177° dec., was obtained. A second crystallization yielded 170 mg. of the compound melting at 188° dec. (lit.[23] *erythro*-, m.p. 182–184° dec.; *threo*-, m.p. 153–155° dec.

(ii) **Ethyl *erythro*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate** (I).—A mixture of 40 mg. of VIII, 28 mg. of sodium acetate and 19 mg. of benzoyl chloride in 4 ml. of water and 4 ml. of chloroform was shaken for 2 hr. About 20 ml. of chloroform was added to the reaction mixture, and the aqueous phase was acidified with a few drops of 2.5 *N* hydrochloric acid. The chloroform phase was separated and dried over anhydrous magnesium sulfate. The chloroform solution was filtered and concentrated *in vacuo*. Crystallization of the residue from ethanol gave 30 mg. of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate, m.p. 154–156°. A second crystallization from ethanol yielded 18 mg. of product melting at 158–160°. There was no depression in melting point on admixture of the product with an authentic specimen of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate.

(23) C. G. Alberti, B. Camerino and A. Vercellone, *Experientia*, **8**, 261 (1952).

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

# A Study of the Action of Acid and Alkali on Certain Purines and Purine Nucleosides[1]

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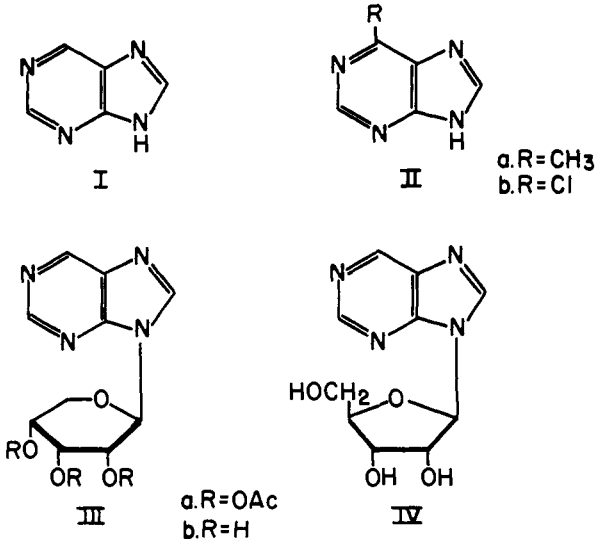
A series of glycosyl derivatives of various purines was found to be extremely labile toward dilute alkali at room temperature. In studies with 9-β-D-ribofuranosylpurine two products resulting from cleavage of the imidazole ring, with loss of carbon-8 but without loss of the ribosyl group, were partially characterized. Purine, its ribofuranosyl and ribopyranosyl derivatives evolve formic acid when heated with *p*-toluenesulfonic acid. Studies of the reaction indicate a complex decomposition pattern, including some loss of carbon-8. Syntheses of 9-D-ribopyranosylpurine and 6-methyl-D-ribofuranosylpurine are described.

In the course of studies of the properties of several derivatives of purine (I to IV), it was observed that the spectrum of 9-β-D-ribofuranosylpurine (IV, nebularine)[3] and of related glycosyl derivatives, underwent drastic and irreversible changes in dilute aqueous alkali at room temperature. It was also observed that 9-(2′,3′,4′-tri-*O*-acetyl-D-ribopyranosyl)-purine (IIIa) and other derivatives of purine yielded anomalous "acetyl" analyses. Such chemical behaviors have not previously been observed with any nucleosides of purines.




One nucleoside, namely, IV, which possesses both of these unusual properties occurs in the mushroom *Agaricus* (*Clitocybe*) *nebularis* Batsch.[4a,b,5] It is inhibitory toward *Mycobacteria*,[4a,b] is highly toxic to mammals[3,6,a,b] and exhibits selective toxicity toward cells of mouse sarcoma 180 in culture.[7] Purine I, IV and other purine derivatives, 6-methylpurine (IIa) and 6-chloropurine (IIb), have some selective inhibitory effects on the growth of experimental tumors.[8]

Investigations of these unusual chemical behaviors, including attempts to elucidate the structural factors responsible and of possible correlations with the pharmacodynamic properties of certain of these compounds are in progress.[9] Consideration of these newly recognized chemical behaviors was necessary in studies of the metabolic fates of IV[10a] and I[10b] as well as in the work described in the following three papers; these properties must be considered when investigating new purine derivatives from natural sources.

## Results and Discussion

**Acid Instability.**—The analysis for the formation of volatile acids involved heating the compound in question with a 25% aqueous solution of *p*-toluenesulfonic acid at 100° for 4 hr., steam distillation

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(2) Post-doctoral Research Fellow of the National Cancer Institute, 1953–1955.

(3) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(4) (a) N. Löfgren and B. Lüning, *Acta Chem. Scand.*, **7**, 225 (1953); (b) N. Löfgren, B. Lüning and H. Hedström, *ibid.*, **8**, 670 (1954).

(5) E. Fischer, "Untersuchungen in der Purin Gruppe," Springer, Berlin, 1907, p. 68, suggested that purine (I) would probably be found in nature.

(6) (a) F. S. Philips and D. A. Clarke, personal communication; (b) A. P. Truant and H. E. D'Amato, *Federation Proc.*, **14**, 391 (1955).

