THE PROOF OF STRUCTURE OF CERTAIN 2,6,8-SUBSTITUTED PURINES

by

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Typed by Betty Bryant
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TO MY WIFE
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THE PROOF OF STRUCTURE OF CERTAIN 2,6,8-SUBSTITUTED PURINES

INTRODUCTION

Purines are nitrogen-containing heterocycles (4, p. 253) formed by the fusion of a pyrimidine ring (I) with the imidazole ring (II) and have the following structure:

These compounds and their derivatives (2, p. 81) are of interest to the chemist because of their association and importance to all the living systems. They are even found "in the twilight zone between the living and the nonliving, the viruses" (2, p. 81). The purines and pyrimidines may be thought of as building blocks for the nucleic acids just as the amino acids are considered building blocks for proteins.

The fundamental investigations which led to the discovery of the nucleic acids were made by Miescher (1844-95) (7, pp. 1-4). In 1868 he isolated the nuclei from pus cells obtained from discarded surgical bandages and showed that the nuclear material contained an unusual phosphorus compound, which we now know was nucleoprotein. He became
interested in salmon sperm as a source of nuclear material and showed that isolated sperm heads contained an acidic compound now recognized as nucleic acid. It was subsequently shown that nucleic acids were normal constituents of all cells and tissues which were examined.

Miller and his co-workers (17, p. 1392) isolated 6-furfurylaminopurine (Kinetin) from herring sperm and found it to be a physiologically highly active chemical. These workers discovered that callus tissue when treated with Kinetin would undergo cell enlargement although with a small increase in weight. Furthermore, it was found that the tissue was incapable of cell division and continued growth unless the cell division factor was supplied. The validity of the observation has been established by cytological examinations and by determinations of small weight increases. This discovery, of the effect of Kinetin on cell division, has caused great activity in the synthesis of other substituted purines.

On the other hand, Hitchings and co-workers (15, pp. 321-327) made an extensive study of analogues of the natural purine and pyrimidine antagonists. These workers studied the mechanism of antagonistic action and the role of nucleic acid biochemistry in embryogenesis.

Howard Skipper and his associates (19, pp. 503-507) reported that the combination of empirical screening
together with screening guided by the metabolite\textsuperscript{1} antagonist theory has provided agents which temporarily inhibit certain types of animal and human cancer. Studies on the mechanism of action of these agents have suggested areas where biochemical differences between normal and neoplastic cells may exist. These workers (19, pp. 503-507) examined the feasibility of potentiating the antileukemic activity of a given agent by administration of a second chemical which might be expected, on the basis of biochemical knowledge, to provide a concurrent block\textsuperscript{2} in a series of biochemical events; for example, they showed that the antileukemic activity of A-methopterim was potentiated by simultaneous administration of ethionine.

Vanderwerff (11, pp. 505-518) tested one hundred purines representing a variety of structural changes of typical purine bases for their effects on the growth of lactobacillus casei in a variety of media.

Certain structural changes such as (1) the introduction of an 8-hydroxyl or 8-chloro group (2) methylation of

\footnotesize

\textsuperscript{1}Metabolite is considered here to be any natural substance involved in a biochemical reaction of a cell (1, p. 43).

\textsuperscript{2}Concurrent block is a term suggested by Elion to describe simultaneous blockade of two or more pathways concerned with the formation of the same end product (12, p. 487).
the imidazole nucleus (3) alkylation of the amino groups of the aminopurines result in a diminution of the growth promoting activity of the parent compound (11, pp. 505-518).

The replacement of the hydroxyl group of guanine and hypoxanthine by the mercapto group results in inhibitory substances (11, pp. 505-518).

The 8-arylpurines, although moderate to strong inhibitors, do not clearly fall in the class of purine antagonists. This conclusion is drawn from the failure of adenine or folic acid to reverse their toxic effects over more than a very narrow range of concentration of the inhibitor, and the relatively negligible effect of the nature of the substituents of the pyrimidine ring on their activities (11, pp. 505-518).

