Purine Studies. Part VII.[1] The Synthesis of Purines as Amplifiers of Phleomycin against *E. coli*.

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Methylaminolysis of 4-chloro-2,6-dimethyl-5-nitropyrimidine and subsequent reduction gave 5-amino-2,5-dimethyl-6-methylaminopyrimidine (4; R = Me), which condensed with orthoesters to give 2,6,9-trimethyl- and 2,6,8,9-tetramethyl-purine (2d and e). Aminolysis or methoxylation of 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine followed by reduction gave 5-amino-2-dimethylamino-(or methoxy-)4-methyl-6-methylamino-pyrimidine (4; R = NMe_2 or OMe) which cyclized with appropriate orthoesters to give 2-dimethylamino-(or methoxy-)6,9-dimethylpurine (2f or h) and their 6,8,9-trimethyl homologues (2g and i). 4,5-Diamino-6-methyl-pyrimidine-2-thione and its 4-*N*-methyl derivative (6; R^1 = Me, R^2 = NHMe), on treatment with carbon disulphide in pyridine gave 6-methyl- and 6,9-dimethyl-purine-2,8-dithione (5; X = S, R = H or Me), which underwent *S*-methylation to give the corresponding 2,8-bismethylthiopurines (2j and k); likewise, appropriate pyrimidine-thiones with orthoesters gave 8-methyl-, 6,8-dimethyl-, and 6,8,9-trimethyl-purine-2-thione and thence the corresponding *S*-methyl derivatives (2l—n). Alkylation of 6,9-dimethylpurine-2-thione gave the 2-ethylthio- and 2-[^{14}C]methylthio-derivatives (2o and a). Administered to mice, the latter thioether reached the urine mainly as the corresponding sulphoxide (2b), identified by unambiguous synthesis from the thioether with *m*-chloroperoxybenzoic acid; the urine contained neither the isomeric 6,9-dimethyl-2-methylthiopurin-8-one (8), made from the dimethyl-2-thioxopurin-8-one (5; X = O, R = Me), nor 5-amino-4-methyl-6-methylamino-2-methylthiopyrimidine (4; R = SMe), made by alkaline fission of the thioether.

The foregoing and related purines were tested as amplifiers of phleomycin against *E. coli*. Activities are discussed; ionization constants and u.v. spectra are recorded.

THE use of phleomycin [2] as an antibiotic is precluded by its mutagenic and chromosome-breaking activity at effective dose levels.[3] However, such levels may be reduced substantially (at least in an *in vitro* test system of *E. coli*) by the addition of caffeine (1), coumarin,

[1] Part VI, D. J. Brown and P. W. Ford, *J. Chem. Soc.* (*C*), 1969, 2620.

[2] T. Takita, K. Maeda, and H. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1959, **12**, 111; T. Takita, *ibid.*, p. 285.

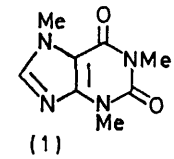
[3] E. Mattingly, *Mutation Res.*, 1967, **4**, 51; B. A. Kihlman, G. Odmark, and B. Hartley, *ibid.*, p. 783.

pyronine Y, or certain thio-substituted purines.[4,5] These amplifiers have no intrinsic antibacterial activity but they have the common characteristic of binding selectively to single stranded DNA,[4-6] a fact of probable significance in their mode of action.[4,5,7,8] Existing data [5] on a few available purines suggest that thioether and *C*-methyl groups may be advantageous, whereas groups anionic at pH 7 and halogeno-substituents may be disadvantageous to activity as a phleomycin amplifier.

In this paper we further explore such structural requirements by the synthesis and testing of a range of methyl-, alkylthio-, methoxy-, and dimethylamino-purines. We also describe the preparation of 6,9-dimethyl-2-[^{14}C]methylthiopurine (2a) and of the corresponding sulphoxide (2b), which is shown to be the principal metabolite of the thioether (2a) in the mouse.

Syntheses.—2,6,8-Trimethylpurine (2c) was made [9] *via* the intermediate pyrimidine (3; R^1 = Me, R^2 = NH_2), prepared by an improved method involving aminolysis of the known [10] chloro-analogue (3; R^1 = Me, R^2 = Cl). The same chloropyrimidine was converted similarly into the methylaminopyrimidine (3; R^1 = Me, R^2 = NHMe) and thence into the diamine (4; R = Me), which reacted with triethyl orthoformate or orthoacetate to give 2,6,9-trimethylpurine (2d) or its tetramethyl homologue (2e). 2-Dimethylamino-6,9-dimethylpurine (2f) and its homologue (2g) were both made from the chloropyrimidine [11] (3; R^1 = Cl, R^2 = NHMe) *via* the intermediates (3; R^1 = NMe_2, R^2 = NHMe) and (4; R = NMe_2), by use of the appropriate orthoester in acetic anhydride for the final cyclization; likewise, the methoxypurines (2h and i) were prepared *via* the intermediates (3; R^1 = OMe, R^2 = NHMe) and (4; R = OMe). The 2,8-bismethylthiopurines (2j and k) were made by methylation of the dithiones (5; R = H or Me, X = S), prepared, respectively, from the pyrimidinethiones [12,13] (6; R^1 = NH_2 or NHMe, R^2 = Me) with carbon disulphide in pyridine. Similarly, the pyrimidinethiones (6; R^1 = NH_2, R^2 = H or Me; and R^1 = NHMe, R^2 = Me) were converted (in the first case with isolation of a 5-acetamido- or 5-ethoxyethylideneaminopyrimidine intermediate, according to the reagent) into the purinethiones (7; R^1 = R^2 = H; R^1 = Me, R^2 = H; and R^1 = H, R^2 = Me) and thence into the thioethers (2l—n).

