## 193. *The Synthesis of Pyrimidin-5-yl Sulphides and Disulphides. Part II.**

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The synthesis of di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide is reported. This compound is also produced during the formation of 6-amino-2 : 4-dihydroxypyrimidin-5-yl purin-6-yl sulphide from 6-mercaptopurine and 6-amino-5-bromouracil. Analogous reactions are described for derivatives of 1-methyluracil. An explanation is sought for the anomalous behaviour of certain pyrimidines.

In Part I,* convenient syntheses of di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide and di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) sulphide were described. We have since discovered early descriptions [2, 3] of "thiodialuric acid" which are of interest in connection with our work. Repetition of the preparations described by the German workers yielded a material which did not correspond to "thiodialuric acid," but was identical spectroscopically and polarographically with our di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide.

In our previous experiments it was found that although thiourea reacts satisfactorily with 6-amino-5-bromouracil, decomposition of the resulting *iso*thiourea yields a monosulphide instead of a disulphide. Since, for the purpose of biological tests, it is desirable to have the two pyrimidine rings joined by a reversibly dissociable linkage, further

* *J.*, 1954, 4206, is to be regarded as Part I.

[1] Barker, Luthy, and Dhar, *J.*, 1954, 4206.
[2] Nencki, *Ber.*, 1871, **4**, 722.
[3] Trzcinski, *Ber.*, 1883, **16**, 1057.

experiments have been carried out and a satisfactory synthesis of di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide, which has already been described briefly,[4] is now reported fully.

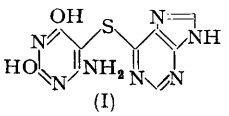
Introduction of the mercapto-group at the 5-position of uracil has recently been effected by displacement of the diazonium residue.[5] We found [1] that the halogen atom in 5-bromouracil is not displaced by thiourea, which is considered a strong nucleophilic reagent [6] and it therefore seemed possible that replacement of a diazonium residue might be effective in bringing about substitution at the 5-position of 6-aminouracil. However, after treatment of 5 : 6-diaminouracil with cold nitrous acid, there was no reaction with sodium disulphide. This may be due to a side reaction leading to the formation of pyrimidinotriazole derivatives.[7] Further experiments indicated that the inability of a reagent to effect substitution is not due simply to lack of susceptibility to nucleophilic attack. Thus, although displacement of halogen by the thiocyanate ion yields satisfactorily a disulphide derived from 5-mercaptobarbituric acid,[3] interaction of 4-amino-5-bromouracil with ammonium thiocyanate was very sluggish, and only small amounts of disulphide were detected polarographically in the resulting solution. Similarly, little or no reaction occurred between the bromo-compound and potassium ethyl xanthate; [8] with potassium hydrogen sulphide, the bromine atom was removed and 6-aminouracil was isolated as the main product. However, 6-amino-5-bromouracil reacts readily with sodium disulphide in 95% ethanol. A dark green colour, initially produced, quickly faded and then crystalline di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide gradually separated. After reprecipitation by acidification of an alkaline solution, the material was characterised as a disulphide by reduction at the dropping-mercury electrode.

There is little doubt that the reaction between sodium disulphide and 6-amino-5-bromouracil is a nucleophilic attack by the disulphide ion. However, a nucleophilic mechanism is difficult to reconcile with the failure to effect substitution by the reagents already mentioned. This may be due to the fact that 6-amino-5-bromouracil behaves in certain circumstances as a "positive-halogen" compound, as it liberates iodine immediately from potassium iodide solution. On the other hand, 5-bromouracil liberates iodine only very slowly. This has the effect of partly neutralising the normal polarisation of the $C_{(5)}$–Br bond, as suggested in analogous cases by Robertson and Waters.[9] This behaviour as a "positive-halogen" compound may explain the replacement of bromine by hydrogen on treatment of 6-amino-5-bromouracil with potassium hydrogen sulphide. Similar considerations may also explain the fission of a $C_{(5)}$–S bond in the conversion of 5-amidinothio-6-amino-2 : 4-dihydroxypyrimidine into a monosulphide.[1] It thus appears that potentially tautomeric groups at the 4- and the 6-position, which were shown in Part I to be necessary to facilitate reaction at the 5-position with thiourea (normally considered to be a nucleophilic substitution), may affect the reactivity at that carbon atom in a complex manner.

