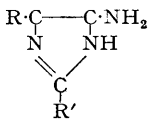
632. *Studies in the Azole Series. Part XXIII. A New Synthesis of 6-Aminopurines.*

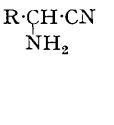
By A. H. Cook and E. Smith.

Representative thiazoles and glyoxalines bearing 5-thioureido- and 4-cyano-substituents have been shown to undergo cyclisation to thiazolopyrimidines and purines, respectively, on treatment with alkali. 9-Methyladenine and some related compounds have been prepared in this way.

The synthesis of purines of the xanthine and hypoxanthine classes bearing a variety of substituents, from glyoxalines (I; R = CO_2Et or $CO{\cdot}NH_2$) has already been described (Parts XIV and XX, this vol., pp. 1071, 2329). In Part XVII (*ibid.*, p. 1440) the possibility was envisaged of cyclising amino-nitriles (I; R = CN), by methods similar to those used in our



(I.)



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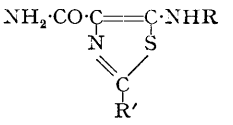


(III.)

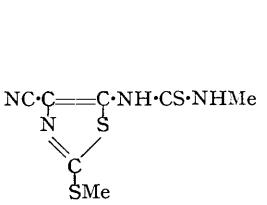
early work to give, readily, 5-membered rings bearing a 5-amino-substituent; this would, when applied to (I), of necessity lead to 6-membered rings and in particular to 6-aminopurines. The requisite amino-nitriles (I; R = CN) might have been obtained by (*a*) cyclising the so-far unknown aminomalononitrile (II; R = CN) in the manner of earlier work or (*b*) dehydrating appropriate amides (I; R = $CO{\cdot}NH_2$).

With regard to (*a*), the formation of aminomalononitrile by polymerising hydrogen cyanide was reported by Lange (*Ber.*, 1873, **6**, 99) but Bedel (*Compt. rend.*, 1923, **176**, 178) showed by means of ebullioscopic measurements that the product was in fact a tetrameride; further work, notably by Hinkel *et al.* (*J.*, 1937, 1433; 1939, 49; 1940, 1206), showed it to consist of aminoiminosuccinonitrile, and authentic aminomalononitrile does not seem to have been prepared. In the present work attempts were made to prepare the required nitrile by reducing oximinomalononitrile or benzeneazomalononitrile by various means. No satisfactory evidence of the formation of aminomalononitrile was obtained, though the reduction of the oximino-compound with aluminium amalgam led to a very small yield αβ-diaminopropionitrile (II; R = $CH_2{\cdot}NH_2$) (isolated as *oxalate* and characterised as *picrate* and *dibenzoyl* derivative).

Turning to the alternative (*b*), we first worked with model compounds, for it was foreseen that the acidic and relatively drastic nature of reagents required to dehydrate the appropriate heterocyclic amides might also necessitate the protection of a free amino-group as in (I; R = $CO{\cdot}NH_2$). As the project required the ultimate conversion of an amino- into a ureido- or thioureido-group, it seemed advantageous to use one of the latter as a permanent protective group and so avoid the complication of using some temporary protection. Accordingly, in these preliminary experiments 5-amino-2-methylthiothiazole-4-carboxyamide (III; R = H, R′ = SMe) was treated with methyl *iso*thiocyanate to give 5-N′-*methylthioureido*-2-*methylthiothiazole*-4-*carboxyamide* (III; R = $CS{\cdot}NHMe$, R′ = SMe) which with phosphorus tribromide in dioxan gave a labile phosphorus *derivative* from which a new compound, formally an anhydride of (III; R = $CS{\cdot}NHMe$, R′ = SMe), was obtained by cold alkali. The same new compound was obtained directly from (III; R = $CS{\cdot}NHMe$, R′ = SMe) by treatment with phosphorus oxychloride.

Now α-amino-nitriles and *iso*thiocyanates give, according to substitution and experimental conditions, thioureido-nitriles, substituted 2 : 5-aminothiazoles, or substituted 5-amino-2-mercaptoglyoxalines (*J.*, 1948, 1262, 1340). Three structures, (IV), (V), and (VI), accordingly came into consideration for the dehydration product. The compound dissolved only with difficulty in boiling dilute acid and was recovered unchanged on cooling; it dissolved in dilute alkali and was recovered unchanged on acidification; and it failed to give the murexide reaction characteristic of purines and thiazolopyrimidines. These properties practically excluded structures (V) and (VI); and, as the compound exhibited absorption in the ultra-violet region similar to that of the parent amide (III; R = $CS{\cdot}NHMe$, R′ = SMe) and markedly different from that of the comparable compound (VII), it was finally regarded as 5-N′-*methylthioureido*-4-*cyano*-2-*methylthiothiazole* (IV). Aqueous alkali or concentrated acid converted it into (VII), almost certainly through the imino-compound (VI); the identity of (VII) was established

beyond doubt by its methylation to the thiazolopyrimidine (VIII), identical with the substance obtained earlier (this vol., p. 1064). The ready emergence of (VII) rather than (VI) must be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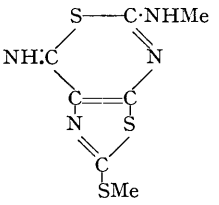
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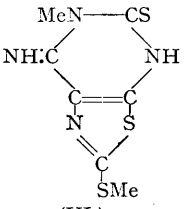
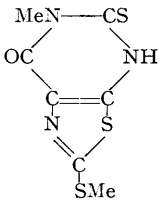

(VI.)

ascribed to the instability of the latter associated with its limited resonance possibilities, which may be correlated with the fact that 6-aminopurines cannot be alkylated in the 1-position.

