The Synthesis of 5-Pyrimidinyl Sulphides and Disulphides.

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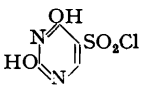
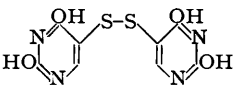
A study has been made of routes for the synthesis of 5-pyrimidinyl disulphides. A successful method has been found for the preparation of di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide from 5-bromo- or 5 : 5-dibromo-barbituric acid and thiourea. 6-Aminouracil on bromination gave 6-amino-5-bromo-2 : 4-dihydroxypyrimidine which with thiourea yielded a monosulphide instead of a disulphide. In both cases, intermediates were isolated, and their structures are discussed. The presence of disulphide linkages in the final products has been confirmed by a study of their behaviour at the dropping-mercury electrode. Ultra-violet and infra-red spectra are also recorded.

Since certain pyrimidines (Barker, Dhar, and Parsons, *Brit. J. Cancer*, 1949, **3**, 427; 1951, **5**, 124) resemble the ribonucleotides and their derivatives (Barakan, Barker, Gulland, and Parsons, *J. Path. Bact.*, 1948, **60**, 441) in exhibiting small but statistically significant acceleratory or inhibitory effects on the growth of grafted tumours in mice, a study has been made of methods for the synthesis of di-5-pyridimidinyl sulphides and disulphides in which hydroxy- or amino-groups, which have previously been shown to be necessary for biological activity, are present at positions 2, 4, and 6. The actions of these compounds on tumour growth will be described elsewhere.

Reduction with zinc and hydrochloric acid of the sulphonyl chloride (I), obtained by the action of chlorosulphonic acid on uracil, gave, in very low yield, the disulphide (II). In one experiment the zinc salt of 2 : 4-dihydroxy-5-mercaptopyrimidine was obtained.

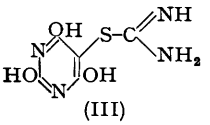
(I) 

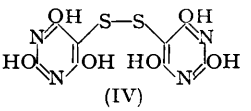
 (II)

However, the same series of reactions, when applied to 6-amino-2 : 4-dihydroxypyrimidine (6-aminouracil), gave a disulphide, also in small yield, which could not be obtained completely free from zinc. This approach was abandoned, however, since difficulty was experienced in the removal of zinc from the reduction product and the preparation of the sulphonyl chloride was not readily reproducible owing to hydrolysis to the sulphonic acid. In some instances the sulphonyl chloride could be converted into a sulphonamidopyrimidine, but in most cases an ammonium salt of the sulphonic acid was obtained.

Of the various methods for introduction of the mercapto-group into the pyrimidine ring, that which seemed most likely to suit the present purpose was interaction of a halogenated pyrimidine with thiourea followed by hydrolysis of the thiuronium residue (Polonovski and Schmitt, *Bull. Soc. chim.*, 1950, **17**, 616; Boarland and McOmie, *J.*, 1951, 1218). This method had not, however, been used for the introduction of the mercapto-group into the 5-position. Polonovski and Schmitt (*loc. cit.*) indeed record that "5-bromo-methyl uracil" (presumably 5-bromo-2 : 4-dihydroxy-6-methylpyrimidine) failed to react with thiourea. They suggested that this was due to the insolubility of the compound and to the unreactive nature of the halogen. In our hands, 5-bromo-2 : 4-dihydroxypyrimidine, in which the 6-methyl group of the above compound is replaced by hydrogen, also failed to react with thiourea at any dilution in alcohol or in aqueous alkaline solution. This suggested the possibility that the presence at both the 4- and the 6-position of groups capable of tautomerism might activate the halogen sufficiently to bring about reaction. In accordance, 5-bromo-2 : 4 : 6-trihydroxypyrimidine (5-bromobarbituric acid) reacted rapidly at room temperature with thiourea in alcohol. A colourless intermediate was formed to which we ascribe the structure (III). When intermediates were obtained by Polonovski and Schmitt and by Boarland and McOmie (*loc. cit.*), these consisted of thiuronium halides, but the intermediate obtained in the present case was free from halogen. It is possible that the *iso*thiourea (III) is stabilised by

internal salt formation or by hydrogen bonding. On hydrolysis with aqueous sodium hydroxide, it gave di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide (IV). This compound was also isolated as a disodium salt (see below).


