84. *Experiments on the Synthesis of Purine Nucleosides. Part V. The Coupling of Pyrimidine Derivatives with Diazonium Salts. A Method for the Preparation of 5-Aminopyrimidines.*

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The work described was undertaken in order to devise a method for the introduction of a 5-amino-group nto 6-amino-4-glycosidaminopyrimidines which would not involve danger of hydrolysing the sugar linkage. In model experiments on sugar-free compounds it is shown that 4 : 6-diaminopyrimidines couple with reactive diazonium compounds, *e.g.*, from *p*-nitroaniline or *p*-chloroaniline, in presence of sodium hydrogen carbonate to give brightly coloured azo-compounds in which the azo-substituent is in position 5; catalytic hydrogenation of these products yields 4 : 5 : 6-triaminopyrimidines. It has also been shown that the coupling of 4-methyluracil with diazotised *p*-chloroaniline yields the 5-*p*-chlorobenzeneazo-derivative. A survey of the structural conditions governing the coupling of pyrimidine derivatives and their relation to those governing nitrosation has been made.

For a successful synthesis of 9-glycosidopurines by the hypothetical route based on the model experiments on 9-alkylpurines described in Part I (Baddiley, Lythgoe, McNeil, and Todd, J., 1943, 383), 5 : 6-diamino-4-glycosidaminopyrimidines would be essential intermediates. It was shown in Part III (Baddiley, Lythgoe, and Todd, J., 1943, 571) that 6-amino-4-glycosidaminopyrimidines may be prepared from suitably substituted 4 : 6-diaminopyrimidines by condensation with sugars. In order to utilise these glycosides for the synthesis of 9-glycosidopurines it was clearly necessary to establish methods whereby an amino-group could be introduced into position 5 of the pyrimidine nucleus under conditions which would not simultaneously cause removal of the sugar residue.

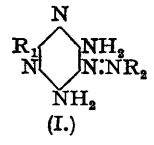
It was decided in the first instance to examine the problem by using sugar-free 4 : 6-diaminopyrimidines, and this paper records our results.

Hitherto the commonest methods used to introduce a 5-amino-group into pyrimidine derivatives have been nitration or nitrosation, followed by reduction. Of these methods, both limited in scope, nitration which involves strongly acid conditions seemed of little value for our purpose. Nitrosation, provided it could be carried out in the absence of mineral acids, might be quite suitable. Unfortunately, however, although 4 : 6-diamino-2-methylthiopyrimidine nitrosates readily in aqueous acetic acid and the method might therefore apply to its glycosides, the presence of mineral acid is necessary in order to nitrosate 4 : 6-diamino-2-methylpyrimidine (Part I; *loc. cit.*) and 4 : 6-diaminopyrimidine.

We therefore turned our attention to the reaction between 4 : 6-diaminopyrimidines and diazonium compounds in the hope that coupling would occur in position 5. Little systematic study has been made of the coupling of pyrimidines with diazonium compounds although there are various reports of its occurrence. Evans (*J. pr. Chem.*, 1893, **48**, 489) mentions the production of a red, rather unstable, substance on treating 2-hydroxy-4 : 6-dimethylpyrimidine with diazotised aniline in presence of sodium acetate; no structure was assigned to this substance. Somewhat later, Steudel (*Z. physiol. Chem.*, 1904, **42**, 170) and Pauly (*ibid.*, p. 512) noted the production of red colours when diazotised sulphanilic acid was coupled with thymine or 6-methyluracil. Johnson and Clapp (*J. Biol. Chem.*, 1908, **5**, 163) used the production or non-production of colour on treating diazotised sulphanilic acid with uracil, thymine, cytosine, and several of their derivatives as a criterion in an endeavour to locate the position of the sugar or phosphate residues in natural pyrimidine nucleotides; these authors record unsuccessful attempts to isolate the coloured product thus obtained from thymine but do not appear to have otherwise investigated the nature of the reaction products. More recently, Bogert and Davidson (*Proc. Nat. Acad. Sci.*, 1932, **18**, 215) coupled *iso*barbituric acid with diazotised aniline and obtained alloxan-6-phenylhydrazone; they also prepared several 5-azo-pyrimidines by indirect methods, *e.g.*, by coupling diazouracil with phenols.