After the above experience had been gained, 5-amino-2-methylthio-1-methylglyoxaline 4-carboxyamide (this vol., p. 1440) was further examined. It failed to react with potassium cyanate or thiocyanate under the usual conditions but with methyl and acetyl *iso*thiocyanate furnished 5-N′-*methylthioureido-* (IX; R = Me, R′ = SMe) and 5-N′-*acetylthioureido*-2-*methylthio*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = SMe), respectively. Incidentally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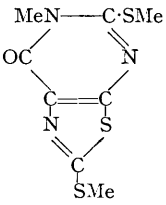
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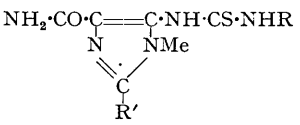
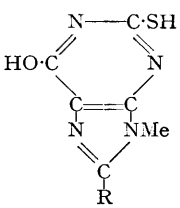

(IX.)

it was found that treating the parent glyoxaline with methyl *iso*thiocyanate in pyridine afforded 6-*hydroxy*-2-*mercapto*-8-*methylthio*-9-*methylpurine* (X; R = SMe) by elimination of methylamine from (IX; R = Me, R′ = SMe) which must have been the primary product; the elimination of methylamine rather than ammonia was proved by the preparation of the same purine (X; R = SMe) by alkaline hydrolysis and simultaneous cyclisation of (IX; R = Me, R′ = SMe). In parallel fashion, 5-amino-1-methylglyoxaline-4-carboxyamide and acetyl *iso*thiocyanate gave 5-N′-*acetylthioureido*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = H), whilst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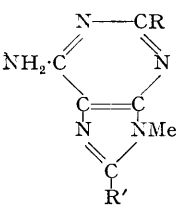
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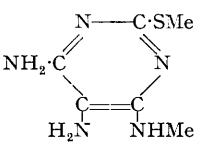

(XII.)

reaction with methyl *iso*thiocyanate in pyridine gave 6-hydroxy-2-mercapto-9-methylpurine (X; R = H), presumably by formation of the intermediate (IX; R = Me, R′ = H) and elimination of methylamine therefrom : the reaction of 5-amino-1-methylglyoxaline-4-carboxyamide with carbon disulphide to give this purine has been recorded earlier (this vol., p. 2329). Phosphorus oxychloride converted the thioureido-compound (IX; R = Ac, R′ = SMe) into a *dichloro*-derivative of incompletely elucidated structure, its precise nature being unimportant in the present connection as on alkaline hydrolysis it underwent deacetylation with concomitant cyclisation to give 6-*amino*-2-*mercapto*-8-*methylthio*-9-*methylpurine* (XI; R = SH, R′ = SMe) which formed a picrate; on methylation with methyl sulphate and alkali this purine gave 6-*amino*-2 : 8-*dimethylthio*-9-*methylpurine* (XI; R = R′ = SMe) which was also characterised as its *picrate*. The justification for these formulations rests on the following considerations.

(*a*) 5 : 6-Diamino-4-methylamino-2-methylthiopyrimidine (XII) (Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 385) and carbon disulphide in pyridine gave a product which was subsequently formulated as 6-*amino*-8-*mercapto*-2-*methylthio*-9-*methylpurine* (XI; R = SMe,

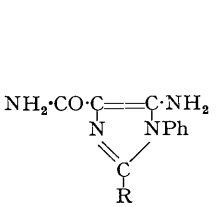
R′ = SH). That this product contained a mercapto-group was demonstrated by its reaction with chloroacetic acid to give 6-*amino*-2-*methylthio*-8-*carboxymethylthio*-9-*methylpurine* (XI; R = SMe, R′ = S·CH_2·CO_2H). This formulation must be correct because on treating the former product with methyl sulphate and alkali the compound postulated above as (XI; R = R′ = SMe) was again obtained. This synthesis clearly establishes the existence of the 6-aminopyrimidine ring in the earlier preparation and incidentally proves that, when completion of the glyoxaline ring is effected by treating (XII) with carbon disulphide, cyclisation occurs between the 5-amino- and 4-methylamino-groups rather than between the 5- and 6-amino-groups. The latter observation is, of course, no more than another illustration of the reaction of the pyrimidine (XII) as the tautomeric 6-imino-1 : 6-dihydropyrimidine (Baddiley, Lythgoe, McNeil, and Todd, *loc. cit.*).

(*b*) Still more direct proof of the structure of (XI; R = R′ = SMe) was obtained by desulphurisation of the compound with Raney nickel to give 9-methyladenine (XI; R = R′ = H), identical with a specimen which had been prepared by methylating adenine from natural sources.

This new aminopurine synthesis starting from the glyoxaline ring appears to be general. Thus 5-amino-2-mercapto-1-methylglyoxaline-4-carboxyamide with benzyl chloride and alkali yielded the 2-*benzylthio*-compound whence acetyl *iso*thiocyanate gave 5-N′-*acetylthioureido*-2-*benzylthio*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = S·CH_2Ph). This on dehydration with phosphorus oxychloride gave a *dichloro*-derivative, hydrolysed by alkali to 6-*amino*-2-*mercapto*-8-*benzylthio*-9-*methylpurine* (XI; R = SH, R′ = S·CH_2Ph), which with methyl sulphate and alkali gave 6-*amino*-8-*benzylthio*-2-*methylthio*-9-*methylpurine* (XI; R = SMe, R′ = S·CH_2Ph) characterised as its *picrate*. The isomeric *purine* (R = S·CH_2Ph, R′ = SMe) was obtained by benzylating (XI; R = SH, R′ = SMe), prepared above, and was also characterised as its *picrate*.

