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A New Heterocyclic Ring System, 8-Acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine, Resulting from the Reaction of Guanine and Deoxyribose*

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ABSTRACT: The white fluorescent material produced on acid hydrolysis of deoxyribonucleic acid is also formed on heating guanine with deoxyribose or furfuryl alcohol in dilute acid.

Complete structural elucidation, establishing the fluorescent compound as 8-acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine (I), was accomplished in the following steps. (1) Degradation of I with alkali gave guanine. (2) A survey of purines and pyrimidines demonstrated that a group such as N(R)C(NHR)=N or =NC-(NHR)=N was essential for production of fluorescent material upon reaction with furfuryl alcohol. (3) Bromine–water degradation of I produced an unstable intermediate which gave, after neutralization, guanine and presumed 2,4-dioxopentanal. The latter was converted to 1,2,4-pentanetriol by borohydride reduction. (4) The reduction product of I, 8-(2′-hydroxypropyl)-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine (II), when subjected to bromine–water degradation gave a stable intermediate, 2-(3′,5′-dideoxy-2′ketopentofuranosyl)amino-6-oxo-1,6-dihydropurine (V) with an ultraviolet spectrum similar to that of 2-methylamino-6-oxo-1,6-dihydropurine-(N^2-methylguanine). Borohydride reduction of V gave an unstable product which decomposed to guanine and 2,4-dihydroxypentanal. (5) Methylation of II by dimethyl sulfate gave a dimethylated derivative (IV) which after bromine–water degradation, borohydride reduction, and neutralization gave 1,7-dimethylguanine and 2,4-dihydroxypentanal. (6) Nuclear magnetic resonance, infrared, and titration measurements confirmed the identity of specific structural features. Reaction sequences are proposed to explain the formation of I and its degradation by bromine–water.

Levy and Snellbaker (1954) originally detected a white fluorescent spot on paper chromatography of dilute acid hydrolysates of DNA and attributed this to "....a hitherto undescribed constituent of DNA." Frick (1956) and Dunn (1955) described the formation of what appears to be the same compound. Dunn presented evidence pointing to guanine as a precursor of the white fluorescent compound, and concluded that the white fluorescent compound is formed by a reaction between guanine and a degradation product of 2-deoxy-D-ribose in the presence of phosphoric acid. Simultaneous work by Hoard (1957) also demonstrated that guanine is a precursor of the white fluorescent compound; however, no requirement for phosphoric acid could be found. Hoard observed that the white fluorescent compound was formed when 2′-deoxyguanylic acid, 2′-deoxyguanosine, an equimolar mixture of 2-deoxy-D-ribose, and guanine, or a mixture of guanine and furfuryl alcohol was submitted to the same conditions used by Levy and Snellbaker for the production of the white fluorescent compound from DNA. None of the other usual purine or pyrimidine derivatives was active in the formation of the white fluorescent compound. It appears, then, that the white fluorescent compound is an artifact formed from guanine and an active fragment arising from 2-deoxy-D-ribose. Although Hoard's data were insufficient to arrive at

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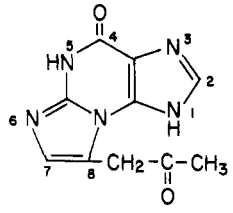
a unique structure, one which seemed very probable was a formula related to imidazo[2,1-*b*]purine.[1]

The complete chemical characterization and structure determination of the white fluorescent compound were undertaken to provide some additional insight into the chemistry of guanine and to ascertain whether the compound was indeed a representative of a new heterocyclic ring system, imidazo[2,1-*b*]purine. In addition there was the possibility that the white fluorescent compound might bear sufficient structural relationship to guanine to have antimetabolite or anticarcinogen activity.[2] The experimental evidence reported herein leads to the formulation of the white fluorescent compound as 8-acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine (I).



I

Materials and Methods

3-Methylguanine (Elion, 1962; Townsend and Robins, 1962) and 9-methylguanine were purchased from Cyclo Chemical Co.; 2-aminopurine, isoguanine, and isocytosine from Calbiochem. 7-Methylguanine (Fischer, 1898), 1,7-dimethylguanine (Fischer, 1897), 1,7,9-trimethylguanine iodide (Pfleiderer, 1961), N^2-methylguanine (Elion *et al.*, 1956), N^2,N^2-dimethylguanine (Elion *et al.*, 1956), 2-methylaminopyrimidine (Overberger and Kogon, 1954), 2-dimethylaminopyrimidine (Overberger and Kogon, 1954), 1,2-dihydro-2-imino-1-methylpyrimidine (Brown *et al.*, 1955), and 2-hydroxypyrimidine (Brown, 1950) were synthesized. Unless otherwise noted, all other chemicals used were purchased from commercial sources.

All analyses were performed by the Microchemical Laboratory, Department of Chemistry, University of California, Berkeley. Unless otherwise indicated the infrared spectra were taken on a Baird-Atomic Model 4-55 using the potassium bromide pellet technique. Nuclear magnetic resonance spectra were measured with a Varian Model A 60 spectrometer, using tetramethylsilane as internal standard.

Experimental Section

Synthesis of 8-Acetonyl-4-oxo-4,5-dihydroimidazo[2,1-b]purine (I). To a 1000-ml erlenmeyer flask were added 5.03 g (0.0333 mole) of guanine and 500 ml of 1 N hydrochloric acid. The mixture was heated with mechanical stirring to a low boil and 33.7 g (0.343 mole) of furfuryl alcohol was added over the course of about 1 hr. The heat was then removed but mechanical stirring was maintained until the reaction mixture had cooled to room temperature. After standing several hours, the reaction mixture was filtered by gravity to remove some black polymeric material and then concentrated to dryness *in vacuo*. The resulting dark brown solid was then extracted with 500 ml of acetone. The residue was collected on a Buchner funnel, washed thoroughly with acetone, and allowed to air dry, giving 8.37 g of an impure, dark brown solid.

The material was crystallized from hot water after treatment with approximately one-twentieth the amount of decolorizing charcoal. Work-up of the mother liquors and a single recrystallization gave 2.14 g (27.8%) of long white needles.

The ultraviolet and infrared spectra of the present preparation were identical with those of the fluorescent compound prepared by Hoard (1957). The ultraviolet spectrum showed, in 0.1 N hydrochloric acid, λ_{max} 220 mμ (ε 25,300) and 257 mμ (ε 9300), λ_{min} 237 mμ (ε 5700); in 0.5 M phosphate, pH 7.5, λ_{max} 220 mμ (ε 28,400) and 265 mμ (ε 11,400), λ_{min} 241 mμ (ε 5100); in 0.1 M phosphate, pH 11.0, λ_{max} 270 mμ (ε 9400), λ_{min} 251 mμ (ε 7700). Infrared spectrum showed principal absorption bands at 2900, 2700, 2550, 1700, 1630, 1580, 1520, 1470, 1430, 1350, 1170, and 860 cm^{-1}. The nuclear magnetic resonance spectrum of the substance in trifluoroacetic acid (tetramethylsilane internal standard) possessed four bands at δ = 2.60, 4.62, 7.50, and 8.37 ppm whose relative areas were in the ratio of 3:2:1:1, respectively. Spectrophotometric evaluation of the dissociation constants gave pK_a values of 2.9 and 9.0 ± 0.2. *Anal.* Calcd for $C_{10}H_8N_5O_2$ (231.21): C, 51.94; H, 3.92; N, 30.29. Found: C, 52.10; H, 4.06; N (by Kjeldahl),[3] 29.53.