To investigate further the usefulness of sodium disulphide in substitution, experiments were carried out with a number of 5-bromopyrimidines. 6-Amino-5-bromo-1-methyluracil, but not 5 : 5-dibromohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine, reacted satisfactorily to yield the corresponding disulphide. This difference between the mono- and di-bromo-compounds was unexpected since both the above compounds react with thiourea to give the same *iso*thiourea. (Hydrolysis of the *iso*thiourea from 6-amino-1-methyluracil gave an intractable oil from which no sulphide or disulphide could be obtained.) As was to be expected 5-bromouracil also failed to react with sodium disulphide; in this case the typical green of the intermediate was produced as with 6-amino-5-bromouracil, but on the disappearance of the colour, the starting material was regenerated. No evidence is available to indicate the nature of these coloured complexes.

[4] Barker and Luthy, *Chem. and Ind.*, 1955, 983.
[5] Bardos, Herr, and Enkoji, *J. Amer. Chem. Soc.*, 1955, **77**, 960.
[6] Pearson, Langer, Williams, and McGuire, *J. Amer. Chem. Soc.*, 1952, **74**, 5130.
[7] Roblin, Lampen, English, Cole, and Vaughan, *ibid.*, 1945, **67**, 290.
[8] Cf. Djerassi, Gorman, Maskley, and Oldenburg, *ibid.*, 1955, **77**, 568.
[9] Robertson and Waters, *J.*, 1947, 492.

To explore further the potentialities as chemotherapeutic agents of sulphides and disulphides of the pyrimidine series, attention was turned to the synthesis of unsymmetrical compounds. In view of the use of 6-mercaptopurine in treating leukæmia, attempts have been made to prepare 6-purinyl 5-pyrimidinyl sulphides. However, interaction of 6-mercaptopurine and 6-amino-5-bromouracil in boiling alcohol yields di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide, identical with the material obtained by interaction of 6-amino-5-bromouracil with sodium disulphide, and a small quantity of 6-amino-2 : 4-dihydroxypyrimidin-5-yl purin-6-yl sulphide (I).



(I)

Similarly, 6-amino-5-bromo-1-methyluracil yielded the corresponding pyrimidinyl disulphide, but it was impossible to isolate any unsymmetrical monosulphide. The formation of the pyrimidinyl disulphides in these reactions is believed to result from the breakdown of the unsymmetrical monosulphide (as I) in which the $C_{(6)}$-atom of the purinyl residue is part of a thioamide system. Such a system would be expected to undergo fission at the $C_{(6)}$–S bond with the formation of a mercaptopyrimidine which would be subsequently converted into a disulphide. Owing to the difficulty of resolving the complex mixture of purines and pyrimidines, it has not been possible to identify with certainty the presence amongst the reaction products of hypoxanthine which would be expected according to the above scheme. However, paper chromatography of the mixture does not exclude its presence, and brief treatment of the pure monosulphide (I) with alkali results in the appearance of a polarographic wave characteristic of disulphides. No such wave is produced on similar treatment of 6-mercaptopurine.

All the disulphides discussed now and in Part I exhibited a unique set of polarographic waves, consisting of two small steps followed by a large reduction wave,[10] and the pattern could be used for identification purposes.

Di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl) disulphide was used as reference, and a quantitative study was made of the most prominent reduction wave which is also the one at the most negative potential. The value of $E_{\frac{1}{2}}$ for this wave is $-0{\cdot}58$ v against the saturated calomel electrode. It is diffusion-controlled in the range of concentration $10^{-3}$ to $10^{-4}$ molar (N/10 sodium hydroxide) since the wave-height is proportional to the concentration and to the ratio of the square roots of two heights of the mercury reservoir in accordance with the Ilkovic equation.

After twelve hours, alkaline solutions of the material show an approximately 5% drop in step-height and a drift in the position of the wave towards more positive reduction potential. This effect is most pronounced with di-(6-amino-2 : 4-dihydroxy-1-methylpyrimidin-5-yl) disulphide and less with the disulphides derived from barbituric acid and 6-aminouracil in descending order. It is believed to be due to the formation of a complex by the thiol at the surface of the mercury.

The other pyrimidinyl disulphides behave similarly to the reference in having half-wave potentials in the region $-0{\cdot}5$ to $-0{\cdot}6$ v against the anodic mercury pool. These waves are preceded by two smaller steps which, for di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl) disulphide are at $E_{\frac{1}{2}}$ $-0{\cdot}05$ and $-0{\cdot}25$ v, respectively, against the saturated calomel electrode. The first, which is often accompanied by a maximum, represents an oxidation and has been observed with a number of pyrimidines which contain no disulphide linkage : the second is not diffusion-controlled but varies irregularly with concentration and time. The nature of these waves will be discussed elsewhere.