Three of the known substances of the purine group that enter into the structure of the nucleic acids are adenine, hypoxanthine, and guanine (16, pp. 75-94). Uric acid, because of its ease of isolation, was the first purine derivative that came to the notice of chemists. This substance was discovered by Karl Wilhelm Scheele in 1776 who described the solubilities of the compound in alkalies and its behavior toward mineral acids.

Fischer (16, pp. 75-94) succeeded in preparing trichloropurine by heating uric acid with phosphorous
oxychloride at 150° C. to 160° C. This reaction was important because of its significant bearing on the theory of structure of uric acid and the establishment of the relationship with the purine bases. This Fischer procedure consisted of two steps and gave very poor yields.

In 1951 J. Davoll and his associates (8, p. 2936) improved the method as well as the yield by reacting uric acid directly with phosphorous oxychloride and dimethyl-aniline in a one step operation to give 2,6,8-trichloropurine. The trichloropurine used in these studies was prepared by this method.

Fischer (13, pp. 2220-2254) used 2,6,8-trichloropurine as an intermediate to successfully synthesize several new purines. Furthermore, by varying the temperature he showed that it was possible to replace one or two of the chlorines with ethoxy groups thus establishing the differences in activities of the chlorine substituents. For example, trichloropurine was reacted with sodium ethoxide first at room temperature and then at 130° C. to obtain 2,8-dichloro-6-ethoxypurine and 8-chloro-2,6-diethoxypurine, respectively.

More recently Breshears (3), of this laboratory, used trichloropurine as an intermediate for the synthesis of some monosubstituted-dichloropurines and trisubstituted purines by aminolytic action. Two compounds which he
prepared were dichloro-?-fururylaminopurine and dichloro-?-dimethylaminopurine. The location of substitution was assumed to be the 6-position in the case where only one chlorine was replaced, as judged by the work of Fischer (13, pp. 2220-2254) and Roberts (18, pp. 3621-3627). This assumption, however, has not been proved.

Wang (21) recently prepared dichloro-?-morpholinopurine from trichloropurine and assumed the 6-position to be the one substituted. In view of the uncertainty of the position of the chloro substituents the problem of establishing the structure of these three compounds was undertaken.

Fischer (16, p. 116) prepared adenine by reducing 6-amine-2,8-dichloropurine with hydriodic acid (sp. gr. 1.96) (9, pp. 2389-2391) and phosphonium iodide (22, pp. 1141-1144) in almost theoretical yield. Furfurylaminodichloropurine, dimethylaminodichloropurine and morpholinodichloropurine were reduced using the method as described by Fischer (16, p. 116). The dimethylaminopurine was converted to its hydrochloride for analysis.

The melting points and ultra-violet data of the dehalogenated furfurylaminoo-?-dichloropurine checked identically with 6-furfurylaminopurine prepared by Bullock and his co-workers (5, pp. 3693-3696). These workers refluxed
6-chloropurine with furfurylamine in methyl cellosolve for two hours to yield a product consisting of white plates, yield 91%.

The dehalogenated dimethylamino-?-dichloropurine gave a product whose melting point and ultra-violet data checked identically with 6-dimethylaminopurine prepared by Elion and her associates (10, pp. 411-414). These workers heated 6-methylmercaptopurine with dimethylamine hydrochloride in a sealed tube for 15 hours at 130° C. to effect amination. The compound was best purified for analysis by conversion to its hydrochloride in absolute ethanol and precipitation with ether.

The physical constants of the dehalogenated morpholine-?-dichloropurine checked identically with 6-morpholinopurine prepared by Daly of this laboratory (6, pp. 177-179). He refluxed 6-chloropurine with morpholine in n-butanol for 2 hours to obtain the product in 63% yield.

All these results confirmed the earlier observations that the 6-position on trichloropurine was the position substituted.

Knowing the position of the non-halogen substituents it was then possible to prepare purine derivatives with mixed substituents for test purposes, without the problem of establishing structures. For this reason 6-morpholine-2,8-dichloropurine (21) was used as the starting material. Piperidine and 6-morpholinodichloropurine were
placed in a small Parr peroxide bomb and heated in an oven at 175° C. The product isolated from the fusion mixture was 6-morpholino-2,8-dipiperidinopurine.

