695. 2-*Phenylpurines, their Chemical and Enzymological Reactivity.*

By F. Bergmann, A. Kalmus, H. Ungar-Waron, and H. Kwietny-Govrin.

2-Phenylpurines are accessible from 4,5-diamino-2-phenylpyrimidine and its 6-hydroxy- and 6-mercapto-derivatives. Thiation of 2-phenylhypoxanthine proceeds as smoothly as that of hypoxanthine itself. 6,8-Dihydroxy-2-phenylpurine is thiated exclusively at position 6, in contrast to the corresponding 2-methyl derivative. Xanthine oxidase is unable to attack position 6 in 2-phenylpurine or its 8-hydroxy-derivative, but does so without difficulty in the corresponding 2-methylpurines. 2-Phenylhypoxanthine is oxidised by the enzyme about 50 times faster than the 2-methyl derivative.

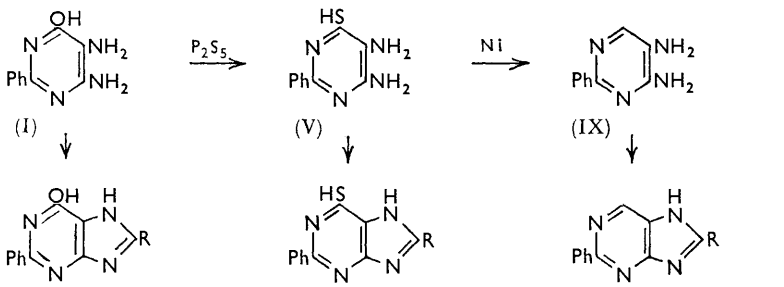
The study of 2-methylpurines has brought to light certain peculiar properties not encountered among purines with a free 2-position.[1] Thus thiation of 2-methylhypoxanthine was much more difficult than that of hypoxanthine, and 6,8-dihydroxy-2-methylpurine gave the 6,8-dithio-derivative as main product, accompanied only by traces of the 6-monomercapto-derivative. In contrast, position 6 was almost exclusively attacked when 6,8-dihydroxypurine itself reacted with phosphorus pentasulphide.[2] Deviations from the reactions established for non-methylated purines may be ascribed to the inductive effect of the 2-methyl group, which is transmitted across the purine ring. It appeared of interest to test the behaviour of 2-phenylpurines for two reasons: (*a*) depending on the character of the other substituents in the purine ring, the phenyl group may serve either as an electron-source or as an electron-sink; and (*b*), on the other hand, by proper

[1] Bergmann and Kalmus, *J.*, 1962, 860.
[2] Bergmann and Kalmus, *J. Org. Chem.*, 1961, **26**, 1660.

substitution of the phenyl group, the latter may become the main source of electron supply or withdrawal in the system and thus may influence reactions in position 6 or 8. Such studies may shed light on the factors determining the different reactivity of the various positions in the purine ring, a problem that is still poorly understood.

The present paper deals with 2-phenylpurines only. The introduction of other aromatic substituents in position 2 will be described separately.

Only 2-phenylhypoxanthine and 2-phenyladenine have been reported previously.[3,4] From 4,5-diamino-6-hydroxy-2-phenylpyrimidine [3] (I) two synthetic routes lead to the various derivatives needed for the present investigation: (*a*) Condensation with formamide, urea, or thiourea gives the purines (II)—(IV). (*b*) In accordance with earlier experience



on 4,5-diaminouracil,[5] the compound (I) can be thiated in position 6. The resulting 4,5-diamino-6-mercapto-2-phenylpyrimidine (V), on reaction with the appropriate components, produces the purines (VI)—(VIII).