(7) J. J. Biesele, M. C. Slautterback and M. Margolis, *Cancer*, **8**, 87 (1955).

(8) (a) D. A. Clarke, F. S. Philips, S. S. Sternberg and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954); (b) F. S. Philips, S. S. Sternberg, L. Hamilton and D. A. Clarke, *ibid.*, **60**, 283 (1954); (c) K. Sugiura, Special Lectures of the 14th Japan Medical Congress, Kyoto, Japan, 697–714 (1955).

(9) G. B. Brown, M. P. Gordon, A. Hampton and D. I. Magrath, "The Chemistry and Biology of Purines," CIBA Symposia, Churchill, London, 1957, p. 192.

(10) (a) M. P. Gordon and G. B. Brown, *J. Biol. Chem.*, **220**, 927 (1956); (b) M. P. Gordon, in preparation.

and titration of the volatile acid produced.[11] The volatile acid obtained from I (*ca.* 0.9 mole/mole I) was identified as formic acid through conversion to the S-benzylisothiouronium salt,[12] by its conversion to benzimidazole and benzimidazole picrate[13] and by its reconversion to I.

When purine-8-$C^{14}$ was similarly treated, the formate carbon of the S-benzylisothiouronium derivative had approximately one-third of the specific activity of the 8-carbon of the parent I. The residue after the completion of the steam distillation was quite dark, evolved ammonia with alkali and gave a test for reducing agents with alkaline phosphomolybdate.[14] When purine-8-$C^{14}$ was treated for 12 hr., 1.8 moles of formic acid was evolved per mole of I, and this formic acid was reconverted to I before determination of the radioactivity. It was again found to have about one-third of the specific activity of the 8-carbon of the starting material.

The residue from the 12 hr. treatment contained 1.9 moles of ammonia per mole of I used. The ninhydrin positive[15] component found in the residue had the same paper chromatographic behavior as glycine in three different chromatographic systems. The presence of a small amount of unchanged I was also detected.

The evolution of formic acid, ammonia and the formation of glycine indicate extensive degradation. The threefold dilution of the radioactivity of carbon atom 8 may well be due to the more rapid liberation of formic acid from other parts of the molecule (*i.e.*, to the initial formation of imidazole derivatives, see below), since a maximum of two moles of acid was evolved on prolonged heating. The positive reactions with the Bratton–Marshall reagent,[16] diazotized sulfanilic acid[17] and alkaline phosphomolybdate,[14] the variable ratio of ammonia produced to formic acid evolved and the marked darkening of the reaction mixture all indicate a complex decomposition pattern. The three color tests were all positive with 4,5-diaminopyrimidine; however, 4-amino-5-imidazolealdehyde, which might result if carbon 2 was lost, would also be expected to react with these color reagents.

It is known that the purine ring system can be cleaved by acids under sufficiently rigorous conditions to yield carbon dioxide, ammonia, carbon monoxide and/or formic acid and glycine. (For a review of the older literature see ref. 18 and 19.) Less drastic hydrolyses of adenine and guanine result in the formation of 4-amino-5-imidazolecarboxamidine[18] and 4-guanidoimidazole,[20] respectively. Although 9-methylpurine and 2-hydroxypurine are decomposed by hot sulfuric acid,[21] I has been reported to be stable toward the action of hot dilute acids[22] or to undergo slight decomposition in more concentrated acid.[21,23] In our hands I appears to be one of the least stable purines toward the action of hot *p*-toluenesulfonic acid or sulfuric acid. In the pteridine series the parent compound was also found to be exceptionally unstable toward acid.[24]

A series of various purines and purine nucleosides were also analyzed for the formation of volatile acid. 9-Methylpurine and nucleosides of I were the only additional compounds that evolved appreciable amounts of volatile acid (Table I).

TABLE I

MOLES VOLATILE ACID EVOLVED PER MOLE OF COMPOUND[a]

| | |
|---|---|
| Purine[b] | 0.87,[f] 1.8,[g] 2.0[h] |
| 9-Methylpurine[b] | 0.63 |
| 6-Methylpurine[c] | Trace |
| 6-Dimethylaminopurine[d] | 0.0 |
| 6-Chloropurine[e] | .0 |
| Adenine | .0 |
| Guanine | .0 |
| Xanthine | Trace |
| 9-β-D-Ribofuranosylpurine | 0.92 |
| 9-D-Ribopyranosylpurine | .84 |
| 9-D-(2′,3′,4′-Tri-*O*-acetylribopyranosyl)-purine | 4.8 |
| Adenosine | 0.0 |
| Guanosine | .0 |
| 6-Dimethylamino-9-β-D-ribofuranosyl-purine[d] | .0 |
| 6-Chloro-9-β-D-ribofuranosylpurine[e] | .0 |
| 6-Dimethylamino-9-β-D-(3′-amino-3′-deoxyribofuranosyl)-purine[d] | .0 |
| 6-Methyl-D-ribofuranosylpurine | Trace |