## Experimental

M. p.s are corrected.

*Di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) Disulphide.*—Sodium disulphide [prepared from sodium sulphide nonahydrate (2·32 g.) and sulphur (0·28 g.) [11]] in hot 95% ethanol (150 c.c.) was added slowly to a stirred solution of 6-amino-5-bromouracil (1·65 g.) in boiling 95% ethanol (4·8 l.). The solution became dark green, quickly fading to pale yellow, and was refluxed for

[10] Cf. Karchmer and Walker, *Analyt. Chem.*, 1954, **26**, 271.

[11] Mellor, " A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Co., London 1941, Vol. II, p. 632.

8 hr. The precipitate was collected after a further 12 hr. at room temperature, washed repeatedly with ethanol and then ether, and dried. It was dissolved in 0·1N-sodium hydroxide (15 c.c.), and the filtered solution (charcoal) brought to pH 2 by addition of N-hydrochloric acid. The *di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide* (1·84 g.) was collected, washed, and dried as before (Found: C, 30·2; H, 2·8. $C_8H_8O_4N_6S_2$ requires C, 30·4; H, 2·5%). It does not melt below 350°, and is sparingly soluble in water and ethanol and insoluble in less polar solvents. Light absorption: $\lambda_{max.}$ 269 and 335 mμ ($\log_{10} \varepsilon_{max.}$ 4·33 and 3·66, respectively); $\lambda_{min.}$ 247 and 306 mμ (0·1N-sodium hydroxide). The compound exhibited an oxidation wave ($E_{\frac{1}{2}}$ −0·05 to −0·1 v) and reduction waves ($E_{\frac{1}{2}}$ −0·32 and −0·52 v) at the dropping-mercury electrode. The crystals exhibit parallel extinction under the polarising microscope. Addition of ethanol to an alkaline solution yields a yellow crystalline disodium salt which shows oblique extinction (average value 51·7°).

*6-Amino-5-bromo-1-methyluracil and 5 : 5-Dibromohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine.*—Bromine (2 c.c.) was added dropwise, with stirring, to a suspension of 6-amino-1-methyluracil (5·6 g.) in water (75 c.c.). Stirring was continued at room temperature for 12 hr. and the solid was then collected, washed with cold water, dried in air, and triturated three times with 95% ethanol (total, 200 c.c.). The residual *6-amino-5-bromo-1-methyluracil* (2 g.) separated from hot water in birefringent crystals showing oblique extinction between crossed Nicols (average value 42°), m. p. 278—280° (Found: C, 27·6; H, 2·85. $C_5H_6O_2N_3Br$ requires C, 27·2; H, 2·7%). Light absorption: $\lambda_{max.}$ 225 and 278 mμ ($\log_{10} \varepsilon_{max.}$ 3·73 and 4·2, respectively); $\lambda_{min.}$ 217 and 244 mμ. A further small quantity of the material slowly separated. The filtrate was concentrated to dryness under reduced pressure. The residual glassy *5 : 5-dibromohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine* crystallised, on the addition of water, and separated from 30% ethanol in prisms (2·8 g.) which sublimed at 100—101° (Found, in material dried at 20°/18 mm.: C, 19·15; H, 1·8. $C_5H_5O_2N_3Br_2,\frac{1}{2}H_2O$ requires C, 19·4; H, 1·9%). They show parallel extinction when observed under the polarising microscope. The mono- and di-bromo-compounds are readily distinguished by the optical properties of the crystals. The use of a large excess of bromine results exclusively in the formation of the dibromo-compound. Silver bromide is produced by warming the 5 : 5-dibromohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine with aqueous silver nitrate, but no test for halide ion is obtained in the cold. This behaviour is similar to that recorded for 5 : 5-dibromohexahydro-6-hydroxy-2 : 4-dioxopyrimidine by Wheeler and Johnson.[12]

*Di(hexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidin-5-yl) Disulphide.*—6-Amino-5-bromo-1-methyluracil (0·44 g.) in 95% ethanol (200 c.c.) was treated with a 15% solution of sodium disulphide in 95% ethanol (150 c.c.) as in the preparation of di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide. The *product* (0·51 g.) formed long acicular crystals which did not melt below 350° and showed parallel extinction under the polarising microscope (Found: C, 35·1; H, 3·8. $C_{10}H_{12}O_4N_6S_2$ requires C, 34·9; H, 3·5%). Light absorption: $\lambda_{max.}$ 267 and 339 mμ ($\log_{10} \varepsilon_{max.}$ 4·29 and 3·60, respectively); $\lambda_{min.}$ 242 and 308 mμ. The compound showed an oxidation wave ($E_{\frac{1}{2}}$ −0·27 and −0·55 v) at the dropping-mercury electrode.