The [^{14}C]methylthiopurine (2a) was obtained by appropriately modified methylation of 6,9-dimethylpurine-2-thione [13] with [^{14}C]methyl iodide. Within 24 h of administering this material to mice, the accumulated urine contained nearly all the radioactivity, of which >95% was associated with a single metabolite easily separated from other constituents by t.l.c. Repetition on a larger scale with unlabelled thioether gave a white solid of composition $C_8H_{10}N_4OS$ (high resolution mass spectrum), indicating a net gain of one oxygen atom as compared with the thioether (2a). The metabolite differed from the purinone (8) [synthesized by fusion of the pyrimidine [13] (6; R^1 = NHMe, R^2 = Me) with urea to give the purinethione (5; R = Me, X = O), followed by *S*-methylation] but was identical with the isomeric sulphoxide (2b) [synthesized by oxidation of the thioether (2a) with *m*-chloroperoxybenzoic acid]. The thioethers (2a and o), of which the latter was prepared by ethylation of the corresponding thione,[13] were stable to acid but in alkali the lower homologue (2a) underwent ring fission to give the pyrimidine (4; R = SMe), which was reconverted into the purine by boiling in formic acid. A preliminary u.v. spectral study of mouse-urine constituents after administration of the other purines suggested that some might have been eliminated unchanged, some gave altered purines or pyrimidines, and some gave gave fragmented metabolites without an appreciable spectrum; the spectra of the original purines are in the Table.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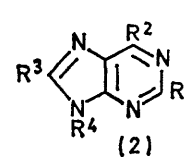
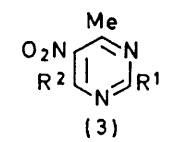

(2)

	R^1	R^2	R^3	R^4
a;	SMe	Me	H	Me
b;	SOMe	Me	H	Me
c;	Me	Me	Me	H
d;	Me	Me	H	Me
e;	Me	Me	Me	Me
f;	NMe_2	Me	H	Me
g;	NMe_2	Me	Me	Me
h;	OMe	Me	H	Me
i;	OMe	Me	Me	Me
j;	SMe	Me	SMe	H
k;	SMe	Me	SMe	Me
l;	SMe	H	Me	H
m;	SMe	Me	Me	H
n;	SMe	Me	Me	Me
o;	SEt	Me	H	Me
p;	H	H	H	H
q;	Me	H	SMe	Me
r;	SMe	H	SMe	H
s;	S·CH_2·CO_2H	Me	H	Me
t;	SMe	H	H	H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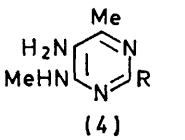
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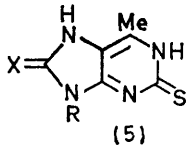
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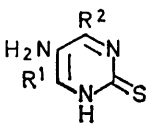
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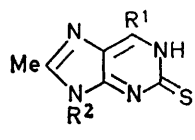
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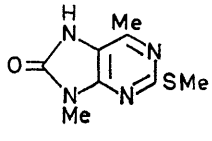
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Ionization, u.v. spectra, and biological activity