(III)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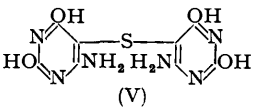
(V)

5 : 5-Dibromo-5 : 6-dihydro-2 : 4-dihydroxy-6-oxopyrimidine (5 : 5-dibromobarbituric acid) gave the same intermediate as the monobromobarbituric acid instead of both bromine atoms being replaced by *iso*thiourea residues. Since the dibromo-compound is more easily obtained, this starting material is preferred for the preparation of the disulphide (IV).

In its reaction with thiourea, 6-amino-5-bromo-2 : 4-dihydroxypyrimidine (6-amino-5-bromouracil), which is described for the first time, behaved differently from 5-bromobarbituric acid in two respects. First, the intermediate which was obtained was not so well characterised. It varied in colour from yellow to red : some samples contained up to approximately 5% of bromine which could not be removed by washing with water, but others analysed correctly for 5-amidinothio-6-amino-2 : 4-dihydroxypyrimidine. Extraction of the bromine-containing material with methanol yielded a small quantity of a yellow compound which was undoubtedly a thiuronium bromide. The red residue remaining after extraction could not be freed from halogen without bringing about hydrolysis. Treatment of any of these intermediates with aqueous sodium hydroxide resulted in the appearance of a purple colour which rapidly faded on heating. After acidification of the resulting solution, almost colourless di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) sulphide (V) was obtained. No disulphide analogous to (IV) was isolated. The same monosulphide can be obtained in good yield, without isolation of an intermediate, by treatment of an alkaline solution of 6-amino-5-bromo-2 : 4-dihydroxypyrimidine with thiourea.

In order to confirm the structures of the monosulphide and disulphides, it was desirable to have some specific method of identifying these classes of compound. Examination of ultra-violet absorption spectra did not distinguish between thiols, monosulphides, and disulphides. Infra-red spectra failed to show a band in the region of 2500 $cm.^{-1}$ which is regarded as characteristic of the mercapto-group in thioacetic acid (*Trans. Faraday Soc.*, 1949, **45**, 693). However, no band which could be assigned to the sulphide or disulphide linkage could be observed : identification of the C–S stretching vibrations would hardly be expected in molecules of this complexity (cf. Sheppard and Sutherland, *ibid.*, 1945, **41**, 261). A cluster of bands which could not be related to particular groupings, but which are useful for purposes of comparison, occur in the double-bond stretching region (cf. Short and Thompson, *J.*, 1952, 168).

In contrast to the failure of spectroscopic methods, the use of the polarograph clearly demonstrated the presence of disulphide linkages. Cavalieri and Lowy (*Arch. Biochem. Biophys.*, 1952, **35**, 83) have studied the reduction of various pyrimidines in acidic solution. According to Kolthoff and Lingane (" Polarography," Interscience Publ., New York, 1952, p. 779) only disulphides in alkaline solution would be expected to be reduced polarographically. Simple pyrimidines which did not contain a disulphide linkage gave no reduction wave. On the other hand, di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl) disulphide (Maggiolo and Hitchings, *J. Amer. Chem. Soc.*, 1951, **73**, 4226) and di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide showed a cathodic wave, which confirms their structures. The sodium salt of the latter compound also was reduced polarographically, indicating that it is the disodium salt of the disulphide and not the sodium salt of the corresponding thiol. After being allowed to stand for several days, however, alkaline solutions of di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide no longer gave a cathodic wave, owing presumably to the destruction of the disulphide linkage. This behaviour is analogous to changes in cystine and glutathione under similar conditons (Stricks and

Kolthoff, *Analyt. Chem.*, 1953, **25**, 1050). As was expected, di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) sulphide showed no reduction wave. Di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide and di-(2 : 4-dihydroxypyrimidin-5-yl) disulphide, obtained in small yields by reduction of the sulphonyl chorides, were, however, reduced polarographically. The zinc salt of 2 : 4-dihydroxy-5-mercaptopyrimidine gave no wave at a potential corresponding to the reduction of a disulphide, but showed only the normal cathodic wave of zinc in alkaline solution.