One milligram of the product and 1 mg of the fluorescent compound prepared by Hoard were each dissolved in 0.1 ml of 1 N hydrochloric acid (Hoard, 1957). Samples of each were chromatographed on Whatman No. 1 paper in two solvent systems, and showed identical R_F values: in isopropyl alcohol–5.7 N hydrochloric acid (65:35) (Wyatt, 1951) (descending), 0.46; in methyl alcohol–formic acid–water (80:15:5) (Hoard, 1957) (descending), 0.67.

Reaction of I with Base. A solution of 3.35 mg of I in 0.1 ml of 1 N potassium hydroxide was heated

[1] For naming the new heterocyclic compounds encountered, the system proposed by Patterson (1928) has been followed. The numbering of the imidazo[2,1-*b*]purine ring system is indicated in the diagram I. While infrared spectral data, to be presented, support the lactam structure at positions 4 and 5, the location of the hydrogen at position 1 is an arbitrary assignment.

[2] Although initial tests performed by Eli Lilly and Co. indicated that the white fluorescent compound possessed activity against a meccalymphosarcoma in mice, subsequent tests did not support this finding.

[3] Considerable difficulty was experienced in measuring the high nitrogen content of the white fluorescent compound. Analyses by the Dumas method resulted in consistently low nitrogen values. Better results were obtained by the Kjeldahl method using mercuric sulfate as a catalyst. Nitrogen analyses of other new compounds encountered in the course of the work also tended to be somewhat low.

TABLE I: R_F Values of Model Purines and Pyrimidines and the Products of Their Reaction with Furfuryl Alcohol.

	R_F Values					
	Solvent 1[a]				Solvent 2[b]	
	Precursor	Product(s)			Precursor	Product(s)
Guanine	0.26		0.42		0.40	0.54
1-Methylguanine	0.25		0.37	0.46	0.47	0.65
7-Methylguanine	0.29	0.18	0.41	0.55	0.49	0.60
1,7-Dimethylguanine	0.27		0.62		0.59	0.76
N^2-Methylguanine	0.44		0.52		0.51	0.57
2-Aminopurine	0.18	0.09	0.30	0.47	0.52	0.69
Isocytosine	0.44		0.48	0.56		
2-Aminopyrimidine	0.50		0.61			
2-Methylaminopyrimidine	0.72		0.73		0.74	0.77
3-Methylguanine	No fluorescent product					
9-Methylguanine	No fluorescent product					
9-Ethylguanine	No fluorescent product					
1,7,9-Trimethylguanine iodide	No fluorescent product					
N^2,N^2-Dimethylguanine	No fluorescent product					
Adenine	No fluorescent product					
Hypoxanthine	No fluorescent product					
Xanthine	No fluorescent product					
Isoguanine	No fluorescent product					
Cytosine	No fluorescent product					
2-Dimethylaminopyrimidine	No fluorescent product					
1,2-Dihydro-2-imino-1-methylpyrimidine	No fluorescent product					
2-Hydroxypyrimidine	No fluorescent product					

[a] Isopropyl alcohol–5.7 N hydrochloric acid (65:35), descending. [b] Methyl alcohol–formic acid–water (85:15:5), ascending.

in a boiling-water bath for 1 hr. Examination of the reaction mixture by chromatography, using the isopropyl alcohol–hydrochloric acid solvent, revealed the presence of at least three products with R_F values of 0.21, 0.28, and 0.37. The product with R_F 0.21 was quenching and migrated at the same rate as guanine; the other two were fluorescent.

The remainder of the reaction mixture was chromatographed on Whatman 3MM paper with the same solvent. The band containing the quenching product was cut out and eluted with 1 N hydrochloric acid. This material was purified further by chromatography on Dowex 50 (H^+) (Cohn, 1955). The eluent containing the desired substance was concentrated at the water pump to a small volume and again subjected to paper chromatography followed by ion-exchange chromatography. The ultraviolet spectrum of the material eluted from the column after the second cycle was virtually identical with that of guanine. It had λ_{max} 247 mμ and λ_{min} 223 mμ, with an inflection point at 270 mμ. In 2 N hydrochloric acid, guanine had λ_{max} 246 mμ and λ_{min} 222 mμ, with an inflection point at 270 mμ.

Reaction of Selected Purines and Pyrimidines with Furfuryl Alcohol. About 1 mg of each substance tested was dissolved in 0.1 ml of 1 N hydrochloric acid and 0.05 ml of furfuryl alcohol was added. The tubes were then heated in a boiling-water bath for 1 hr, cooled, and samples were removed for paper chromatography. Chromatograms were examined for the presence of new fluorescent derivatives under an ultraviolet lamp. The substances tested and the results are summarized in Table I.

Reaction mixtures containing 2-aminopyrimidine and 2-methylaminopyrimidine were neutralized with 1 N ammonium hydroxide and examined by paper electrophoresis at various pH values using an apparatus similar to that described by Crestfield and Allen (1955). Spots were located on dry electrophoretograms with an ultraviolet lamp. The results are shown in Table II, from which it can be seen that the fluorescent product derived from 2-aminopyrimidine is cationic at pH 3.5 but uncharged at pH 6.8, while that derived from 2-methylaminopyrimidine still bears a partial positive charge at pH 10.5.

Demonstration of the Presence of a Reactive Carbonyl Group in I. A. FORMATION OF A 2,4-DINITROPHENYLHYDRAZONE. To 0.025 g of I in a solution of 5 ml of 5 N hydrochloric acid in 10 ml of 95% ethanol was added a solution containing 0.1 g of 2,4-dinitrophenylhydrazine in 30 ml of 95% ethanol. After 48 hr, the

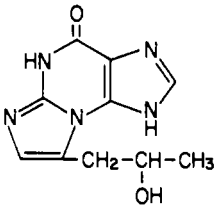
TABLE II: Electrophoretic Migration of the Products Derived from the Reaction of 2-Aminopyrimidines with Furfuryl Alcohol.

Compd	Migration toward Cathode, cm (rel to caffeine)[a]			
	pH 3.5[b]	pH 6.8[c]	pH 10.5[d]	pH 11.0[d]
2-Aminopyrimidine	1.2	−0.3	−0.9	
Fluorescent product from 2-aminopyrimidine	12.8	−0.3	−0.9	
2-Methylaminopyrimidine		−0.3	0.1	0.1
Fluorescent product from 2-methylaminopyrimidine		9.4	6.9	−0.4

[a] Potential gradient: 49 v/cm for 0.5 hr. [b] 0.02 M ammonium formate. [c] 0.01 M sodium phosphate. [d] 0.01 M methylamine.

crystalline solid was collected on a filter, washed successively with ethanol and water, and dried: mp 201–203° dec. Attempts to recrystallize the derivative were unsuccessful. *Anal.* Calcd for $C_{16}H_{13}N_9O_5 \cdot HCl \cdot H_2O$ (468.8): C, 41.25; H, 3.46; N, 27.08. Found: C, 41.31; H, 3.55; N, 26.44.