In preliminary experiments it was found that, although diazotised aniline would not couple with 4 : 6-diaminopyrimidines, coupling proceeded smoothly in presence of sodium hydrogen carbonate with more reactive components such as diazotised *p*-nitroaniline or *p*-chloroaniline, yielding high-melting, brightly-coloured azo-compounds. In each case examined coupling occurred at position 5 in the pyrimidine nucleus. Thus 4 : 6-diamino-2-methylpyrimidine coupled with diazotised *p*-nitroaniline to yield 4 : 6-*diamino*-5-p-*nitrobenzeneazo*-2-*methylpyrimidine* (I; $R_1 = Me$, $R_2 = C_6H_4 \cdot NO_2$). The structure of this product follows from

its reduction by hydrogen in presence of Raney nickel to 4 : 5 : 6-*triamino*-2-*methylpyrimidine*, identical with the reduction product from 4 : 6-*diamino*-5-*benzeneazo*-2-*methylpyrimidine* (I; R_1 = Me, R_2 = Ph) (synthesised from acetamidine and benzeneazomalononitrile). An attempt to synthesise (I; R_1 = Me, R_2 = $C_6H_4{\cdot}NO_2$) directly from p-*nitrobenzeneazomalononitrile* and acetamidine was unsuccessful, extensive decomposition occurring on condensation in presence of sodium ethoxide. In similar fashion 4 : 6-diamino-2-methylpyrimidine coupled with diazotised *p*-chloroaniline gave 4 : 6-*diamino*-5-p-*chlorobenzeneazo*-2-*methylpyrimidine* (I; R_1 = Me, R_2 = C_6H_4Cl), identical with the condensation product of acetamidine and p-*chlorobenzeneazomalononitrile*. By coupling with the appropriate components, 4 : 6-diaminopyrimidine gave in the same way 4 : 6-*diamino*-5-p-*nitrobenzeneazopyrimidine* (I; R_1 = H, R_2 = $C_6H_4{\cdot}NO_2$) and 4 : 6-*diamino*-5-p-*chlorobenzeneazopyrimidine* (I; R_1 = H, R_2 = C_6H_4Cl).


(I.)

Reductive fission of the azo-linkage in these compounds proceeds smoothly, and coupling followed by reduction thus offers a route for the introduction of a 5-amino-group, which, if it could be applied to a 6-amino-4-glycosidaminopyrimidine, would meet all our requirements since there is little danger of hydrolysis at any stage. A practical point worthy of note is that in applying this method to the preparation of 4 : 5 : 6-triaminopyrimidines the use of the *p*-nitrobenzeneazo-compounds is not recommended; owing to the similarity in their solubilities the separation of the triamino-compounds from the *p*-phenylenediamine simultaneously formed on reduction is difficult and wasteful. *p*-Chlorobenzeneazo-compounds have not this disadvantage.

In view of the potential importance of this method for preparing 5-aminopyrimidines a series of experiments was made to ascertain the structural requirements for successful coupling. In the majority of cases the dyes were not isolated, colour being taken as an indication of coupling. In order to confirm that coupling occurs at position 5 in the uracils we did, however, isolate 2 : 6-*dihydroxy*-5-p-*chlorobenzeneazo*-4-*methylpyrimidine* from the reaction between 4-methyluracil and diazotised *p*-chloroaniline; the location of the azo-substituent was confirmed by reduction of the product to 2 : 6-dihydroxy-5-amino-4-methylpyrimidine.

The results of these coupling experiments are set out in the table; for comparison, the behaviour of the same compounds towards nitrous acid is included.

Reaction of Some Pyrimidine Derivatives with Diazotised p-*Nitroaniline and with Nitrous Acid.*