The formation of a monosulphide from 6-amino-5-bromouracil instead of a disulphide is not without precedent. Monosulphide formation has been observed in the thiazole (Watt, *J. Org. Chem.*, 1939, **4**, 436), pyridine (Surrey and Lindwall, *J. Amer. Chem. Soc.*, 1940, **62**, 1697), and quinoline series (Rosenhauer, Hoffmann, and Heuser, *Ber.*, 1929, **62**, 2730; Renfrew, *J. Amer. Chem. Soc.*, 1946, **68**, 1433), and also by Polonovski and Schmitt (*loc. cit.*) in the pyrimidine series. The reaction was believed to be due to either (*a*) interaction of excess of bromopyrimidine with a thiuronium derivative or a thiol or (*b*) interaction of a thiol and a thiuronium derivative. In the present experiments, since a sulphide was obtained from a halogen-free intermediate, reactions of type (*a*) can be excluded. A full explanation of the difference in behaviour between 5-bromobarbituric acid and 6-amino-5-bromouracil must await the elucidation of the structures of the coloured intermediates formed from the latter.

Experimental

M. p.s are corrected.

Chlorosulphonations (with M. M. Dhar).—The material (10 g.) was added gradually, with stirring, to chlorosulphonic acid (50 c.c.) and the temperature was not allowed to rise above 40°. The mixture was then heated on the steam-bath for ½ hr., and, after being cooled to room temperature, was poured on crushed ice (200 g.). The precipitate was collected and washed with water (50 c.c.) at 0°. For conversion into the sulphonamide, this material was added gradually to liquid ammonia (300 c.c.), the excess of ammonia was allowed to evaporate, and the residue was crystallised from hot water. Although in some experiments only an ammonium salt was obtained by this method, there were obtained, from uracil, 2 : 4-*dihydroxypyrimidine-5-sulphonamide* as needles, m. p. 305° (decomp.) (Found: C, 25·1; H, 2·6; N, 22·1. $C_4H_5O_4N_3S$ requires C, 25·2; H, 2·6; N, 22·0%), and, from 6-aminouracil, 6-*amino*-2 : 4-*dihydroxypyrimidine-5-sulphonamide* as needles, m. p. 268° (Found: C, 23·6; H, 2·9; N, 27·2. $C_4H_6O_4N_4S$ requires C, 23·3; H, 2·9; N, 27·2%).

Reduction of Sulphonyl Chlorides (with M. M. Dhar)—The sulphonyl chloride (from 5 g. of the parent pyrimidine) was suspended in 3N-hydrochloric acid (90 c.c.) and cooled in ice–salt. Zinc dust (9 g.) was added and the mixture was stirred overnight. The precipitate was collected and suspended in water, and 2N-sodium hydroxide was added, care being taken to avoid redissolving the precipitated zinc hydroxide. The zinc hydroxide was removed and the filtrate was neutralised with 2N-hydrochloric acid. The precipitate was collected and crystallised from hot water. By this method, the sulphonyl chloride from uracil gave *di*-(2 : 4-*dihydroxypyrimidin-5-yl*) *disulphide* which did not melt below 360° (Found: C, 33·0; H, 2·7; N, 18·6. $C_8H_6O_4N_4S_2$ requires C, 33·6; H, 2·1; N, 19·6%) [infra-red spectrum: peaks at 1769(m), 1715(s) (broad), 1660(s), and 1603(m) cm.$^{-1}$]. The sulphonyl chloride from 6-aminouracil, in one experiment, yielded impure di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) disulphide which did not melt below 360° (Found: C, 28·0; H, 3·0; N, 24·2; Ash, 2. Calc. for $C_8H_8O_4N_6S_2$: C, 30·4; H, 2·5; N, 26·6%) [infra-red spectrum: peaks at 1785(sh), 1730(s) (broad), 1640(s) (broad), 1567(ms), and 1520(m) cm.$^{-1}$], but in other experiments the material corresponded approximately to the zinc salt of 6-amino-2 : 4-dihydroxy-5-mercaptopyrimidine (Found: C, 26·1; H, 2·3. Calc. for $C_8H_6O_4N_4S_2Zn$: C, 27·3; H, 1·7%).