Reaction of 6,8-dihydroxy-2-phenylpurine (III) with phosphorus pentasulphide gave the 6-mercapto-derivative (VII) in 75% yield; the dimercapto-derivative (VIII) was sought but was not identified with certainty. In this reaction the diol (III) behaves like 6,8-dihydroxypurine [2] or uric acid [6] but differs from 6,8-dihydroxy-2-methylpurine.[1] As with the examples studied previously, the 6,8-dimercapto-derivative (VIII) was obtained readily by thiation of 6-hydroxy-8-mercapto-2-phenylpurine (IV). On the other hand, the isomer (VII) was resistant to attack at position 8, again in accordance with earlier observations on 8-hydroxy-6-mercaptopurine and its 2-methyl derivative.[1]

Catalytic desulphuration of compound (V) led to 4,5-diamino-2-phenylpyrimidine (IX), from which 2-phenylpurine (X) and its 8-hydroxy-derivative (XI) were obtained. The direct synthesis of compounds (X) and (XI) by this route proved superior to the alternative desulphuration of the purines (VI) and (VII). The latter process gave mixtures of starting material, sulphur-free constituents, and hydrolytic products, resulting from the instability of the 6-mercapto-group in these derivatives to alkali.

Condensation of 4,5-diamino-2-phenylpyrimidine (IX) with benzamidine [7] gave 2,8-diphenylpurine (XII).

Like the pyrimidine (I) and the purines (III) and (IV), 2-phenylhypoxanthine (II) undergoes thiation smoothly. This excludes the possibility that substitution reactions at C-6 may be sterically hindered by groups attached to C-2, and supports the hypothesis that a 2-alkyl substituent retards thiation at C-6 by virtue of its inductive effect. On the other hand, a 2-phenyl group does not alter materially the electron distribution in the π-deficient purine system.

[3] Traube and Herrmann, *Ber.*, 1904, **37**, 2267.
[4] Andrisano and Maioli, *Gazzetta*, 1953, **83**, 264.
[5] Levin, Kalmus, and Bergmann, *J. Org. Chem.*, 1960, **25**, 1752.
[6] Elion, Müller, and Hitchings, *J. Amer. Chem. Soc.*, 1959, **81**, 3042.
[7] Bergmann and Tamari, *J.*, 1961, 4468.

It has not yet been established whether thiation involves the 6-carbonyl group as such or as its enolised form.[1] In the latter case, although N-1 does not participate directly in the reaction, the aromatic substituent at C-2 may influence the mobility of the proton in the neighbouring 1-NH group. However, the present results do not reveal any such effect.

It was hoped to obtain information on this problem from the dissociation constants. However, Table 1 does not give a clear picture. Thus, in the hypoxanthine series, a 2-phenyl substituent does not alter the acid pK, while a 2-methyl group raises it. On the other hand, 2-methylpurine exhibits the same pK value as purine itself, while the pK of 2-phenylpurine is 0·6 unit higher. An even greater difference is found in the series of 6,8-dihydroxypurine, a 2-phenyl group raising pK by 1·6 units. Finally, among the three 6-hydroxy-8-mercaptopurines in Table 1, no marked difference in the values of the

TABLE 1.

Physical properties of purines.

(*a*) Compound	$\lambda_{max.}$ (mμ) of neutral molecule	pK_a	R_F in solvent * 1	2	3	Fluorescence
Purine	263	9·0	—	0·6	—	Black-violet
2-Methyl-	268	9·1	0·76	0·75	—	Black-violet
2-Phenyl-	236, 282	9·6	0·82	0·77	—	Black-violet
Hypoxanthine	251	8·8	—	0·52	—	Black-violet
2-Methyl-	250	9·9	0·71	0·6	—	Black-violet
2-Phenyl-	262, 279	8·9	0·73	0·66	—	Blue-violet
8-Hydroxypurine	275	7·9	—	0·6	—	Violet
2-Methyl-	280	8·6	0·74	0·68	—	Blue-violet
2-Phenyl-	288·5	8·3	—	0·73	—	Bright blue
6,8-Dihydroxypurine	256	8·1	—	0·34	—	Black-violet
2-Methyl-	255	8·0	0·64	0·45	—	Black-violet
2-Phenyl-	268	9·7	0·53	0·55	—	Greenish-blue
6-Hydroxy-8-mercaptopurine	289	6·8	—	0·42	—	Violet
2-Methyl-	290	7·1	—	0·48	—	Violet
2-Phenyl-	296	7·2	0·61	0·61	0·74	Violet

(*b*) Compound	$\lambda_{max.}$ (mμ) at pH 8·0	R_F in solvent * 1	2	3	Fluorescence
6-Mercapto-2-phenylpurine	261, 318	0·77	0·66	—	Black-violet
8-Hydroxy-6-mercapto-2-phenylpurine	259, 315/316	0·78	0·57	—	Blue-yellow
6,8-Dimercapto-2-phenylpurine	226, 278, 343	0·68	0·64	0·69	Violet