Purine	pK_a [a]	$\lambda_{max.}$ [b] (log ε) [pH; ionic species]	Biological activity [c]
Unsubstituted [d]	8·9; 2·4 [d]	*e*	1·5
2,6,8-Me_3 [f]	9·90 ± 0·01 (290) 4·49 ± 0·01 (270)	277 (3·95), 211 (4·28) [12·0; −] 270 (3·92), 243 (3·52), 204 (4·26) [7·0; 0] 271 (3·85), 211 (4·31) [2·0; +]	13
2,6,9-Me_3	4·02 ± 0·03 (267)	266 (3·87), *246* (3·59), 206 (4·28) [7·0; 0] 267 (3·77), 210 (4·37) [2·0; +]	0·5
2,6,8,9-Me_4	4·53 ± 0·03 (285)	275 (3·93), 205 (4·15) [7·0; 0] 281 (3·86), *206* (4·05) [2·0; +]	6
2-SMe [d]	8·9; 1·9 [d]	*e*	23 (20)
8-Me-2-SMe	9·58 ± 0·04 (245) 2·83 ± 0·03 (255) −1·03 ± 0·03 (272)	302 (3·87), 240 (4·28) [12·0; −] 305 (3·85), *253* (3·96), *248* (3·97), 231 (4·22) [7·0; 0] 312 (3·72), 254 (4·07), 227 (4·11), 207 (4·19) [0·8; +] 320 (3·45), 268 (4·26), 236 (4·26) [−2·5; mainly ++]	(120)
6,7-Me_2-2-SMe [g]	2·3 [g]	*g*	1000 (90)
6,8-Me_2-2-SMe	9·70 ± 0·06 (240) 3·04 ± 0·04 (300) −0·60 ± 0·03 (271)	299 (3·92), *265* (3·70), 239 (4·26) [12·0; −] 299 (3·89), 254 (3·96), 227 (4·23) [7·0; 0] 305 (3·79), 254 (4·06), 225 (4·14) [1·2; +] 318 (3·51), 268 (4·23), 234 (4·25) [−2·8; ++]	(80)
6,9-Me_2-2-SMe [g]	2·65 [g]	*g*	750 (95)
6,8,9-Me_3-2-SMe	3·49 ± 0·03 (248)	297 (3·90), 261 (3·88), 232 (4·22) [7·0; 0] 306 (3·75), *275* (3·76), *256* (3·79), 244 (4·12), 230 (4·13) [1·0; +]	(33)
2,8-$(SMe)_2$ [h]	7·7; 2·2 [h]	*h*	(70)
6-Me-2,8-$(SMe)_2$	7·94 ± 0·01 (245) 2·74 ± 0·02 (280) −1·36 ± 0·02 (279)	315 (4·23), 247 (4·33), 220 (4·21) [12·0; −] 314 (4·22), *264* (4·06), 248 (4·20), 224 (4·19) [5·5; 0] 324 (4·07), *285* (4·19), 264 (4·35), 237 (4·04) [0·7; +] 334 (3·98), 278 (4·57), 226 (3·93), 215 (3·95) [−3·6; ++]	(70)
6,9-Me_2-2,8-$(SMe)_2$	2·92 ± 0·03 (257)	313 (4·23), 268 (4·01), 236 (4·29), 209 (4·12) [7·0; 0] 321 (4·02), 284 (4·19), 259 (4·38) [0·5; +]	(4)
2,9-Me_2-6-SMe [g]	2·1 [g]	*g*	25
9-Me-8-SMe [e]	3·0 [e]	*e*	(8)
2,9-Me_2-8-SMe [g]	3·8 [g]	*g*	470
2-SEt-6,9-Me_2	2·64 ± 0·05 (273)	298 (3·91), 260 (3·93), 235 (4·23) [7·0; 0] *302* (3·70), *274* (3·97), 245 (4·28) [0·5; +]	80
2-S·CH_2·CO_2H-6,9-Me_2 [i]	2·03 ± 0·05 (244) *ca.* −2	297 (3·88), 258 (3·87), 234 (4·27) [7·0; −] 300 (3·77), *274* (3·71), *258* (3·93), 242 (4·16), 229 (4·20) [0·5; 0]	0·2 (0·2)
9-CH_2Ph-8-SMe [g]	2·8 [g]	*g*	
8-OH-6,9-Me_2-2-SMe [j]	9·41 ± 0·04 (279) 2·62 ± 0·03 (275)	308 (4·04), 275 (4·06), 229 (4·21) [12·0; −] 301 (4·00), 261 (4·08), 221 (4·19) [5·5; 0] 308 (3·88), 272 (4·11), 248 (4·46) [0·5; +]	(2)
2-SH-6,9-Me_2 [g,j]	8·0; 0 [g]	*g*	34 (23)
2-OMe-6,9-Me_2	3·05 ± 0·04 (279)	279 (3·96), 238 (3·39), 209 (4·38) [7·0; 0] 282 (3·85), *245* (3·32), 213 (4·36) [1·0; +]	55
2-OH-8-CF_3 [h]	10·9; 5·4 [h]	*h*	0·5
2-NMe_2-6,9-Me_2 [k,l]	4·71 ± 0·03 (321) −0·23 ± 0·04 (255)	324 (3·77), 254 (3·97), 227 (4·44) [7·0; 0] 333 (3·56), *255* (3·88), 233 (4·52) [2·0; +] 354 (3·50), *262* (3·83), 254 (3·98), 228 (4·53) [−3·0; ++]	85 (35)
2-NMe_2-6,8,9-Me_3 [l]	5·19 ± 0·03 (235)	323 (3·83), 255 (4·01), 228 (4·46) [8·0; 0] 330 (3·62), 252 (3·95), 232 (4·56) [2·0; +]	225
6,9-Me_2-2-SOMe	0·54 ± 0·05 (277)	269 (3·89) [7·0; 0] 266 (3·76), *234* (3·73) [−2·8; +]	2·5
Caffeine	<1 [m]	*n*	30 (11)
2-OMe-6,8,9-Me_3	3·73 ± 0·03 (302)	281 (4·05), 238 (3·50), 211 (4·42) [7·0; 0] 282 (3·97), *233* (3·42), *206* (4·36) [1·0; +]	90
6-OH-2-SMe [o,p]		*o*	30
8-OH-2-SMe [p,q]		*q*	(19)
2-SEt-6-OH [p,r]		*r*	(7)

[a] Analytical wavelength (nm) in parentheses. [b] Inflexions in italics; data for absorption <210 nm approximate. [c] Adjusted activities (see Experimental section) at 8mM; figures in parentheses at 2mM. [d] A. Albert and D. J. Brown, *J. Chem. Soc.*, 1954, 2060. [e] D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 1957, 682. [f] Ref. 9. [g] Ref. 13. [h] A. Albert, *J. Chem. Soc.* (*B*), 1966, 438. [i] Ref. 5. [j] For convenience, OH and SH is used for purinone and purinethione, respectively. [k] Ref. 16. [l] Showed an intrinsic mild antibacterial action towards *E. coli*. [m] C. E. O'Rourke, L. B. Clapp, and J. O. Edwards, *J. Amer. Chem. Soc.*, 1956, **78**, 2159. [n] W. Pfleiderer and G. Nübel, *Annalen*, 1961, **647**, 155. [o] G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Amer. Chem. Soc.*, 1956, **78**, 217. [p] Supplied by Dr. H. Maguire, University of Sydney. [q] C. W. Noell and R. K. Robins, *J. Org. Chem.*, 1959, **24**, 320. [r] G. Gouch and M. H. Maguire, *J. Medicin. Chem.*, 1967, **10**, 475.