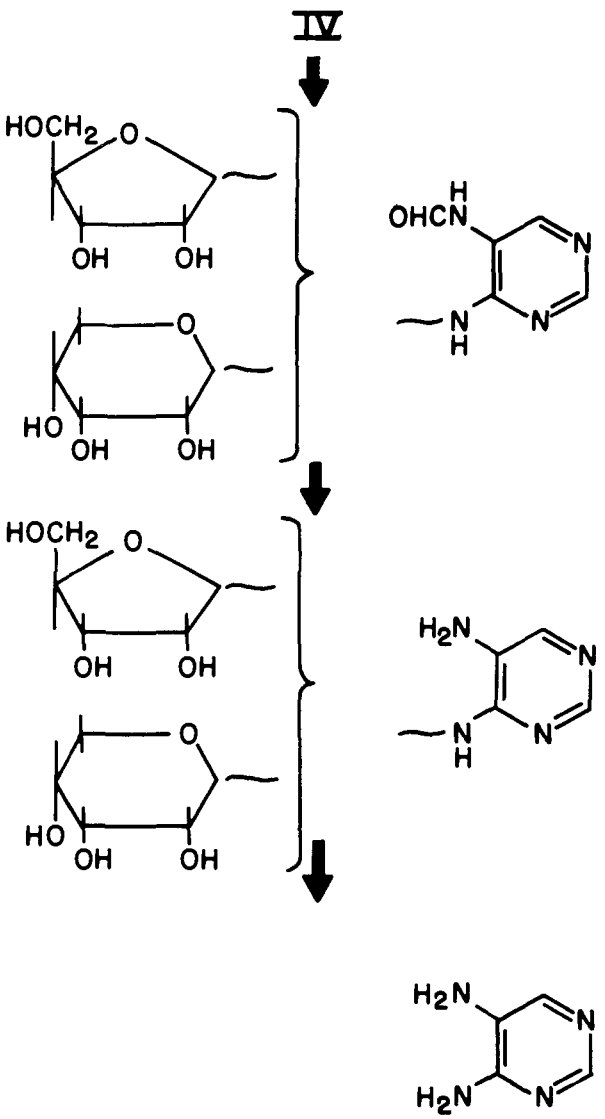
[a] Analyses by J. F. Alicino, Metuchen, N. J.; H. B. Clark, Urbana, Ill.; and by this Laboratory, duplicates in most cases. [b] Obtained from A. Bendich of these Laboratories or (in the case of I) also purchased from Francis Earle Laboratories, Peekskill, N. Y. [c] Obtained from G. B. Elion and G. H. Hitchings, The Wellcome Research Laboratories, Tuckahoe 7, N. Y. [d] Obtained from B. R. Baker, Southern Research Institute, Birmingham, Ala. [e] In these two cases, silver sulfate was added to the distillation flask to trap any hydrogen chloride generated. No chloride ion was detected in the distillate. [f] Heated for 4 hr. 1.35 moles of $NH_3$ evolved per mole I. [g] Heated for 12 hr. 1.93 moles of $NH_3$ evolved per mole I. [h] Heated for 24 hr. 2.25 moles of $NH_3$ evolved per mole I.

I and IV do behave similarly to other members of the purine series in that the purine base is stable in 70% perchloric acid under conditions which are used to hydrolyze nucleic acids.[25]

**Alkaline Instability.**—A study of the spectral changes induced by the action of dilute alkali at room temperature on IV (see Fig. 1) showed that a compound (or compounds) were formed that exhibited two maxima in alkali and one in acid. Eventually the solution showed only non-specific end absorption. The similarity of the intermediate

(11) F. Pregl, "Quantitative Organic Microanalysis," revised by J. Grant, The Blakiston Co., Philadelphia, Pa., 1945, p. 161.
(12) J. J. Donleavy, THIS JOURNAL, **58**, 1004 (1936).
(13) E. L. Brown and N. Campbell, *J. Chem. Soc.*, 1699 (1937).
(14) A. Bendich and G. C. Clements, *Biochem. et Biophys. Acta*, **12**, 462 (1953).
(15) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).
(16) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).
(17) B. N. Ames and H. J. Mitchell, THIS JOURNAL, **74**, 252 (1952).
(18) L. F. Cavalieri, J. F. Tinker and G. B. Brown, *ibid.*, **71**, 3973 (1949).
(19) Reference 5, p. 69.
(20) G. Hunter, *Biochem. J.*, **30**, 1183 (1936).
(21) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).
(22) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).
(23) E. Fischer, *Ber.*, **31**, 2564 (1898).
(24) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 4219 (1952).
(25) A. Marshak and H. J. Vogel, *J. Biol. Chem.*, **189**, 597 (1951).

spectra with those of 4,5-diaminopyrimidine and 4-methylamino-5-aminopyrimidine strongly suggested that cleavage of the imidazole ring had occurred with the resulting formation of a derivative of 4,5-diaminopyrimidine. The solution was strongly reducing toward alkaline phosphomolybdate[14] which is also true for 4,5-diaminopyrimidine.




The addition of picric acid to a solution of the alkaline degradation products of IV resulted in the formation of 4,5-diaminopyrimidine picrate. Paper chromatography of the original reaction mixture indicated the presence of three components, each of which had spectra very similar to those of 4,5-diaminopyrimidine in acid and alkali. The fastest moving component, which was present in trace amounts, migrated with the $R_f$ of 4,5-diaminopyrimidine. The two slower components gave positive tests for *cis*-glycol groups,[26] and these

(26) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950). The test for vicinal hydroxyls, or $\alpha$-glycols, can distinguish between *cis* and *trans* configurations in nucleosides when the rapidity of the oxidation is considered: D. M. Brown, A. Todd and S. Varadarajan, *ibid.*, 2388 (1956); J. J. Fox, N. Yung and A. Bendich, THIS JOURNAL, **79**, 2775 (1957).