* Solvent 1, 95% EtOH–dimethylformamide–H_2O 60 : 20 : 20 (v/v). Solvent 2, 95% EtOH–H_2O–AcOH 85 : 10 : 5 (v/v). Solvent 3, 95% EtOH–25% NH_3 80 : 20 (v/v).

first acid dissociation constants is found. However, it should be recalled that pK values are comparable only if in all members of a given series the same NH group is involved in the ionisation process. At present, no information is available on the localisation of the first dissociation step in the derivatives under consideration.

Although in most of the purines studied the 2-phenyl substituent does not influence the dissociation of NH groups, it facilitates excitation of the uncharged molecules markedly. The bathochromic effect of the 2-phenyl group (7—28 mμ) is much larger than that of a 2-methyl substituent. In fact, the latter may produce small shifts of $\lambda_{max.}$ in either direction ($\Delta\lambda_{max.} = -1$ to $+5$ mμ).

Striking differences between 2-methyl- and 2-phenyl-purines are observed in their behaviour towards mammalian xanthine oxidase. 2-Methylpurine is converted with

TABLE 2.

Compound	$\lambda_{max.}$ (mμ) at pH 8·0	log ε	Wavelengths used for rate measurements	Enzyme diln.	Oxidation at postn.	Relative rate *
2-Phenylpurine	236	4·38	283	1/500	8	2·8
	265	4·15	303			
	282	4·16				
2-Phenylhypoxanthine	262	4·10	325	1/500	8	1·7
	279	4·10	330			
			335			
			340			
8-Hydroxy-2-phenylpurine	292	4·26	—	1/200	Not attacked	—
2-Methylpurine	268	3·91	240	1/2000	6	12
			250			
			275			
2-Methylhypoxanthine	250	4·04	275	1/200	8	~0·03
			280			
8-Hydroxy-2-methylpurine	281	3·88	255	1/500	6	0·5
			260			

* All rates measured were recalculated for an enzyme dilution of 1 : 5000; a linear relation between rate and enzyme concentration was assumed. The results are expressed as percentage of the rate of oxidation of xanthine.

considerable speed, first, into the corresponding hypoxanthine, while 2-phenylpurine is attacked exclusively at C-8 (Table 2). In accordance with this divergence in the reactivity of position 6, the enzyme oxidises 8-hydroxy-2-methylpurine at C-6 at a relative rate of 0·5, while 8-hydroxy-2-phenylpurine is refractory. On the other hand, 2-phenylhypoxanthine is attacked at position 8 about 50 times faster than 2-methylhypoxanthine.

The strong inhibitory influence of the 2-phenyl group on enzymic oxidation at C-6 suggests that the enzymic mechanism involves, *inter alia*, attachment of the nitrogen of the group $C^6{=}N^1$ to the active surface, a process which is strongly opposed by the bulky aromatic substituent at C-2. Conversely, a 2-methyl group has only a slight retarding influence, as the oxidation of 2-methylpurine to 2-methylhypoxanthine proceeds at about half the rate at which purine is converted into hypoxanthine.[8] The effect of 2-substituents on enzymic attack at C-6 thus contrasts sharply with their role in chemical interactions at position 6.

Likewise, the different rates of oxidation of the respective hypoxanthines (Table 2) are of great interest. It has been pointed out previously [9] that hypoxanthine is the only 6-monosubstituted purine in which enzymic attack is directed towards position 2. This exceptional behaviour was ascribed to the formation of an enzyme–substrate complex in which the hypoxanthine is activated by shift of a proton from N-1 to N-3.[8] However, in kinetic studies on the conversion of hypoxanthine into xanthine it could not be excluded that a small percentage of the substrate may pass through the stage of 6,8-dihydroxypurine to uric acid.[10] The data in Table 2 suggest that such an alternative pathway is indeed possible because both 2-methyl- and 2-phenyl-hypoxanthine are attacked at position 8. It is remarkable that in this series the rate of oxidation of the 2-phenyl derivative is considerably higher than that of the 2-methyl compound. Neither substituent can exert a steric effect on reactions taking place at C-8. On the other hand, it appears probable that the 2-phenyl substituent prevents tautomerisation of hydrogen from N-1 to N-3 more effectively than does a 2-methyl group. Combination of 2-phenyl-hypoxanthine with the enzyme in a manner similar to complex formation by hypoxanthine itself, is now largely suppressed and an alternative mode of attachment, leading to activation of C-8, becomes possible. On the other hand, with 2-methylhypoxanthine there