Substituents in pyrimidine.					Substituents in pyrimidine.				
2.	4.	6.	Coupling.	Nitrosation.	2.	4.	6.	Coupling.	Nitrosation.
SH	OH	OH	+ ($NaHCO_3$)	+ ($CH_3{\cdot}CO_2H$)	SH	OH	Me	+ ($NaHCO_3$)	—
SH	NH_2	NH_2	+ ,,	+ ,,	NH_2	OH	Me	+ ,,	—
SMe	OH	NH_2	+ ,,	+ ,,	OH	OH	H	+ (Na_2CO_3)	—
SMe	NH_2	NH_2	+ ,,	+ ,,	OH	OH	Me	+ ,,	—
OH	OH	OH	+ ,,	+ ,,	OH	OH	Et	+ ,,	—
OH	OH	NH_2	+ ,,	+ ,,	SMe	OH	Me	—	—
OH	NH_2	NH_2	+ ,,	+ ,,	SMe	Cl	NH_2	—	—
NH_2	OH	NH_2	+ ,,	+ ,,	NH_2	Cl	Cl	—	—
Me	OH	OH	+ ,,	+ ,,	Cl	Cl	NH_2	—	—
Me	OH	NH_2	+ ,,	+ ,,	Me	Cl	NH_2	—	—
α-Furyl	OH	OH	+ ,,	+ ,,	Me	Me	NH_2	—	—
Me	NH_2	NH_2	+ ,,	+ (HCl)	OH	Me	Me	—	+ (on heating nitrite with water)
H	OH	OH	+ ,,	+ (HCl followed by Na_2CO_3)					
H	NH_2	NH_2	+ ,,	+ (HCl followed by Na_2CO_3)					

From the tabulated results it would appear that in general only those pyrimidines nitrosate in which both positions 4 and 6 are occupied by such groups as OH or NH_2. For coupling with a diazonium compound two such substituents are also necessary, but these may be in the 2 : 4- or 4 : 6-positions, and in the former case the substituent at 2 may be OH, NH_2, or SH. The only apparent exception to these rules so far noted is the remarkable case of 2-hydroxy-4 : 6-dimethylpyrimidine, which yields with nitrous acid a nitrite converted into a " nitroso-compound " on boiling with water (Majima, *Ber.*, 1908, **41**, 185). No evidence for the structure of this product has been presented other than an elementary analysis. In contrast to the finding of Evans (*loc. cit.*), we were unable to couple 2-hydroxy-4 : 6-dimethylpyrimidine with diazotised *p*-nitroaniline. A point of interest in the coupling of pyrimidines is Johnson and Clapp's observation (*loc. cit.*) that coupling, or at any rate development of colour, occurs between diazotised sulphanilic acid and, *e.g.*, thymine (2 : 6-dihydroxy-5-methylpyrimidine) in which the 5-position is already substituted. We have confirmed their observation, and in the case of 2 : 6-dihydroxy-4-methyl-5-*n*-butylpyrimidine treated with diazotised *p*-chloroaniline we have endeavoured to isolate the deep red products. Identification of the reaction products is not yet complete, but present indications are that the reaction is more complex than that which occurs with compounds containing a free 5-position. The development of colour on mixing with the diazo-solution is appreciably slower, and chromatographic analysis indicates the presence in the product of at least two coloured substances; these products, which are formed in poor yield, show unexpectedly high solubility and have not been obtained crystalline.

As regards the mechanism of the coupling and nitrosation reactions of pyrimidines, several interpretations would seem possible; the evidence at present available does not, however, permit of a decision between them

Experimental.

p-*Nitrobenzeneazomalononitrile.*—Aqueous sodium acetate (140 g. in 250 c.c.) was added to a solution of malononitrile (10 g.) in alcohol (200 c.c.), and the mixture cooled in ice. *p*-Nitroaniline (20·9 g.) was dissolved in dilute hydrochloric acid (85 c.c., *d* 1·16, diluted with 200 c.c. of water), diazotised in the usual manner, and the ice-cold diazonium salt solution added slowly with stirring to the above malononitrile–sodium acetate mixture. After a few minutes the yellow *azo-compound* was collected, washed with water, and recrystallised from alcohol; orange-yellow needles (24·5 g.), m. p. 222° (decomp.) (Found: C, 50·3; H, 2·6; N, 32·4. $C_9H_5O_2N_5$ requires C, 50·2; H, 2·3; N, 32·6%).

p-*Chlorobenzeneazomalononitrile.*—This was prepared in precisely similar fashion from diazotised *p*-chloroaniline; yellow needles, m. p. 188—190° (decomp.) (Found: C, 53·0; H, 2·6; N, 27·3; Cl, 17·4. $C_9H_5N_4Cl$ requires C, 52·8; H, 2·5; N, 27·4; Cl, 17·3%).