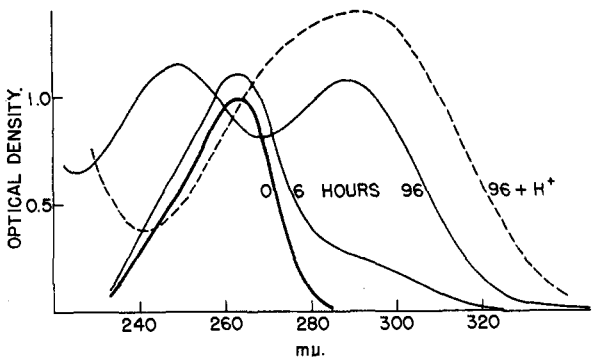



Fig. 1.—Spectral changes, with time, of 9-$\beta$-D-ribofuranosylpurine in 0.04 *N* sodium hydroxide.

materials, when eluted from paper, gave strongly positive orcinol reactions.[27] The relatively large yield of the picrate of 4,5-diaminopyrimidine and the small amount of the free base in the reaction mixture indicate that some of the ribose-containing diamino compounds were cleaved by the aqueous picric acid.

The reaction which the nucleoside undergoes in alkaline solution may be related to the Bamberger reaction,[28] or it may be viewed as a reversal of the Traube purine synthesis.[29]

The reaction is depicted as in Scheme I.

Little evidence was obtained for intermediate formyl compounds, but they are logically expected.[30] The two ribosyl-4,5-diaminopyrimidines are depicted as furanose and pyranose forms, but the possibility of $\alpha$- and $\beta$-isomers cannot be excluded.[32] Further decomposition of such products could lead to the formation of 4,5-diaminopyrimidine and its eventual decomposition products. The possibility that the two postulated ribosyl compounds could actually be Amadori-type rearrangement products was excluded by the following considerations: (a) the ready liberation of 4,5-diaminopyrimidine by picric acid, (b) the Amadori rearrangement is acid catalyzed,[33] whereas the reaction in question takes place in alkaline solution, (c) the intensities of the orcinol reactions of the glycosyl decomposition products of IV indicated the presence of *ca.* one mole of pentose per mole of 4,5-diaminopyrimidine. Products resulting from an Amadori rearrangement would be expected[34] to give very low values in a color test of this type, which depends upon a dehydration to a furfural derivative.

The ribofuranosyl derivatives of the IIa and IIb, as well as pyranosyl I (IIIb), were unstable in dilute aqueous alkali at room temperature. The

(27) Z. Dische and K. Schwartz, *Mikrochim. Acta*, **2**, 13 (1937).

(28) K. Hoffman, "Imidazole and Its Derivatives," Part I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 273.

(29) W. Traube, *Ber.*, **33**, 3035 (1900).

(30) A formyl compound has been isolated among the products resulting from the action of alkali on 9-$\beta$-D-ribofuranosylpurine-5'-phosphate.[31]

(31) D. I. Magrath and G. B. Brown, THIS JOURNAL, **79**, 3252 (1957).

(32) Interconversion reactions of $\alpha$- and $\beta$-aniline ribosyl derivatives have been described; G. A. Howard, G. W. Kenner, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 855 (1946).

(33) F. Weygand, *Ber.*, **73**, 1259 (1940).

(34) A. Abrams, P. H. Lowy and H. Borsook, THIS JOURNAL, **77**, 4794 (1955).

TABLE II

EFFECTS OF 0.04 *N* ALKALI ON THE SPECTRA OF CERTAIN PURINE NUCLEOSIDES

| Compound | Alkali[a] Max., mμ | | Alkali[a] Min., mμ | | Acid[b] Max., mμ | | Acid[b] Min., mμ | |
|---|---|---|---|---|---|---|---|---|
| 9-β-D-Ribofuranosylpurine[c,d] | 262.5 | | 230 | | 262.5 | | 230 | |
| 9-β-D-Ribofuranosylpurine, alkali treated for 76 hr. | 244 | 290 | 225 | 269 | 289 | | 242 | |
| 4,5-Diaminopyrimidine | 245.5 | 288 | 226.5 | 266.5 | 285 | | 237.5 | |
| 6-Chloro-9-β-D-ribofuranosylpurine | 264 | | 232 | | 264 | | *ca.* 227 | |
| 6-Chloro-9-β-D-ribofuranosylpurine, alk. treated for 16 hr.[d] | 254 | 290 | *ca.* 232 | 274 | 250.5 | 292 | *ca.* 230 | *ca.* 275 |
| 6-Chloro-4,5-diaminopyrimidine | 253.5 | 290.5 | 230 | 270 | 270 | 306 | 237.5 | 282 |
| | | | | | 255 | 292 | 226 | 270[e] |
| 6-Methyl-D-ribofuranosylpurine | 261 | | 227.5 | | 265 | | 235 | |
| 6-Methyl-D-ribofuranosylpurine, alkali treated for 150 hr.[d,f] | 245 | 284 | Indefinite | | Indefinite | | | |
| 4.5-Diamino-6-methylpyrimidine | 248 | 286 | 264 | | 288 | | 238 | |