[8] Bergmann, Kwietny, Levin, and Brown, *J. Amer. Chem. Soc.*, 1960, **82**, 598.
[9] Bergmann, Ungar-Waron, Goldberg, and Kalmus, *Arch. Biochem. Biophys.*, 1961, **94**, 94.
[10] Bergmann and Dikstein, *J. Biol. Chem.*, 1956, **223**, 765.

may still be formed an appreciable amount of an activated complex resembling that of hypoxanthine. Formation of this complex decreases the probability of attack at C-8 considerably. Indeed conversion of 2-methylhypoxanthine into 6,8-dihydroxy-2-methylpurine is extremely slow and can be measured only because no other reaction can take place.

In conclusion, it may be stated that in enzymic oxidations the 2-phenyl group exerts a pronounced effect either by virtue of steric hindrance, as exemplified by obstruction of the attack at C-6, or by its influence on tautomerisations, as shown by the relatively rapid oxidation at position 8.

Experimental

Absorption spectra were measured in a Beckman spectrophotometer. Dissociation constants were determined by the spectrophotometric method,[11] as modified by Bergmann and Dikstein.[12] The following buffers were used for these measurements: pH −3, 13·5N-sulphuric acid; pH −1, 5N-sulphuric acid; pH 0—3, perchloric acid; pH 4—6, 0·1M-acetate; pH 6—8, 0·1M-phosphate; pH 8—11, 0·1M-borate adjusted with sodium hydroxide; pH 11—14, sodium hydroxide.

Paper chromatograms were developed by the descending method, with the solvents specified in Table 1. Spots were located by their fluorescence under a Mineralight ultraviolet lamp (λ ~255 mμ).

4-*Amino*-6-*hydroxy*-2-*phenylpyrimidine*.[3,4]—For a successful condensation, it is necessary to dehydrate benzamidine hydrochloride in a vacuum-desiccator over concentrated sulphuric acid until a m. p. of 167—169° is reached.

To a solution of sodium (6 g.; 0·26 g.-atom) in absolute ethanol (120 ml.) was added first benzamidine hydrochloride (10·4 g., 0·06 mole), then ethyl cyanoacetate (7·6 g., 0·06 mole). After 3 hours' refluxing, the solvent was evaporated, and the residue dissolved in water and acidified with acetic acid. Recrystallisation from dilute acetic acid gave colourless plates (8·8 g., 70%), m. p. 264—265°, $\lambda_{max.}$ (pH 8·0) 223 and 264—265 mμ, R_F (solvent 1) 0·71.

The 5-nitroso-derivative, prepared as described by Traube and Herrmann,[3] crystallised from dilute acetic acid in green plates, m. p. 255° (yield, 81%), $\lambda_{max.}$ (pH 8·0) 282 and 346—347 mμ.

4,5-*Diamino*-6-*hydroxy*-2-*phenylpyrimidine* (I).[3,4]—The above nitroso-compound (9·3 g.) was suspended in water (300 ml.) at 60° and sodium dithionite added portionwise until the green-blue colour had disappeared. The diamino-derivative (I) crystallised from water in needles (58%), m. p. 228—230°, $\lambda_{max.}$ (pH 8·0) 229, 283, and 318 mμ, R_F (solvent 2) 0·64, (solvent 3) 0·79.

2-*Phenylhypoxanthine* (III).[3,4]—Heating the sulphate of the diamino-derivative (I) with formamide at 195—200° during 1 hr. gave 2-phenylhypoxanthine in 90% yield. The product crystallised from dimethylformamide or acetic acid in needles, decomp. >300°.

6,8-*Dihydroxy*-2-*phenylpurine* (III).—A mixture of the free pyrimidine (I) (4 g.) and urea (6 g.) was heated at 170—175° for 1 hr. The water-insoluble part of the product was dissolved in N-sodium hydroxide, and the hot solution added slowly to boiling acetic acid, to yield the *purine* (III) (4·2 g., 95%) that crystallised from dilute acetic acid in rods, decomp. >300° (Found: C, 57·9; H, 3·7; N, 24·6. $C_{11}H_8N_4O_2$ requires C, 57·9; H, 3·5; N, 24·6%).