It can be seen that the foregoing amino-purines contain a methyl group in the 9-position, which was a consequence of using methyl *iso*thiocyanate in building up the glyoxaline ring (Part XVII). Accordingly the use of other appropriate *iso*thiocyanates in this connection should lead to 9-substituted aminopurines in general: as described below, some 9-phenyl analogues have now been prepared.

Aminocyanoacetamide (II; R = CO·NH_2) with 1 mol. of phenyl *iso*thiocyanate gives 5-*amino*-2-*anilinothiazole*-4-*carboxyamide* (III; R = H, R′ = NHPh), but with an excess of the *iso*thiocyanate gives 2-*anilino*-5-N′-*phenylthioureidothiazole*-4-*carboxyamide* (III; R = CS·NHPh, R′ = NHPh). The isomerisation of 2 : 5-diaminothiazoles with alkali to give 2-mercaptoglyoxalines has been described earlier (*J.*, 1948, 1262, 1340) although rearrangement of the 2-anilinothiazoles has not hitherto been reported. It was found that 5-amino-2-anilinothiazole-4-carboxyamide (III; R = H, R′ = NHPh) in refluxing aqueous alkali gave a diazotisable isomeride, formulated as 5-*amino*-2-*mercapto*-1-*phenylglyoxaline*-4-*carboxyamide* (XIII; R = SH). As expected, this product dissolved in alkali and when this solution was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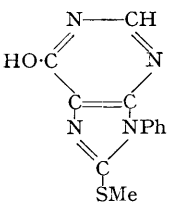
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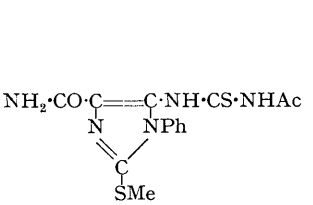
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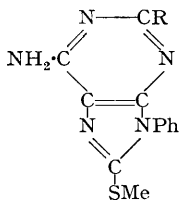
(XVI.)

shaken with methyl sulphate a diazotisable monomethyl derivative separated from solution. This was formulated as 5-*amino*-2-*methylthio*-1-*phenylglyoxaline*-4-*carboxyamide* (XIII; R = SMe); condensation with formic acid (cf. Cook and Smith, this vol., p. 2329) gave what must be 6-*hydroxy*-8-*methylthio*-9-*phenylpurine* (XIV), whilst reaction with acetyl *iso*thiocyanate gave 5-N′-*acetylthioureido*-2-*methylthio*-1-*phenylglyoxaline*-4-*carboxyamide* (XV). Dehydration of (XV) with phosphorus oxychloride gave a *dichloro*-derivative of the 4-*cyano*-compound, deacetylated by alkali to 6-*amino*-2-*mercapto*-8-*methylthio*-9-*phenylpurine* (XVI; R = SH), Methylation then gave 6-*amino*-2 : 8-*dimethylthio*-9-*phenylpurine* (XVI; R = SMe) (characterised as its *picrate*), and treatment with benzyl chloride gave 6-*amino*-2-*benzylthio*-8-*methylthio*-9-*phenylpurine* (XVI; R = S·CH_2Ph).

Experimental.

Reduction of Oximinomalononitrile.—The silver salt of oximinomalononitrile (290 g.), dried over phosphoric oxide *in vacuo* and thoroughly powdered, was suspended in ether (1 l.), and hydrogen sulphide was passed in for 1½ hours with vigorous stirring. The solution was filtered and the filtrate evaporated to a small bulk *in vacuo*. The residual brown oil was filtered from a little sulphur, dissolved in ether (200 c.c.), and added dropwise during 2 hours to amalgamated aluminium foil strips (90 g.) in ether (2 l.) containing water (80 c.c.) with vigorous stirring, care being taken to prevent any undue rise in temperature. The solution was stirred for a further hour and filtered. The alumina was extracted with boiling ethanol (600 c.c.), and to the combined filtrates was added oxalic acid (120 g.) in ether (400 c.c.), precipitating a crystalline solid. The solution was kept overnight at 0° to complete precipitation, and the product (35 g.), m. p. 145°, was collected and crystallised from aqueous ethanol (charcoal) in small colourless rods, m. p. 161° (decomp.), of *αβ-diaminopropionitrile oxalate* (Found : C, 34·5; H, 4·95. $C_5H_9O_4N_3$ requires C, 34·3; H, 5·1%). To the oxalate (1 g.) in water (25 c.c.) containing sodium hydrogen carbonate (3 g.) was added benzoyl chloride (1·5 g.) in ether (25 c.c.). The solution was shaken for 30 minutes, and the product which separated (2·0 g.) was collected, washed with water and then ether, and crystallised from ethanol, to give colourless needles of *αβ-dibenzamidopropionitrile*, m. p. 238—239° (Found : C, 69·0; H, 5·2; N, 13·7. $C_{17}H_{15}O_2N_3$ requires C, 69·6; H, 5·2; N, 14·3%). The base was extracted from the oxalate as described above, and the *dipicrate*, prepared by the usual procedure, was crystallised from aqueous ethanol, giving rods, m. p. 138—139° (decomp.) (Found : C, 31·3; H, 3·3; N, 22·2. $C_{15}H_{13}O_{14}N_9,2H_2O$ requires C, 31·1; H, 2·9; N, 21·8%).