[a] 0.04 *N* sodium hydroxide. [b] 0.1 *N* hydrochloric acid. [c] 9-D-Ribopyranosylpurine gave similar spectral changes upon treatment with alkali. [d] The parent purine was stable under these conditions. [e] Values in water (*p*H 5) which agree more closely with those of the ribose derivative in acid. [f] There is also a maximum of 261 mμ due to unchanged starting material. A further study of the alkali lability of this compound is being carried out by Dr. A. Hampton. Some formyl compound has been detected among the reaction products.

absorption spectra of the decomposition products again indicated that the imidazole ring had been cleaved (Table II). A possible mechanism of this cleavage is considered in an accompanying paper.[31]

Examples of the instability of the 9-methylpurine[21] and of methylated oxypurines[35] in hot alkali have been reported. The formation of the compound $C_6H_7N_4Cl$ (which could be 4-amino-2-chloro-5-methylaminopyrimidine), by the action of hot potassium hydroxide on 2-chloro-7-methylpurine, could be explained by this same type of reaction.[36] Other known instances of the rupture of the purine ring system such as the cleavage of the imidazole ring of 2-methylmercaptopurine by mono- or dimethylamine[21] and the decomposition of 6-amino-2-methylmercaptopurine by alkylamines[37] are possibly due to similar mechanisms. The alkaline disruption of the pyrimidine ring of 3,5′-cyclo-6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine-2′-3′-carbonate methanesulfonate[38] is not related. The stability[39] of most common naturally occurring purine nucleosides in dilute alkali at room temperature has been confirmed.[40]

TABLE III

ABSORPTION MAXIMA OF DERIVATIVES OF PURINE

| | Max. in acid | Max. in water | Max. in base |
|---|---|---|---|
| Purine[a] | 260 | 262.5 | 270 |
| 9-Methylpurine[a] | 262.5 | 264 | 264 |
| 7-Methylpurine[a] | 257.5 | 266.5 | 266.5 |
| 9-β-D-Ribofuranosylpurine[b] | 262.5 | 262.5 | 262.5 |
| 9-D-Ribopyranosylpurine[c] | 262.5 | 262.5 | 262.5 |

[a] Ref. 22. [b] Ref. 3. [c] See Experimental.

**Syntheses.**—Purine-8-$C^{14}$ prepared from hypoxanthine-8-$C^{14}$[10] was diluted with non-radioactive I. The 6-methyl-D-ribofuranosylpurine and the 9-D-ribopyranosylpurine (IIIb) were synthesized by the general method of Davoll and Lowy.[41] The spectra of IIIb and those of IV, 7-methylpurine and 9-methylpurine (Table III) indicate that the glycosyl substituent of IIIb is on the 9-nitrogen, in analogy to evidence used to establish the position of substitution in puromycin[42] and adenosine.[43]

## Experimental

All m.p.'s are corrected. Radioactivity determinations were carried out on infinitely thin samples plated on 10 cm.$^2$ aluminum planchets with an internal Geiger–Müller flow counter (Radiation Counter Laboratories, Mark 12, model 1, helium isobutane gas), probable error ±5%. Ultraviolet absorption spectra were determined with the Beckman model DU spectrophotometer with matched 1-cm. cells. Optical rotations were determined with a polarimeter, model D, attachment to the Beckman model DU spectrophotometer, calibrated with standard sucrose solutions.[44]

**Paper Chromatography.**—Solvent systems (descending, Schleicher and Schuell No. 597 paper) were (vol./vol.): A = glacial acetic acid 20%, *n*-butyl alcohol 50%, water 30%, B = 1% aqueous ammonium sulfate 33⅓%, isopropyl alcohol 66⅔%,[45] C = 5% aqueous monobasic sodium phosphate layered with isoamyl alcohol (ascending)[46]; D = *n*-butyl alcohol 77 parts, 88% formic acid 10 parts, water 13 parts,[47]; E = *n*-butyl alcohol saturated with water at room temperature.[48] Chromatograms were inspected under ultraviolet light and examined for the formation of mercuric sulfide precipitates.[49]

**Identification of the Volatile Acid from Purine (I).**—A 200-mg. (1.66 mmoles) sample of I was heated under reflux with 10 ml. of 25% (w./vol., *ca.* 1.3 *M*) *p*-toluenesulfonic acid in a boiling water-bath for 4 hr.[11] Then 6 ml. of *ca.* 0.8 *N* sodium hydroxide was added to partially neutralize the *p*-toluenesulfonic acid. The volatile acid was steam distilled, and 50-ml. fractions were titrated to the phenolphthalein end-point with sodium hydroxide until a constant titer was obtained. This large-scale procedure yielded 1.44 mmoles of volatile acid. The solutions of the sodium salt of