6-*Hydroxy*-8-*mercapto*-2-*phenylpurine* (IV).—A mixture of the pyrimidine (I) (3 g.), thiourea (3 g.), and anhydrous sodium acetate (1 g.) was heated at 190—195° during 1 hr. The product was worked up as described for the condensation with urea. The *product* (2·7 g., 75%) formed plates (from dilute acetic acid), decomp. >300° (Found: C, 54·4; H, 3·4; N, 23·4. $C_{11}H_8N_4OS$ requires C, 54·1; H, 3·3; N, 23·0%).

4,5-*Diamino*-6-*mercapto*-2-*phenylpyrimidine* (V).—To freshly distilled, boiling β-picoline (140 ml.) was added, first, 4,5-diamino-6-hydroxy-2-phenylpyrimidine (I) (7 g.), then phosphorus pentasulphide (28 g.). After 40 minutes' refluxing, the solvent was removed *in vacuo* and the residue stirred with water (150 ml.) at 70—80° during 1 hr. The insoluble portion was removed. From the filtrate, a second crop of yellow crystals was obtained on cooling in the refrigerator. The combined crystals were dissolved in N-sodium hydroxide, the solution was decolorised with dithionite, and the *product* precipitated with acetic acid. Recrystallisation from acetic acid

[11] Robinson and Pekrul, *J. Amer. Chem. Soc.*, 1945, **67**, 1186.
[12] Bergmann and Dikstein, *J. Amer. Chem. Soc.*, 1955, **77**, 691.

gave rods (3·9 g., 51%), decomp. ~270°, $\lambda_{max.}$ (pH 8·0) 247 and 333—334 mμ (Found: C, 55·1; H, 4·9; N, 26·1. $C_{10}H_{10}N_4S$ requires C, 55·1; H, 4·6; N, 25·7%).

4,5-*Diamino*-6-*methylthio*-2-*phenylpyrimidine*.—The pyrimidine (V) (1 g.) was dissolved in N-sodium hydroxide (5 ml.) and stirred with methyl iodide (0·5 ml.) at room temperature for 2 hr. The mixture was acidified with acetic acid to yield the 6-*methylthio-derivative* (0·5 g., 40%). From water, acidulated with a few drops of acetic acid, yellowish plates, m. p. 97°, $\lambda_{max.}$ (pH 8·0) 229 and 306 mμ, R_F (solvent 2) 0·84, (solvent 3) 0·9 (Found: C, 52·9; H, 5·6. $C_{11}H_{12}N_4S,H_2O$ requires C, 52·8; H, 5·6%), were obtained.

6-*Mercapto*-2-*phenylpurine* (VI).—(*a*) *Condensation of* 4,5-*diamino*-6-*mercapto*-2-*phenylpyrimidine with formamide*. A mixture of the diamino-derivative (V) (0·6 g.) and formamide (4 ml.) was heated at 190° for 30 min. After cooling, a solid mass was obtained which was first extracted with hot water. The insoluble portion was then dissolved in hot N-sodium hydroxide, from which acetic acid precipitated the expected *purine* (VI). From acetic acid this formed yellowish boat-shaped crystals (0·6 g., 83%), decomp. >300° (Found: C, 57·7; H, 3·15; N, 24·5. $C_{11}H_8N_4S$ requires C, 57·9; H, 3·5; N, 24·6%).

(b) *Thiation of* 2-*phenylhypoxanthine* (II). 2-Phenylhypoxanthine (4 g.) and phosphorus pentasulphide (16 g.) in anhydrous pyridine (200 ml.) were refluxed for 3·5 hr. After removal of the solvent *in vacuo*, the residue was decomposed with water (35 ml.) at 70° during 1 hr. The insoluble portion was dissolved in sodium hydroxide, the reddish solution decolorised with charcoal and a small amount of dithionite, and the filtrate acidified with acetic acid. The product (3·8 g., 89%), crystallised from acetic acid, was identical with the foregoing compound.

