156. *Potential Anti-purines. Part I. The Synthesis of Derivatives of 8-Thiapurine (2-Thia-1 : 3 : 4 : 6-tetra-azaindene) by a New Reaction.*

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A new reaction is described for the synthesis of derivatives of 8-thiapurine (2-thia-1 : 3 : 4 : 6-tetra-azaindene) from 4-amino-5-nitrosopyrimidines and thiourea, and its mechanism is discussed.

Of recent years the conception has been developed that, by the use in chemotherapy of substances with anti-purine activity, an interference with nucleic acid biosynthesis might be caused which might be usefully reflected in the inhibition of the growth of viruses and possibly even in some partially selective inhibition of malignant growth. This conception is supported by the incorporation of 2-amino-6-hydroxy-8-azapurine [1] (8-azaguanine) and 6-mercaptopurine [2] into the nucleic acids of mice and rats and of 8-azaguanine into tobacco

[1] Mitchell, Skipper, and Bennett, *Cancer Res.*, 1950, **10**, 647.
[2] Elson, Bieber, and Hitchings, *Ann. New York Acad. Sci.*, 1954, **60**, 297.

mosaic virus causing loss of infectivity.[3] 6-Mercaptopurine is useful in the treatment of leukæmias.[4] A new reaction has now yielded derivatives of 8-thiapurine (2-thia-1 : 3 : 4 : 6-tetra-azaindene) (I) which are potential anti-purines because of a degree of structural similarity to purine.

Thiourea and the appropriate 4-amino-5-nitrosopyrimidine (II; shown in the tautomeric form) were fused together and yielded, with evolution of steam, the corresponding " thiapurine " (I), usually in yields of over 50%. The temperature required for the reaction varies from 145° to 185° according to the solubility and reactivity of the nitrosopyrimidine. The best solvent for the reaction is, in general, molten thiourea used in the necessary excess. Thiourea and 4-amino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 4-dioxopyrimidine, dispersed in tetrahydronaphthalene, reacted satisfactorily, however, with the evolution of only a trace of ammonia and no hydrogen sulphide. The reaction has been applied to the synthesis of 8-thiatheophylline and 6-hydroxy-3-methyl-2-oxo-2 : 3-dihydro- and 2 : 6-diamino-8-thiapurine (I; R = R′ = NH_2).

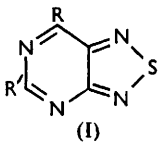
The diaminothiapurine, on hydrolysis with aqueous hydrochloric acid, yielded 2-amino-6-hydroxy-8-thiapurine (I; R = OH, R′ = NH_2) which was purified by sublimation under reduced pressure. The structure was confirmed by synthesis from 2 : 4 : 5-triamino-6-hydroxypyrimidine and thionyl chloride. The structures of the three thiapurines made by the new method were similarly confirmed. The conditions used by Blicke and Godt [5] for the known method were modified by the use of thionyl chloride without any solvent and by using the conveniently prepared bisulphite salt of the 4 : 5-diaminopyrimidine in some cases instead of the base. The structures were similarly confirmed of 6-amino- and 2 : 6-dihydroxy-8-thiapurine, previously obtained in only poor yield by the action of sulphur dichloride on 4 : 5 : 6-triaminopyrimidine, and of sulphuryl chloride on 4 : 5 : 6-triamino-2-methylthiopyrimidine respectively.[6]

The advantage of the new method over the old is that one stage, reduction of the 4-amino-5-nitrosopyrimidine to the diamine, is avoided with a consequent saving of time and improvement in yield.

Since the new reaction must involve an intermediate stage, the three alternative possibilities for its structure have been tested in preliminary experiments and two of them eliminated, thus pointing to the probable mechanism of the reaction. Thus the dihydro-imino-2-thiatetra-azanaphthalene (III) might have been formed by condensation of *iso*thiocyanic acid (from the thiourea) with the imino-group of the imino-oxime (II) and subsequent cyclodehydrogenation effected in some way by the oxygen of the oxime group with consequent loss of water. Similarly the isomer (IV) could conceivably arise by initial condensation of *iso*thiocyanic acid with the oxime group. Conversion of these structures into an 8-thiapurine would require loss of hydrocyanic acid, but this could not be detected in the gaseous products of the reaction either by the delicate benzidine cupric acetate colour reaction or by examination of the infrared spectrum. The remaining possibility involves the reaction of *iso*thiourea and the imino-oxime (II), probably by condensation between the mercapto- and the oxime groups to form the compound (VII), with loss of water. Analogous known reactions which support this suggested condensation are the formation of 5-anilino-3-phenyl-1 : 2 : 4-thiadiazole [7] (VI) from the condensate [8] (V) of benzamidoxime and phenyl *iso*thiocyanate, the conversion (by a Beckmann rearrangement) of *O*-acetylbenzaldoxime into thiobenzamide by the action of sodium hydrogen sulphide [9] and formation of 5 : 6-dihydro-7 : 8 : 8-trimethyl-4 : 7-methanobenz*iso*thiadiazole from

