coupled product. Reduction of the red compound gave an amino-4,5-dihydroxypyrimidine which was different from the known 2-amino-compound. It must therefore be 6-amino-4,5-dihydroxypyrimidine (V; R = H) and this was confirmed by comparison with an authentic specimen which was made by the Elbs persulphate oxidation of 4-amino-6-hydroxypyrimidine (VI) (cf. ref. 10).

Attempts to nitrosate 4,5-dihydroxypyrimidine were unsuccessful, while attempted bromination caused profound decomposition, ammonium bromide being the only isolable product.

4-Amino-5-hydroxypyrimidine [1] coupled with diazotised *p*-nitroaniline to give a red compound which is probably analogous to the similar compound from 4,5-dihydroxypyrimidine. 5-Benzyloxy-4-hydroxy-2-mercapto- and 5-benzyloxy-4-hydroxy-2-methylthio-pyrimidine failed to couple with diazotised *p*-chloroaniline.

Experimental

2-Amino-5-benzyloxy-4-hydroxypyrimidine (I; R = NH_2).—A mixture of benzyl benzyloxyacetate [11] (51·2 g.) and ethyl formate (14·8 g.) was added to a well-stirred suspension of powdered sodium (4·6 g.) in toluene (50 ml.) during 2 hr. The mixture was stirred for a further 2 hr., then kept overnight. A solution of sodium ethoxide (made from 4·6 g. of sodium and 80 ml. of ethanol) was added to a suspension of guanidine carbonate (36 g.) in ethanol (80 ml.), and this mixture was added to the above solution of benzyl sodio-α-benzyloxy-β-hydroxyacrylate in toluene. The whole was stirred for 1 hr., then boiled under reflux for 4·5 hr. After being cooled, the mixture was poured into water (300 ml.) and acidified with acetic acid. The resulting buff powder (20·8 g., 48%), m. p. 247—249° (decomp.), was well washed with water and ethanol and dried at 60°. A sample recrystallised from ethanol gave needles, m. p. 253—254° (decomp.), lit.,[6] 245—246° (decomp. >200°) (Found: C, 61·2; H, 5·1; N, 19·2. Calc. for $C_{11}H_{11}O_2N_3$: C, 60·8; H, 5·1; N, 19·4%).

2-Amino-4,5-dihydroxypyrimidine (II; R = NH_2).—The above-named ether (10 g.) was boiled with 3N-hydrochloric acid (280 ml.) for 0·5 hr., then the solution was concentrated to 100 ml. under reduced pressure. On cooling, the pyrimidine hydrochloride separated and was collected. Concentration of the mother-liquor gave a further crop of the hydrochloride (total 6·0 g.). The free base (4·6 g., 80%) was obtained by neutralising a saturated aqueous solution of the hydrochloride with potassium carbonate. A sample of the 2-amino-4,5-dihydroxypyrimidine was recrystallised from water (Found: C, 37·5; H, 4·3; N, 33·1. Calc. for $C_4H_5N_3O_2$: C, 37·8; H, 4·0; N, 33·1%); it did not melt even at 300°.

The amine (0·1 g.) was boiled with acetic anhydride (10 ml.) and a crystal of sodium acetate for 2 hr. The clear solution was allowed to cool and next day the crystals which formed were collected. They were suspended in water and the pH was adjusted to 8—9 by addition of dilute sodium hydroxide. The solid was crystallised from ethanol giving pure *2-acetamido-5-acetoxy-4-hydroxypyrimidine* as a crystalline powder, m. p. *ca.* 200° (decomp.) (Found: C, 45·3; H, 4·0. $C_8H_9N_3O_4$ requires C, 45·3; H, 4·3%).

[8] Chang and Wu, *Sci. Sinica*, 1957, **6**, 279.
[9] Chang and Chiang, *Sci. Sinica*, 1957, **6**, 293.
[10] Hull, *J.*, 1956, 2033.
[11] Chesterfield, McOmie, and Tute, *J.*, 1960, 4590.