Biological Activity.—Figures for the activity of each purine, as an amplifier to the antibacterial action of phleomycin towards *E. coli*, are given in the Table; the testing procedure and necessary definitions are in the Experimental section. Such activity figures become more meaningful on considering that the viable-cell count for an untreated culture of *E. coli* increases *ca.* 4-fold during 2 h; however, for a phleomycin-treated culture growing in a medium containing, for example, 8mM-caffeine (activity 30), this increase is replaced by a 30-fold decrease: hence the final count is only 1/120th of that under normal conditions. It is evident that the ratio of adjusted activity at 8mM concentration to adjusted activity at 2mM concentration varies widely for different purines, probably because the (yet unknown) optimum ratio of [purine] to [phleomycin] also varies widely.

When compared with caffeine at similar molarity, the tested purines fell into the following order within four categories.

(*a*) Very highly active (>10 × caffeine): 6,7-Me_2-2-SMe; 6,9-Me_2-2-SMe; 2,9-Me_2-8-SMe; 8-Me-2-SMe.*

(*b*) Highly active (3—10 × caffeine): 2-NMe_2-6,8,9-Me_3; 6,8-Me_2-2-SMe; * 2,8-$(SMe)_2$; * 6-Me-2,8-$(SMe)_2$; * 6,8,9-Me_3-2-SMe; * 2-SEt-6,9-Me_2; 2-NMe_2-6,9-Me_2; 2-OMe-6,8,9-Me_3.

(c) Moderately active (0·3—3 × caffeine): 2-OMe-6,9-Me_2; 8-OH-2-SMe; * 2-SH-6,9-Me_2; caffeine; 6-OH-2-SMe; 2-SMe; 2,9-Me_2-6-SMe; 9-Me-8-SMe; * 2-SEt-6-OH; * 2,6,8-Me_3; 6,9-Me_2-2,8-$(SMe)_2$.*

(d) Slightly active (<0·3 × caffeine): 2,6,8,9-Me_4; 8-OH-6,9-Me_2-2-SMe; * 6,9-Me_2-2-SOMe; unsubst. purine; 2,6,9-Me_3; 2-OH-8-CF_3; 2-S·CH_2·CO_2H-6,9-Me_2.

Because of their limited solubility, purines marked with an asterisk could be measured only at 2mM concentration and were classified by comparison with 2mM-caffeine; for practical antibacterial purposes, they were better compared with 8mM-caffeine and thereupon all except one fell into the next lower category.

The slight activity of unsubstituted purine (2p) was improved by appropriate methylation to the point of moderate activity in the trimethylpurine (2c). The addition of a methylthio-group was far more effective, especially in the 2- or 8-position and if accompanied by one or two methyl groups: *e.g.* the very highly active thioethers (2a, l, and q); the dimethylated ethylthiopurine (2o) was more akin in activity to the isomeric trimethylated thioether (2n) than to the very highly active dimethylated analogue (2a). The high activities of the bismethylthiopurine (2r) and its monomethyl derivative (2j) decreased to moderate in the case of the dimethyl derivative (2k). A 2-methoxy- or 2-dimethylamino-group strongly increased the activity of purine: the highly active trimethylated methoxypurine (2i) proved better than its moderately active dimethylated homologue (2h); the same relationship pertained in the case of the di- and tri-methylated dimethylaminopurines (2f and g), of which the second was the more active homologue, although they both fell in the highly active category. The very high activity of the thioether (2a) was practically eliminated by conversion into the carboxylic acid (2s), the sulphoxide (2b), or the purinone (8). The only tested purine fully ionized in the test medium (pH 7·0) was the foregoing inactive carboxylic acid (pK_a 2·0); partial ionization in the purines (2p and t) (each 1% anion), (2j) (11% anion), (2r) (17% anion), (2g) (2% cation), 8-trifluoromethylpurin-2-one (2·5% anion), and 6,9-dimethylpurine-2-thione (10% anion) appeared to be unimportant in relation to activity.

From these results, and in view of the high activity associated with lipophilic methyl and methylthio-groups, we suggest tentatively that the low activity of many purines bearing ionizing groups (7/9-NH, OH, SH) may be due more to poor lipophilicity than to ionization. Preliminary work on the partition coefficients (between n-octanol and water) of such purines appears to support this hypothesis.

EXPERIMENTAL

Ionization constants were measured spectrometrically at 20° and concentrations below 10^{-4}M in buffers [14] of 10^{-2}M ionic strength by methods outlined in ref. 15. U.v. spectra were recorded on an SP 800 spectrophotometer; peaks were checked with a manual SP 500 instrument.