[3] Matthews, *J. Gen. Microbiol.*, 1954, **10**, 521.
[4] Burchenal, Ellison, Murphy, Karnofsky, Sykes, Tan, Mermann, Yuceoglu, Myers, Krakoff, and Alberstadt, *Ann. New York Acad. Sci.*, 1954, **60**, 359.
[5] Blicke and Godt, *J. Amer. Chem. Soc.*, 1954, **76**, 2798.
[6] Schrage and Hitchings, *J. Org. Chem.*, 1951, **16**, 207.
[7] Koch, *Ber.*, 1891, **24**, 394.
[8] Kruger, *Ber.*, 1884, **17**, 1060.
[9] Anger and Billy, *Compt. rend.*, **136**, 556.

hydrogen sulphide and β-camphorquinone dioxime.[10] The fact that a substantial amount of water is liberated in the reaction, coupled with a yield of the thiapurine of over 50%, argues against its formation from (VII) by hydrolysis of the formamidino-group as urea and subsequent cyclodehydrogenation of the resulting oxime. The remaining alternative to be considered is therefore fission of formamidine from (VII) and simultaneous ring closure. Since formamidine itself was unlikely to be isolable from the reaction mixture,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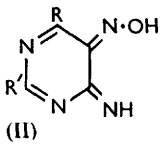
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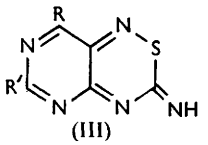
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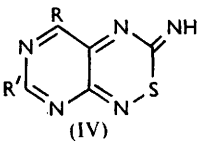
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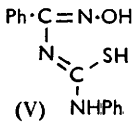
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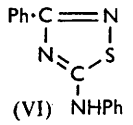
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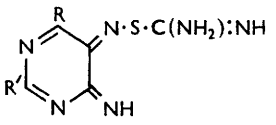
(VII)

further evidence was sought by treating 5-ethyl-5-1′-methylbutyl(thiobarbituric) acid with 4-amino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 6-dioxopyrimidine since the cyclised formamidine structure (pyrimidine) presumably formed would be more stable than formamidine and therefore isolable; but only a small yield of 8-thiatheophylline could be isolated after reaction at the abnormally high temperature required for this condensation.

Experimental

Analyses are by Mr. P. R. W. Baker and by the Microanalytical Laboratory, Imperial College of Science and Technology, London.

Synthesis of 8-Thiapurines (2-Thia-1 : 3 : 4 : 6-tetra-azaindenes) from 4-Amino-5-nitroso-pyrimidines and Thiourea.—Thiourea (1·8 g.) and 4-amino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 4-dioxopyrimidine (0·9 g.) were finely powdered, mixed, and heated in an oil-bath until a bath temperature of about 160° was reached; the mixture melted, the red colour of the pyrimidine disappeared, and brisk effervescence occurred; the temperature was held constant for 2—3 min., *i.e.*, until the reaction was finished. The light yellow melt was cooled and triturated with cold water (7 ml.), to remove unchanged thiourea, and the solid collected, dried, and sublimed at 140°/1 mm. The white microcrystalline sublimate of 8-thiatheophylline (0·7 g., 72%), m. p. 185°, gave, on recrystallisation from water, needles (0·53 g., 55%), m. p. 186° (Found: C, 36·6; H, 2·9; N, 28·3; S, 15·9. Calc. for $C_6H_6O_2N_4S$: C, 36·4; H, 3·0; N, 28·3; S, 16·1%). This compound was also obtained as needles, m. p. 154° (cf. Blicke and Godt [7]), and the two forms were interconvertible sometimes merely on storage. Water vapour evolved during the reaction condensed in the upper part of the tube. The condensate was shown to be water (containing ammonia), by boiling it off on heating the tube at 104°, by contact with anhydrous copper sulphate which became blue and by determination of the refractive index.