6-Morpholino-2,8-dihydrazinopurine was prepared readily by amination of 6-morpholino-2,8-dichloropurine by merely refluxing the compound with hydrazine hydrate.

Reaction of 6-morpholinodichloropurine with furfurylamine at reflux temperatures yielded 6-morpholino-2,8-difurfurylaminopurine. The reaction mixture was poured into water to extract the excess furfurylamine. The compound was precipitated as a monohydrate.

6-Morpholino-2,8-di-n-hexylaminopurine, on the other hand, was prepared by heating n-hexylamine with 6-morpholinodichloropurine in a Parr bomb at 180° C. for 12 hours. The mixture was steam distilled to remove the excess n-hexylamine. The product isolated was found to be the monohydrate.

Studies were made on the reduction of trichloropurine in an attempt to dehalogenate it. Fischer (14, pp. 2550-2574) first made an attempt to chemically reduce trichloropurine by reacting it with hydriodic acid only to get 2,6-diiodopurine as the product. The chemical reduction of Fischer was repeated in this laboratory with similar results.

Attention in the laboratory was then directed toward catalytic reduction. Breshears (3, p. 14) used glacial
acetic acid as a solvent and palladized charcoal to reduce trichloropurine to tetrahydropurine. The reduction was repeated and the results were confirmed.

Smith (20, pp. 829-838) reduced chloropyrimidines with 10% palladium by using a two phase ether-sodium hydroxide media; attempts were made in this laboratory to reduce trichloropurine in the same media but without success.
EXPERIMENTAL

6-Furfurylaminopurine: Two hundred mg. of pulverized furfurylaminodichloropurine were added to 2 g. of hydriodic acid (Sp. gr. 1.96) (7, pp. 2389-2391). The mixture became warm with a considerable portion of the compound going into solution; the reaction was evident by the strong brown color in the liquid. Pulverized phosphonium iodide (17, pp. 141-144) in excess was added and the mixture stirred for two hours at room temperature. The reduction was nearly complete by this time as judged by the disappearance of the solid phase. The mixture was then heated to boiling until a clear, almost colorless solution was obtained; if separation of iodine occurs, further addition of phosphonium iodide was necessary. The colorless solution was evaporated under vacuum to almost dryness. The free base was precipitated from a warm water solution by the addition of 28% ammonium hydroxide. The tan precipitate was recrystallized from 95% alcohol, yield 20.1 mg. (13.3%), m. p. 269°-271° C.

6-Dimethylaminopurine: 6-Dimethylamino-2,8-dichloropurine was reduced with hydriodic acid to 6-dimethylamino- purine by the same procedure. Two hundred mg. of pulverized dimethylaminodichloropurine were added to 2 g. of hydriodic acid (Sp. gr. 1.96). The mixture again became warm with a considerable portion of the compound going into the solution;
the reaction was evident by the strong brown color in the liquid. Pulverized phosphonium iodide in excess was then added and the mixture was stirred for two hours at room temperature. The reduction was nearly complete by this time. The mixture was then heated to boiling until a clear, almost colorless solution was obtained. The colorless solution was evaporated under vacuum to almost dryness. The free base was prepared by the addition of 28% ammonium hydroxide. Since dimethylaminopurine was soluble in water the solution was allowed to evaporate to dryness at room temperature. The residue was purified by sublimation, giving a white fluffy material. The compound was best purified for analysis by conversion to its hydrochloride in absolute ethanol and precipitated with ether, yield 99.6 mg. (71%) m. p. 248.5°-250° C.