2,6,8-*Trimethylpurine.*— 4-Chloro-2,6-dimethyl-5-nitropyrimidine was prepared from the corresponding pyrimidinone according to the method of Urban and Schnider,[10] but using *NN*-diethyl- instead of *NN*-dimethyl-aniline with the phosphoryl chloride, thereby increasing the yield from 78 to 90%. To a stirred solution of the chloropyrimidine (7 g) in ethanol (15 ml) at 0—5°, saturated ethanolic ammonia (40 ml) was added dropwise. After a further 15 min at 20—30° and 30 min at 0° the yellow solid was filtered off and gave 4-amino-2,6-dimethyl-5-nitropyrimidine (80%), m.p. 160° (from water) (lit.,[9] 160°). This was converted [9] into the trimethylpurine, m.p. 220—222° (lit.,[9] 222°).

2,6,9-*Trimethylpurine.*—Ethanolic 33% methylamine (40 ml) was added in drops to an ice-cold stirred solution of 4-chloro-2,6-dimethyl-5-nitropyrimidine (12 g) in ethanol (25 ml). After 30 min longer at 0°, the solid was removed and gave yellow 2,4-*dimethyl*-6-*methylamino*-5-*nitropyrimidine* (77%), m.p. 107—108° (from water) (Found: C, 46·3; H, 5·6; N, 31·0. $C_7H_{10}N_4O_2$ requires C, 46·2; H, 5·5; N, 30·8%). The nitropyrimidine (5·0 g) was hydrogenated over Raney nickel in methanol (200 ml) at atmospheric pressure. The filtered solution was evaporated to dryness to give 5-*amino*-2,4-*dimethyl*-6-*methylaminopyrimidine* (96%), m.p. 185—186° (decomp.) (from toluene) (Found: C, 55·3; H, 7·9; N, 36·55. $C_7H_{12}N_4$ requires C, 55·3; H, 7·9; N, 36·8%). This diamine (1·0 g), triethyl orthoformate (25 ml), and acetic anhydride (15 ml) were heated under reflux for 3 h. The residue from removal of volatile materials was recrystallized (charcoal) from light petroleum (b.p. 60—80°) and then sublimed to give the hygroscopic 2,6,9-*trimethylpurine* (75%), m.p. 75—76° (Found: C, 59·0; H, 6·3. $C_8H_{10}N_4$ requires C, 59·2; H, 6·2%).

2,6,8,9-*Tetramethylpurine.*— 5-Amino-2,4-dimethyl-6-methylaminopyrimidine (1·5 g), triethyl orthoacetate (25

[14] D. D. Perrin, *Austral. J. Chem.*, 1963, **16**, 572.

[15] A. Albert and E. P. Serjeant, 'The Determination of Ionization Constants,' Chapman and Hall, London, 2nd edn., 1971.

ml), and acetic anhydride (15 ml) were heated under reflux for 4 h. The residue from evaporation was put on to a silica column in a little methanol and eluted with ether–methanol (4 : 1). Recrystallization of the appropriate fraction gave the hygroscopic *tetramethylpurine* (35%), m.p. 57—58° (Found: C, 60·7; H, 7·0. $C_9H_{12}N_4$ requires C, 61·3; H, 6·9%).

2-*Dimethylamino*-6,9-*dimethylpurine*.—Ethanolic 33% dimethylamine (15 ml) was added slowly to a stirred ice-cold suspension of 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine [11] (5 g) in ethanol (10 ml). A vigorous reaction gave a yellow solution from which crystals separated. The mixture was stirred for 2 h at 25° and then chilled. Filtration (and partial evaporation of the filtrate for a second crop) gave 2-*dimethylamino*-4-*methyl*-6-*methylamino*-5-*nitropyrimidine* (94%), m.p. 147° (from cyclohexane) (Found: C, 45·45; H, 6·45; N, 33·6. $C_8H_{13}N_5O_2$ requires C, 45·5; H, 6·2; N, 33·2%). Hydrogenation at 50° (Raney nickel; methanol) of the nitropyrimidine gave 5-*amino*-2-*dimethylamino*-4-*methyl*-6-*methylaminopyrimidine* (98%), m.p. 109—110° (after recrystallization from light petroleum and subsequent sublimation at 85° and 0·2 mmHg) (Found: C, 53·2; H, 8·3; N, 38·5. $C_8H_{15}N_5$ requires C, 53·0; H, 8·3; N, 38·6%). This 5-aminopyrimidine (1·4 g) and 98% formic acid were heated under reflux for 2 h. Evaporation and recrystallization of the residue from ethanol gave 2-*dimethylamino*-5-*formamido*-4-*methyl*-6-*methylaminopyrimidine* (>90%), m.p. 229° (decomp.) (Found: C, 51·6; H, 7·4; N, 33·0. $C_9H_{15}N_5O$ requires C, 51·6; H, 7·2; N, 33·4%). The same 5-aminopyrimidine (2·5 g), triethyl orthoformate (25 ml), and acetic anhydride (15 ml) were heated under reflux for 3 h. The residue from evaporation *in vacuo* was extracted with boiling light petroleum. After treatment with charcoal, the extract was evaporated. Purification by t.l.c. [silica; chloroform–light petroleum (9 : 1)] followed by sublimation (90° and 1 mmHg) gave 2-dimethylamino-6,9-dimethylpurine (0·8 g), m.p. 106° (lit.,[16] 106—109°) (Found: C, 56·8; H, 7·1; N, 36·9. Calc. for $C_9H_{13}N_5$: C, 56·5; H, 6·85; N, 36·6%).