(35) E. Fischer, *Ber.*, **31**, 3266 (1898).
(36) E. Fischer, *ibid.*, **31**, 2558 (1898).
(37) G. B. Elion, W. H. Lange and G. H. Hitchings, THIS JOURNAL, **78**, 217 (1956).
(38) B. R. Baker and J. P. Joseph, *ibid.*, **77**, 15 (1955).
(39) P. A. Levene and L. W. Bass, "Nucleic Acids," The Chemical Catalog Co. (Reinhold Publ. Corp.), New York, N. Y., 1931, p. 144.
(40) A. Hampton, private communication.
(41) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).
(42) B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).
(43) J. M. Gulland and E. R. Holliday, *J. Chem. Soc.*, 765 (1936).
(44) Standard Polarimeter Co., New York, N. Y.; see A. S. Keston, Abstr. 125th Meeting, Am. Chem. Soc., Cincinnati, Ohio, 1955, p. 18-C.
(45) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).
(46) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).
(47) R. Markham and J. D. Smith, *Biochem. J.*, **45**, 294 (1949).
(48) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).
(49) E. Vischer and E. Chargaff, *ibid.*, **168**, 781 (1947).

the volatile acid were combined and concentrated *in vacuo* to 2.0 ml. One-half of the sodium formate was converted to S-benzylisothiouronium formate.[12] The m.p. was 149–149.5°, mixed with the known formate derivative 149–149.5°, mixed with reagent 122–142° (capillary). The remaining sodium formate was converted to benzimidazole,[13] m.p. 172–173°, picrate 230–232°, no depression on admixture with known samples.

**Action of Concentrated Perchloric Acid on I and the Purine Moiety of IV.**—A 9.0-mg. sample of I was heated with 1 ml. of 70% perchloric acid for 1 hr. at 100°. The solution was diluted to 500 ml. with water, and the spectra in acidic and basic solutions were found to be unchanged. The values at the maximum and at the isosbestic point, 275 mμ,[22] indicated recoveries of 97 to 98%.

A 7.0-mg. sample of IV was hydrolyzed as above. The carbonaceous precipitate, resulting from the sugar moiety, was finely ground and thoroughly extracted with hot water. Spectrophotometric assay at the maximum and at the isosbestic point indicated recoveries of the purine moiety of 100 to 103%, respectively.

**Action of Sulfuric Acid.**—Two hundred mg. (1.66 mmoles) of I was heated 4 hr. at 100° with 15.0 ml. of 1.3 *N* sulfuric acid. The solution became bright yellow in 10 minutes and later brownish. Steam distillation, as above, yielded a total of 1.04 mmoles (0.62 mole per mole of I) of volatile acid. The residue contained 1.3 moles of ammonia per mole of I and gave positive tests with alkaline phosphomolybdate, the Bratton–Marshall, and the Ehrlich reagents. The spectrum of the diluted residue, at *p*H 4, indicated the presence of *ca.* 50% of unchanged I. A shoulder at 280 mμ indicated the presence of decomposition products.

**Recovery of Formic Acid from *p*-Toluenesulfonic Acid.**—A mixture of 3.41 mmoles of formic acid in 15 ml. of 25% aqueous *p*-toluenesulfonic acid was treated as in the analysis for acetyl groups, with the recovery of 3.47 mmoles of volatile acid (101%).

**Formation of Radioactive Formic Acid from Purine-8-C$^{14}$.**

**A. Heating Period of 4 Hours.**—Purine-8-C$^{14}$, 321 mg. (2.68 mmoles, 28.0 c.p.m./μmole) was treated with *p*-toluenesulfonic acid as described above. A total of 0.87 mole of formic acid and 1.35 moles of ammonia was found per mole of I. The sodium formate obtained was converted to S-benzylisothiouronium formate, which was recrystallized from aqueous ethyl alcohol, m.p. 149–149.5°.

*Anal.* Calcd. for $C_9H_{12}N_2O_2S$ (212.3): C, 50.9; H, 5.70; N, 13.2; S, 15.1. Found: C, 51.2; H, 5.66; N, 13.6; S, 15.3; specific activity, 8.2 c.p.m./μmole.

**B. Heating Period of 12 Hours.**—A 250-mg. sample of purine-8-C$^{14}$ (2.08 mmoles, 86 c.p.m./μmole) yielded 3.72 mmoles of volatile acid. The neutralized steam distillate was concentrated and brought to exactly 5.00 ml. A 1.25-ml. (0.93 mmole) aliquot was placed in a 25-ml. flask with 102 mg. (0.93 mmole) of 4,5-diaminopyrimidine and 0.20 ml. of 5 *N* hydrochloric acid. The solution was refluxed 1 hr. at 100° and evaporated to dryness under nitrogen. The yellowish residue was heated at 210° for 5 minutes in a nitrogen atmosphere. The residue was dissolved in 15 ml. of water and 0.2 ml. of 70% perchloric acid, and excess 10% silver nitrate was added. The mixed silver chloride and silver salt of I were collected by centrifugation and washed twice with 10 ml. of water. The salts were decomposed by two hot 10-ml. portions of 1 *N* hydrochloric acid. The acidic solution of I was evaporated to dryness *in vacuo*, redissolved in water, adjusted to *p*H 7 and evaporated in a sublimation apparatus. Compound I was sublimed at 0.01 mm. pressure at a bath temperature of 120°, giving 39 mg. (0.33 mmole, 35% yield) of material melting at 213–215°, admixed with an authentic sample of I, 214–215°. The spectrum was identical with that of I except for slight end absorption. Samples of the material were purified by chromatography on Whatman No. 3 MM paper in solvent systems D and E, with good separation from trace amounts of both a slower moving yellow and a fluorescent blue impurity. The spectra of aqueous solutions of samples thus purified were identical with that published. Radioactivity measurements gave values of 29.2 and 29.6 c.p.m./μmole for materials purified in solvents D and E, respectively (an average of 34% of the specific activity of the starting material).