4 : 6-*Diamino*-5-p-*nitrobenzeneazo*-2-*methylpyrimidine.*—*p*-Nitroaniline (6·9 g.) was dissolved in dilute hydrochloric acid (35 c.c., *d* 1·16, diluted with 200 c.c. of water), cooled in ice, and diazotised in the usual way. 4 : 6-Diaminopyrimidine (6·2 g.; Part I, *loc. cit.*) was dissolved in water (50 c.c.), cooled in ice, and the above diazonium salt solution added, followed by aqueous sodium hydrogen carbonate (29·4 g. in 400 c.c.). The mixture immediately became red, and after 30 minutes the scarlet solid (11·2 g.) which had separated was collected, washed, and dried. A sample of the product, which did not melt below 360°, was recrystallised for analysis from pyridine (Found: C, 48·6; H, 4·3; N, 36·4. $C_{11}H_{11}O_2N_7$ requires C, 48·3; H, 4·1; N, 35·9%).

4 : 6-*Diamino*-5-*benzeneazo*-2-*methylpyrimidine.*—Acetamidine hydrochloride (4·8 g.) was added to benzeneazomalononitrile (8·5 g.; Hantzsch and Thompson, *Ber.*, 1905, **38**, 2266) dissolved in alcoholic sodium ethoxide (1·2 g. of sodium in 50 c.c. of alcohol). After standing for 1½ hrs., the mixture was refluxed for 1½ hrs., left overnight, and the separated solid collected and washed with water. Recrystallised from pyridine, 4 : 6-*diamino*-5-*benzeneazo*-2-*methylpyrimidine* formed brownish-yellow prisms (3·8 g.), m. p. 311° (decomp.) (Found: C, 58·0; H, 5·6; N, 36·9. $C_{11}H_{12}N_6$ requires C, 57·9; H, 5·3; N, 36·8%).

4 : 5 : 6-*Triamino*-2-*methylpyrimidine.*—(1) 4 : 6-Diamino-5-*p*-nitrobenzeneazo-2-methylpyrimidine (2·5 g.) was suspended in alcohol (250 c.c.) and hydrogenated during 7 hours at 100° under a pressure of 60 atm. in presence of Raney nickel. The almost colourless solution was filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in a little alcohol and precipitated with benzene; the product was purified by recrystallisation from *n*-butanol and sublimation at 120°/10^{-2} mm. 4 : 5 : 6-*Triamino*-2-*methylpyrimidine* was thus obtained in colourless needles, m. p. 252—254° (Found: C, 43·4; H, 6·5; N, 49·9. $C_5H_9N_5$ requires C, 43·2; H, 6·5; N, 50·3%).

(2) 4 : 6-Diamino-5-benzeneazo-2-methylpyrimidine (2·5 g.), suspended in alcohol (300 c.c.), was hydrogenated at 100°/60 atm., and the product worked up as above. The 4 : 5 : 6-triamino-2-methylpyrimidine obtained had m. p. 252—255°, not depressed by that prepared by route (1).

4 : 6-*Diamino*-5-p-*chlorobenzeneazo*-2-*methylpyrimidine.*—(a) *From* 4 : 6-*diamino*-2-*methylpyrimidine.* To an ice-cold solution of 4 : 6-diamino-2-methylpyrimidine (2 g.) in water (20 c.c.), diazotised *p*-chloroaniline (prepared in the usual manner from 2·55 g. of base in dilute hydrochloric acid) was added, followed by aqueous sodium hydrogen carbonate (5 g. in 70 c.c. of water). After standing overnight, the *azo-compound* (3·1 g.) was collected. Recrystallised from pyridine, it formed golden-yellow plates, m. p. 340—342° (decomp.) (Found: C, 50·5; H, 4·2; N, 32·2; Cl, 13·4. $C_{11}H_{11}N_6Cl$ requires C, 50·3; H, 4·2; N, 32·0; Cl, 13·5%).

(b) *From* p-*chlorobenzeneazomalononitrile.* A solution of acetamidine hydrochloride (2·9 g.) in alcohol (60 c.c.) was mixed with alcoholic sodium ethoxide (0·8 g. of sodium in 20 c.c. of alcohol), sodium chloride removed by filtration, and *p*-chlorobenzeneazomalononitrile (4·1 g.) added. After standing for an hour, the mixture was refluxed for 6 hours and set aside at room temperature. At the end of 2 days water was added, and the resulting yellow solid (1·0 g.) collected. Recrystallised from pyridine, it had m. p. 340—342° (decomp.) undepressed in admixture with the product prepared by method (a).