5-N′-Methylthioureido-2-methylthiothiazole-4-carboxyamide (III; R = H, R′ = SMe).—5-Amino-2-methylthiothiazole-4-carboxyamide (cf. Part XVII, *loc. cit.*) (0·9 g.) in pyridine (2·5 c.c.) was heated under reflux for 2 hours with excess of methyl *iso*thiocyanate (0·8 g.). The solution was cooled and diluted with water (10 c.c.), precipitating a colourless *product* (1·1 g.), m. p. 172—174°. This was collected, washed with water and ether, and recrystallised from aqueous ethanol giving needles, m. p. 179° (Found : C, 32·0; H, 4·1. $C_7H_{10}ON_4S_2$ requires C, 32·1; H, 3·8%). Light absorption (ethanol) : Max. at 2680 and 3280; inflexion at 2800 A.; ε = 15,210, 14,960, 13,650, respectively.

Dehydration.—(*a*) *With phosphorus tribromide.* The preceding compound (0·2 g.) in anhydrous boiling dioxan (35 c.c.) was treated with phosphorus tribromide (2 c.c.). The solution was heated under reflux with exclusion of moisture for 30 minutes, and the yellow product (0·15 g.) which had separated from the hot solution was filtered off and recrystallised from ethanol in yellow felted needles, m. p. 234° (Found : C, 26·5; H, 2·7; N, 16·7%). This product (0·8 g.) was dissolved in 2N-sodium hydroxide solution and set aside at room temperature for 1 hour. The solution was acidified with 2N-hydrochloric acid to give a creamy precipitate (0·7 g.) of *4-cyano-5-N′-methylthioureido-2-methylthiothiazole* (IV), m. p. 222°, which was filtered off and recrystallised from glacial acetic acid in microprisms (Found : C, 34·6; H, 3·6; N, 22·3; S, 39·0. $C_7H_8N_4S_3$ requires C, 34·4; H, 3·3; N, 22·9; S, 39·3%). Light absorption (ethanol) : Max. at 2420, 2760, and 3080 A.; ε = 11,970, 17,590, 12,220, respectively.

(*b*) *With phosphorus oxychloride.* 5-*N′*-Methylthioureido-2-methylthiothiazole-4-carboxyamide (0·6 g.) was covered with phosphorus oxychloride (12 c.c.) and heated under reflux for 1½ hours. The solution was evaporated to dryness, and to the brown oily residue was cautiously added 2N-sodium carbonate (7 c.c.), thereby precipitating a greenish product. This product was filtered off, quickly dissolved in the minimum quantity of cold glacial acetic acid, filtered from a little insoluble matter, and treated with 2N-sodium carbonate in slight excess, precipitating a white solid. This was collected and the filtrate combined with the first sodium carbonate filtrate above, and set aside at 0°; more product separated. The combined products (0·2 g.) crystallised from glacial acetic acid in colourless prisms, m. p. 222° undepressed on admixture with 4-cyano-5-*N′*-methylthioureido-2-methylthiothiazole, prepared by route (*a*). This product was gently evaporated with concentrated hydrochloric acid and a little potassium chlorate; the residue remained colourless on treatment with ammonia or sodium hydroxide solution, indicating a negative murexide reaction.

Hydrolysis of the Above Product.—(*a*) *With alkali.* The preceding compound (0·2 g.) in 10% sodium hydroxide solution (5 c.c.) was heated under reflux for 1 hour. The solution was cooled and acidified with 10% hydrochloric acid, colourless microprisms (0·2 g.) of 7-keto-5-thio-2-methylthio-6-methyl-4 : 5 : 6 : 7-tetrahydrothiazolo[4 : 5-*d*]pyrimidine (VII) (cf. this vol., p. 1064) separating. These were crystallised from glacial acetic acid and had m. p. 265° (Found : C, 34·6; H, 3·3. Calc. for $C_7H_7ON_3S_3$: C, 34·4; H, 3·3%). Light absorption (0·2N-NaOH) : Max. at 2280 and 3400; ε = 19,370 and 20,110, respectively. This compound (0·2 g.) was dissolved in N-sodium hydroxide solution (5 c.c.) and shaken for 1 hour with excess of methyl sulphate (1 c.c.). The solid compound (VIII) which separated was washed with water and then ether and crystallised from ethanol in needles; on admixture with authentic material (cf. Part XII, *loc. cit.*) the m. p. was undepressed.

(*b*) *With acid.* 4-Cyano-5-*N′*-methylthioureido-2-methylthiothiazole (0·3 g.) covered with concentrated hydrochloric acid (3 c.c.) was heated under reflux for 0·5 hour, cooled, diluted with water (10 c.c.), made just alkaline to litmus with 10% sodium hydroxide, and then treated as above to give the same thiazolopyrimidine. Both this thiazolopyrimidine and its methylation product gave positive murexide colours by the method outlined above.

5-N′-Methylthioureido-2-methylthio-1-methylglyoxaline-4-carboxyamide (IX; R = Me, R′ = SMe).—5-Amino-2-methylthio-1-methylglyoxaline-4-carboxyamide (0·2 g.) dissolved in boiling ethyl acetate (30 c.c.) was heated under reflux with excess of methyl *iso*thiocyanate (1 g.) for 45 minutes. The solution was evaporated to dryness *in vacuo*, and the residue (0·3 g.) crystallised from ethanol in colourless prisms of the *thioureido*-derivative, m. p. 193° (Found : C, 37·3; H, 5·0. $C_8H_{13}ON_5S_2$ requires C, 37·1; H, 5·0%).