The dark brown residue from the steam distillation of the formic acid was neutralized with 10 *N* sodium hydroxide. A determination of ammonia[50] showed the presence of 1.93 moles of ammonia per mole of I. The residue gave a dark blue color with alkaline phosphomolybdate,[14] a pink in the Bratton–Marshall test[16] and a brownish-yellow with diazotized sulfanilic acid.[17] In the above color tests solutions of *ca.* 1 mg./ml. in 25% aqueous *p*-toluenesulfonic acid of I reacted uniformly negatively, whereas all of the tests were positive with similar solutions of 4,5-diaminopyrimidine. Glycine and purine were detected in the residue by ascending chromatograms, in which reference samples of purine and glycine and the unknowns gave identical $R_f$ values: in solvent A, 0.73, 0.37; in B, 0.71, 0.29; and in C, 0.67, 0.84, respectively.

**C. Heating Period of 24 Hours.**—A 151-mg. sample of I was heated with 60 ml. of 25% aqueous *p*-toluenesulfonic acid for 24 hr. at 100°. The mixture was analyzed for volatile acid and base as above giving 2.0 moles of volatile acid and 2.25 moles of ammonia per mole of I, respectively. It was noticed that with the large excess of *p*-toluenesulfonic acid the solution did not darken appreciably.

**Spectral Studies of Alkaline Instability.**—A solution of IV with an optical density of about 1 at 260 mμ was prepared in 0.04 *N* sodium hydroxide. The solution was kept tightly stoppered in the dark, and at appropriate intervals the spectrum was determined on aliquots against the appropriate blank (Fig. 1). After 96 hr. the spectrum of an aliquot was determined both in acid and alkaline solution. A similar procedure was used to determine the alkali instability of the compounds listed in Table II.

**Isolation of 4,5-Diaminopyrimidine from the Degradation of IV.**—A 500-mg. sample of IV was treated with 40 ml. of saturated barium hydroxide for 4 days. At this time the spectrum in alkali exhibited the two peaks characteristic of 4,5-diaminopyrimidine. The solution was neutralized with carbon dioxide, filtered, the precipitate washed and the filtrate and washings combined to give 60 ml. of a clear yellow solution. This solution gave a deep blue color when tested with alkaline phosphomolybdate. A 10-ml. portion (estimated to contain 0.33 mmole of free and combined 4,5-diaminopyrimidine) was treated with 10 ml. of saturated aqueous picric acid, and a yellow crystalline precipitate formed immediately. This was collected, recrystallized from 15 ml. of 1:1 ethyl alcohol:water to yield 51 mg. (0.15 mmole, 47%) of material, m.p. 264–266° dec.; admixture with an authentic sample[51] of 4,5-diaminopyrimidine picrate did not lower the melting point.

*Anal.* Calcd. for $C_{10}H_9O_7N_7$ (339.2): N, 28.9. Found: N, 28.8.

**Paper Chromatographic Studies.**—A 10.7-mg. sample of IV was dissolved in 1.0 ml. of saturated barium hydroxide. Aliquots withdrawn at 0, 24, 72, 96 and 120 hr. were chromatographed (ascending, solvent E) in parallel with control spots of starting material, $R_f$ 0.32, and 4,5-diaminopyrimidine, $R_f$ 0.40. Upon visual inspection under ultraviolet light, the riboside spot was dark while that of the diaminopyrimidine was a fluorescent blue. After 24 hr. some starting material remained; however, two additional fluorescent blue spots of $R_f$'s 0.084 and 0.17, respectively, had appeared. After 72 hr., these latter two spots had increased in intensity, and a small amount of material at the position of 4,5-diaminopyrimidine was evident. All three of these components showed ultraviolet absorption spectra identical to that of 4,5-diaminopyrimidine. The parent nucleoside was no longer detectable. Chromatograms at 96 and 120 hr. were similar to that after 72 hr.

Chromatograms prepared from the 96-hr. reaction mixture were examined more closely with the results shown in Table IV. If it is assumed that the molar extinction coefficients of these three components are equal, it may be concluded that about 90% of the 4,5-diaminopyrimidine was combined with a pentose moiety.

No evidence was seen for any formylated derivatives on the above chromatograms. When 0.04 *N* sodium hydroxide was used and the solution tested after 4 hr., the trailing edges of the two pentose-containing spots had a dark appearance characteristic of 5-formamido-4-aminopyrimidine; however, the separation from other components was very poor.

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TABLE IV

PROPERTIES OF THE DEGRADATION PRODUCTS

| Rf | *cis*-Glycol test[26] | Orcinol test[27] | Optical density at 288 mμ, *p*H 5.2[a] |
|---|---|---|---|
| 0.084 | + | + | 0.78 |
| .17 | + | + | .75 |
| .40 | − | − | .16 |

[a] Determined on the material eluted from one lane with 4 ml. of water. Assuming an $\epsilon_{max}$ of 7250 for the products ($\epsilon_{max}$ at 289 = 7250 for the uncharged molecule of 4,5-diaminopyrimidine[52]), the two slow moving compounds each contained *ca.* one mole of ribose per mole of 4,5-diaminopyrimidine.