Thiourea (3 g.) and 2 : 4 : 6-triamino-5-nitrosopyrimidine (1·5 g.) were mixed and heated in a bath at 170—175°. The reaction was completed in 4 min., the melt triturated with water, the solid collected, extracted with 0·5N-sodium hydroxide, washed, and extracted again with boiling 20% aqueous acetic acid, and the extract was basified with ammonia solution, yielding a brick-red crystalline precipitate (0·8 g.), m. p. 353°. Sublimation at 230°/1 mm. yielded yellow microcrystalline 2 : 6-*diamino-8-thiapurine* (0·7 g.), which crystallised from water as yellow needles, m. p. 355° (Found: C, 28·3; H, 2·6; N, 49·8; S, 18·8. $C_4H_4N_6S$ requires C, 28·6; H, 2·4; N, 50·0; S, 19·0%). Identity with the product synthesised from tetra-aminopyrimidine and thionyl chloride was indicated by a mixed m. p.

Thiourea (1 g.) and 4-amino-2 : 3-dihydro-6-hydroxy-3-methyl-5-nitroso-2-oxopyrimidine (0·5 g.) were heated together at 185—190° until the reaction was finished (3 min.). After

[10] Sen, *J. Indian Chem. Soc.*, 1938, **15**, 537.

trituration with water (5 ml.) the solid was collected, dried, and sublimed at 150°/1 mm., yielding white needles (0·24 g.), m. p. 217°. Recrystallisation from water yielded 2 : 3-*dihydro*-6-*hydroxy*-3-*methyl*-2-*oxo*-8-*thiapurine* as either long slender or very short prisms, m. p. 219° (Found: C, 32·6; H, 2·1; N, 30·7; S, 17·0. $C_5H_4O_2N_4S$ requires C, 32·6; H, 2·2; N, 30·4; S, 17·4%), identified by mixed m. p. with the product from thionyl chloride and the diaminopyrimidine (see below).

Thiourea (0·42 g., 1·1 mol.) and 4-amino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-5-nitroso-2 : 4-dioxopyrimidine (0·9 g.) were dispersed in tetrahydronaphthalene (10 ml.), all materials being anhydrous, and heated in an oil-bath at 160—165° for 15 min.; water and a trace of ammonia were evolved but no hydrogen sulphide. After cooling, the liquid was separated and mixed with light petroleum (b. p. 40—60°; 30 ml.); crude 8-thiatheophylline (0·35 g.) separated. Also, the sticky solid deposited from the mixture was washed with ether and triturated with water, to yield crude thiapurine. On sublimation the crude material yielded pure 8-thiatheophylline (0·4 g.), m. p. 154°, identified by mixed m. p. and analysis (Found: C, 36·5; H, 3·2; N, 28·0%).

Under fusion conditions the reaction appears to be catalysed by the admixture of 10% of anhydrous sodium acetate with the nitroso-compound, since the required temperature is then lowered by about 10°.

Reaction of 5-*Ethyl*-5-1′-*methylthiobarbituric Acid and* 4-*Amino*-1 : 2 : 3 : 4-*tetrahydro*-1 : 3-*dimethyl*-5-*nitroso*-2 : 4-*dioxopyrimidine*.—A mixture of the thiobarbituric acid (2·2 g.) and the pyrimidine (1·62 g.) with anhydrous sodium acetate (0·3 g.) was heated at 200—204° until the reaction ceased (3 min.). The melt was triturated with ether (50 ml.), and the remaining solid after trituration with methanol (30 ml.) was extracted with 0·5N-sodium hydroxide, the extract acidified with glacial acetic acid, and the precipitate recrystallised from ethanol, forming needles (0·1 g.), m. p. 334°. Analyses were not consistent with 5-ethyl-3 : 4 : 5 : 6-tetrahydro-5-1′-methylbutyl-4 : 6-dioxopyrimidine. The methanol solution was evaporated to dryness and the residue extracted with chloroform (200 ml.), leaving a brown resin (0·6 g.). 8-Thiatheophylline (0·1 g.) was isolated from the resinous impurities in the chloroform solution by passing it through alumina, evaporating the solution to dryness, and subliming the residue at 140°/1 mm. Concentration of the ether solution first yielded crude 8-thiatheophylline which, after sublimation, had m. p. 184° (0·3 g.), and finally, unchanged thiobarbituric acid (0·45 g.) which was identified by mixed m. p. (157°) and analysis (Found: S, 13·0. Calc. for $C_{11}H_{18}O_2N_2S$: S, 13·2%).