6-Hydroxy-2-mercapto-8-methylthio-9-methylpurine (X; R = SMe).—5-Amino-2-methylthio-1-methylglyoxaline-4-carboxyamide (0·2 g.) in pyridine (2 c.c.) was heated under reflux for 2 hours with excess of methyl *iso*thiocyanate (1 g.). The solution was cooled and diluted with water (10 c.c.), and the product

which separated (0·1 g.) was purified by recrystallisation from pyridine, followed by dissolution in 10% sodium hydroxide and precipitation with 10% hydrochloric acid, whereupon microprisms of the *purine* separated. These decomposed above 300° (Found: C, 37·3; H, 3·1. $C_7H_6ON_4S_2$ requires C, 36·8; H, 3·5%).

5-N′-*Acetylthioureido*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = H).—5-Amino-1-methylglyoxaline-4-carboxyamide (Part IX) (1 g.), suspended in pyridine (20 c.c.), was treated under reflux with acetyl *iso*thiocyanate (0·5 c.c.). Pyridine, which was the only suitable non-hydroxylic solvent for this compound, tended to decompose the *iso*thiocyanate. After 30 minutes, more acetyl *iso*thiocyanate (0·5 c.c.) was added, and two more such additions were made at half-hourly intervals for 1½ hours, the toal period of reflux being 2½ hours. The solution was evaporated under reduced pressure, and the residual oil was dissolved in boiling ethanol. Evaporation of this to smaller bulk (*ca.* 20 c.c.), followed by cooling to 0° and addition of ether (10 c.c.), gave a product (0·5 g.), m. p. 230°. This was recrystallised several times from ethanol to give colourless laths of the *thioureido*-derivative, m. p. 245° (decomp.) (Found: C, 40·2; H, 4·6. $C_8H_{11}O_2N_5S$ requires C, 39·8; H, 4·6%).

6-*Hydroxy*-2-*mercapto*-9-*methylpurine* (X; R = H).—5-Amino-1-methylglyoxaline-4-carboxyamide (0·2 g.), suspended in pyridine (30 c.c.), was heated under reflux for 8 hours with methyl *iso*thiocyanate (1 c.c.), the purine (0·2 g.) gradually separating from the hot solution. This was filtered off and recrystallised from water as the hemihydrate in rosettes of colourless laths, m. p. 310—320° (Found: C, 38·0; H, 3·8; N, 29·3. Calc. for $C_6H_6ON_4S,\frac{1}{2}H_2O$: C, 37·7; H, 3·7; N, 29·3%) (cf. Part XX). The anhydrous *purine* of unchanged m. p. was obtained by drying the hydrate over phosphoric oxide for 1 hour *in vacuo* at 100° (Found: N, 30·7. $C_6H_6ON_4S$ requires N, 30·8%).

5-N′-*Acetylthioureido*-2-*methylthio*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = SMe).—5-Amino-2-methylthio-1-methylglyoxaline-4-carboxyamide (2·4 g.) was suspended in boiling ethyl acetate (100 c.c.) and on being heated under reflux for 30 minutes with acetyl *iso*thiocyanate (1·5 g.) gradually dissolved. The solution was evaporated to dryness *in vacuo*, and the residue crystallised from ethanol to give slightly yellow prisms (3·1 g.) of the *thioureido*-derivative, m. p. 199° (decomp.) (Found: C, 37·5; H, 6·6; N, 24·2. $C_9H_{13}O_2N_5S_2$ requires C, 37·6; H, 4·6; N, 24·4%).

Alkaline Hydrolysis of the Above Compound.—The above compound (0·5 g.) in 10% aqueous sodium hydroxide (10 c.c.) was heated under reflux for 30 minutes. The solution was cooled and acidified with 10% hydrochloric acid, precipitating a white solid (0·4 g.). This was collected, crystallised from pyridine–ether, dissolved in 10% aqueous sodium hydroxide, and precipitated with glacial acetic acid in colourless scintillating plates of 6-*hydroxy*-2-*mercapto*-8-*methylthio*-9-*methylpurine* (X; R = SMe) which decomposed gradually above 300° (Found: C, 36·7; H, 3·6. $C_7H_6ON_4S_2$ requires C, 36·8; H, 3·5%).

Action of Phosphorus Oxychloride on the Above Compound.—The above compound (1·5 g.), dried over phosphoric oxide *in vacuo*, was covered with phosphorus oxychloride (20 c.c.) and heated under reflux for 1 hour, giving a clear brown solution after *ca.* 15 minutes, from which crystals were deposited. The solution was evaporated to dryness *in vacuo*, and the residue was cautiously treated at 0° with ice-water (20 c.c.) to give a yellow creamy precipitate. This was dissolved in 10% aqueous sodium hydroxide, filtered from a little insoluble residue, and acidified with 10% hydrochloric acid, giving a product which was collected and dissolved in boiling glacial acetic acid (25 c.c.). Filtration, followed by cooling and dilution with ether (30 c.c.), gave yellow microprisms (1·4 g.) of the *dichloro*-derivative, m. p. 225—226° (effervescence), of 4-cyano-5-*N*′-acetylthioureido-2-methylthio-1-methylglyoxaline (?) which were recrystallised from glacial acetic acid (Found: C, 32·1; H, 3·5; N, 20·2. $C_9H_9ON_5Cl_2S_2$ requires C, 32·0; H, 2·7; N, 20·7%).