**6-Methyl-D-ribofuranosylpurine.**—A 402-mg. (3 mmoles) sample of IIa was converted to its chloromercuri derivative[41] giving 0.900 g. (2.44 mmoles, 81.5%) of product. The chloromercuri-6-methylpurine was condensed with 2,3,5-tri-*O*-acetyl-D-ribosyl chloride[53] prepared from 0.945 g. (3.0 mmoles) of tetraacetylribofuranose,[54] and the product was worked up as described below, except that the intermediate triacetyl compound was not obtained in crystalline form. Two recrystallizations of the crude 6-methyl-D-ribofuranosylpurine from ethyl alcohol gave 100 mg. (0.38 mmole, 12.5%) of product in the form of fine needles, m.p. 209–210°. In another preparation, from 2.6 g. of IIa, a yield of 21% was obtained.

*Anal.* Calcd. for $C_{11}H_{14}O_4N_4$ (266.3): C, 49.6; H, 5.29; N, 21.0. Found: C, 49.7; H, 5.50; N, 21.2.

The absorption maxima were: $\epsilon_{265}$ = 6340 at *p*H 1, $\epsilon_{261}$ = 7640 at *p*H 5.5 and 11 (when determined immediately).

**9-D-Ribopyranosylpurine (IIIb).**—A 1.00-g. (8.3 mmoles) sample of I was converted to its chloromercuri derivative (2.74 g., 7.8 mmoles),[3] and the dried, powdered chloromercuri salt was condensed with 2.8 g. (9.5 mmoles) of crystalline 2,3,4-tri-*O*-acetyl-D-ribosyl chloride[55] by refluxing in xylene for 4 hr. By previously described procedures,[41] 3.19 g. of crude crystalline 9-D-triacetylribopyranosylpurine (IIIa) was obtained, which, when recrystallized from ethyl alcohol, gave 1.1 g. of product (2.9 mmoles, 35%) melting at 169–171°.

Treatment of 570 mg. of IIIa (1.45 mmoles) for 16 hr. at 5° with methanolic ammonia gave 410 mg. of IIIb. After two recrystallizations from *n*-butyl alcohol, the product, 280 mg. (1.1 mmoles), melted at 250–252° (76% yield from the acetyl derivative, 13% over-all yield from I). A sample recrystallized from ethyl alcohol was analyzed.

*Anal.* Calcd. for $C_{10}H_{12}O_4N_4$ (252.2): C, 47.5; H, 4.80; N, 22.2. Found: C, 47.6; H, 4.73; N, 21.7; $[\alpha]^{20}_{546m\mu}$ −33.8°; $[\alpha]^{20}_{589m\mu}$ −28.7° (0.5% in water).

The absorption spectrum possessed a maximum at 262.5 mμ, the position of which did not change in acid or alkali (when determined immediately). The $\epsilon_{max}$ at *p*H 0.3 was 5.65 × 10³, at *p*H 7.7 was 6.91 × 10³ and at 12.3 was 7.06 × 10³. There were two isosbestic points: one at 231.5 mμ with ε 2.64 × 10³ and one at *ca.* 268 mμ with ε 4.67 × 10³. The apparent $pK_a$ was found to be 1.80 ± 0.05 by the procedure outlined by Fox and Shugar.[56]

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[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

# Synthesis of an Isopropylidene Derivative of an Alkali-labile Nucleoside: 2′,3′-O-Isopropylidene-9-β-D-ribofuranosylpurine[1]

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2′,3′-*O*-Isopropylidene-9-β-D-ribofuranosylpurine can be prepared in good yield by condensation of 9-β-D-ribofuranosylpurine with acetone in the presence of zinc chloride or in the presence of *p*-toluenesulfonic acid. The techniques should be useful for the preparation of isopropylidene derivatives of other alkali-labile purine nucleosides. With *p*-toluenesulfonic acid the conversion is rapid and quantitative at room temperature and the method may be applicable to nucleosides in general.

2′,3′-*O*-Isopropylidene-9-β-D-ribofuranosylpurine was desired for the synthesis of 9-β-D-ribofuranosylpurine-5′-phosphate,[2] and a number of procedures for its preparation have been examined.

The 2′,3′-*O*-isopropylidene derivatives of many nucleosides[3-6] have been prepared by condensations with acetone in the presence of zinc chloride. The resulting complex of the product with zinc chloride was in each case decomposed with warm barium hydroxide. 9-β-D-Ribofuranosylpurine[7] and its 2′,3′-*O*-isopropylidene derivative were found to be alkali-labile, and application of the usual work-up led to considerable losses. Milder conditions for breakdown of the zinc chloride complex were investigated. Ion-exchange chromatography of an aqueous solution of the reaction products using Dowex-50 resin ($NH_4^+$) effected the removal of the zinc ions without decomposition of the isopropylidene derivative, but a large excess of resin was required and 60% of the product was retained on the column. A more satisfactory procedure was treatment of the reaction mixture at 0° with aqueous sodium carbonate, whereupon the 2′,3′-*O*-isopro-

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