[CONTRIBUTION OF THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Potential Purine Antagonists. XXX. Purine Betaines and Related Derivatives Prepared by Direct Methylation of the Simple Purines[1]

BY JESSE W. JONES AND ROLAND K. ROBINS

RECEIVED NOVEMBER 18, 1961

Guanine, xanthine and hypoxanthine have been methylated with dimethyl sulfate in dimethylacetamide to give a good yield of the corresponding 7,9-dimethylpurine betaine. Methylation of 6-hydroxypurine-2-thiol (VI) and 2-aminopurine-6-thiol under similar conditions resulted in alkylation of the sulfur in addition to the introduction of the 7- and 9-methyl groups. Direct methylation of adenine with methyl *p*-toluenesulfonate in dimethylacetamide gave a good yield of 3-methyladenine (XVIII). Methylation of purine-6-thiol (6-mercaptopurine) under similar conditions gave 3-methyl-6-methylthiopurine (XVI). The biochemical implication of these studies is discussed.

In recent years a rather substantial number of methylated purines such as 1-methylhypoxanthine,[2] 1-methylguanine,[2-4] 6-hydroxy-2-methylaminopurine,[2-4] 2-amino-8-hydroxy-7-methylpurine,[2] 6-methylaminopurine,[3,5] 6-dimethylaminopurine,[6] 7,9-dimethylguanine[7,8] and 1-methyladenine[9,10] have been discovered in material of biological origin. These methylated purines are known in addition to the naturally-occurring methylated xanthine derivatives, caffeine, theophylline, theobromine and paraxanthine found in plants. Salmon and Kruger,[11,12] as early as 1898, isolated 7-methylguanine, 7-methylxanthine and 1-methylxanthine from human urine. Although the biochemical role of these methylated purines is as yet obscure, it seems likely that in general they might well arise by *in vivo* methylation of the natural purines or purine nucleosides. Most of the laboratory methylation studies of purine derivatives have to date been achieved in basic media. Since the *in vivo* methylation of the natural purines presumably occurs close to neutral *p*H, the present study was initiated to approximate more closely these physiological conditions. Dimethylacetamide was chosen as the reaction media since most of the natural purines show at least some solubility in this polar solvent which also acts as a buffer in the presence of dimethyl sulfate and other alkylating agents. In most instances methylation in this solvent occurred at a *p*H of 5 to 6 without the addition of a basic reagent. In a number of cases methylation studies were made with the simple purines and methyl *p*-toluenesulfonate in dimethylacetamide because the initial tosylate salt could often be readily isolated from the reaction mixture. In all purines studied both dimethyl sulfate and methyl *p*-toluenesulfonate provided the same methylated purine derivative.

(1) Supported by Research grant CY-4008(C2) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) B. Weissman, P. A. Bromberg and A. B. Gutman, *J. Biol. Chem.*, **224**, 407 (1957).

(3) M. Adler, B. Weissman and A. B. Gutman, *ibid.*, **230**, 435 (1958).

(4) J. D. Smith and D. B. Dunn, *Biochem. J.*, **72**, 294 (1959).

(5) J. D. Dunn and D. B. Smith, *ibid.*, **68**, 627 (1958).

(6) J. W. Littlefield and D. B. Dunn, *ibid.*, **70**, 642 (1958).

(7) D. Ackermann and P. H. List, *Z. physiol. Chem.*, **309**, 286 (1957).

(8) D. Ackermann and P. H. List, *ibid.*, **318**, 281 (1960).

(9) D. B. Dunn, *Biochim. et Biophys. Acta*, **46**, 198 (1961).

(10) D. Ackermann and P. H. List, *Naturwissenschaften*, **48**, 74 (1961).

(11) M. Kruger and G. Salmon, *Z. physiol. Chem.*, **24**, 364 (1898).

(12) M. Krüger and G. Salmon, *ibid.*, **26**, 350 (1898).

Brookes and Lawley[13] have recently studied the methylation of adenosine in dimethylformamide with dimethyl sulfate. Biltz and Beck[14] report that caffeine results from the methylation of xanthine with dimethyl sulfate in the presence of sodium hydroxide. Biltz and Strufe[15] report that the methylation of 9-methylxanthine in the presence of sodium hydroxide and dimethyl sulfate gave 3,9-dimethylxanthine. Later Biltz and Sauer[16] corrected this original structure and reassigned the product from this methylation as 8,9-dimethylxanthine. This rather unusual reported alkylation of the 8-carbon in the purine molecule was reinvestigated in our laboratory. Following the directions of Biltz and co-workers,[15,16] 9-methylxanthine[17] was treated with dimethyl sulfate. A small amount of product was obtained which melted in the range described.[15,16] However, the product exhibited two spots when chromatogrammed and was found to consist of unreacted starting material and a dimethylxanthine derivative which has now been identified as 7,9-dimethylxanthine[17a] (VIII). Further examination of the reaction conditions of Biltz revealed that during the methylation of 9-methylxanthine, the solution had become acidic and the product, VIII, resulted from the action of dimethyl sulfate under neutral or at slightly acidic conditions. An extended study of the methylation of xanthine (VII) revealed that dimethyl sulfate in dimethylacetamide at 150° gave 7,9-dimethylxanthine (VIII) in excellent yield. The preparation of VIII has been reported[18] in poor yield by the methylation of xanthine with methyl iodide in a sealed tube. The structure of VIII was established by alkylation of 9-methylxanthine[17] and 7-methylxanthine[19] independently under similar conditions which resulted in each instance in the same product, 7,9-dimethylxanthine.