6-*Amino*-2-*mercapto*-8-*methylthio*-9-*methylpurine* (XI; R = SH, R′ = SMe).—The preceding compound (0·6 g.) in N-sodium hydroxide solution (5 c.c.) was heated under reflux for 15 minutes. The solution was cooled and made just acid to litmus with 10% hydrochloric acid, giving a creamy white solid (0·4 g.). This was filtered off and dissolved in 10% hydrochloric acid (5 c.c.) with warming. The solution was filtered and 10% sodium hydroxide solution was added until the mixture was just acid to litmus. The purine was filtered off and purified for analysis by dissolution in 10% sodium hydroxide and precipitation with 10% hydrochloric acid as colourless microprisms, m. p. 270° (Found: C, 37·0; H, 4·0; N, 30·4. $C_7H_9N_5S_2$ requires C, 37·0; H, 4·0; N, 30·8%). The purine was heated under reflux with methanolic picric acid until it had dissolved, and the picrate which separated on cooling crystallised from dilute methanolic picric acid in dun-coloured needles, m. p. 244—246°.

6-Amino-2-mercapto-8-methylthio-9-methylpurine (0·1 g.) in N-sodium hydroxide (2 c.c.) was shaken for 30 seconds with methyl sulphate (0·2 c.c.). The crystals which separated (0·1 g.) were filtered off, washed with water and then ether, and crystallised from ethanol in colourless prismatic laths, m. p. 236°, of 6-*amino*-2 : 8-*dimethylthio*-9-*methylpurine* (XI; R = R′ = SMe) (Found: C, 39·9; H, 4·7; N, 29·2; S, 25·5. $C_8H_{11}N_5S_2$ requires C, 39·8; H, 4·6; N, 29·1; S, 26·5%). The *picrate*, prepared by the usual method, crystallised from a large volume of methanol in yellow needles, m. p. 259—260° (decomp.) (Found: C, 36·2; H, 2·9. $C_{14}H_{14}O_7N_8S_2$ requires C, 35·8; H, 3·0%). 6-Amino-2-mercapto-8-methylthio-9-methylpurine (0·2 g.) in N-sodium hydroxide (5 c.c.) was shaken for 30 minutes with benzyl chloride (1 c.c.), whereupon a white solid gradually separated (0·3 g.). This was filtered off, washed as above, and recrystallised from ethanol in colourless prisms, m. p. 198°, of 6-*amino*-2-*benzylthio*-8-*methylthio*-9-*methylpurine* (XI; R = S·CH_2Ph, R′ = SMe) (Found: C, 52·9; H, 4·8; N, 21·9; S, 19·9. $C_{14}H_{15}N_5S_2$ requires C, 53·0; H, 4·8; N, 22·1; S, 20·2%). The *picrate* crystallised from methanol in fine needles, m. p. 203—204° (Found: C, 44·1; H, 3·6; N, 20·8. $C_{20}H_{18}O_7N_8S_2$ requires C, 44·0; H, 3·3; N, 20·5%).

5-*Amino*-2-*benzylthio*-1-*methylglyoxaline*-4-*carboxyamide*.—5-Amino-2-mercapto-1-methylglyoxaline-4-carboxyamide (Part XX) (2·0 g.) in N-sodium hydroxide (5 c.c.) was shaken for 1 hour with excess of benzyl chloride (3 c.c.). The *product* (2·75 g.) which separated was washed with water and then ether, and crystallised from ethanol in colourless hexagonal prisms, m. p. 225° (Found: C, 54·7; H, 5·5; N, 21·7. $C_{12}H_{14}ON_4S$ requires C, 54·9; H, 5·4; N, 21·4%).

5-N′-*Acetylthioureido*-2-*benzylthio*-1-*methylglyoxaline*-4-*carboxyamide* (IX; R = Ac, R′ = S·CH_2Ph).—5-Amino-2-benzylthio-1-methylglyoxaline-4-carboxyamide (2·0 g.), suspended in boiling ethyl acetate

(250 c.c.), was treated with excess of acetyl *iso*thiocyanate (2 c.c.) and on being heated under reflux for 45 minutes gradually dissolved. The solution was evaporated to dryness *in vacuo*, and when the residual brown oil was rubbed with ether a bright yellow solid (2·5 g.) gradually separated. This *compound* recrystallised from ethanol in colourless monoclinic prisms, m. p. 192° (Found: C, 49·7; H, 4·6; N, 19·6. $C_{15}H_{17}O_2N_5S_2$ requires C, 49·6; H, 4·7; N, 19·3%).

Reaction of the Above Compound with Phosphorus Oxychloride.—The above thioureido-derivative (1·5 g.), dried over phosphoric oxide *in vacuo*, was covered with phosphorus oxychloride (10 c.c.) and heated under reflux for 35 minutes with exclusion of moisture. The material had dissolved within 10 minutes to give a clear brown solution. This was evaporated to dryness *in vacuo*, and to the brown residual oil at 0° was cautiously added ice-water (30 c.c.) in small portions. The product (1·3 g.) was allowed to crystallise at 0°, filtered off, and recrystallised from methanol–ether, whereupon the *dichloro*-derivative of 4-cyano-5-*N'*-acetylthioureido-2-benzylthio-1-methylglyoxaline (?) separated in brownish prisms, m. p. 226—228° (Found: N, 16·6. $C_{15}H_{13}ON_5Cl_2S_2$ requires N, 16·9%).

6-*Amino*-2-*mercapto- and* 6-*Amino*-2-*methylthio*-8-*benzylthio*-9-*methylpurine* (XI; R = SMe, R' = S·CH_2Ph).—The preceding compound (0·75 g.) in N-sodium hydroxide (10 c.c.) was heated under reflux for 10 minutes, and the solution was cooled, filtered, and made just acid to litmus by glacial acetic acid, giving a white solid (0·5 g.). The *purine* was filtered off and purified for analysis by dissolution in 10% sodium hydroxide solution and precipitation with 10% acetic acid as colourless microprisms, which decomposed at 190—195° (Found: N, 23·1. $C_{13}H_{13}N_5S_2$ requires N, 23·1%).