6-*Methylthio*-2-*phenylpurine*.—6-Mercapto-2-phenylpurine (VI) (1 g.) was stirred with methyl iodide (0·5 ml.) in 0·2N-sodium hydroxide (30 ml.) at room temperature for 30 min. The white precipitate resulting from addition of acetic acid recrystallised from dilute acetic acid, forming needles, m. p. 257°, $\lambda_{max.}$ (pH 8·0) 258 and 297—298 mμ, R_F (solvent 2) 0·79, yellow-red fluorescence (Found: C, 59·6; H, 4·05; N, 23·2. $C_{12}H_{10}N_4S$ requires C, 59·5; H, 4·1; N, 23·1%).

Desulphuration of 6-mercapto-2-phenylpurine or its 6-methylthio-derivative was attempted in ethanolic ammonia. Only starting material was recovered. The same reaction, when carried out in 0·5N-sodium hydroxide, gave an inseparable mixture of starting material, 2-phenylhypoxanthine, and 2-phenylpurine.

8-*Hydroxy*-6-*mercapto*-2-*phenylpurine* (VII).—(*a*) *Condensation of* 4,5-*diamino*-6-*mercapto*-2-*phenylpyrimidine with urea*. A mixture of the pyrimidine (V) (1 g.) and urea (1·5 g.) was heated at 175—180° for 30 min. The *product* was dissolved in warm sodium hydroxide, treated with charcoal, and acidified. The precipitate was purified by reprecipitation, forming yellowish needles (1 g., 88%), decomp. 300° (Found: C, 54·1; H, 3·6; N, 22·8. $C_{11}H_8N_4OS$ requires C, 54·1; H, 3·3; N, 22·95%).

(*b*) *Thiation of* 6,8-*dihydroxy*-2-*phenylpurine* (III). To a suspension of the diol (1 g.) in boiling pyridine (50 ml.) was added phosphorus pentasulphide (3 g.) with stirring. After 4 hours' refluxing, the solvent was distilled *in vacuo* and the residue treated at 70—80° with water (30 ml.) during 1 hr. The water was decanted and the insoluble portion dissolved in N-sodium hydroxide. Acidification gave 8-hydroxy-6-mercapto-2-phenylpurine (VII) (0·95 g., 90%), identical with the foregoing product.

The mother-liquors were concentrated and the residue subjected to paper chromatography. However, there was no spot corresponding to the dithio-derivative (VIII) (see below).

6,8-*Dimercapto*-2-*phenylpurine* (VIII).—(*a*) *Condensation of* 4,5-*diamino*-6-*mercapto*-2-*phenylpyrimidine* (V) *with thiourea*. A mixture of the sulphate of the diamine (V) (1 g.) and thiourea (1·6 g.) was kept at 190—200° during 30 min. The dark mixture was dissolved in hot sodium hydroxide and added dropwise to boiling glacial acetic acid with stirring. Recrystallisation of the precipitate from dilute acetic acid gave the *dithiol* as yellow plates (0·6 g., 73%), decomp. >300° (Found: C, 47·65; H, 3·5; N, 20·3. $C_{11}H_8N_4S_2,H_2O$ requires C, 47·5; H, 3·6; N, 20·1%).

(*b*) *Thiation of the purine* (IV). The purine (IV) (2 g.) and phosphorus pentasulphide (8 g.) in anhydrous pyridine (100 ml.) was refluxed for 3·5 hr. After removal of the solvent *in vacuo*, the residue was decomposed with water (25 ml.) at 70° during 1 hr. The insoluble portion was dissolved in a minimal amount of N-sodium hydroxide, decolorised with charcoal and a small amount of dithionite, and acidified with acetic acid. Recrystallisation from dilute acetic acid gave yellow plates (53% yield), identical with the dithio-derivative described above.

4,5-Diamino-2-phenylpyrimidine (IX).—4,5-Diamino-6-mercapto-2-phenylpyrimidine (V) (10 g.) was dissolved in 10% aqueous ammonia (100 ml.) with gentle heating. After addition of Raney nickel (12 g. wet) the mixture was refluxed for 2 hr. with stirring. The catalyst was filtered off and the solution concentrated *in vacuo*. The residual oil solidified on trituration with a few drops of water (yield, 5 g., 60%). From benzene, plates, m. p. 146°, $\lambda_{max.}$ (pH 8·0) 298 mμ, R_F (solvent 2) 0·82, (solvent 3) 0·85, violet fluorescence (Found: C, 64·2; H, 5·6; N, 30·35. $C_{10}H_{10}N_4$ requires C, 64·5; H, 5·4; N, 30·1%), were obtained.