B. REDUCTION WITH SODIUM BOROHYDRIDE. To 0.201 g of I dissolved in 60 ml of 0.01 N sodium hydroxide was added (portionwise) 0.2 g of sodium borohydride. After standing overnight at room temperature, the reaction mixture was adjusted to pH 7 by addition of 1 N hydrochloric acid. The fine silky precipitate which formed was filtered and recrystallized from 100 ml of hot water, giving white needles: yield 0.176 g (86.7%). Measurement of ultraviolet spectra gave, in 1 N hydrochloric acid, λ_{max} 222 mμ (ε 24,700) and 258 mμ (ε 9040) and λ_{min} 238 mμ (ε 5690); in N sodium hydroxide, λ_{max} 231 mμ (ε 33,500) and 273 mμ (ε 8780) and λ_{min} 243 mμ; isosbestic point between pH 1 and 7, 257 mμ; between pH 7 and 14, 251 mμ. The pK_a values, determined spectrophotometrically, were 2.8 ± 0.1 and 9.9 ± 0.32 (Flexser *et al.*, 1935). In isopropyl



II

alcohol–hydrochloric acid compound II had an R_F value of 0.51 and in methanol–formic acid–water an R_F of 0.68. As in the case of I, the reduced fluorescent compound exhibits a white fluorescence on paper chromatograms.

The infrared spectrum of the reduced fluorescent compound possesses a peak at 3250 cm^{-1} which is not present in the unreduced compound. Furthermore, a peak in the carbonyl region in the unreduced compound at 1700 cm^{-1} is absent in the reduced compound.

Anal. Calcd for $C_{10}H_{11}N_5O_2$ (233.23): C, 51.49; H, 4.75; N, 30.35. Found: C, 51.3; H, 4.6; N, 29.8.

Iodoform Test on I. To a solution of 0.1 g of I in 5 ml of water and 1 ml of 10% sodium hydroxide was added, dropwise, 30% potassium iodide–iodine solution until a definite dark color remained: required, 2.5 ml. The dark color was discharged by addition of a few drops of 10% sodium hydroxide and 10 ml of water was added. Yellow crystals of iodoform, which were deposited on the bottom of the tube, were filtered by suction and allowed to air dry: yield 0.009 g (5.4%), mp 119–121° (lit. (Shriner and Fuson, 1948) mp 119°).

Degradation of I by Successive Treatment with Bromine–Water and Sodium Borohydride. A. FORMATION OF GUANINE. To 200 ml of 1 N hydrochloric acid was added with stirring 13.10 g (0.0567 mole) of I. After all material had dissolved, bromine–water (saturated, *ca.* 400 ml) was added over the course of 0.5 hr with continuous stirring. When the brown color persisted, addition was stopped and the solution was aerated to remove excess bromine. Near the end of the addition, a sample of the reaction mixture was removed and chromatographed in methanol–formic acid–water, giving rise to two absorbing components, R_F 0.33 and 0.55. The slower moving compound had the same R_F as a guanine marker and was indistinguishable from guanine on cochromatography.

Near the end of the addition of bromine–water a white solid began to precipitate and was collected on a filter and dried. Comparison of the infrared spectrum with that of guanine revealed that the two were identical.

The filtrate was adjusted to pH 6 by the addition of 6 N sodium hydroxide, causing the color to change from a very light yellow to orange. Portionwise addition of 2.5 g of sodium borohydride led to a reversal of the color change and further precipitation. After 18 hr at 0–5° the solid was collected on a Buchner funnel and dried. The infrared spectrum of this compound was also identical with that of guanine. The total yield of guanine was 7.92 g (92.4%).

B. FORMATION OF 1,2,4-PENTANETRIOL. The presence

of an alcohol in the filtrate was suggested by the red coloration produced when a sample of the filtrate was treated with ceric nitrate solution (Duke and Smith, 1940). The filtrate was concentrated to dryness, and the dark brown residue was dried *in vacuo* over phosphorus pentoxide and calcium chloride.

Borate in the residue was next removed by repeated addition and evaporation of 1% hydrochloric acid in methanol. The residue, consisting of a white solid mixed with a small amount of dark brown oil, was dissolved in 50 ml of water and placed on a column of Amberlite MB-3 (*ca.* 300 ml). The column was washed with water, and the salt-free eluate (300 ml) was concentrated at the water pump to yield about 1.5 ml of a light brown syrup. After addition and immediate removal by distillation of 15 ml of methanol, the residue was distilled under reduced pressure to yield 1.28 g of a viscous syrup: bp 125–130° (0.5 mm), $n_D^{24.4°}$ 1.4656.

The infrared spectrum of the syrup (III) was compared to that of synthetic 1,2,4-pentanetriol and the

$$CH_3CHOHCH_2CHOHCH_2OH$$
III

two were found to be identical. Moreover the isolated and synthetic material were found to have identical R_F values on paper chromatography in ethyl acetate–pyridine–saturated boric acid (60:25:20, descending). Chromatograms were developed with benzidine–periodate spray (Viscontini *et al.*, 1955); R_F 0.73. *Anal.* Calcd for $C_5H_{12}O_5$ (120.15): C, 49.95; H, 10.07. Found: C, 48.2; H, 10.1.

Synthesis of 1,2,4-Pentanetriol. To 100 ml of acetone was added 2.5 g (3 ml, 0.0208 mole) of once-distilled 4-penten-2-ol (Levene and Haller, 1928). The solution was chilled, and oxidation was carried out in the cold room at 5° in the following manner. To the solution, stirred with a magnetic stirrer, 4.6 g (0.0291 mole) of potassium permanganate in 210 ml of water was added over the course of 6 hr. The pH was then adjusted to 7 with sulfuric acid, the reaction mixture was filtered to remove manganese dioxide, and about 20 g of Amberlite MB-3 resin was added to the filtrate. The flask was mechanically swirled for an hour and the resin was removed by filtration. The filtrate was concentrated at the water pump to a syrup, which was taken up in 20 ml of methanol. Solvent was removed by flash distillation, and 1,2,4-pentanetriol was vacuum distilled to give 0.940 g (27.0%) of a viscous, colorless liquid: bp 123–130° (0.55 mm); infrared spectrum showed principal bands at 3250, 2800, 1380, 1100, 1050, and 1020 cm^{-1}.

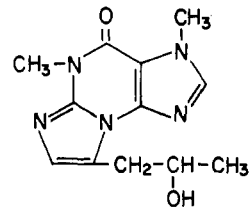
The phenylurethans of the isolated and synthetic 1,2,4-pentanetriols were prepared in the standard way by reaction with phenyl isocyanate at 120° for 4 hr in a sealed tube. After recrystallization from benzene, the melting point of the 1,2,4-tri-*O*-phenylurethan derived from authentic 1,2,4-pentanetriol was 145–149°, while that originating from III had mp 147–150°. A mixture of the two derivatives had mp 146–150° (lit. (Pariselle, 1911) mp 145–150°).

Periodate Oxidation of 1,2,4-Pentanetriol (III). A. IDENTIFICATION OF ONE OF THE PRODUCTS AS FORMALDEHYDE. Fifty milligrams of the syrup (III) was dissolved in 4 ml of water and to this solution was added 1 ml of 0.5 N sodium periodate. After 15 min the reaction mixture was distilled, water being added to the distillation flask as the distillate was collected. To the first 5 ml of distillate was added 60 mg of 5,5-dimethylcyclohexane-1,3-dione in 1 ml of 50% ethanol. After standing overnight the crystals were collected and recrystallized twice from 50% ethanol: mp 187–188°. A mixture melting point with the derivative obtained from authentic formaldehyde showed no depression. The infrared spectra of the two derivatives were identical.