This purine (0·2 g.) in N-sodium hydroxide was shaken for 30 seconds with methyl sulphate (0·5 c.c.). The crystals which separated (0·2 g.) were filtered off, washed with water and then ether, and crystallised from ethanol in colourless microprisms of 6-*amino*-8-*benzylthio*-2-*methylthio*-9-*methylpurine* (XI; R = SMe, R' = S·CH_2Ph), m. p. 199—200° (Found: C, 52·7; H, 4·8; N, 21·8. $C_{14}H_{15}N_5S_2$ requires C, 53·0; H, 4·8; N, 22·1%). The *picrate* crystallised from ethanol in fine needles, m. p. 200—201° (Found: C, 42·9; H, 3·7. $C_{20}H_{18}O_7N_8S_2,H_2O$ requires C, 42·6; H, 3·6%).

6-*Amino*-2 : 8-*dimethylthio*-9-*methylpurine* (XI; R = R' = SMe).—(*a*) *Alternative synthesis.* 5 : 6-Diamino-4-methylamino-2-methylthiopyrimidine (Baddiley, Lythgoe, McNeil, and Todd, *loc. cit.*) (0·3 g.) in pyridine (10 c.c.) was heated under reflux for 6 hours with carbon disulphide (2 c.c.). The solution was evaporated to dryness *in vacuo*, and the residue (0·3 g.) was washed out with water and purified for analysis by dissolution in 10% sodium hydroxide solution, filtration, and then precipitation with 10% hydrochloric acid as colourless microprisms of 6-*amino*-8-*mercapto*-2-*methylthio*-9-*methylpurine* (XI; R = SMe, R' = SH), m. p. 280—282° (Found: N, 30·8. $C_7H_9N_5S_2$ requires N, 30·8%). This compound, dissolved in the minimum quantity of pyridine, was heated under reflux for 10 minutes with a slight excess of chloroacetic acid. The solution was cooled and diluted with water (4 vols.), and the product which separated was washed with water and then ether and crystallised from glacial acetic acid–ether in colourless prisms, m. p. 244°, of 6-*amino*-2-*methylthio*-8-*carboxymethylthio*-9-*methylpurine* (XI; R = SMe, R' = S·CH_2·CO_2H) (Found: N, 24·2. $C_9H_{11}O_2N_5S_2$ requires N, 24·55%). 6-Amino-8-mercapto-2-methylthio-9-methylpurine was treated as in the methylation of 6-amino-2-mercapto-8-methylthio-9-methylpurine (see above), and the product, m. p. 236°, on admixture with 6-amino-2 : 8-dimethylthio-9-methylpurine prepared by the route shown above gave no depression of m. p.

(*b*) *Desulphurisation.* 6-Amino-2 : 8-dimethylthio-9-methylpurine (0·4 g.) in methanol (20 c.c.) was heated under reflux for 4 hours with Raney nickel (7 c.c. of settled suspension in methanol, prepared according to Mozingo, *J. Amer. Chem. Soc.*, 1943, **65**, 1013). The nickel was filtered and extracted (Soxhlet) with the filtrate for *ca.* 2 days. Evaporation of the extract yielded 9-methyladenine (XI; R = R' = H) (20 mg.) which crystallised from a large volume of methanol in colourless microprisms, m. p. 296—297° (uncorr.), undepressed on admixture with an authentic specimen (kindly supplied by Prof. A. R. Todd, F.R.S. to whom we express our thanks).

Reaction of Aminocyanoacetamide with Phenyl iso*Thiocyanate.*—(*a*) *With one equivalent of* iso*thiocyanate.* Aminocyanoacetamide (1 g.) in boiling ethyl acetate (80 c.c.) was heated under reflux for 30 minutes with phenyl *iso*thiocyanate (1·4 g.). The solvent was removed *in vacuo*, and the residue recrystallised from ethyl acetate in clusters of colourless micro-laths (1·2 g.) of 5-*amino*-2-*anilinothiazole*-4-*carboxyamide* (III; R = H, R' = NHPh), m. p. 163° (Found: C, 51·7; H, 3·9. $C_{10}H_{10}ON_4S$ requires C, 51·3; H, 4·3%).

(*b*) *With two equivalents of* iso*thiocyanate.* Aminocyanoacetamide (0·8 g.) in pyridine (5 c.c.) was heated under reflux for 30 minutes with an excess of phenyl *iso*thiocyanate (3 g.). The solvent was removed *in vacuo*, and the residue was recrystallised from methanol to give 2-*anilino*-5-N'-*phenylthioureidothiazole*-4-*carboxyamide* (III; R = CS·NHPh, R' = NHPh) (0·5 g.) in colourless laths, m. p. 207° (Found: C, 54·9; H, 4·0. $C_{17}H_{15}ON_5S_2$ requires C, 55·3; H, 4·1%).

5-*Amino*-2-*mercapto*-1-*phenylglyoxaline*-4-*carboxyamide* (XIII; R = SH).—5-Amino-2-anilinothiazole-4-carboxyamide (5·0 g.), suspended in 10% sodium carbonate solution, was heated under reflux for 1½ hours. The material dissolved completely within 30 minutes. The solution was cooled and made just acid to litmus by the addition of concentrated hydrochloric acid. The creamy yellow solid (4·5 g.) which separated was filtered off, washed with ether, and recrystallised from methanol to give colourless laths of the *glyoxaline*, m. p. 229° (decomp.) (Found: N, 24·1. $C_{10}H_{10}ON_4S$ requires N, 23·9%). This product, on diazotisation in dilute acid solution, followed by coupling with β-naphthol in alkali, gave a deep-red dye.