2-Phenylpurine (X).—4,5-Diamino-2-phenylpyrimidine (IX) (0·5 g.) was heated with formamide (3 ml.) at 180—190° for 1 hr. The precipitate was washed with water and recrystallised from methanol, with addition of charcoal. The *purine* formed colourless plates, m. p. 228° (0·6 g., 64%) (Found: C, 67·0; H, 4·1; N, 28·6. $C_{11}H_8N_4$ requires C, 67·3; H, 4·1; N, 28·6%).

8-Hydroxy-2-phenylpurine (XI).—A mixture of the diamine (IX) (1 g.) and urea (2 g.) was kept at 145—150° for 30 min. A clear melt resulted which soon solidified. The mixture was treated with water, and the insoluble portion dissolved in hot aqueous sodium carbonate. The solution was acidified with acetic acid and treated with charcoal while still hot. On cooling, *8-hydroxy-2-phenylpurine* (0·5 g., 44%) crystallised. After two recrystallisations from dioxan, it sublimed at ~340° without decomposition (Found: C, 62·7; H, 3·85; N, 26·2. $C_{11}H_8N_4O$ requires C, 62·3; H, 3·8; N, 26·4%).

2,8-Diphenylpurine (XII).—A mixture of the pyrimidine (IX) (1·2 g.), benzamidine hydrochloride (2·5 g.), and anhydrous sodium acetate (1·2 g.) was heated at 160° for 20 min. The mixture was cooled and the resulting mixture dissolved in N-sodium hydroxide. Acidification with acetic acid precipitated the diphenyl derivative (XII) (1 g., 60%). This crystallised from dioxan in prisms, m. p. 259°, $\lambda_{max.}$ (pH 8·0) 244 and 317 mμ, R_F (solvent 2) 0·8 (Found: C, 75·1; H, 4·7; N, 20·4. $C_{17}H_{12}N_4$ requires C, 75·0; H, 4·4; N, 20·6%).

2-Methylpurine,[13] 2-methylhypoxanthine,[14] 8-hydroxy-2-methylpurine,[1] and 6,8-dihydroxy-2-methylpurine [15] were synthesised according to known procedures.

Experiments with Milk Xanthine Oxidase.—Highly purified milk xanthine oxidase was a gift of Professor F. Bergel and Dr. D. A. Gilbert of the Chester Beatty Cancer Research Institute, London, England. At a dilution of 1 : 5000, the enzyme produced under standard conditions (see below) 1 μg. ml.$^{-1}$ min.$^{-1}$ of uric acid, when $6·5 \times 10^{-5}$M-xanthine served as substrate.

Catalase (Worthington) was added in all enzyme experiments at a concentration of 1 unit/ml. This amount is sufficient to decompose 1 mg. of hydrogen peroxide per ml. per min., when the substrate concentration is ~1·5 mM.

All enzyme reactions were carried out at 28° and pH 8·0, in the presence of 0·01M-phosphate buffer. Kinetic measurements were performed by observing the initial change of optical density at the wavelengths, specified in column 4 of Table 2. Substrate, buffer, and catalase were mixed in a quartz cell, and the enzyme was added at zero time.

The reaction was stopped after a suitable period by immersion of the vessel into boiling water for 10 min. The mixture was then concentrated *in vacuo* and the products were identified by paper chromatography.

This research was supported by the National Institutes of Health of the United States of America. The authors thank Professor F. Bergel for the xanthine oxidase.

Department of Pharmacology, The Hebrew University–Hadassah Medical School, Jerusalem, Israel. [*Received, December 10th*, 1962.]

[13] Prasad, Noell, and Robins, *J. Amer. Chem. Soc.*, 1959, **81**, 193.
[14] Robins, Dille, Willits, and Christensen, *J. Amer. Chem. Soc.*, 1953, **75**, 263.
[15] Noell and Robins, *J. Org. Chem.*, 1959, **24**, 320.