*5-Amidinothiohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine.*—Thiourea (0·065 g.) in ethanol (15 c.c.) was added gradually to 5 : 5-dibromohexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidine (0·34 g.) in ethanol (15 c.c.), and the mixture refluxed for 1 hr., and then set aside at room temperature overnight. The microcrystalline *product* was collected, washed with ethanol and then ether, and recrystallised from 50% ethanol. The material sublimed at *ca.* 180°, forming thick prisms which did not melt below 350° and showed parallel extinction under the polarising microscope (Found: C, 30·35; H, 4·7. $C_6H_9O_2N_5S,H_2O$ requires C, 30·9; H, 4·7%). Treatment of an equivalent of 6-amino-5-bromo-3-methyluracil in the same way yielded an identical product.

*Interaction of 6-Amino-5-bromouracil and 6-Mercaptopurine.*—6-Mercaptopurine (0·155 g.) in warm 95% ethanol (100 c.c.) was added to a stirred boiling solution of 6-amino-5-bromouracil (0·2 g.) in 95% ethanol (500 c.c.). The solution was refluxed for 24 hr., a precipitate gradually appearing. The volume of the suspension was reduced to 300 c.c. by distillation of the ethanol; the remainder was cooled to room temperature, and di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide (0·07 g.) was collected and washed with ethanol and with ether. It was purified as described above (Found: C, 30·55; H, 2·5. Calc. for $C_8H_8O_4N_6S_2$: C, 30·4; H, 2·5%). The compound had the same ultraviolet absorption spectrum and exhibited the same polarographic behaviour as that recorded above. The filtrate from this material was concentrated to 100 c.c.,

[12] Wheeler and Johnson, *J. Biol. Chem.*, 1907, **3**, 187.

and 6-*amino*-2 : 4-*dihydroxypyrimidin*-5-*yl purin*-6-*yl sulphide* (0·022 g.) slowly separated and was collected and purified by acidification of a cold solution in N-sodium hydroxide (Found : C, 37·4; H, 3·0; S, 11·3. $C_9H_7O_2N_7S,0{\cdot}5H_2O$ requires C, 37·8; H, 2·8; S, 11·2%). Light absorption : $\lambda_{max.}$ 268 mμ; $\lambda_{min.}$ 245 mμ ($\log_{10} \varepsilon_{max.}$ 4·19; $\log_{10} \varepsilon_{min.}$ 3·0). The material did not melt below 350° and gave no polarographic reduction wave. It formed a yellow crystalline hydrobromide which was precipitated from an alcoholic solution by addition of ether, and which was reconverted into the free base by addition of sodium hydroxide solution.

*Interaction of* 6-*Amino*-5-*bromo*-1-*methyluracil and* 6-*Mercaptopurine*.—6-Mercaptopurine (0·152 g.) and 6-amino-5-bromo-1-methyluracil (0·22 g.) were refluxed in ethanol (150 c.c.) and di(hexahydro-6-imino-1-methyl-2 : 4-dioxopyrimidin-5-yl) disulphide (0·13 g.) was isolated as described in the previous experiment (Found : S, 19·4; N, 23·3. Calc. for $C_{10}H_{12}O_4N_6S_2$ : S, 18·6; N, 24·1%). It had the same ultraviolet absorption spectrum and exhibited the same polarographic behaviour as recorded above. Concentration of the mother liquors yielded only a mixture from which no pure monosulphide could be isolated.

All polarographic measurements were made with a Tinsley pen-recording polarograph, Mark 15. Fresh solutions ($10^{-4}$ molar) of di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl) disulphide in 0·1N-potassium hydroxide (Polaritan grade) were used as standards and measurements were made with two heights of the mercury reservoir (36 cm. and 64 cm.), the standard calomel half-cell being used as the reference anode. Measurements with other pyrimidinyl disulphides were made against the anodic mercury pool, to which the half-wave potentials are referred.

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