2-*Dimethylamino*-6,8,9-*trimethylpurine*.— 5-Amino-2-dimethylamino-4-methyl-6-methylaminopyrimidine was treated with triethyl orthoacetate and acetic anhydride as above. The silica column was eluted with light petroleum containing increasing amounts of diethyl ether to give the *dimethylaminotrimethylpurine* (49%), m.p. 127° (from light petroleum) (Found: C, 58·9; H, 7·6; N, 34·2. $C_{10}H_{15}N_5$ requires C, 58·5; H, 7·4; N, 34·1%).

2-*Methoxy*-6,9-*dimethylpurine*.— 2-Chloro-4-methyl-6-methylamino-5-nitropyrimidine [11] (5 g) was added in small portions to sodium methoxide [from sodium (1·5 g)] in anhydrous methanol (50 ml) at 0—5°. The mixture was then stirred at *ca.* 10° for 60 min. The solid was washed with cold methanol and recrystallized from cyclohexane to give 2-*methoxy*-4-*methyl*-6-*methylamino*-5-*nitropyrimidine* (49%), m.p. 130—131° (from water) (Found: C, 42·3; H, 5·05; N, 28·1. $C_7H_{10}N_4O_3$ requires C, 42·4; H, 5·1; N, 28·3%). Hydrogenation of the nitropyrimidine (1·75 g) in methanol (60 ml) over Raney nickel followed by filtration and evaporation gave 5-*amino*-2-*methoxy*-4-*methyl*-6-*methylaminopyrimidine* (88%), m.p. 134—135° (from carbon tetrachloride) (Found: C, 49·7; H, 7·3; N, 33·7. $C_7H_{12}N_4O$ requires C, 50·0; H, 7·2; N, 33·3%). Treatment of this diamine with triethyl orthoformate and acetic anhydride followed by sublimation of the crude product gave the *methoxydimethylpurine* (69%), m.p. 125—126° (from light petroleum) (Found: C, 54·2; H, 6·0; N, 31·1. $C_8H_{10}N_4O$ requires C, 53·9; H, 5·7; N, 31·45%).

2-*Methoxy*-6,8,9-*trimethylpurine*.—Made like the foregoing homologue (but with triethyl orthoacetate) and purified by t.l.c. [silica; chloroform–ethanol (50 : 1)] the *methoxytrimethylpurine* (22%) had m.p. 95—96° (after sublimation) (Found: C, 56·3; H, 6·3; N, 29·45. $C_9H_{12}N_4O$ requires C, 56·25; H, 6·3; N, 29·15%).

6,9-*Dimethyl*-2-[^{14}C]*methylthiopurine*.—A solution of 6,9-dimethylpurine-2-thione [13] (89·7 mg, 0·5 mmol) in 0·20M-sodium hydroxide (2·5 ml) was introduced into an ampoule of [^{14}C]methyl iodide (0·5 mCi; *ca.* 1·5 mg) cooled in liquid nitrogen. The ampoule was sealed and shaken at room temperature for 15 min. Methyl iodide (67 mg) was then introduced and the mixture was shaken for a further 20 min. The residue from evaporation was extracted with chloroform. The extract was dehydrated (sodium sulphate) and evaporated to give the purine (85%; *ca.* 2% labelled) which on t.l.c. showed only one (radioactive) spot, identical in position and appearance with that from the non-radioactive purine.[13]

2-*Ethylthio*-6,9-*dimethylpurine*.— 6,9-Dimethylpurine-2-thione [13] (0·9 g), ethanolic 0·5M-sodium ethoxide (10 ml), and ethyl iodide (0·8 g) were stirred at 25° until the solid had dissolved (*ca.* 20 min). The residue from evaporation was extracted with chloroform. Removal of the solvent and recrystallization from light petroleum and then water gave the *ethylthiopurine* (89%), m.p. 93° (Found: C, 51·9; H, 6·0; N, 27·05. $C_9H_{12}N_4S$ requires C, 51·8; H, 5·8; N, 26·9%).

6-*Methyl*-2,8-*bismethylthiopurine*.—4,5-Diamino-6-methylpyrimidine-2-thione [12] (1·8 g), carbon disulphide (12·5 ml), and pyridine (38 ml) were heated under reflux for 6 h. The residue from evaporation, dissolved in the minimum quantity of hot M-sodium hydroxide, was treated with charcoal and then acidified to give 6-*methylpurine*-2,8(1H,7H)-*dithione* (or tautomer) (78%), m.p. <360° (from water) (Found: C, 36·6; H, 3·4. $C_6H_6N_4S_2$ requires C, 36·4; H, 3·1%). The dithione (1·0 g), M-sodium hydroxide (10 ml), and methyl iodide (1·5 g) were stirred at 25° for 30 min. The solution was adjusted to pH <5 (if necessary) and the solid was filtered off. Recrystallization from ethanol–light petroleum gave the *methylbismethylthiopurine* (83%), m.p. 236° (Found: C, 42·5; H, 4·5; N, 24·6. $C_8H_{10}N_4S_2$ requires C, 42·5; H, 4·5; N, 24·8%).