5-*Amidinothio*-2 : 4 : 6-*trihydroxypyrimidine* (III).—(*a*) To a solution of 5-bromobarbituric acid (Bock, *Ber.*, 1922, **55**, 3400) (1 g.) in ethanol (40 c.c.), thiourea (0·385 g.) in ethanol (25 c.c.) was added dropwise with stirring. The precipitate, which was formed immediately, was collected after the mixture had been warmed on the steam-bath for ½ hr. The *product* (1 g.) was washed with ethanol and dried at room temperature. It did not melt below 325° and gave a negative test for bromide ion after sodium fusion (Found: C, 28·8; H, 3·2; N, 26·4; S, 14·9. $C_5H_6O_3N_4S,\frac{1}{2}H_2O$ requires C, 28·8; H, 2·9; N, 26·5; S, 15·2%).

(*b*) To a solution of 5 : 5-dibromobarbituric acid (Bock, *loc. cit.*) (2·0 g.) in ethanol (15 c.c.), thiourea (0·65 g.) in ethanol (35 c.c.) was added dropwise with stirring. The product was isolated as described above. It did not melt below 325° and had the same ultra-violet absorption spectrum as the product obtained from 5-bromobarbituric acid (see Table 1).

TABLE 1. *Ultra-violet absorption spectra of substituted pyrimidines.*

Subst. at posn. 2	4	6	5	Solvent	$\lambda_{min.}$ (mμ)	$\lambda_{max.}$ (mμ)	$\log_{10} \varepsilon_{max.}$
OH	OH	—	Br	N-NaOH	249—250	281	3·95
OH	OH	NH_2	Br	N-NaOH	247	274	4·02
OH	OH	NH_2	–S–	N-NaOH	230	267	4·03
OH	OH	OH	$H_2N{\cdot}C(:NH)S$– [1]	H_2O	232—234	253—255	3·75
OH	OH	OH	–S–S– [1, 2]	N-NaOH	238—240	262—263	4·20
				H_2O	294—297	258—262	5·33
						315—320	4·84
OH	OH	OH	Br [3]	H_2O	238	269	5·18
				N-NaOH	242	269	4·03
OH	OH	CH_3	–S–S–	N-NaOH	250	285	4·24

[1] The same results were obtained with materials prepared from both mono- and di-bromobarbituric acid. [2] The same results were obtained with the disulphide and its yellow sodium salt obtained by precipitation with alcohol. [3] Dibromobarbituric acid in aqueous solution showed only end absorption.

The products obtained by both methods had identical infra-red spectra (Nujol mull), with peaks at 1780(sh), 1700(s), 1674(s), 1650(ms), 1625(s), 1597(s) (broad) cm.$^{-1}$.

Di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) Disulphide.—A solution of 5-amidinothio-2 : 4 : 6-trihydroxypyrimidine (0·9 g.) in N-sodium hydroxide (15 c.c.) was heated on the steam-bath for ½ hr. The hot solution was clarified by filtration and, after cooling, an equal volume of ethanol was added. The yellow crystalline *disodium* salt of di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) disulphide was collected and, after being dried at room temperature, did not melt below 320° (Found, by flame photometry : Na, 13·2. $C_8H_4O_6N_4S_2Na_2$ requires Na, 12·7%).