By using the procedure of Marinetti and Rouser (1955), a quantitative study of periodate uptake was made. In duplicate determinations on 16-mg samples an uptake of 0.828 mole of periodate/mole of degradation product (III) (assumed molecular weight of 120.15) occurred within 15 min. Glycerol (11-mg samples) controls were found to react with 1.89 moles of periodate/mole by this procedure.

B. IDENTIFICATION OF 3-HYDROXYBUTYRIC ACID AS A PRODUCT. Into two separate 25-ml erlenmeyer flasks were placed 20.7 mg of 3-hydroxybutyraldehyde and 26.9 mg of the degradation product (III). The samples were dissolved in 2 ml of water and 1 ml of 0.5 N sodium periodate was added to each flask. After the reaction mixtures had stood 20 min, about 3 g of Amberlite MB-3 was added to each flask and the mixtures were allowed to stand for 2 hr. The resin was filtered, and to each filtrate were added 1 drop of 1 N sodium hydroxide and 0.6 ml of 3% hydrogen peroxide solution. After 30 min a few milligrams of 5% palladium on charcoal was added to destroy excess hydrogen peroxide, and the reaction mixtures were filtered. Samples of each filtrate were chromatographed individually and cochromatographed on sheets of Whatman No. 1 filter paper in ascending fashion. In each of four solvent systems the spot originating from III traveled at the same rate as that originating from 3-hydroxybutyraldehyde, giving the following R_F values: 0.82 in *n*-butyl alcohol–glacial acetic acid–water (4:1:1.67), 0.71 in ethyl alcohol–concentrated ammonia–water (16:1:3), 0.78 in ether–glacial acetic acid–water (13:3:1), and 0.84 in methyl alcohol–formic acid–water (Smith, 1958; Cheftel *et al.*, 1952; Denison and Phares, 1952). Spots were located with an aniline–xylose spray (Saarnio *et al.*, 1952).

Synthesis of 3,5-Dimethyl-8-(2'-hydroxypropyl)-4-oxo-4,5-dihydroimidazo[2,1-b]purine (IV) by Methylation of II. To 1 g of II dissolved in 50 ml of 1 N sodium hydroxide was added 5 ml of dimethyl sulfate. The reaction mixture was mechanically swirled until the solution became acid; then 5 ml more of dimethyl sulfate and 6 ml of 6 N sodium hydroxide were added, and the swirling was continued. After 16 hr the pH of the solution was adjusted to 12–13 with 6 N sodium hydroxide, and the accumulated precipitate was collected by



IV

suction filtration, washed with water, and air dried: yield 0.419 g (37.4%). The compound is later shown to be 3,5-dimethyl-8-(2′-hydroxypropyl)-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine (IV). A sample of the product submitted to paper chromatography in two solvent systems revealed a single fluorescent spot with the following R_F values: in methyl alcohol–formic acid–water, 0.82; and in isopropyl alcohol–hydrochloric acid, 0.78.

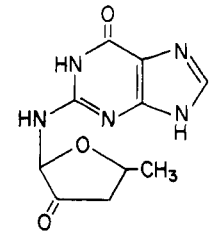
It exhibited the following ultraviolet spectral properties: in 1 N hydrochloric acid, λ_{max} 258 mμ and λ_{min} 240 mμ; in water, λ_{max} 270 mμ and λ_{min} 247 mμ; in 1 N sodium hydroxide, λ_{max} 270 mμ and λ_{min} 247 mμ. *Anal.* Calcd for $C_{12}H_{15}N_5O_2$ (261.28): C, 55.16; H, 5.79; N, 26.81. Found: C, 55.51; H, 5.91; N, 26.58.

Reaction of IV with Bromine–Water. A solution of 0.4 g of the methylated product (IV) in 25 ml of 1 N hydrochloric acid was mechanically stirred, and a saturated aqueous solution of bromine (9.7 ml) was added (dropwise) until 1 drop caused the solution to change from colorless to light brown. After aeration to remove excess bromine, the reaction mixture was neutralized with 6 N sodium hydroxide and 0.5 g of sodium borohydride was added. After 15 min the excess borohydride was destroyed with 1N hydrochloric acid.[4] A sample of the reaction mixture was examined by chromatography in ethyl acetate–pyridine–saturated boric acid. The benzidine–periodate spray gave a single spot of R_F 0.84. A sample of 2,4-dihydroxypentanal (preparation described below) had an identical R_F in the same solvent.

Further chromatographic examination of the reaction mixture in three solvent systems revealed the presence of a major ultraviolet-absorbing component migrating with the same R_F values as 1,7-dimethylguanine: 0.47 in methyl alcohol–formic acid water (80:15:5), 0.29 in *n*-butyl alcohol saturated with 0.6 N ammonium hydroxide and 0.28 in *n*-butyl alcohol–water (86:14). The chromatographically purified material had ultraviolet spectral characteristics which were identical with those of authentic 1,7-dimethylguanine taken in the same solvents: presumed 1,7-dimethylguanine in 1 N hydrochloric acid, λ_{max} 253 mμ, shoulder 270 mμ, and λ_{min} 232 mμ; in 1 N sodium hydroxide, λ_{max} 250 mμ and 284 mμ and λ_{min} 243 mμ and 263 mμ; authentic 1,7-dimethylguanine in 1 N hydrochloric acid λ_{max} 253 mμ, shoulder 271 mμ, and λ_{min} 231 mμ; in 1 N NaOH, λ_{max} 250 mμ and 284 mμ and λ_{min} 240 mμ and 263 mμ.

Over the course of about 1 week, the reaction mixture (after bromine oxidation and borohydride reduction) deposited a white solid. After crystallization from the minimum amount of hot water, the infrared spectrum was measured and found to be identical with that of 1,7-dimethylguanine.

Synthesis of 2-(3′,5′-Dideoxy-2′-ketopentofuranosyl)-amino-6-oxo-1,6-dihydropurine[5] (*V*). A 2.18-g sample of



V

II was dissolved in 100 ml of 1 N hydrochloric acid. A saturated solution of bromine in water (*ca.* 55 ml) was added with stirring until the addition of 1 more drop caused the reaction mixture to change from colorless to light brown. Excess bromine was then removed by aeration for 5 min. The reaction mixture was neutralized to pH 7 with 6 N sodium hydroxide and allowed to stand overnight at room temperature. The accumulated precipitate was removed by suction filtration, washed successively with water and ethanol, and allowed to air dry. The yield of white crystalline solid was 1.58 g (67.8%): mp >260°. Recrystallization from the minimum amount of hot water resulted in a 61.5% recovery.

The following ultraviolet data were obtained: in 1 N hydrochloric acid, λ_{max} 247 mμ (ε 12,500) and 277 mμ (ε 6020) and λ_{min} 225 mμ (ε 4300) and 267 mμ (ε 5500); in 1 N sodium hydroxide, λ_{max} 256 mμ (ε 6730) and 278 mμ (ε 7700) and λ_{min} 242 mμ (ε 5490) and 265 mμ (ε 6480); isosbestic point between pH 1 and 7 at 271 mμ; isosbestic point between pH 7 and 14 at 264 mμ. The pK_a values, determined spectrophotometrically, were 2.7 ± 0.1 and 10.0 ± 0.2.