The alkylation of guanine with dimethyl sulfate in dimethylacetamide gave 7,9-dimethylguanine (XI)[20] in over 90% yield. The synthesis of XI

(13) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).

(14) H. Biltz and A. Beck, *J. prakt. Chem.*, [2] **118**, 198 (1928).

(15) H. Biltz and K. Strufe (in part), E. Topp, M. Heyn and R. Robl, *Ann.*, **423**, 200 (1921).

(16) H. Biltz and J. Sauer, *Ber.*, **64**, 752 (1931).

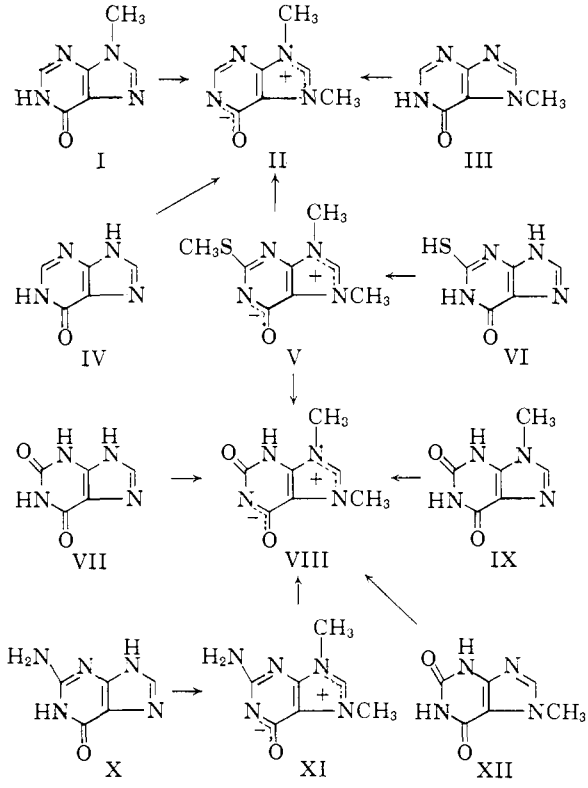
(17) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).

(17a) *Note Added in Proof.*—This same structural reassignment recently has been reported independently by W. Pfleiderer, *Ann.*, **647**, 161 (1961).

(18) H. Bredereck, G. Kupsch and H. Wieland, *Chem. Ber.*, **92**, 566 (1959).

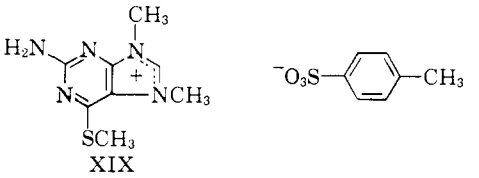
(19) R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6401 (1957).

REACTION SCHEME I



has not previously been reported by the direct alkylation of guanine. This reaction is of interest since 7,9-dimethylguanine (herbipolin) has recently[7,8] been isolated from natural sources. Treatment of XI with nitrous acid gave 7,9-dimethylxanthine, thus verifying the assigned structure and relationship of VIII and XI. Hypoxanthine, methylated with dimethyl sulfate in dimethylacetamide at 160–165°, gave a quantitative yield of 7,9-dimethylhypoxanthine (II). The structure assigned II was verified by unambiguous synthesis of 7,9-dimethylhypoxanthine from 7-methylhypoxanthine (III)[19] and 9-methylhypoxanthine (I)[21] by methylation with methyl *p*-toluenesulfonate in dimethylacetamide. The general nature of 7,9-dimethylation under these reaction conditions was further illustrated by methylation of 6-hydroxypurine-2-thiol (VI) which yielded 7,9-dimethyl-2-methylthio-6-purinone (V). The structure of V was established by conversion to 7,9-dimethylxanthine (VIII) with aqueous hydrogen peroxide. Raney nickel and V provided 7,9-dimethylhypoxanthine (II) which furnished additional structural proof for V.

The synthesis of a 7,9-dimethylpurine betaine by alkylation of the simple purine was successful only when a keto group was present in position 6. Methylation of 2-aminopurine-6-thiol with methyl *p*-toluenesulfonate in dimethylacetamide gave 2-amino-7,9-dimethyl-6-methylthiopurine *p*-toluenesulfonate (XIX) which, as expected, could not be successfully freed from the tosylate anion. The structure of XIX was established since 2-amino-7-methylpurine-6-thiol,[19] 2-amino-9-methyl-6-methylthiopurine, 2-amino-9-methylpurine-6-thiol[17] and 2-amino-6-methylthiopurine[22] all gave the same product, XIX, when methylated with methyl *p*-toluenesulfonate in dimethylacetamide.



XIX

Attempts to form the 7,9-dimethyl betaine of purine-6-thiol (6-mercaptopurine) were unsuccessful. Methyl *p*-toluenesulfonate and XV in dimethylacetamide gave a dimethyl derivative in approximately 50% yield which was identified as 3-methyl-6-methylthiopurine (XVI). The synthesis of XVI has recently been reported by Bergmann[23] by methylation of 3-methylpurine-6-thiol, although no structure proof of the methylated product was given. The structure of XVI was established in our laboratory by its conversion to 3-methylhypoxanthine[23] with hydrogen peroxide.

Adenine and methyl *p*-toluenesulfonate gave a good yield of 3-methyladenine (XVIII) uncontaminated by other isomers. 3-Methyladenine was also prepared from XVI with methanolic ammonia at 100°, which constitutes an unambiguous synthesis. Brookes and Lawley[13] report the isolation of 3-methyladenine from the methylation of adenosine and adenylic acid. The alkylation of adenosine at the 3-position was first reported by Clark, Todd and Zussman[24] from 5′-tosyl-2′,3′-isopropylidene adenosine which forms a cyclonucleoside derivative.