2-Amino-4,5-dihydroxy-6-nitrosopyrimidine (III).—2-Amino-4,5-dihydroxypyrimidine (3·8 g.), sodium nitrite (2·5 g.), and sodium hydroxide (2·5 g.) were dissolved in water (20 ml.). The cooled solution was added dropwise, with stirring, to acetic acid (11 ml.) and ice. During the addition, small pieces of ice were added from time to time. After being stirred for 1 hr. more, the mixture was filtered and the precipitate was collected. It was washed with water and ethanol, then dried in a vacuum desiccator. The orange *nitrosopyrimidine* (III) (4·4 g., 94%) decomposed slowly above 300° (Found: C, 30·7; H, 2·7; N, 35·7. $C_4H_4N_4O_3$ requires C, 30·8; H, 2·6; N, 35·9%).

Divicine (V; R = NH_2).—(*a*) Sodium dithionite (1·8 g.) was added gradually to a suspension of the above nitroso-compound (0·78 g.) in water (10 ml.) at 60—65°. The solid gradually dissolved giving a pale yellow solution from which a yellow solid rapidly separated. When the addition was complete, the mixture was cooled to 0° and the divicine (2,4-diamino-5,6-dihydroxypyrimidine) was collected. After having been washed with water and ethanol and dried in a vacuum desiccator, the divicine (4·4 g., 94%) formed pale yellow microcrystals, which decomposed above 300° (Found: C, 33·5; H, 4·4; N, 39·5. Calc. for $C_4H_6N_4O_2$: C, 33·8; H, 4·3; N, 39·4%).

(*b*) A suspension of the nitroso-compound (0·78 g.) in 5% acetic acid (30 ml.) was reduced at room temperature and pressure, using 10% palladium–charcoal (0·3 g.) as catalyst. As the reduction proceeded, the orange colour was discharged. The mixture was filtered and the solids were extracted with hot 5% acetic acid as quickly as possible, and the divicine (0·35 g., 49%) was obtained by cooling the extracts to 0°.

(*c*) Sodium nitrite (0·73 g.) in water (5 ml.) was added to a solution of sulphanilic acid (1·85 g.) and anhydrous sodium carbonate (0·5 g.) in water (10 ml.). The mixture was cooled to 10° and added to concentrated hydrochloric acid (5 ml.) and ice (15 g.). After 0·5 hr., the suspension of diazotised sulphanilic acid was added gradually to a well-stirred solution of 2-amino-4,6-dihydroxypyrimidine (1·3 g.) and sodium hydroxide (3·0 g.) in water (5 ml.). The dark red solution was kept for 0·5 hr., and was then neutralised with acetic acid. The nearly black product (IV; R = NH_2; Ar = *p*-$C_6H_4SO_3Na$) (3·05 g., 90%) was collected, washed with water and acetone, and dried in a vacuum desiccator. It was too hygroscopic for a satisfactory analysis to be obtained.

The azo-compound (IV) (0·5 g.) was dissolved in water at 90—95° and sodium dithionite (1·0 g.) was added in portions. The colourless solution was concentrated under reduced pressure to *ca.* 5 ml. and then cooled to 0°. The solid was collected and recrystallised from 5% acetic acid giving divicine (15 mg., 7%).

The divicine prepared by these three methods gave the same colour tests with ferric chloride, phosphomolybdic acid, and 2,6-dichlorophenol-indophenol, and it had the same ultraviolet absorption spectrum in N/4-hydrochloric acid ($\lambda_{max.}$ 281 mμ, $\log_{10}$ ε 4·20) as those recorded for divicine from natural material.[7]

Divicine Diacetate (2,4-*Diacetamido*-5,6-*dihydroxypyrimidine*).—(*a*) Synthetic divicine (0·85 g.) was heated with acetic anhydride (12 ml.) under reflux for 2·5 hr. The sparingly soluble divicine was gradually replaced by a colourless crystalline powder. The mixture was cooled to 0° and next day the solid was collected. This was probably a triacetyl derivative; it had m. p. 272—274° and gave no colour with ferric chloride, but it could not be obtained pure. It was suspended in water (10 ml.), and 5N-ammonium hydroxide was added until the solid dissolved. The solution was then acidified with acetic acid, and the *diacetate* (0·68 g., 51%) was collected; it had m. p. 309—312° (decomp.) and gave an intense blue colour with ferric chloride. A sample was recrystallised from water for analysis (Found: C, 42·45; H, 4·8; N, 25·4. $C_8H_{10}N_4O_4$ requires C, 42·5; H, 4·5; N, 24·8%).