A solution at room temperature of the sodium salt was quickly brought to pH 1 by the addition of a few drops of concentrated hydrochloric acid, and then cooled to 0° (if the acidification was not carried out quickly, slight decomposition indicated by a smell of thiol was observed). The *disulphide* was collected by filtration and reprecipitated once more from alkaline solution. It formed colourless crystals, m. p. 263—264° (Found, in material dried at 50°/0·1 mm. : C, 27·9; H, 2·9; S, 18·9. $C_8H_6O_6N_4S_2,2H_2O$ requires C, 27·6; H, 2·9; S, 18·6. Found, in material dried at 100°/0·1 mm. : C, 30·1; H, 2·3. $C_8H_6O_6N_4S_2$ requires C, 30·2; H, 1·9%). The pointed needle-like crystals are highly birefringent and show both parallel and oblique extinction between crossed nicols of a polarising microscope. The oblique extinction angle was 20°. This disulphide was obtained in the same way from the *iso*thiourea derivative prepared from dibromobarbituric acid. The two products had the same m. p. which was not depressed by admixture. They had identical ultra-violet absorption spectra (see Table 1), and their infra-red spectra (Nujol mull) both showed peaks at 1690(s) (broad), and 1610(s) (broad) cm.$^{-1}$.

6-Amino-5-bromo-2 : 4-dihydroxypyrimidine.—6-Amino-2 : 4-dihydroxypyrimidine (Conrad, *Annalen*, 1905, **340**, 312) (12·7 g.) was suspended in carbon disulphide (125 c.c.), and bromine (16 g.) was added dropwise with stirring. After 12 hr. at room temperature, the mixture was boiled under reflux for 3 hr., and the reddish-brown product was freed under reduced pressure from carbon disulphide and excess of bromine. The pale yellow residue was washed first by trituration with warm water and filtered, then, on the filter, with further quantities of water until the filtrate was free from bromide ions, and finally with alcohol. The material (19·5 g.) was recystallised from hot water (1 g. from 625 c.c.) giving cream-coloured dendritic crystals of the *bromopyrimidine* which sublimed at 270° but did not melt below 350° (Found : N, 20·4. $C_4H_4O_2N_3Br$ requires N, 20·4%).

Di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) Sulphide.—(*a*) 6-Amino-5-bromo-2 : 4-dihydroxypyrimidine (5·6 g.) was dissolved in boiling ethanol containing 10% of water (4·5 l.), and a solution of thiourea (2·07 g.) in ethanol (150 c.c.) was added with stirring. The formation of a yellow precipitate began immediately, and during the addition the colour changed to deep orange. The mixture was boiled under reflux for 24 hr., cooled to room temperature, and filtered. The solid (2·3 g.), which was essentially 5-amidinothio-6-amino-2 : 4-dihydroxypyrimidine contained up to 5% of bromine which could not be removed by washing with