The fact that adenine can be readily alkylated at position 3 is of considerable significance,[24a] especially in view of the recent work of Forrest, *et al.*,[25] which indicates the existence of uric acid-3-riboside. Of the common nucleic acid purines studied, adenine is the only purine which is alkylated at position 3 in neutral media. The currently proposed mechanism of *in vivo* utilization of preformed nucleic acid purines for ribonucleic acid biosynthesis involves alkylation of the appropriate purine with 5-phospho-1-α-D-ribofuranose phosphate.[26] It is therefore quite possible that the as yet unknown adenine-3-riboside is the biological precursor of the purine-3-ribosides.

3-Methyl-6-methylthiopurine (XVI) was treated with aqueous methylamine on the steam-bath to

(20) H. Bredereck, O. Christmann and W. Koser, *Chem. Ber.*, **93**, 1206 (1960).

(21) R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957).

(22) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel and A. G. Beaman, *ibid.*, **82**, 2633 (1960).

(23) F. Bergmann, *et al.*, *J. Org. Chem.*, **26**, 1504 (1961).

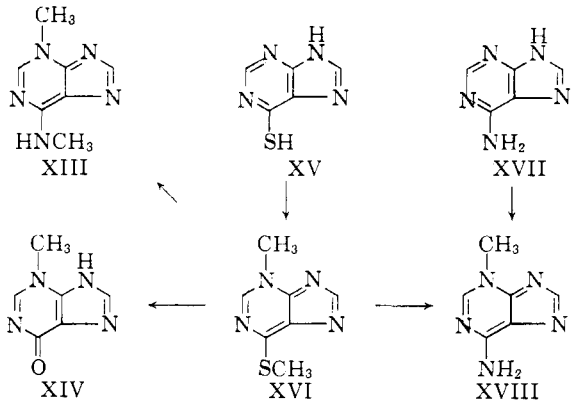
(24) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(24a) *Note Added in Proof.*—N. J. Leonard and J. A. Deyrup *J. Am. Chem. Soc.* (in press) have also alkylated adenine at position 3. These authors and R. Denayer, A. Cavé and R. Goutarel, *Compt. Rend.*, **253**, 2994 (1961), recently have shown the alkaloid triacanthine to be a 3-substituted derivative of adenine.

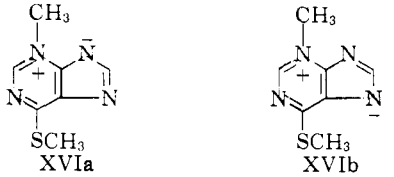
(25) H. S. Forrest, D. Hatfield and J. M. Lagowski, *J. Chem. Soc.*, 963 (1961).

(26) G. R. Barker, in "Chemistry of Natural Products," Proceedings of a Symposium under Auspices of IUPAC, August, 1960, (Australia), London, 1961, p. 125.

REACTION SCHEME II



give 3-methyl-6-methylaminopurine (XIII). The greater mobility of the methylthio group over that of 6-methylthiopurine,[27] when attacked by nucleophilic reagents, can be explained by the assumption that such resonance forms as XVIa and XVIb contribute significantly to the lowering of the electron density in the pyrimidine ring.



Such structures could be considered as a favorable distribution of electrons in an effort to maintain aromatic character of both the pyrimidine and imidazole ring. 3-Amino-1-propanol and *p*-chlorobenzylamine with XVI in refluxing ethanol gave 3-methyl-6-1-propanolaminopurine and *p*-chlorobenzylamino-3-methylpurine, respectively. The fact that 2 *N* sodium hydroxide at room temperature converted XVI to 3-methylhypoxanthine is additional support for structures XVIa and XVIb. 3-Methyladenine could not be changed to 3-methylhypoxanthine under the standard diazotization conditions, which suggests a lack of the usual basicity of the 6-amino group.

On the basis of present evidence it is not possible to make a definite assignment of the position of the negative charge in the 7,9-dimethylpurine betaines. The negative charge has been designated on the oxygen atom at position 6 by Bredereck, *et al.*,[18,20] and on nitrogen 1 by Brookes and Lawley[28] who have recently described the synthesis of 7,9-di-(2-hydroxyethyl)-guanine from 7-(2′-hydroxyethyl)-guanine and ethylene oxide. The strong carbonyl absorption observed in the infrared spectrum (KBr or Nujol mull) of the tosylate salt of 7,9-dimethylhypoxanthine in the region 1720 cm.$^{-1}$ is shifted to 1650 cm.$^{-1}$ with 7,9-dimethylhypoxanthine betaine (VIII).

The ultraviolet absorption spectra of the 3-methylpurines and purine betaines are recorded in Table I.

(27) G. B. Elion, E. Burgi and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

(28) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 3923 (1961).