The preceding compound (3·5 g.), dissolved in 10% sodium hydroxide solution (10 c.c.), was shaken for 5 minutes with an excess of methyl sulphate (3 c.c.), whereupon a colourless solid (3·3 g.) rapidly separated. This was filtered off, washed with water, dried at 80°, and recrystallised from benzene; 5-*amino*-2-*methylthio*-1-*phenylglyoxaline*-4-*carboxyamide* (XIII; R = SMe) separated in laths, m. p. 154° (Found: C, 53·7; H, 4·7. $C_{11}H_{12}ON_4S$ requires C, 53·2; H, 4·9%). This product could also be diazotised.

6-*Hydroxy*-8-*methylthio*-9-*phenylpurine* (XIV).—5-Amino-2-methylthio-1-phenylglyoxaline-4-carboxyamide was dissolved in a mixture of formic acid (3 c.c.; 98%) and acetic anhydride (3 c.c.). The solution was heated under reflux for 3 hours and then evaporated to dryness *in vacuo*. The residue was

dissolved in boiling methanol (*ca.* 15 c.c.): this solution was filtered, evaporated to a smaller bulk (*ca.* 10 c.c.), cooled, and diluted with ether (10 c.c.); colourless laths (0·1 g.) of the purine, m. p. 304—307°, separated when this solution was kept (Found: C, 55·4; H, 3·9; N, 21·9. $C_{12}H_{10}ON_4S$ requires C, 55·8; H, 4·0; N, 21·7%).

5-N'-*Acetylthioureido*-2-*methylthio*-1-*phenylglyoxaline*-4-*carboxyamide* (XV).—5-Amino-2-methylthio-1-phenylglyoxaline-4-carboxyamide (3·3 g.), suspended in boiling ethyl acetate (100 c.c.), was heated under reflux with acetyl *iso*thiocyanate (2 c.c.) for 30 minutes. The glyoxaline dissolved within 10 minutes, and shortly afterwards colourless crystals began to separate from the hot solution. The solution was cooled and the *thioureido*-derivative (3·7 g.) was filtered off and recrystallised from methanol in colourless plates, m. p. 204° (decomp.) (Found: N, 19·95. $C_{14}H_{15}O_2N_5S_2$ requires N, 20·05%). A further crop (0·3 g.) of less pure material was obtained by evaporation of the filtrate.

6-*Amino*-2 : 8-*dimethylthio*-9-*phenylpurine* (XVI; R = SMe).—5-*N'*-Acetylthioureido-2-methylthio-1-phenylglyoxaline-4-carboxyamide (1 g.), covered with phosphorus oxychloride (10 c.c.), was heated under reflux for 35 minutes with exclusion of moisture. A clear solution resulted after 10 minutes. The solution was evaporated to dryness, and the residual oil was treated at 0°, cautiously at first, with ice–water (10 c.c.). This solution was filtered from a little insoluble material and made slightly alkaline to litmus by treatment with 5% aqueous sodium hydroxide at 0° and then acidified with 10% hydrochloric acid, to give a *dichloro*-derivative of 4-cyano-5-*N'*-acetylthioureido-2-methylthio-1-phenylglyoxaline (1 g.). This was filtered off and recrystallised from glacial acetic acid–ether several times, to give an almost colourless product, m. p. 232—233° (Found: N, 17·0. $C_{14}H_{11}ON_5Cl_2S_2$ requires N, 17·5%). This compound (1 g.) was dissolved in 10% aqueous sodium hydroxide (10 c.c.), and the solution was heated under reflux for 10 minutes and then cooled, filtered, and made just acid to litmus by the addition of 10% acetic acid. The colourless product which separated was purified by dissolution in 10% aqueous sodium hydroxide, followed by acidification as above, giving 6-*amino*-2-*mercapto*-8-*methylthio*-9-*phenylpurine* (XVI; R = SH) as laths (0·7 g.) which decomposed at 230—235° (Found: N, 24·0. $C_{12}H_{11}N_5S_2$ requires N, 24·2%).

The preceding purine (0·1 g.) in 10% sodium hydroxide (10 c.c.) was shaken with benzyl chloride (0·5 c.c.) for 15 minutes, whereupon colourless crystals (0·1 g.) of 6-*amino*-2-*benzylthio*-8-*methylthio*-9-*phenylpurine* (XVI; R = S·CH_2Ph) separated. These were collected, washed with 10% sodium hydroxide, water, and ether, and recrystallised from methanol in rods, m. p. 174° (Found: N, 18·5. $C_{19}H_{17}N_5S_2$ requires N, 18·5%).

5-Amino-2-mercapto-8-methylthio-9-phenylpurine (0·1 g.) in 10% sodium hydroxide (10 c.c.) was shaken with methyl sulphate (0·5 c.c.) for 5 minutes, whereupon colourless crystals (0·1 g.) of 6-*amino*-2 : 8-*dimethylthio*-9-*phenylpurine* (XVI; R = SMe) separated. These were filtered off, washed with 10% sodium hydroxide, water, and ether, and recrystallised from ethanol in needles, m. p. 228—229° (Found: C, 51·55; H, 4·5. $C_{13}H_{13}N_5S_2$ requires C, 51·5; H, 4·3%). The *picrate*, prepared by the usual method, recrystallised from a large volume of methanol in yellow needles, m. p. 239—240° (decomp.) (Found: C, 42·9; H, 2·9. $C_{19}H_{16}O_7N_8S_2$ requires C, 42·9; H, 3·0%).

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