The infrared spectrum of V shows an absorption band at 1720 cm^{-1} which is not seen in the starting material, II. The substance migrated as a single ultraviolet-absorbing spot in two solvent systems: isopropyl alcohol–hydrochloric acid, R_F 0.46; methyl alcohol–formic acid–water, 0.70. *Anal.* Calcd for $C_{10}H_{11}N_5O_3$

[4] The initial product resulting from borohydride reduction is unstable in neutral solution and breaks down to give 1,7-dimethylguanine and 2,4-dihydroxypentanal. Excess borohydride remaining after the reduction was destroyed, therefore, to prevent further reduction of 2,4-dihydroxypentanal.

[5] We have chosen to write the structure of V as a glycosylamine rather than as a Schiff base. This is supported by the ultraviolet and infrared spectra which provide no evidence for a Schiff base structure. Thus, the ultraviolet spectrum is similar to that of N^2-methylguanine and shows no bathochromic shift as would be expected from the extension of the conjugated system in the Schiff base structure.

(249.23): C, 48.19; H, 4.45; N, 28.10. Found: C, 48.09; H, 4.52; Br, neg; N, 27.62.

Compound V reacted with 2,4-dinitrophenylhydrazine to yield a product with mp 209–210° dec. Attempts to recrystallize the derivative were unsuccessful.

Reaction of V with Sodium Borohydride. To a solution of 0.294 g of V in 10 ml of 1 N sodium hydroxide was added (portionwise) 0.118 g of sodium borohydride. The reaction was allowed to proceed for 15 min and the pH was then adjusted to 7 with 1 N hydrochloric acid. Upon standing overnight the reaction mixture deposited a white solid which was filtered, washed with water, and dried at 120° for 1 hr. The material was purified by dissolving it in 10 ml of 1 N hydrochloric acid, then adjusting the pH to 6 with 1 N sodium hydroxide. The white solid was filtered, washed with water, and dried at 120° for 1 hr. The infrared spectrum of this product was identical with that of guanine: yield 0.163 g (91.9%).

The filtrate from the reaction mixture was deionized with Amberlite MB-3 and tested with fuchsin–aldehyde reagent. The test was weakly positive.

By paper chromatography in ethyl acetate–pyridine–saturated boric acid, it could be shown that the new compound had a higher R_F (0.80) than 1,2,4-pentanetriol (0.67). Both compounds were detected by spraying successively with periodate and benzidine (Viscontini *et al.*, 1955). However, if 1 ml of the filtrate from the original 15-min borohydride reduction was treated with 10 mg of sodium borohydride for an additional 15 min, the fuchsin-positive compound was converted to 1,2,4-pentanetriol. This was demonstrated by chromatography in the ethyl acetate–pyridine–saturated borate system and by periodate oxidation.

Reaction of 2,4-Dihydroxypentanal with Sodium Periodate. A solution of the presumed 2,4-dihydroxypentanal was prepared from 0.306 g of V by the procedure described above. The filtrate was poured into a weighed flask and concentrated to dryness at the water pump. The flask was placed in a vacuum dessicator overnight over phosphorus pentoxide. On reweighing, the flask was found to contain 25.5 mg (17.6%) of the compound assumed to be 2,4-dihydroxypentanal. This was dissolved in exactly 10 ml of water and an excess of periodate was added. The periodate uptake was found to be 0.88 μmole/ml or 0.684 mole of periodate/mole of 2,4-dihydroxypentanal.

The reaction mixture was next quantitatively analyzed for formate by the enzymatic procedure of Rabinowitz and Pricer (1957). Inhibition of the enzyme by periodate did not occur at the concentration present in the reaction mixture. The value measured was 0.88 mole of formate/ml. This is the value expected from the previous procedure, if it is assumed that for every mole of periodate consumed a mole of formate is produced.

Degradation of 2,4-Dihydroxypentanal to 3-Hydroxybutyric Acid. To a solution of 0.203 g of V in 10 ml of 0.5 N sodium hydroxide was added 50 mg of sodium borohydride. After the reaction mixture had stood overnight at room temperature, the pH was adjusted to 7. The precipitated guanine was filtered with suction and the filtrate was treated with periodate and peroxide as described previously. Paper chromatography in the same four solvent systems established the identity of the product as 3-hydroxybutyric acid.

Results and Discussion

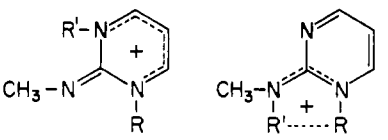
When I was degraded with 1 N sodium hydroxide at 100° for 1 hr, a product was detected in the reaction mixture whose paper chromatographic behavior and ultraviolet spectrum were nearly identical with those of guanine. The experiment suggests that the guanine nucleus is intact in I.

A study of the reactions of furfuryl alcohol with various purine and pyrimidine derivatives in 1 N acid revealed a definite pattern. Thus, a fluorescent product could only be detected in reaction mixtures in which the purine or pyrimidine derivative, employed as a starting material, possessed an NH_2 or NHR group. Furthermore, there appeared to be a requirement that the amino group be attached to C-2 of the base. For example, guanine, 2-aminopurine, isocytosine, and 2-aminopyrimidine all gave rise to fluorescent derivatives; whereas, in the case of isoguanine, adenine, cytosine, and 2-hydroxypyrimidine, no such fluorescent derivatives were detected. If both hydrogens of the amino group were substituted, as in the case of N^2,N^2-dimethylguanine and 2-dimethylaminopyrimidine, formation of a fluorescent compound also appeared to be prevented; but partial substitution of the amino group, as exemplified by N^2-methylguanine and 2-methylaminopyrimidine, did not prevent the reaction. Derivatives of guanine with substituents on positions 1 and/or 7, *i.e.*, 1-methylguanine, 7-methylguanine, and 1,7-dimethylguanine, readily reacted with furfuryl alcohol. However, alkyl substituents at positions 3 or 9 prevented the occurrence of such a reaction. Finally, all compounds which reacted with furfuryl alcohol had as a common structural feature the substituted guanidine group, *i.e.*, N(R)C(NHR)=N or =NC(NHR)=N, where R=H or CH_3. There is a strong implication from these experiments that the amino group of guanine is involved in the formation of I.

Further information concerning the structure of I was deduced from model experiments in which paper electrophoresis was carried out on products of the reaction of furfuryl alcohol with 2-aminopyrimidine and certain of its methylated derivatives. The compounds that gave rise to a detectable fluorescent derivative were 2-aminopyrimidine and 2-methylaminopyrimidine. The derivative arising from 2-aminopyrimidine migrated as a cation at pH 3.5 and thus is protonated at this pH. Electrophoresis further demonstrated that this material at pH 6.8 and 10.5 is in the neutral form. In contrast, the fluorescent derivative arising from 2-methylaminopyrimidine was found to migrate as a cation at pH 6.8 and 10.5, but not at pH 11.0. The fact that this compound exhibits a positive charge even at pH 10.5 suggests that a quaternary nitrogen is present. The lack of charge at pH 11.0 is probably due to loss of a proton through enolization of a carbonyl group

present in the side chain derived from the furfuryl alcohol (see below).

Two possible structures for the quaternary nitrogen compound were considered: (1) one in which both ring nitrogens are substituted, and (2) one in which one ring nitrogen and the amino group are substituted. That the first possibility is unlikely may be inferred from the fact that 2-hydroxypyrimidine fails to react with furfuryl alcohol. Furthermore, structure 1 would be labile at neutral pH and above, a property not observed for the fluorescent compounds. Since the amino group must be present and possess at least one unsubstituted hydrogen for reaction to occur, it may be



(1) (2)

concluded that it is involved in the formation of the fluorescent derivative. Further substitution at the ring N, as in 2, would provide the quaternary N.