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF METHYLATED PURINES

Compound	*p*H 1 λ_{max}, mμ	*p*H 1 ε	*p*H 11 λ_{max}, mμ	*p*H 11 ε
6-Amino-3-methylpurine (XVIII)	274	17,900	273	13,100
3-Methyl-6-methylaminopurine (XIII)	281	19,620	287	14,500
3-Methyl-6-methylthiopurine[23] (XVI)	235	9,200	237	10,250
	274	4,860	311	17,200
	317	25,200		
Tosylate salt of 2-amino-7,9-dimethyl-6-methylthiopurine (XIX)	248	10,100		
	320	11,100		
7,9-Dimethyl-2-methylthio-6-purinone (V)	269	17,200	241	17,850
			275	10,900
7,9-Dimethyl-6-purinone (II)	251	10,800	265	11,200
7,9-Dimethylguanine	255	6,650	262	6,550
	270–280(s)	4,600		
3-Methyl-6-*n*-propanolaminopurine	283	21,600	287	16,400
6-*p*-Chlorobenzylamino-3-methylpurine	285	24,800	289	18,600

Experimental[29]

7,9-Dimethyl-6-hydroxy-2-methylthiopurine (V).—Ten grams of 6-hydroxypurine-2-thiol (VI)[30] was stirred in 100 ml. of N,N-dimethylacetamide and treated with 30 g. of methyl *p*-toluenesulfonate in one portion. The mixture was heated at 115–120° for 2.5 hours and then allowed to cool at room temperature for 6 hours. The precipitate that separated was filtered and washed with cold methanol. After drying under an infrared lamp, the tosylate salt weighed 13 g. A portion of the crude product (6.5 g.) was then dissolved in 75 ml. of hot methanol (95%). The filtered solution was allowed to crystallize at 15° for 15 hours, and the tosylate salt weighed 4.5 g. after filtering and drying (infrared lamp). The pure product melted at 255–257°.

Anal. Calcd. for $C_{15}H_{18}N_4O_4S_2 \cdot H_2O$: C, 45.05; H, 5.00; N, 14.03. Found: C, 45.04; H, 5.01; N, 13.94.

The tosylate salt of 7,9-dimethyl-2-methylthio-6-purinone (6.5 g.) was dissolved in 150 ml. of hot methanol, and the solution was adjusted to *p*H 8 with concentrated aqueous ammonium hydroxide. The white precipitate that separated was filtered after 15 hours at 15° and dried to constant weight of 3 g., m.p. 330–332°.

Anal. Calcd. for $C_8H_{10}N_4OS$: C, 45.6; H, 4.76; N, 26.62. Found: C, 45.12; H, 4.76; N, 26.7.

2-Amino-7,9-dimethyl-6-methylthiopurine Toluenesulfonate (XIX). Method 1.—A mixture of 10 g. of 2-aminopurine-6-thiol,[22] 35 g. of methyl *p*-toluenesulfonate and 25 ml. of N,N-dimethylacetamide was heated at 170° for 105 minutes. The reaction mixture was cooled to room temperature and diluted to 250 ml. with cold methanol. The mixture was allowed to stand at room temperature for 8 hours, then the product was filtered, washed with cold methanol, and air-dried to yield 10 g. A small sample was recrystallized from methanol to give a product with a melting point of 291–294°.

Method 2.—9-Methyl-6-thioguanine[17] (1.5 g.) was suspended in 25 ml. of N,N-dimethylacetamide and treated with 2.0 g. of methyl *p*-toluenesulfonate. The mixture was stirred at 120–121° for 2 hours. The reaction mixture was then chilled at 10–15° for 5 hours and filtered. The product was washed with cold methanol and dried under a drying lamp to yield 1.8 g. Recrystallization from methanol gave a product with a melting point of 289–292°.

(29) Paper chromatography was accomplished on Whatman No. 1 filter paper, the following solvents being used: A, 5% aqueous ammonium bicarbonate; B, isopropyl alcohol–water (6:4); C, 1-butanol–acetic acid–water (5:2:3); D, 5% disodium phosphate saturated with isoamyl alcohol.

(30) A. G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).

Anal. Calcd. for $C_{15}H_{19}N_5O_3S_2$: C, 47.25; H, 5.0; N, 18.38. Found: C, 46.88; H, 4.86; N, 18.15.

Method 3.—One gram of 7-methyl-6-thioguanine[19] was stirred in 20 ml. of N,N-dimethylacetamide and treated with 2 g. of methyl *p*-toluenesulfonate. The mixture was heated at 140° for 2 hours and then cooled at room temperature for 5 hours. The precipitate that separated was filtered, washed with acetone, and dried under an infrared lamp to give a crude yield of 1.5 g., m.p. 264–268°. A small sample was recrystallized from methanol to give a product that melted at 289–291°. The ultraviolet and infrared spectra were identical to those of the products isolated by methods 1 and 2.

Method 4.—2-Amino-6-methylthiopurine[22] (1.5 g.) was suspended in 25 ml. of N,N-dimethylacetamide at 120–125° and treated with 3.5 g. of methyl *p*-toluenesulfonate. The mixture was allowed to stir at 125° for 2 hours. The reaction mixture was chilled at 15° for 2.5 hours, and the precipitate that separated was collected, washed with cold methanol, and dried under an infrared drying lamp to yield 1 g. of product. A small sample was recrystallized from methanol to provide a product which melted at 293–294°. The infrared and ultraviolet spectra were identical to those of the products prepared by methods 1, 2 and 3.

Anal. Calcd. for $C_{15}H_{19}N_5O_3S_2$: C, 47.25; H, 5.0; N, 18.38. Found: C, 46.98; H, 4.75; N, 18.05.

Method 5.—9-Methyl-6-methylthioguanine[17] (0.5 g.) was suspended in 10 ml. of N,N-dimethylacetamide, treated with 2 g. of methyl *p*-toluenesulfonate, and stirred for 3 hours at 120–125°. The reaction mixture was cooled to room temperature and filtered. The yellow product was washed with cold methanol and dried under an infrared drying lamp to yield 1 g. Recrystallization from methanol gave a product which melted at 293–294°. The infrared and ultraviolet spectra were identical to those of the products obtained by methods 1, 2, 3 and 4.