6-Morpholinodichloropurine: 6-Morpholino-2,8-dichloropurine was reduced with hydriodic acid to 6-morpholinopurine. Two hundred mg. of pulverized morpholinodichloropurine were added to 2 g. of hydriodic acid (Sp. gr. 1.96). The mixture as usual became warm and a considerable part of the compound went into solution. Pulverized phosphonium iodide in excess was added and the mixture was shaken for two hours at room temperature. The mixture was then heated to boiling until a clear, almost colorless solution was obtained. The colorless solution was evaporated under vacuum to almost
dryness. The free base was precipitated from a warm water solution by the addition of 28% ammonium hydroxide. The white precipitate was recrystallized from 50% alcohol, yield 124 mg. (83%) m. p. 299°-300.5° C.
<table>
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<tr>
<th>NAME OF</th>
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<th>PH</th>
<th>MAX E</th>
<th>MIN E</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Furfurylaminopurine</td>
<td>C_{10}H_{9}N_{5}O</td>
<td>1.0</td>
<td>274</td>
<td>15,930</td>
</tr>
<tr>
<td>6-Dimethylaminopurine</td>
<td>C_{7}H_{9}N_{5}</td>
<td>1.0</td>
<td>277</td>
<td>14,710</td>
</tr>
<tr>
<td>6-Morpholinopurine</td>
<td>C_{9}H_{11}N_{5}O</td>
<td>distilled water</td>
<td>282</td>
<td>17,740</td>
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6-Morpholino-2,8-dipiperidinopurine: 6-morpholino-2,8-dichloropurine (2 g.) and 10 ml. of piperidine were put into a Parr bomb (20 ml. capacity) and heated in an oven at 175° C. for 20 hours. The reaction product was then dissolved in a minimum amount of absolute alcohol and then 100 ml. of water added. The solution was neutralized with acetic acid and then filtered, to remove insoluble matter. The compound was precipitated by the addition of ammonium hydroxide to the filtrate. The product, after filtering, was immediately dissolved in hot 95% ethanol and precipitated by diluting with water, yielding a blue colored material, yield 2.3 g. (85.3%) m. p. gradual decomposition above 117° C.

**Anal.** Calc'd. for C_{19}H_{29}N_{7}O: C, 61.4; H, 7.84.
Found: C, 61.2; H, 7.81.

6-Morpholino-2,8-dihydrazinopurine: 6-morpholino-2,8-dichloropurine (2 g.) and 8 ml. of 100% hydrazine hydrate were refluxed together for two hours. The solution was diluted with 50 ml. of water and cooled overnight. A yellow tan precipitate formed which was filtered and washed. The material was resuspended in water and washed, filtered and dried, yield 1.7 g. (88%) m. p. gradual decomposition above 172° C.

**Anal.** Calc'd for C_{9}H_{15}N_{9}O: C, 40.75; H, 5.67.
Found: C, 40.7; H, 5.67.
6-Morpholino-2,8-difurfurylaminopurine: 6-morpholino-2,8-dichloropurine (2g.) and 15 ml. of furfurylamine were refluxed together for 6 hours. The solution was then diluted with 100 ml. of water and then neutralized with acetic acid. A precipitate formed which was filtered and washed with water. The grey colored product was immediately recrystallized from 95% ethanol to give a light yellow amorphous substance, yield 1.5 g. (52%) m. p. gradual decomposition above 137° C.

**Anal.** Calc'd for C\(_{19}H_{21}N_{7}O_{3}\cdot H_{2}O\): C, 55.2; H, 5.57. Found: C, 55.2; H, 5.44.

6-Morpholino-2,8-di-n-hexylaminopurine: 6-Morpholino-2,8-dichloropurine (2g.) and 12 ml. of n-hexylamine were put into a Parr bomb (20 ml. capacity) and heated in an oven at 180° C. for 12 hours. The reaction mixture was then steam distilled until all the excess n-hexylamine was removed. The resulting mixture was cooled and the water decanted. The residue was recrystallized twice from absolute ethanol. The product was washed each time with cold ethanol, yield 2.1 g. (69%), m. p. decomposed above 216° C.