If it is assumed that the reaction of furfuryl alcohol with guanine takes a course similar to that observed with 2-aminopyrimidine and 2-methylaminopyrimidine, it may be concluded that in the fluorescent compound (I) the purine ring is substituted at the amino group and the nitrogen atom at position three. This conclusion is supported by our failure to detect any fluorescent derivative as a product of a reaction between 3-methylguanine and furfuryl alcohol. In contrast, 1-methylguanine reacted positively in the same test.

Failure of 1,2-dihydro-2-imino-1-methylpyrimidine to produce a fluorescent compound may be due to the increased basicity of this substance, $pK_a = 10.75$, compared to 2-aminopyrimidine, $pK_a = 3.54$, and 2-methylaminopyrimidine, $pK_a = 3.82$ (Brown *et al.*, 1955). As will be discussed later, a plausible mechanism for the reactions yielding the fluorescent derivatives is one involving nucleophilic attack by the heterocyclic base on an acid degradation product of furfuryl alcohol. Since this reaction is normally conducted in the pH range 0–1, the fraction of unprotonated base would be *ca.* 10^{-4} in the case of the aminopyrimidines but only *ca.* 10^{-11} in the case of 1,2-dihydro-2-imino-1-methylpyrimidine.

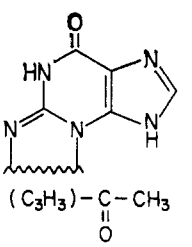
It should be noted that attempts to detect a fluorescent compound as a reaction product of furfuryl alcohol with 9-methylguanine, 9-ethylguanine, or 1,7,9-trimethylguanine all failed. The failure in this case may be related to observations made in a study of the alkylation of 9-alkyladenines (Leonard and Fujii, 1964). In the latter case, a specific directive effect of the nine substituent (to position 1) was observed which was reciprocal in nature, suggesting that the lack of alkylation at N-3 might be due to electronic rather than steric factors. While the present data do not permit a decision between a steric or an electronic effect, it is noteworthy that inspection of an atomic model of I indicates that the substitution of a methyl group at position 1 of I would result in close contact with the 8-acetonyl group.

The presence of a carbonyl group in I was suggested by the formation of a 2,4-dinitrophenylhydrazone. Previously Hoard (1957) had shown that, in addition to the elements of guanine, a five-carbon moiety is present in the fluorescent compound ($C_{10}H_9N_5O_2$). Presumably the carbonyl group resides in this five-carbon residue, since guanine itself will not react with 2,4-dinitrophenylhydrazine. The presence of a carbonyl group was further demonstrated by the reduction of I with sodium borohydride to give a product in high yield that analyzed for $C_{10}H_{11}N_5O_2$, *i.e.*, contained two hydrogen atoms more than the original fluorescent compound (I). The infrared spectrum of I showed a strong carbonyl absorption band near 1700 cm^{-1}. Reduction eliminated this band and gave rise to a new absorption peak at 3250 cm^{-1}. This hydroxyl peak was not present in the spectrum of the unreduced material. In addition, the unreduced fluorescent compound was found to give a positive iodoform test, suggesting the presence of an acetyl function as a part of the nonpurine five-carbon moiety.

Evidence which is consistent with the presence of an acetyl function was furnished by the nmr spectrum of I. Four sharp bands occur in the spectrum at δ values of 2.62, 4.62, 7.50, and 8.37 ppm with relative areas in the ratio of 3:2:1:1, respectively. We ascribe the peak at 2.62 ppm to an uncoupled methyl group, presumably the methyl group of the acetyl function. The peak at 4.62 ppm suggests the presence of a methylene group in the nonpurine five-carbon residue of I.

The two bands at 7.50 and 8.37 ppm are in the region characteristic of a vinylic proton adjacent to an electronegative element. By analogy to the position of resonance of the H-8 proton of purine derivatives (Jardetzky and Jardetzky, 1960), we assign the peak at 8.37 ppm to the proton at position 2 of I. The other band at 7.50 ppm we assign to a CH proton in the five-carbon moiety of I. The latter two assignments are supported by our observation of a small coupling (<1 cycle/sec) of the band at 7.5 ppm.

The evidence, thus far accumulated, supports the following partial structure for I.



Additional evidence concerning the nature of the five-carbon moiety was obtained when bromine–water was found to react rapidly with a solution of I in 1 N hydro-

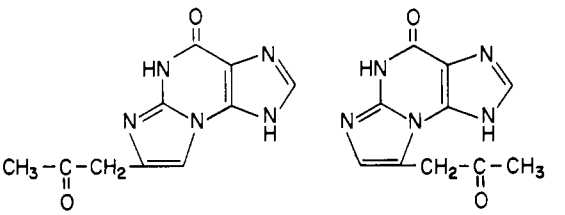
chloric acid, giving rise to an ultraviolet-absorbing product whose R_F in methyl alcohol–formic acid–water was nearly the same as that of the starting material. Partial neutralization of the reaction mixture with 6 N sodium hydroxide was followed by precipitation of a white solid which was identified as guanine by infrared spectroscopy. The nearly quantitative recovery of guanine from the reaction mixture (A) suggested that the new absorbing compound detected by paper chromatography was an intermediate in the degradation of I, and (B) required that any additional product(s) of the reaction have their origin in the five-carbon, nonpurine moiety. Sodium borohydride reduction of the reaction mixture, followed by an attempt to recover such a reaction product, led to the isolation of a syrup (III) which was identified as 1,2,4-pentanetriol. That the identification was correct may be seen from the following considerations. (1) The syrup consumed 0.828 mole of periodate/mole, implying the presence of two adjacent hydroxyl groups. (2) Formaldehyde, identified as the dimedon derivative, was found in the periodate oxidation reaction mixture. The presence of a primary alcohol function in the glycol grouping is suggested by this result. (3) The remaining product of periodate oxidation, when further oxidized with hydrogen peroxide, was identified as 3-hydroxybutyric acid by paper chromatography in four solvent systems. The identification of 3-hydroxybutyric acid indicates that the product of periodate oxidation must have been 3-hydroxybutyraldehyde. This result, considered together with the previous two, serves to identify the syrup as 1,2,4-pentanetriol.

Additional evidence strongly supports the identification. For example, the infrared spectrum of the syrup (III) was identical with that of authentic 1,2,4-pentanetriol. Moreover, III and authentic 1,2,4-pentanetriol had identical paper chromatographic behavior. The 1,2,4-tri-*O*-phenylurethan of III had the same melting point as the derivative prepared from synthetic 1,2,4-pentanetriol, and a mixture of the two preparations caused no depression in melting point. Finally, the infrared spectra of the two derivatives were also identical. These results leave little doubt that III is actually 1,2,4-pentanetriol.

The isolation of 1,2,4-pentanetriol provides valuable clues to the structure of I. Thus, carbon atoms four and five of the triol must be derived from the same ones responsible for the acetyl group previously shown to be present in the fluorescent derivative. Furthermore, the presence of the adjacent hydroxyl group at positions one and two of the triol tends to support the conclusion reached before that the purine ring of I is disubstituted, *i.e.*, attached at two points to the five-carbon residue.