Anal. Calcd. for $C_{15}H_{19}N_5O_3S_2$: C, 47.25; H, 5.0; N, 18.38. Found: C, 46.82; H, 4.94; N, 17.85.

7,9-Dimethylguanine (XI).—Fifty grams of guanine (X) was suspended in 500 ml. of N,N-dimethylacetamide, and the stirred suspension was treated at 90° with 100 g. of dimethyl sulfate. The temperature was allowed to increase to 140–145° during the addition which required 20 minutes. Stirring was continued at 145° for 105 minutes, and then the resulting solution was allowed to cool to room temperature. One liter of methanol was added to the solution, followed immediately by the addition of concentrated aqueous ammonium hydroxide until the solution was *p*H 8. A heavy precipitate separated and was filtered and washed with 250 ml. of methanol. The resulting white product was then suspended in 1 l. of hot methanol, stirred for 30 minutes, and finally filtered and dried at 85° for 15 hours to yield 48 g. of white product. A small sample was recrystallized from a mixture of methanol and water to give a product with a melting point of 310–312°. This product was found to be identical to that prepared by Bredereck, *et al.*,[20] as judged on the basis of comparison with an authentic sample. There was no depression in the mixed melting point, and the ultraviolet spectra were identical.

Preparation of 3-Methylhypoxanthine (XIV) from 3-Methyl-6-methylthiopurine (XVI).—One gram of 3-methyl-6-methylthiopurine (XVI) was suspended in 75 ml. of water and then treated dropwise with 20 ml. of 30% hydrogen peroxide over a period of 30 minutes. The mixture was stirred for 8 hours at room temperature after the final addition. The solution was then allowed to evaporate to 10–15 ml. in an open beaker at room temperature. Acetone (200 ml.) was added, and the solid that immediately separated was filtered and washed with 15 ml. of acetone. The white solid was transferred to a beaker containing 25 ml. of isopropyl alcohol, and the resulting mixture was treated with 3 ml. of concentrated aqueous hydrochloric acid and stirred for 20 minutes at room temperature. The crystalline solid that resulted was filtered, washed with excess isopropyl alcohol, and air-dried. The ultraviolet spectra and R_f values in solvents C (0.21) and D (0.60) were identical to those of 3-methylhypoxanthine.[23]

Anal. Calcd. for $C_6H_6N_4 \cdot 1/2H_2O \cdot HCl$: C, 36.85; H, 4.09; N, 28.6. Found: C, 37.2; H, 3.99; N, 28.3.

3-Methyl-6-methylthiopurine (XVI).—Twenty grams of purine-6-thiol (XV) was added at 135–140° to 20 ml. of N,N-dimethylacetamide containing 50 g. of methyl *p*-toluenesulfonate (in 4 equal portions). The vigorously-stirred solution was heated at 140° for 2.5 hours and cooled to room temperature. The resulting gummy residue was extracted three times with 100-ml. portions of boiling ethyl ether. The ether extract was discarded, and the residue that remained was dissolved in hot acetone (250 ml.). The solution was allowed to stand at 15° for 15 hours, and the precipitate (15 g.) that separated was filtered and washed with 50 ml. of acetone. Recrystallization from methanol gave the tosylate salt, m.p. 269–271°.

Anal. Calcd. for $C_{14}H_{16}N_4O_3S_2$: C, 47.7; H, 4.55; N, 15.9. Found: C, 47.94; H, 4.48; N, 15.75.

The tosylate salt of 3-methyl-6-methylthiopurine (15 g.) was dissolved in hot water and cooled to 30°. The solution was then adjusted to *p*H 8 with concentrated aqueous ammonium hydroxide. The precipitate (5 g.) that separated at 15° for 15 hours was filtered and washed with a small quantity of cold water. A small sample was recrystallized once from water, followed by recrystallization from acetone, to provide a product which melted at 163–165°.

Anal. Calcd. for $C_7H_8N_4S \cdot 2H_2O$ (air-dried): C, 38.9; H, 5.56; N, 25.9. Found: C, 38.91; H, 5.16; N, 26.1. Calcd. for $C_7H_8N_4S$ (after drying at 112° for 48 hours): C, 46.7; H, 4.44; N, 31.1. Found: C, 46.7; H, 4.9; N, 31.1.

3-Methyl-6-methylaminopurine (XIII).—One gram of 3-methyl-6-methylthiopurine (XVI) was dissolved in a mixture of 20 ml. of 40% aqueous methylamine and 50 ml. of methanol. The solution was allowed to stand at room temperature for 15 hours in a tightly-sealed flask and was then evaporated to dryness on a steam-bath under reduced pressure. The resulting residue was dissolved in 50 ml. of methanol and again evaporated as before. The solid residue was suspended in 50 ml. of acetone, filtered, and washed with 10 ml. of cold acetone. The air-dried product yielded 0.8 g. A small sample was recrystallized from a mixture of methanol and acetone to provide a product which melted at 314–315° dec.

Anal. Calcd. for $C_7H_9N_5$: C, 51.5; H, 5.52; N, 42.9. Found: C, 51.69; H, 5.54; N, 42.5.

3-Methyladenine (XVIII). Method 1.—Ten grams of adenine (XVII) was added to a mixture of 40 g. of methyl *p*-toluenesulfonate and 20 ml. of N,N-dimethylacetamide. The mixture was heated on the steam-bath for 1 hour, then cooled to room temperature. The resulting viscous mass was extracted three times with 500 ml. of acetone. The acetone extract was allowed to evaporate at room temperature in an open dish to approximately 350 ml. and then chilled at 10–15° for 8 hours. A white crystalline precipitate (5.5 g.) was collected and air-dried. Recrystallization from methanol (95%) gave a product with a melting point of 209–210°.