6,9-*Dimethyl*-2,8-*bismethylthiopurine*.— 5-Amino-4-methyl-6-methylaminopyrimidine-2-thione [13] was converted similarly into 6,9-*dimethylpurine*-2,8(1H,7H)-*dithione* (46%), m.p. <360° (from water) (Found: C, 39·7; H, 3·8; N, 26·1. $C_7H_8N_4S_2$ requires C, 39·6; H, 3·8; N, 26·4%) and thence into the *dimethylbismethylthiopurine* (97%), m.p. 151° (from light petroleum) (Found: C, 45·1; H, 4·9; N, 23·1. $C_9H_{12}N_4S_2$ requires C, 45·0; H, 5·0; N, 23·3%).

8-*Methyl*-2-*methylthiopurine*.—The intermediate purinethione proved unexpectedly difficult to prepare in high yield. (*a*) 4,5-Diaminopyrimidine-2-thione [17] (1 g) was heated under reflux in acetic anhydride for 2 h. After evaporation the residue was dissolved in water and again evaporated. Recrystallization from water gave 5-*acetamido*-4-*aminopyrimidine*-2(1H)-*thione* (84%), gradually decomposing on heating above 260° (Found: C, 38·8; H,

[16] E. S. Chaman and E. S. Golovchinskaya, *Zhur. obshchei Khim.*, 1966, **36**, 1608.
[17] D. J. Brown, *J. Appl. Chem.*, 1952, **2**, 239.

4·7; N, 29·8. $C_6H_8N_4OS$ requires C, 39·1; H, 4·4; N, 30·4%). This amide (0·3 g) was heated under reflux with 2M-sodium hydroxide (5 ml) for 30 min. The hot solution was acidified to pH 5—6. After chilling, the solid was filtered off and extracted with a little boiling water. The residual solid was recrystallized from a large volume of water to give 8-*methylpurine*-2(1H)-*thione* (22%), m.p. ⩽310° (Found: C, 43·4; H, 3·7. $C_6H_6N_4S$ requires C, 43·4; H, 3·6%); the extract yielded 4,5-diaminopyrimidine-2-thione (0·15 g).

(*b*) The same diamine (1·42 g) and acetamidine hydrochloride (2·0 g) were heated at 180—200° until evolution of ammonia ceased. The cooled mixture, dissolved in M-sodium hydroxide, was treated with charcoal and acidified to pH 6. The solid was separated as in (*a*) into starting material (0·6 g) and the purinethione (0·7 g).

(*c*) The diaminopyrimidine (1·42 g), triethyl orthoacetate (20 ml), and acetic anhydride (2·05 ml) were heated under reflux for 4 h. The resulting slurry was evaporated to dryness. The solid, dissolved in M-sodium hydroxide, was decolourized with charcoal and acidified to pH 6. A white precipitate was removed whereupon the filtrate deposited the yellow purinethione (13%). The white solid proved to be 4-*amino*-5-(1-*ethoxyethylidene*)*aminopyrimidine*-2(1H)-*thione* (40%), m.p. ⩽310° (from water) (Found: C, 45·1; H, 5·75; N, 26·7. $C_8H_{12}N_4OS$ requires C, 45·3; H, 5·7; N, 26·4%), which was converted almost quantitatively into the purinethione by boiling in 2M-sodium hydroxide for 30 min followed by acidification.

Shaking a solution of the purinethione (0·6 g) and methyl iodide (0·6 g) in M-sodium hydroxide (3·7 ml) gave 8-*methyl*-2-*methylthiopurine* (92%), m.p. 270° (decomp.) (from ethanol, then water) (Found: C, 46·6; H, 4·4; N, 31·1. $C_7H_8N_4S$ requires C, 46·6; H, 4·5; N, 31·1%).

6,8-*Dimethyl*-2-*methylthiopurine*.—4,5-Diamino-6-methylpyrimidine-2-thione [12] (1·56 g), triethyl orthoacetate (20 ml), and acetic anhydride (2·1 g) were heated under reflux for 4 h. The residue from evaporation was boiled in 2M-sodium hydroxide for 30 min. Decolourization and acidification gave 6,8-*dimethylpurine*-2(1H)-*thione* (30%), m.p. ⩽330° (from water) (Found: C, 46·2; H, 4·7; N, 30·9. $C_7H_8N_4S$ requires C, 46·6; H, 4·5; N, 31·1%); it was made also by the acetamidine method [(*b*) above] in 24% yield.

The thione was *S*-methylated like its homologue to give the *dimethyl*-2-*methylthiopurine* (66%), m.p. 213° (from water, then ethanol–light petroleum) (Found: C, 49·6; H, 5·2; N, 28·9. $C_8H_{10}N_4S$ requires C, 49·5; H, 5·2; N, 28·85%).

6,8,9-*Trimethyl*-2-*methylthiopurine*.— 5-Amino-4-methyl-6-methylaminopyrimidine-2-thione [13] (2·0 g) and acetic anhydride (12 ml) were heated under reflux for 1 h. The residue from evaporation was heated under reflux in 2M-sodium hydroxide (10 ml) for 1 h. The solution was decolourized and acidified to give 6,8,9-*trimethylpurine*-2(1H)-*thione* (22%), m.p. 288—292° (decomp.) (from water) (Found: C, 49·3; H, 5·1; N, 28·9. $C_8H_{10}N_4S$ requires C, 49·5; H, 5·2; N, 28·85%). A similar experiment with only triethyl orthoacetate failed. *S*-Methylation of the thione as before gave the *trimethylmethylthiopurine* (93%), m.p. 103—104° (from light petroleum) (Found: C, 51·9; H, 6·05; N, 27·3. $C_9H_{12}N_4S$ requires C, 51·9; H, 5·8; N, 26·9%).