water. The filtrate was concentrated under reduced pressure to 400 c.c., and a further quantity of material (0·6 g.) was obtained. The combined solids were extracted with boiling methanol (100 c.c.). After filtration the yellow supernatant liquid was concentrated to 30 c.c. under reduced pressure and filtered from a small amount of insoluble reddish material. Addition of an equal volume of ether gave a yellow flocculent precipitate which was collected, washed with a few small portions of ether, and dried in air. The yellow crystalline product sublimed at approx. 290° but did not melt below 300°. It gave a positive test for bromine after sodium fusion (Found: C, 22·1; H, 3·4; S, 10·9; N, 24·4. $C_5H_8O_2N_5SBr$ requires C, 21·3; H, 2·8; S, 11·3; N, 24·8%). The orange-red residue left after extraction with methanol still gave a faint positive test for halogen after sodium fusion, but was not investigated further at this point. In one experiment on a small scale, an intermediate was obtained which was free from halogen. It formed anisotropic crystals which did not melt below 350° (Found: C, 29·7; H, 3·7; S, 16·5. $C_5H_7O_2N_5S$ requires C, 29·8; H, 3·5; S, 15·9%) [infra-red spectrum: peaks at 1785(w), 1720(s), 1620(s) (broad), and 1515(m) cm.$^{-1}$]. This *isothiourea* derivative (or the crude material containing a trace of bromine) (1·85 g.) was dissolved and boiled in N-sodium hydroxide (40 c.c.) for 3 hr. A deep purple colour was initially formed and quickly faded to light yellow. The hot solution was filtered (charcoal), cooled, and treated with acetic acid until precipitation was complete. The *sulphide* (1·63 g.), which tended to form a colloidal suspension, was collected and reprecipitated as white prisms by addition of acetic acid to its solution in sodium phosphate buffer at pH 9 (Found: C, 33·8; H, 3·3; N, 29·6; S, 11·6. $C_8H_8O_4N_6S$ requires C, 33·8; H, 2·8; N, 29·6; S, 11·3%).

(*b*) 6-Amino-5-bromo-2 : 4-dihydroxypyrimidine (1 g.) was suspended in water (50 c.c.) and dissolved by addition of a few drops of 5% aqueous sodium hydroxide. A solution of thiourea (0·38 g.) in water (25 c.c.) was added and the mixture was boiled under reflux for 1½ hr. The initial yellow colour faded and a cream-coloured microcrystalline precipitate was formed. It was collected, washed with water, ethanol, and ether, dried, and recrystallised from 5% aqueous disodium phosphate with the addition of a few drops of N-sodium hydroxide to effect dissolution. The sulphide was readily soluble in water at pH 11, but was reprecipitated at pH 8. It did not melt below 350° (Found: C, 33·6; H, 3·6%).

The products obtained by methods (*a*) and (*b*) had identical ultra-violet absorption (see Table 1) and infra-red spectra (Nujol mull), showing peaks at 1790(w), 1740(m), 1700(s), 1625(s) (broad), 1590(sh), and 1515(w) cm.$^{-1}$. For comparison, measurements were made of the infra-red spectra of 6-amino-2 : 4-dihydroxypyrimidine [peaks at 1727(m), 1695(ms), 1655(sh), 1625(s), 1597(s), and 1530(m) cm.$^{-1}$], and di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl) disulphide (Maggiolo and Hitchings, *loc. cit.*) [peaks at 1730(s), 1706(s), 1665(s), and 1590(m) cm.$^{-1}$].

TABLE 2. *Polarographic behaviour of substituted pyrimidines in alkaline solution.*

Disulphide	Solvent	$E_{\frac{1}{2}}$ (volts)
Di-(2 : 4-dihydroxy-6-methylpyrimidin-5-yl)	0·05N-KOH, 0·1N-NaOH	−0·35
Di-(2 : 4 : 6-trihydroxypyrimidin-5-yl) [a]	0·05N-KOH	−0·5
Di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl)	0·1N-NaOH	−0·25
Di-(2 : 4-dihydroxypyrimidin-5-yl)	0·1N-NaOH	−0·3

[a] The same result was obtained with the sodium salt of this compound obtained by precipitation with alcohol. The identical cathodic half-wave potential was observed in 0·05N-sulphuric acid with the addition of gelatin.

Polarographic Determinations (see Table 2).—Measurements were made with a Tinsley automatic recording polarograph (capillary drop-time, T, = 3·5 sec.) Concentrations were approx. 10^{-4}M. The zinc salt of 5-mercaptouracil gave only the expected reduction wave of the zinc ion in alkaline solution at −1·2 v. The following compounds gave no cathodic wave in 0·1N-sodium hydroxide: 6-aminouracil, 6-methyluracil, barbituric acid, 4 : 6-dimercaptopyrimidine, di-(6-amino-2 : 4-dihydroxypyrimidin-5-yl) sulphide.

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