Anal. Calcd. for $C_{13}H_{15}N_5O_3S \cdot 1/4H_2O$: C, 48.0; H, 4.76; N, 21.5. Found: C, 48.13; H, 4.98; N, 21.1.

A small sample of the tosylate salt of 3-methyladenine was dissolved in hot water and adjusted to *p*H 7.5 with concentrated aqueous ammonium hydroxide. Recrystallization was effected from water. The product which began to sublime at 250° melted at 310–313°.

Method 2.—Two grams of 3-methyl-6-methylthiopurine (XVI) in 50 ml. of methanolic ammonia, saturated at 0°, was heated at 100° for 2.5 hours in a high-pressure reaction vessel. The resulting solution was boiled with charcoal, filtered, and evaporated to dryness on the steam-bath under reduced pressure. The crude product (0.8 g.) was recrystallized twice from water to give a product which melted at 309–312°. No depression of the melting point was observed when the pure products prepared by methods 1 and 2 were mixed. Identical ultraviolet spectra and R_f values (R_f = 0.18 in solvent C) were observed.

Anal. Calcd. for $C_6H_7N_5$: C, 48.2; H, 4.81. Found: C, 48.3; H, 4.7.

7,9-Dimethylhypoxanthine (II). Method 1.—Ten grams of hypoxanthine (V) was suspended in 100 ml. of N,N-dimethylacetamide and treated in one portion with 20 g. of methyl *p*-toluenesulfonate. The mixture was then heated

to 120–125° and stirred for 1.5 hours at 125°. A clear solution resulted during the first 30 minutes of stirring, and later a precipitate began to separate. After the solution was allowed to cool at room temperature for 2 hours, a white solid was collected and washed with 10 ml. of cold methanol. The product was dried to constant weight (7.5 g.) under an infrared drying lamp. Chilling the combined filtrates at 15° for 20 hours yielded an additional 1.5 g. Recrystallization of the tosylate salt from methanol gave an analytically pure sample, m.p. 258–260°.

Anal. Calcd. for $C_{14}H_{16}N_4O_4S$: C, 50.0; H, 4.76; N, 16.7. Found: C, 50.49; H, 5.1; N, 16.4.

Method 2.—7-Methylhypoxanthine[19] (5 g.) in 50 ml. of N,N-dimethylacetamide containing 5 g. of methyl *p*-toluenesulfonate was heated at 120–125°. The mixture was stirred for 1.5 hours at 125° and then chilled at 15° for 15 hours. The resulting precipitate was filtered and washed with cold methanol. The tosylate salt was recrystallized from methanol to give a melting point of 258–260°.

Method 3.—9-Methylhypoxanthine[21] (2 g.) in 50 ml. of N,N-dimethylacetamide containing 5 g. of methyl *p*-toluenesulfonate was heated at 120–125° for 1.5 hours. The mixture was then cooled at 15° for 6 hours. The product that separated was filtered and washed with cold acetone. The crude tosylate salt (3.5 g.) was recrystallized from hot methanol. The pure crystalline product melted at 258–260°.

Anal. Calcd. for $C_{14}H_{16}N_4O_4S$: C, 50.0; H, 4.76; N, 16.7. Found: C, 49.77; H, 5.11; N, 16.25.

The tosylate salt of 7,9-dimethyl-6-purinone (5 g.) was dissolved in 100 ml. of hot methanol, and the solution was adjusted to *p*H 8–9 with concentrated aqueous ammonium hydroxide. The mixture was allowed to stand at 15° for 15 hours and was then filtered and washed with cold methanol. The crude product (1.5 g.) was recrystallized from methanol to give a product with a melting point of 330–332°. (The tosylate salts of 7,9-dimethylpurine prepared by methods 1, 2 and 3 were each treated as described immediately above and gave identical products.)

Anal. Calcd. for $C_7H_8N_4O$: C, 51.1; H, 4.88; N, 34.1. Found: C, 51.03; H, 4.88; N, 34.2.

Method 4.—Fifty grams of hypoxanthine (IV) was added to a mixture of 100 g. of dimethyl sulfate and 300 ml. of N,N-dimethylacetamide at 140°. The temperature of the mixture increased rapidly to 160–165° while stirring vigorously. The solid dissolved rapidly. The solution was allowed to cool to 135–140°, and stirring was continued for 1.5 hours. After cooling to room temperature, the solution was adjusted to *p*H 8–9 with concentrated aqueous ammonium hydroxide. The resulting precipitate was filtered and washed with excess acetone. The white product was dried to constant weight (60 g.) under an infrared heat ray lamp. A small sample was recrystallized from methanol. There was no depression of the melting point when this product was mixed with pure samples isolated by methods 1, 2 or 3. The ultraviolet and infrared spectra were identical for the products isolated by each of the four methods. Identical R_f values were observed for these products: solvent A, R_f 0.80; solvent B, R_f 0.59.