Preparation of 2-*Amino*-6-*hydroxy*-8-*thiapurine from* 2 : 6-*Diamino*-8-*thiapurine*.—2 : 6-Diamino-8-thiapurine (2 g.), boiled for 45 min. with concentrated hydrochloric (10 ml.) and water (10 ml.), gave, on cooling, a precipitate which was collected and dissolved in 0·5N-sodium hydroxide (50 ml.). Aqueous 2N-acetic acid (*ca.* 2·3 ml.) was cautiously stirred in and a brown amorphous precipitate removed. The clear filtrate, acidified (litmus) with acetic acid, gave a yellow amorphous precipitate which, crystallised from 2N-hydrochloric acid, gave buff-coloured prisms (0·8 g.), m. p. 380° (decomp.), of 2-*amino*-6-*hydroxy*-8-*thiapurine hydrochloride* (Found: C, 22·9; H, 2·2; N, 32·3; S, 14·5; Cl, 16·0. $C_4H_3ON_5S,HCl,\frac{1}{2}H_2O$ requires C, 22·4; H, 2·3; N, 32·6; S, 14·9; Cl, 16·5%). Identity with the compound synthesised from 2 : 4 : 5-triamino-6-hydroxypyrimidine (see below) was established by mixed m. p. and ultraviolet absorption spectrum. In 0·1N-hydrochloric acid $\lambda_{max.}$ was 309 mμ (ε 9700); $\lambda_{min.}$ 265 (ε 765).

Synthesis of 8-*Thiapurines from* 4 : 5-*Diaminopyrimidines and Thionyl Chloride*.—2 : 4 : 5 : 6-Tetra-aminopyrimidine hydrogen sulphite (1 g.) was dispersed in thionyl chloride (30 ml.), boiled for 1½ hr., then cooled, the solid was collected, washed with ether, and triturated with warm 2N-ammonia, and the solid crystallised from water, yielding yellow needles, m. p. 355°, of 2 : 6-diamino-8-thiapurine (Found: C, 28·3; H, 2·6; N, 49·7; S, 18·5%).

4 : 5-Diamino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-2 : 4-dioxopyrimidine and thionyl chloride was boiled for 20 min., the solid dissolving completely. After evaporation to dryness, trituration of the residue with 2N-ammonia, and recrystallisation from water gave needles (0·4 g.), m. p. 154°, of 8-thiatheophylline, identified by mixed m. p. with the product made according to Blicke and Godt.[5]

4-Amino-2 : 3-dihydro-6-hydroxy-3-methyl-5-nitroso-2-oxopyrimidine (1 g.), suspended in water (6 ml.) and 2N-ammonia (2 ml.), was stirred with addition of sodium dithionite until all the nitroso-compound had disappeared; spontaneous warming to about 45° occurred. Cooling, filtration, and recrystallisation of the solid from water yielded 4 : 5-diamino-2 : 3-dihydro-6-hydroxy-3-methyl-2-oxopyrimidine (0·5 g.). The diamine was dispersed in thionyl chloride (20 ml.), the mixture boiled for 4½ hr., then cooled, and the solid collected, extracted with 20%

aqueous ammonia (20 ml.), and acidified with acetic acid. The dried precipitate sublimed at 150°/1 mm., yielding 2 : 3-dihydro-6-hydroxy-3-methyl-2-oxo-8-thiapurine (0·16 g.), m. p. 218°; recrystallised from water, this had m. p. 219° (0·10 g.) (Found: C, 32·3; H, 2·4; N, 30·3; S, 16·9%).

2 : 4 : 5-Triamino-6-hydroxypyrimidine (0·45 g.) and thionyl chloride (9 ml.) were boiled for 6½ hr., the whole evaporated to dryness, and the residue recrystallised from 2N-hydrochloric acid in buff-coloured prisms of 2-amino-6-hydroxy-8-thiapurine hydrochloride, m. p. (decomp.) 380° (Found: C, 22·5; H, 2·5; N, 32·8; S, 14·8; Cl, 16·2%).

4 : 5-Diamino-2 : 6-dihydroxypyrimidine (0·5 g.) and thionyl chloride (15 ml.) were boiled for 7½ hr., all the solids dissolving. After evaporation to dryness the residue was washed with water and recrystallised to purity (indicated by paper chromatography) from water, yielding stout buff prisms (0·2 g.) of 2 : 6-dihydroxy-8-thiapurine (Found: C, 28·4; H, 1·5; N, 33·1; S, 18·5. Calc. for $C_4H_2O_2N_4S$: C, 28·2; H, 1·2; N, 32·9; S, 18·8%).

4 : 5 : 6-Triaminopyrimidine hydrogen sulphite (0·8 g.) and thionyl chloride (30 ml.) were boiled together for 2 hr. After cooling, the precipitate was collected, washed with ether and very dilute ammonia solution, and recrystallised from water in buff needles (0·30 g.), m. p. 248°, of 6-amino-8-thiapurine (Found: C, 31·8; H, 2·0; N, 45·2; S, 20·4. Calc. for $C_4H_3N_5S$: C, 31·4; H, 2·0; N, 45·7; S, 20·9%).

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