**Anal.** Calc'd for C\(_{21}H_{37}N_{7}O_{2}\cdot H_{2}O\): C, 59.8; H, 9.26. Found: C, 59.7; H, 9.31.
<table>
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<tr>
<th>NAME OF COMPOUND</th>
<th>EMPIRICAL FORMULA</th>
<th>PH</th>
<th>MAX E</th>
<th>MIN E</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Morpholino-2,8-dipiperidinopurine</td>
<td>C₁₉H₂₉N₇O</td>
<td>1.0</td>
<td>226</td>
<td>23,540</td>
</tr>
<tr>
<td>6-Morpholino-2,8-dihydrazinopurine</td>
<td>C₉H₁₅N₉</td>
<td>1.0</td>
<td>295</td>
<td>17,220</td>
</tr>
<tr>
<td>6-Morpholino-2,8-difurfurylaminopurine</td>
<td>C₁₉H₂₁N₇O₃·H₂O</td>
<td>95%</td>
<td>233</td>
<td>28,870</td>
</tr>
<tr>
<td>6-Morpholino-2,8-din-hexylaminopurine</td>
<td>C₂₁H₃₇N₇O₂·H₂O</td>
<td>95%</td>
<td>235</td>
<td>24,900</td>
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TABLE II
ULTRA-VIOLET DATA
2,6-Diodopurine: One g. of trichloropurine was added to 10 g. of hydriodic acid (Sp. gr. 1.96) (7, pp. 2389-2391). The reaction was evident by the strong brown color in the liquid. Pulverized phosphonium iodide (17, pp. 141-144) in excess was added and the mixture stirred intermittently for 8 hours at 0° C. The mixture was cooled in the refrigerator for 12 hours and then added to 20 ml. of ice cold water during which an orange colored precipitate formed. The product was filtered and washed with cold water. Four ml. of hot water were added to the compound and then 28% ammonium hydroxide until complete solution was obtained. The solution was treated with norite and filtered while still hot. The filtrate was cooled and colorless crystals of the ammonium salt precipitated which were filtered and washed. The compound was dissolved in dilute ammonium hydroxide and 6 N. sulfuric acid was added giving a colorless crystalline powder (11, pp. 2550-2574), yield 290 mg. (18%), m. p. decomposes above 224° C.

 Anal. Calc'd. for C_{5}H_{2}N_{4}I_{2}: C, 16.1; H, 0.54.
Found: C, 16.3; H, 0.71.

Tetrahydropurine dihydrochloride: 2,6,8-Trichloropurine (2 g. 0.0085 mole), 1 g. 10% palladium on charcoal, 75 ml. glacial acetic acid and just enough water to wet the palladium catalyst were mixed together in a low
pressure hydrogenation bottle and reacted at 42 pounds of hydrogen for 24 hours. A hydrogen uptake of 0.04 mole was noted at this time. The reaction mixture was filtered and evaporated to 3 ml. and the tetrahydrogen dithiochloride was precipitated by the addition of 50 ml. of dry ether. The compound was filtered and dried (3, p. 14), yield 1g. (53%), m.p. gradual decomposition above 160° C.

**Anal.** Calc'd. for C₅H₁₀N₄Cl₂: C, 30.4; H, 5.08.

Found: C, 30.7; H, 4.91.
SUMMARY

It was shown that the 6 position in trichloropurine was the most reactive by the reduction of 6-aminodichloro-purines with hydriodic acid (Sp. gr. 1.96) to 6-aminopurines. The ultra-violet studies were compared with the corresponding 6-aminopurines found in the literature which had been prepared from 6-chloropurine and 6-methylmercapto-purine. The results were identical giving a proof of structure of 6-aminopurines.

The use of 6-morpholino-2,8-dichloropurine prepared from 2,6,8-trichloropurine for the synthesis of some mixed amino trisubstituted purines by amination was shown here. It was shown that temperatures greater than reflux temperatures were needed to prepare 6-morpholino-2,8-dipiperidino-purine and 6-morpholino-2,8-di-n-hexylaminopurine. Steric hindrance and decreased reactivity were factors contributing to the use of higher temperatures.

It was shown that trichloropurine gave 2,6-diiodopurine when reduced chemically with hydriodic acid; but when trichloropurine was reduced catalytically with palladized charcoal it was dehalogenated, but not without an accompanying nuclear reduction.

A total of five compounds were prepared and submitted for testing.