Method 5.—Three grams of V was dissolved in 100 ml. of water, and the solution was made alkaline with 6 ml. of concentrated aqueous ammonium hydroxide. Raney nickel (4 g., wet) was added, and the mixture was refluxed for 5 hours. The hot solution was filtered and the Raney nickel washed with hot water. The filtrate was evaporated to dryness, and the residue was recrystallized from methanol. There was no depression of melting point when this product was mixed with the tosylate salt of 7,9-dimethyl-6-purinone. This identification was further substantiated by identical ultraviolet and infrared spectra with the tosylate salt prepared by methods 1 through 4. The product isolated from an ammoniacal methanolic solution of the dethiated material was identical to the betaine of 7,9-dimethyl-6-purinone (II) as shown by mixed melting point, ultraviolet spectra and paper chromatography (solvent B, R_f 0.59).

7,9-Dimethylxanthine (VIII). Method 1.—Fifty grams of xanthine (VII) in 500 ml. of N,N-dimethylacetamide was heated to 150°. The mixture was then treated with 100 g. of dimethyl sulfate. The temperature of the mixture increased to 165° during the addition which required 30 minutes. The temperature was then lowered to 140° and stirring continued at this temperature for 2 hours. The resulting solution was cooled to room temperature and adjusted to *p*H 8 with concentrated aqueous ammonium hydroxide. The precipitate that separated was filtered, washed with excess acetone, and air-dried to yield 42 g. of white product. A small sample was recrystallized from a mixture of water and methanol to provide a product that melted at 378–379° dec.[18] The ultraviolet absorption maxima were identical to those previously reported.[18]

Method 2 (According to Biltz's Procedure).[15,16]—Five grams of 9-methylxanthine (IX)[17] was dissolved in 190 ml. of water containing 10 ml. of 10% sodium hydroxide. The mixture was treated with 8 g. of dimethyl sulfate at room temperature, and the *p*H of the resulting solution decreased to the acid region during the first 30 minutes of stirring. After the first hour an additional 4 g. of dimethyl sulfate was added. The solution was allowed to stand at room temperature for 15 hours. The volume of the solution was then reduced to 50 ml. The solution was first made ammoniacal with concentrated aqueous ammonium hydroxide and then acidified to *p*H 5–6 with glacial acetic acid. A precipitate separated after 15 hours at 15°. The solid was collected, washed with cold water, and dried at 80°. The crude product melted at 343–345° and chromatogrammed to show two spots—one identical to 9-methylxanthine (IX) (R_f 0.66, solvent D). Repeated recrystallizations from a mixture of water and methanol increased the melting point to 376–378° dec., and the product chromatogrammed (R_f 0.81) as a single spot in solvent D. These data are identical to those of 7,9-dimethylxanthine (VIII) prepared by Bredereck, *et al.*,[18] which also gave identical ultraviolet spectra.

Method 3.—Two grams of 7-methylxanthine (XII)[19] was suspended in 100 ml. of N,N-dimethylacetamide and treated with 2.5 g. of dimethyl sulfate in one portion at room temperature. The mixture was stirred at 120–125° for 1 hour. The solution was then chilled to 15°, neutralized to *p*H 8 with concentrated aqueous ammonium hydroxide, and filtered after 30 minutes. The white product was washed with methanol and air-dried. The ultraviolet spectra were identical to those of VIII.

Method 4 (from 7,9-Dimethylguanine (XI)).—One gram of 7,9-dimethylguanine (XI) was dissolved in 5 ml. of 10% aqueous hydrochloric acid at 38–40°. The resulting solution was treated at this temperature with 1 g. of solid sodium nitrite in one continuous portion. The reaction mixture was stirred for 1 hour at 40°, cooled to room temperature, and adjusted to *p*H 8 with concentrated aqueous ammonium hydroxide. Glacial acetic acid was then added until the solution was *p*H 6. Finally the solution was reduced to one-half its volume and allowed to stand at 15° for 24 hours. The precipitate that separated was filtered and recrystallized from a mixture of water and methanol to provide a product which melted at 380° dec. There was no depression of the melting point when this product was mixed with the products isolated by methods 1, 2, or 3. Furthermore, the ultraviolet spectra were identical to those of 7,9-dimethylxanthine prepared by the other methods.

Method 5.—The betaine of 2-methylthio-6-purinone (V, 0.5 g.) was stirred in 10 ml. of 30% hydrogen peroxide for 48 hours. The solution was allowed to evaporate to dryness at room temperature. The residue was then suspended in ethanol and filtered. The product was identical to 7,9-dimethylxanthine (VIII) prepared by the other methods.

3-Methyl-6-*n*-propanolaminopurine.—3-Methyl-6-methylthiopurine (XVI, 1.5 g.) was dissolved in a solution of 3 g. of 3-amino-1-propanol, 45 ml. of ethanol and 5 ml. of water. The solution was heated to gentle reflux for 2.5 hours and then allowed to stand at room temperature for 15 hours. The solvent was then removed under reduced pressure at 70°. Methanol (50 ml.) was added, and the volume of the resulting solution was reduced as before. This process was repeated twice more. To the final residue was added 50 ml. of acetone and the suspension allowed to stand for 1 hour at room temperature. The white precipitate was collected (1.2 g.), washed with acetone, and dried. A small sample was recrystallized from ethanol to yield a pure product, m.p. 213–216°.

Anal. Calcd. for $C_9H_{13}N_5O$: C, 52.2; H, 6.3; N, 33.9. Found: C, 52.2; H, 6.5; N, 33.6.

6-(*p*-Chlorobenzylamino)-3-methylpurine.—A solution of 3-methyl-6-methylthiopurine (XVI, 1 g.) and 3 g. of *p*-chlorobenzylamine dissolved in 50 ml. of 90% ethanol was refluxed for 2 hours and finally cooled and filtered. The white product was washed with methanol and dried at 45° for 10 hours to yield 1.2 g. Recrystallization from methanol gave a pure product that melted at 263–265°.