Further evidence for the site of attachment of the five-carbon residue to the purine ring came from the demonstration that positions one and seven, which are two possible points of attack for furfuryl alcohol, or a reactive species derived from it, are not involved in the formation of I. When the reduced compound (II) was treated with dimethyl sulfate in alkaline solution, a product (IV) was obtained which on analysis was found to have two methyl groups substituted for two hydrogens of II. This material was subjected to bromine–water degradation and borohydride reduction in a manner similar to that employed for the degradation of I. A compound was isolated from the reaction mixture with the same chromatographic behavior and ultraviolet and infrared spectra as authentic 1,7-dimethylguanine. This leaves ring nitrogens 3 and 9 and the amino nitrogen as the only possible points of attachment and, of these, N-9 is unlikely because of the ready reaction of various pyrimidine derivatives which lack this position.

A question arises concerning the orientation of the five-carbon moiety with respect to the purine ring, *i.e.*, whether, in I, carbon one of the five-carbon fragment is bound to the amino group of guanine and carbon two to the ring nitrogen or *vice versa*. Of the



two alternatives, b was considered the more likely based on the observation that 9-methyl-, 9-ethyl-, and 1,7,9-trimethylguanines failed to react with furfuryl alcohol. This failure might be ascribed to steric hindrance if orientation b were correct.

A more convincing answer to this question was obtained by a study of the first detectable reaction product resulting from bromine-water oxidation of II. This product (V) would appear to be related to the unstable intermediate which was detected in the reaction mixture of I upon bromine–water oxidation. In contrast, V appeared to be stable and could be dissolved in acid or base without appreciable decomposition. Compound V absorbed ultraviolet light and had an ultraviolet spectrum markedly different from that of II but very similar to that of guanine at all pH values except those in the region of pH 13–14. Besides forming a 2,4-dinitrophenylhydrazone, V had an infrared spectrum which possessed a carbonyl peak at 1720 cm^{-1}. This peak was absent in the spectrum of the parent compound (II). Reducing the new carbonyl group with borohydride for 15 min gave a product that was unstable, even at pH 7. On standing several hours this reaction mixture deposited a solid which was identified as guanine from its infrared spectrum.

Remaining in solution was a product that was identified as 2,4-dihydroxypentanal. Identification was based on a positive aldehyde test and the stoichiometry and products of its reaction with periodate. The identification was confirmed by showing that the product of borohydride reduction had the same chromatographic behavior as 1,2,4-pentanetriol.

In summary, it is concluded that treatment of II

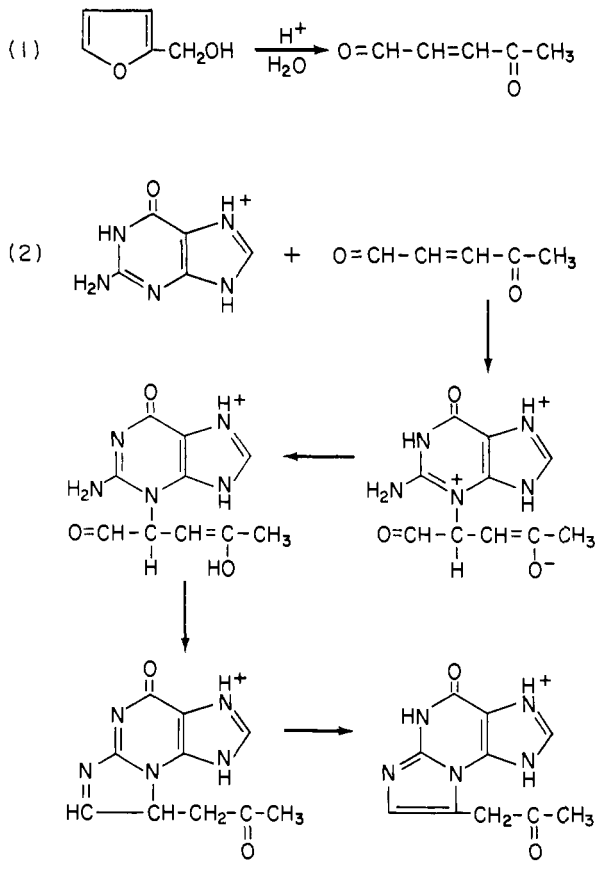


FIGURE 1: Formation of 8-acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine.

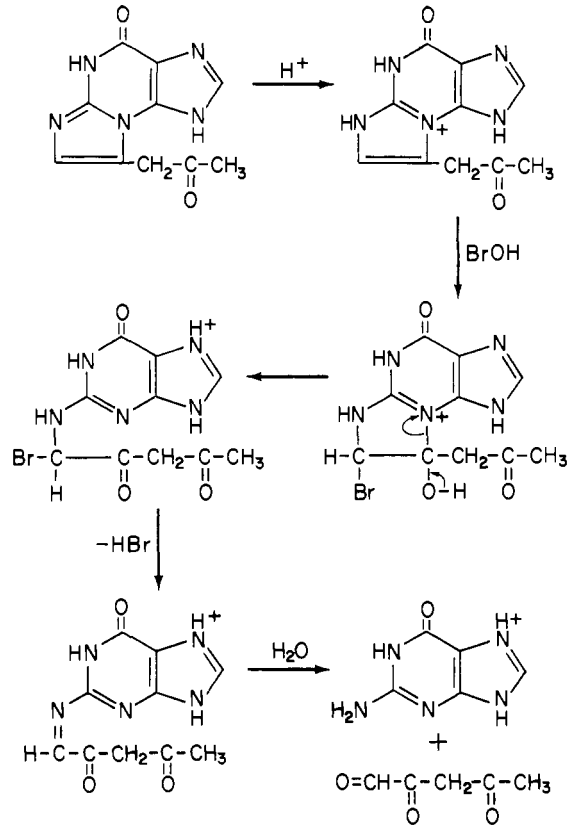


FIGURE 2: Bromine–water degradation of 8-acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine.

with bromine–water breaks one of the bonds holding the five-carbon moiety to guanine, and in the process a carbonyl group is produced, resulting in the formation of a compound of empirical formula, $C_{10}H_{11}N_5O_3$ (V). Reduction of the new carbonyl group in V gives a product which is unstable and breaks down to guanine and 2,4-dihydroxypentanal. Production of 2,4-dihydroxypentanal from a compound which had previously been treated with borohydride unequivocally shows that carbon one of the five-carbon residue was bound to guanine in V. The protection, thus afforded, explains the presence of the aldehyde group at C-1. The formation of 2,4-dihydroxypentanal also demonstrates that the carbonyl group of V is at carbon two of the non-purine moiety.