4 : 6-*Diamino*-5-p-*chlorobenzeneazopyrimidine.*—(a) *From* 4 : 6-*diaminopyrimidine.* 4 : 6-Diaminopyrimidine (2·0 g.; Part IV, J., 1943, 574) was coupled with diazotised *p*-chloroaniline (from 2·4 g. of base) in the manner described for 4 : 6-diamino-2-methylpyrimidine. Recrystallised from pyridine, the *azo-compound* formed yellow prisms, m. p. 301—302° (decomp.) (Found: C, 48·5; H, 3·8; N, 33·8; Cl, 14·5. $C_{10}H_9N_6Cl$ requires C, 48·3; H, 3·6; N, 33·8; Cl, 14·3%).

(b) *From* p-*chlorobenzeneazomalononitrile.* A solution of formamidine hydrochloride (2·4 g.) in alcohol (40 c.c.) was mixed with alcoholic sodium ethoxide (0·65 g. of sodium in 10 c.c. of alcohol), and *p*-chlorobenzeneazomalononitrile (4·1 g.) added. The mixture was stirred until the nitrile dissolved, left for 2 hours, refluxed for 3 hours, and set aside; after 4 days it was diluted with an equal volume of water, and the azo-compound (0·6 g.) collected and recrystallised from pyridine. It had m. p. 301—303°, undepressed in admixture with a sample prepared by method (a).

4 : 5 : 6-*Triaminopyrimidine.*—4 : 6-Diamino-5-*p*-chlorobenzeneazopyrimidine [2·0 g. prepared by method (a) above] was suspended in alcohol (200 c.c.) and hydrogenated during 2 hours at 100°/50 atm. by means of a Raney nickel catalyst. The almost colourless solution was filtered, evaporated under reduced pressure and, after removal of *p*-chloroaniline by washing with ether, the residue was recrystallised from alcohol. 4 : 5 : 6-Triaminopyrimidine (0·65 g.) was obtained as colourless needles, m. p. 255—257°; the mixed m. p. with an authentic specimen, m. p. 257° (Part II, *loc. cit.*), was 255—257°.

4 : 6-*Diamino*-5-p-*nitrobenzeneazopyrimidine.*—When 4 : 6-diaminopyrimidine (1·0 g.) was coupled with diazotised *p*-nitroaniline by the procedure used in the case of 4 : 6-diamino-2-methylpyrimidine, the *product* crystallised from nitrobenzene in bright red plates (1·0 g.) which did not melt below 360° (Found: C, 46·7; H, 3·4; N, 37·2. $C_{10}H_9O_2N_7$ requires C, 46·3; H, 3·5; N, 37·8%).

2 : 6-*Dihydroxy*-5-p-*chlorobenzeneazo*-4-*methylpyrimidine.*—*p*-Chloroaniline (10 g.), dissolved in N-hydrochloric acid (200 c.c.), was diazotised in the usual manner with sodium nitrite (6 g.). After addition of sodium hydrogen carbonate (15 g.) the diazo-solution was added to an aqueous solution of 4-methyluracil (10 g. in 1500 c.c.). Sodium carbonate solution (20 c.c. of 15%) was now added, and after 30 mins. the mixture was acidified with hydrochloric acid, and the orange-yellow *azo-compound* (4 g.) collected. Recrystallised from pyridine, it formed small prisms, m. p. 235° (decomp.) (Found: C, 49·6; H, 3·8; N, 21·4. $C_{11}H_9O_2N_4Cl$ requires C, 49·9; H, 3·4; N, 21·2%). In the preparation of this compound it is necessary to avoid adding large amounts of sodium carbonate solution; if this precaution is not taken the product is very impure.

When the above azo-compound was hydrogenated in the normal way by using Raney nickel it yielded 5-amino-2 : 6-dihydroxy-4-methylpyrimidine, m. p. 270° (decomp.) (yield, 50%), identical with a specimen prepared by hydrogenation of 2 : 6-dihydroxy-5-nitro-4-methylpyrimidine.

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