Anal. Calcd. for $C_{13}H_{12}N_5Cl$: C, 57.1; H, 4.4; N, 25.6. Found: C, 57.3; H, 4.5; N, 25.4.

[CONTRIBUTION FROM THE DIVISION OF PLANT INDUSTRY, C.S.I.R.O., CANBERRA, AUSTRALIA]

Studies on Phytoalexins. V. The Structure of Pisatin from *Pisum sativum* L.[1,2]

BY DAWN R. PERRIN AND W. BOTTOMLEY

RECEIVED OCTOBER 27, 1961

Chemical and physicochemical studies of pisatin and some of its degradation products indicate that pisatin has the chromanocoumarane ring skeleton and is 3-hydroxypterocarpin (IIb).

In the course of a study of the chemical basis of disease resistance in plants, an antifungal substance, named pisatin, was isolated from the pods of garden peas (*Pisum sativum*) which had been inoculated with fungal spores.[3] Some biological properties of pisatin have been discussed elsewhere.[3] No other substance with antifungal activity was detected in the active extracts from which pisatin was isolated, and the activity of pisatin accounted for all the activity in those diffusates. At concentrations around 10^{-4} *M* pisatin possessed antifungal activity toward a wide range of plant fungi.[2,3]

Pisatin has the molecular formula $C_{17}H_{14}O_6$ and is optically active. It is sparingly soluble in water, but is soluble in organic solvents. Its ease of extraction by solvents from aqueous alkaline solutions indicates the absence of phenolic or acidic groups. Pisatin is stable in neutral or alkaline solutions but is very acid labile; for example, when solutions of pisatin in alcohol, acetone or water are made weakly acid and allowed to stand in the cold, pisatin loses a molecule of water and is transformed to anhydropisatin. Anhydropisatin is optically inactive. A comparison of the ultraviolet absorption spectra showed that the conversion of pisatin to anhydropisatin was accompanied by a marked shift to longer wave lengths and an intensification of the absorption maxima, indicating that dehydration had considerably extended the conjugation pathway in the molecule. Both pisatin and anhydropisatin contained one alkoxyl (probably methoxyl) group and both compounds gave a positive color test for a methylene dioxy group.[4] Pisatin could not be methylated using either methyl iodide and potassium carbonate or methyl iodide and silver oxide, and attempts to methylate with methyl sulfate, or to acetylate, resulted in its dehydration to anhydropisatin. Both pisatin and anhydropisatin were extremely resistant to hydrogenation using Adams catalyst.

No chemical evidence could be obtained for the presence of a carbonyl grouping in pisatin or anhydropisatin, nor were any strong bands observed in the 1630–1800 cm.$^{-1}$ region of their infrared spectra (Fig. 1). The band at 3610 cm.$^{-1}$ in the infrared spectrum of pisatin showed the presence of an alcoholic hydroxyl group. An integration of the area under this peak and a comparison with other hydroxyl-containing substances strongly suggested that only one hydroxyl group was present.[5] This band is absent from the infrared spectrum of anhydropisatin. According to Briggs, *et al.*,[6] a methylene dioxy group attached to an aromatic ring gives twelve major bands, but most of these are also given by a methoxyl group; the strong band around 930–940 cm.$^{-1}$ is the only one given by the methylene dioxy group alone. Other workers[7] attribute absorption bands near 1037 and 1165 cm.$^{-1}$ to methylene dioxy groupings. All three bands are present in pisatin (945, 1047, 1163 cm.$^{-1}$) and anhydropisatin (950, 1045, 1140 or 1170 cm.$^{-1}$) and also in the two degradation products identified below as V and VI. The inertness of the two remaining oxygen atoms suggested that they were present in other ether linkages.

When an ethanolic solution of anhydropisatin was exposed to diffuse daylight or mercury 365 mμ light, a phenol was produced with a molecular formula corresponding to the addition of one molecule of ethanol to the anhydropisatin molecule. This phenol was presumably formed by the fission of an aryl ether moiety. On the other hand, if an alcoholic or aqueous solution of pisatin was irradiated briefly with mercury 253.7 mμ light, a substance was formed whose ultraviolet spectrum differed from that of pisatin only by the absence of the maximum at 280 mμ and by a shift of the 309 mμ maximum to 312 mμ. This substance had no anti-fungal activity[3] nor was it dehydrated under the same conditions as those which transformed pisatin into anhydropisatin. The compound could not be isolated because further irradiation or attempted evaporation gave a yellow material having two visible absorption maxima (λ_{max} 425 mμ, log ε 4.0; λ_{max} 350 mμ, log ε 4.2). In strongly acid solutions the color changed to a bright cherry-red, but the solution became yellow once more on dilution or neutralization. The yellow material could be extracted quantitatively

(1) Presented, in part, at the International Symposium on The Chemistry of Natural Products (International Union of Pure and Applied Chemistry), Sydney, Australia, August, 1960.

(2) Part IV of the series: I. A. M. Cruickshank, *Aust. J. Biol. Sci.*, in press, (1962).

(3) I. A. M. Cruickshank and D. R. Perrin, *ibid.*, **14**, 336 (1961).

(4) J. A. Labat, *Bull. soc. chim. biol.*, **15**, 1344 (1933).

(5) E. Spinner, private communication.

(6) L. H. Briggs, L. D. Colebrook, H. M. Fales and W. C. Wildman, *Anal. Chem.*, **29**, 904 (1957).

(7) A. Robertson and W. B. Whalley, *J. Chem. Soc.*, 1440 (1954).