(*b*) Vicine (1·2 g.) was isolated from the seeds of *Vicia sativa* (1 kg.) by the method of Bendich and Clements.[7] It formed needles, m. p. 240—245° (decomp.), lit.,[7] 243—244° (decomp.). The vicine was hydrolysed as described by Davoll and Laney [6] giving divicine sulphate which was acetylated as above (but with the addition of sodium acetate) to give divicine diacetate, m. p. 309—312°, alone or mixed with the above material (Found: C, 42·5; H, 4·6; N, 24·5%). The infrared spectra of the two samples were identical.

Diazo-coupling with 4,5-*Dihydroxypyrimidine.*—*p*-Chloroaniline (1·07 g.) in 20% hydrochloric acid (25 ml.) was diazotised at 5° by additon of sodium nitrite (1·0 g.) in water (15 ml.). The diazo-solution was added dropwise, with stirring, to an ice-cooled solution of 4,5-dihydroxypyrimidine [1] (1·0 g.) in 10% sodium hydroxide (10 ml.). The deep red precipitate (1·85 g.,

83%) was collected and recrystallised from ethanol giving 4-p-*chlorophenylazo*-5,6-*dihydroxypyrimidine* (IV; R = H, Ar = *p*-Cl·C_6H_4) as a red crystalline powder which decomposed above 250° (Found: C, 48·1; H, 2·7; N, 22·6. $C_{10}H_7ClN_4O_2$ requires C, 47·8; H, 2·8; N, 22·3%).

4-*Amino*-5,6-*dihydroxypyrimidine* (V; R = H).—(*a*) The above-named compound (0·5 g.) was suspended in hot water (15 ml.) and sodium dithionite (1—2 g.) was added with stirring until the red colour had gone. The cold mixture was extracted with ether to remove *p*-chloroaniline and then cooled to 0°. The solid (0·14 g., 60%) was collected and recrystallised from water giving 4-*amino*-5,6-*dihydroxypyrimidine* as felted needles which decomposed above 290° (Found: C, 38·1; H, 4·1; N, 32·7. $C_4H_5N_3O_2$ requires C, 37·8; H, 4·0; N, 33·1%). Acetylation of the amine (0·1 g.) with acetic anhydride (10 ml.) containing a little acetic acid and a crystal of sodium acetate gave 6-*acetamido*-5-*acetoxy*-4-*hydroxypyrimidine* as compact crystals which did not melt below 240° (Found: C, 45·3; H, 4·0; N, 19·2. $C_8H_9N_3O_4$ requires C, 45·5; H, 4·3; N, 19·9%).

(*b*) Ammonium persulphate (7·7 g.) in water (16 ml.) was added dropwise to a stirred ice-cold solution of 6-amino-4-hydroxypyrimidine [12] (2·5 g.) in 3N-sodium hydroxide (50 ml.) during 1·5 hr. After being stirred overnight, the mixture was acidified with concentrated hydrochloric acid, then cooled to 0°, and the 6-amino-4-hydroxy-5-pyrimidinyl hydrogen sulphate (1·0 g., 20%) was collected. The sulphate (0·5 g.) was boiled with N-hydrochloric acid (10 ml.) for 0·5 hr. The solution was cooled and the 6-amino-4,5-dihydroxypyrimidine (0·2 g., 65%) was recrystallised from water (Found: C, 37·7; H, 4·1%). Both samples of the amine gave a blue colour with ferric chloride and both samples had identical infrared spectra.

5-*Benzyloxy*-2,4-*dihydroxypyrimidine* (I; R = OH).—(*a*) A solution of 5-benzyloxy-4-hydroxy-2-methylthiopyrimidine [13] (0·4 g.) in ethanol (15 ml.) and 6N-hydrochloric acid (16 ml.) was boiled for 45 min. The solution was concentrated under reduced pressure and, after cooling, the solid was collected and recrystallised from ethanol giving the dihydroxypyrimidine, m. p. above 270° (Found: C, 60·5; H, 4·5%).