A return to the question of how the five-carbon moiety is oriented in I can now be made. From previous considerations, it is known that the two points of attachment of guanine to the five-carbon residue in I and II, are N-3 and the amino nitrogen. After bromine–water oxidation one of these bonds is broken leaving a single linkage to the one position of the five-carbon residue. To establish whether the remaining bond was to N-3 or the amino nitrogen, the spectra of suitable guanine derivatives bearing a substituent at N-3 or the amino nitrogen were compared with that of V. The validity of this comparison rests on the assumption that the effect of the five-carbon substituent would be similar to that of an alkyl group (Goos *et al.*, 1930). The spectrum of V proved to be remarkably similar to that of N^2-methylguanine, indicating an attachment of the five-carbon residue to the amino nitrogen of the guanine moiety (Elion *et al.*, 1956). The absence of any marked bathochromic shift showed that there had been no extension of the conjugated bond system of guanine and, in part, justified our assumption that the five-carbon residue could be equated to an alkyl group for the purpose of spectral comparison. When the spectra are measured at pH 7, the principal effect of a methyl substituent on the amino group of guanine is an increase in absorption at the major band and a decrease in absorption at the shoulder, with the general shape of the spectrum unchanged (guanine λ_{max} 247 mμ and 276 mμ; N^2-methylguanine, λ_{max} 247 mμ and 281 mμ). At the same pH, V has maxima at 245 mμ and 280 mμ. The ratio of absorbancies of the maxima (245:280) is 1.75 for V and 1.66 for N^2-methylguanine (247:281), while the same ratio (247:276) for guanine is 1.32.

In contrast to the above noted similarities in spectra was the striking difference observed when spectral comparison was made of V and 3-methylguanine. At pH 7, 3-methylguanine has maxima at 234 mμ and 268

mμ and an $A_{234}:A_{268}$ ratio of 0.721, thus differing from guanine, guanosine, and N^2-methylguanine.

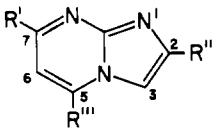
Having established, in the case of V that the linkage is *via* C-1′ of the five-carbon residue and the amino nitrogen of the guanine residue, we can write the structure of V as 2-(3′,5′-dideoxy-2′-ketopentofuranosyl)-amino-6-oxo-1,6-dihydropurine.

By deduction, we conclude that bromine–water treatment of II caused the rupture of the C-8 to N-9 linkage. Thus II can be represented as 8-(2′-hydroxypropyl)-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine.

Finally, since II is produced from the original fluorescent compound (I) by borohydride reduction, I can be formulated as 8-acetonyl-4-oxo-4,5-dihydroimidazo[2,1-*b*]purine.

In addition, the product of reaction of II with dimethyl sulfate is established as 3,5-dimethyl-8-(2′-hydroxypropyl)-4-oxo-4,5-dihydroimidazo[2,1-*b*]-purine.

Since the completion of this study, the related synthesis has been reported (Paudler and Kuder, 1966) of some imidazo[1,2-*a*]pyrimidines by condensation of properly substituted 2-aminopyrimidines with the



appropriate α-halocarbonyl compounds. Of particular relevance to the bromine–water degradation described in the present work is the observation that the imidazo-[1,2-*a*]pyrimidines are protonated at position 1.

Even though the experimental data provide an insufficient basis on which to propose a detailed mechanism for the formation of I, a possible mode of synthesis is offered in Figure 1. As proposed by Dunlop and Peters (1953), the furfurylcarbonium ion, postulated to occur in the acid-catalyzed degradation of furfuryl alcohol, may add water and eventually form 3-acetylacrolein. The next reaction could be pictured as an attack by N-3 of the guanine nucleus on the α-carbon of 3-acetylacrolein leading to a hypothetical zwitterion. Proton transfer from N-1 to the enolate oxygen, followed by Schiff base formation and another proton shift, completes the sequence of events. Although this mechanism by no means exhausts the possible ways of forming I, it does serve to show that a plausible mechanism for the formation of the fluorescent derivative can be written.

The manner in which bromine–water effects the cleavage of the new imidazole ring in I requires further experimentation in order to be resolved. A tentative hypothesis for this breakdown is shown by the sequence of reactions pictured in Figure 2. At the low pH of the solution in which degradation takes place, I is a cation. The resulting modification in the resonance of the new imidazole ring may be sufficient to allow the carbon–carbon double bond to act enough like an isolated double bond so that hypobromous acid can add across it. The α-hydroxy quaternary amine would readily revert to 2-(1′-bromo-2′,4′-dioxopentyl)amino-6-oxo-1,6-dihydropurine. Dehydrobromination and cleavage of the resulting labile Schiff base linkage results in the complete removal of the five-carbon chain to form 2,4-dioxopentanal and guanine. While 2,4-dioxopentanal was not isolated, its presence was inferred from the pale yellow color of the reaction mixture after bromine oxidation (with subsequent aeration) and the intense yellow color and decomposition (slow darkening) accompanying alkalinization. As previously mentioned, the addition of sodium borohydride to the alkaline solution resulted in an immediate decrease in the intensity of the yellow color and permitted the isolation of 1,2,4-pentanetriol.

Acknowledgments

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Chemical Studies on Riboflavin and Related Compounds. I. Oxidation of Quinoxaline-2,3-diols as a Possible Model for the Biological Decomposition of Riboflavin*

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ABSTRACT: In connection with the biological decomposition of riboflavin, the oxidation of three quinoxaline-2,3-diol derivatives, *i.e.*, 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I), 6,7-dimethylquinoxaline-2,3-diol (II), and quinoxaline-2,3-diol (IV), was examined by chemical means. The former two compounds are known to be catabolic intermediates of riboflavin. On oxidation with alkaline ferricyanide, IV gave *cis,trans*-muconic acid and probably a dimer VII, II gave 3,4-dimethyl-4-carboxymethyl-Δ^1-butenolide (VIII) and 6-carboxy-7-methylquinoxaline-2,3-diol (IX), and I gave II, VII, and IX. Whereas IV was resistant to photosensitized oxidation, both I and II were degraded destructively under similar conditions. However, II was found to be an intermediate in the photooxidation of I. Mechanisms of these oxidation reactions are discussed in connection with the enzymatic degradation of riboflavin.

The first significant observation on the biological decomposition of riboflavin was reported by Foster *et al.* (Foster, 1944; Yanagita and Foster, 1956), who demonstrated that *Pseudomonas riboflavinus* catalyzes the hydrolysis of riboflavin to lumichrome and ribitol, followed by the oxidation of the latter to carbon dioxide. Miles and Stadtman (1955) reported that a microorganism degrades riboflavin to 6,7-dimethyl-9-(2′-hydroxyethyl)isoalloxazine under anaerobic conditions. Recently the isolation of 6,7-dimethyl-9-(2′-carboxyethyl)isoalloxazine, related to the above isoalloxazine, from urine of sheeps was reported (Owen and Montgomery, 1962). Such types of the degradation of the side chain of riboflavin seem to be related to the photochemical degradation (for a review, see Hemmerich *et al.*, 1965) of riboflavin.

Another type of the biological decomposition of riboflavin has been investigated by Stadtman and collaborators (Smyrniotis and Stadtman, 1957; Smyrniotis *et al.*, 1958; Miles *et al.*, 1959; Tsai *et al.*, 1963; Harkness *et al.*, 1964). They demonstrated that a different strain of *P. riboflavinus* degrades the isoalloxazine portion of the molecule. Thus riboflavin is oxidized to 3,4-dimethyl-6-carboxy-α-pyrone (III) *via* 2,3-diketoquinoxaline derivatives, I and II, as shown in Scheme I.

Although the mechanism of this microbial degradation of riboflavin has not yet been established, it has been suggested that the metabolic conversion of I to II may involve a mixed function oxygenase enzyme and that the conversion of II to III is a multistep process in which the presence of molecular oxygen plus any one of several cosubstrates capable of pyridine nucleotide linked oxidation are required (Harkness *et al.*, 1964). In order to contribute to the elucidation of the mechanism of this biological oxidation, we have investigated the oxidation of the metabolic intermedi-

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