6,9-*Dimethyl*-2-*methylthiopurin*-8(7H)-*one*.— 5-Amino-4-methyl-6-methylaminopyrimidine-2-thione [13] (0·68 g) and urea (0·68 g) were heated at 185° for 15 min. The cooled mixture, dissolved in aqueous 1·5M-ammonia, was decolourized and acidified to give 1,2-*dihydro*-6,9-*dimethyl*-2-*thioxopurin*-8(7H)-*one* (64%), m.p. ⩽300° (Found: C, 42·4; H, 4·2; N, 28·2. $C_7H_8N_4OS$ requires C, 42·85; H, 4·1; N, 28·55%). Subsequent *S*-methylation gave the *dimethylmethylthiopurine* (85%), m.p. 279—280° (Found: C, 45·6; H, 4·9; N, 26·7. $C_8H_{10}N_4OS$ requires C, 45·7; H, 4·8; N, 26·65%).

Hydrolyses of 6,9-*Dimethyl*-2-*methylthiopurine*.—The purine (0·15 g) was boiled with M-sodium hydroxide for 1 h under reflux. The solution was adjusted to pH 5—6 and cooled, whereupon the unchanged purine (0·06 g) crystallized out and was filtered off. Evaporation of the filtrate and extraction of the residue with chloroform gave, on removal of the solvent, 5-*amino*-4-*methyl*-6-*methylamino*-2-*methylthiopyrimidine* (94% net), m.p. 160° (from chloroform–light petroleum, then sublimed) (Found: C, 45·9; H, 6·9; N, 30·5. $C_7H_{12}N_4S$ requires C, 45·65; H, 6·6; N, 30·4%), identified (mixed m.p.) with material prepared (60%) unambiguously by *S*-methylation of the corresponding thione [13] under the usual conditions. This pyrimidine recyclized during boiling for 2 h in 98% formic acid under reflux to give the starting purine (75%), which was unaffected by boiling in 2M-hydrochloric acid for 1 h.

6,9-*Dimethyl*-2-*methylsulphinylpurine*.—(*a*) 6,9-Dimethyl-2-methylthiopurine [13] (0·49 g) and 80% *m*-chloroperoxybenzoic acid (0·53 g) were stirred in chloroform, first at 0° for 2 h and then at 25° for 16 h. After washing with saturated aqueous sodium hydrogen carbonate, the solution was evaporated to dryness. Sublimation (130° at 0·2 mmHg) gave the *sulphoxide* (70%), m.p. 162° [δ($CDCl_3$) 3·06 (s) and 3·13 (s) (6-Me and SMe), 4·13 (s, 9-Me), and 7·47 p.p.m. (s, 8-H)] (Found: C, 45·8; H, 5·0; N, 26·2. $C_8H_{10}N_4OS$ requires C, 45·7; H, 4·8; N, 26·6%).

(*b*) A 0·1% solution of 6,9-dimethyl-2-[^{14}C]methylthiopurine (*ca.* 2% labelled) in safflower oil (2·5 ml) was administered orally to mice. More than 95% of the radioactivity appeared within 24 h in the urine, which showed only one radioactive spot on t.l.c. [silica; chloroform–ethanol (12 : 1)]. The whole process was repeated with a larger amount of non-radioactive purine. The crude material derived from chloroform extraction of the combined urine was submitted to preparative t.l.c. (Merck, 2 mm silica plates; chloroform–ethanol). The appropriate zone was eluted; the derived solid, recrystallized from light petroleum and then sublimed, was identical with the foregoing sulphoxide (u.v. and i.r. spectra) (Found: M^+, 210·05754. $C_8H_{10}N_4OS$ requires *M*, 210·05753). It differed markedly from the isomeric 6,9-dimethyl-2-methylthiopurin-8-one.

Measurement of Biological Activity.—In summary, the cultues of *E. coli B* were grown overnight to stationary phase. Washed cells were resuspended in fresh glucose-salts medium containing phleomycin (1 or 2 μg ml^{-1}) at 37° for 30 or 45 min. Samples were then membrane-filtered and resuspended in fesh medium containing the purine (8 or 2 mM according to solubility). Samples were removed at the outset and at intervals during 120 min for estimating the number of viable cells present. Because batches of phleomycin varied appreciably in their intrinsic activity,[5] an experiment with 8mM-caffeine was run as a standard in parallel with each purine.

The activity of each tested purine was then expressed (Table) as an 'adjusted activity', relative to the mean

activity of caffeine (30) as a standard, and hence reasonably independent of small variations in conditions or in the intrinsic activity or amount of phleomycin used.

Thus $A_m = N_0/N_{120}$ and $A_{ad} = 30A_m/A_{caf}$ where A_m is the measured activity of the purine under test, A_{ad} is the adjusted activity, N_0 and N_{120} are the numbers of viable cells at the outset and after 120 min, respectively, and A_{caf} is the measured activity of 8mM-caffeine in the parallel experiment.

Results were reproducible within $\pm 7\%$; manipulative details have been given elsewhere.[4,5]

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