(*b*) Benzyl benzyloxyacetate (25·6 g.) was formylated as described above for the amino-compound (I; R = NH_2). Urea (6·0 g.) and ethanol (40 ml.) were added to the solution of the formyl derivative, the mixture being stirred for 1·5 hr. at room temperature, then 10 hr. at the boiling point. The cooled mixture was poured into water (150 ml.) and brought to pH 5 with hydrochloric acid. The product was collected and recrystallised from 50% aqueous ethanol giving leaflets (2·7 g., 12%), m. p. 294—296° (decomp.) (in preheated bath; lit.,[9] m. p. 283° decomp.) (Found: C, 60·6; H, 4·5; N, 12·7. Calc. for $C_{11}H_{10}N_2O_3$: C, 60·6; H, 4·6; N, 12·8%).

2,4,5-*Trihydroxypyrimidine* (*Isobarbituric Acid*) (II; R = OH).—The above dihydroxypyrimidine (0·5 g.) and 6N-hydrochloric acid (15 ml.) were boiled for 1 hr. The resulting solution was cooled and the product was recrystallised from water containing a little sodium dithionite. The trihydroxypyrimidine (0·22 g., 75%) had m. p. >300° (Found: C, 37·1; H, 3·2; N, 22·0. Calc. for $C_4H_4N_2O_3$: C, 37·5; H, 3·1; N, 21·9%).

5-*Benzyloxy*-2,4-*dichloropyrimidine*.—5-Benzyloxy-2,4-dihydroxypyrimidine (0·5 g.), phosphoryl chloride (6 ml.), and dimethylaniline (0·3 ml.) were boiled under reflux for 3 hr. After being cooled, the mixture was poured on to ice, and starting material (0·2 g.) was removed by filtration. The filtrate was extracted with ether, thereby giving an oil which was purified by sublimation at 125—130°/17 mm. The *dichloropyrimidine* (30 mg., 5%) formed needles, m. p. 88—90° (Found: C, 52·1; H, 3·0; N, 11·0. $C_{11}H_8Cl_2ON_2$ requires C, 51·8; H, 3·1; N, 11·0%).

5-*Benzyloxy*-4-*hydroxy*-2-*methylpyrimidine* (I; R = Me).—Benzyl benzyloxyacetate (25·6 g.) was formylated as for (I; R = NH_2), and to the formyl derivative acetamidine hydrochloride (9·5 g.) and ethanol (40 ml.) were added. The mixture was boiled for 8 hr., then poured into water (150 ml.) and brought to pH 5 by addition of hydrochloric acid. The resulting precipitate was recrystallised from water giving the pyrimidine hemihydrate (9·0 g., 40%) as needles, m. p. 187—190° (lit.,[9] 186°) (Found: C, 63·8; H, 6·2; N, 12·5. Calc. for $C_{12}H_{12}N_2O_2,\frac{1}{2}H_2O$: C, 64·0; H, 5·8; N, 12·4%).

[12] Brown, *J. Soc. Chem. Ind.*, 1950, **69**, 353.
[13] Part XIV, Hurst and McOmie, *J.*, 1964, in the press.

4,5-Dihydroxy-2-methylpyrimidine (II; R = Me).—The above-named compound (0·3 g.) and 6N-hydrochloric acid (10 ml.) were boiled for 20 min. The solution was concentrated under reduced pressure and neutralised with sodium hydrogen carbonate. The precipitate was collected and recrystallised from water, giving the dihydroxypyrimidine (0·12 g., 71%) as needles, m. p. >300° (Found: C, 47·9; H, 4·7; N, 22·4. Calc. for $C_5H_6N_2O_2$: C, 47·6; H, 4·8; N, 22·2%).

Paper Chromatography.—The purity and identity or otherwise of samples of 2- and 6-amino-4,5-dihydroxypyrimidine were shown by downward chromatography in three solvent mixtures, the spots being detected by examination under ultraviolet light. (*a*) Acetic acid, water, ethyl acetate (1 : 2 : 3 by volume); (*b*) benzene, water, t-butyl alcohol (1 : 3 : 4); (*c*) water, ethanol, benzene (1 : 3 : 11).

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