INFRARED SPECTRUM OF PURINE AND CERTAIN SUBSTITUTED PURINE DERIVATIVES

by

CHARLES HAINES WILLITS

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1956

APPROVED:

Redacted for Privacy

Professor of Chemistry

In Charge of Major

Redacted for Privacy

Chairman of Chemistry Department

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 23, 1955

Typed by Mary Willits

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Bert E. Christensen for his patient guidance of this work. He also wishes to thank Dr. J. C. Decius for his assistance in obtaining and interpreting the infrared spectra.

DEDICATION

To Mary

TABLE OF CONTENTS

INFRARED SPECTRUM OF PURINE AND CERTAIN SUBSTITUTED PURINE DERIVATIVES

INTRODUCTION

Among chemists Karl Wilhelm Scheele is probably best known for his discovery of oxygen and chlorine. But to those active in the study of pyrimidine and purine chemistry he is known for his discovery, in 1776, of the purine derivative uric acid. It was this discovery by Scheele and by Bergmann, before the dawn of organic chemistry as an organized discipline, that set the stage for the studies begun by Liebig and Wohler in 1834 and continued by Baeyer in 1863. The work of Liebig, Wohler and Baeyer led to the establishment of the cyclic structure of uric acid and guanine by Mendicus in 1875 and of the pyrimidine nucleus by Pinner in 1884.

The torch of research was picked up by Emil Fischer whose monumental work in this field in the years 1882 to 1907 included the characterization and synthesis of the pyrimidines and purines obtainable from natural sources as well as the synthesis of many not found in nature.

The investigation of the chemistry of pyrimidines and purines is of special interest to man because compounds with these structures are found closely associated with all living matter. It was only natural that the study of these compounds turn to an investigation of their synthesis in

nature and their role in the biosynthesis of derivatives of nucleic acids. Although this is primarily work for the biochemist, the organic chemist has contributed to this investigation by studying the relationship between chemical structure and the role that pyrimidines and purines play in growth.

This work was initiated by Hitchings (19, pp.1318-1334) in 1942 and resulted in the discovery that 2,6-diaminopurine had biological activity as an antagonist. With the discovery of one such compound there began a search for others of closely related structure which might also be biologically active. In 1953 Burchenal (12, pp.7-8) reported that 6-mercaptopurine showed promise in clinical trials on cancer patients. This discovery has stimulated even greater interest in the field of purine chemistry.

During World War II there was also considerable interest in pyrimidine derivatives as anti-malarial compounds. A few were found to have marked activity but the primary value of this work was the publication of data concerning the chemical characteristics of pyrimidine compounds. This work coupled with that in the purine field has made available a large number of pyrimidine and purine derivatives for further study.

While the work of Liebig, Wohler and Baeyer established the cyclic structure of these compounds, those who

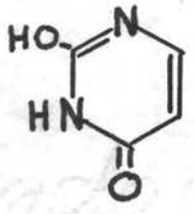
have worked in this field have found that the chemical reactivity of pyrimidines and purines is quite different from that of analogous benzene derivatives. Lythgoe (21, pp.181-207), in a review of the chemistry of pyrimidines and purines, noted that the chemical activity of the pyrimidine nucleus was more like that of pyridine or nitrobenzene than like benzene. He attributed this to the electro-negativity of the ring nitrogen atoms in the pyrimidine nucleus.

Of particular note are the increase in activity of the chloropyrimidines (21, p.199) and the decrease in activity of the amino- and hydroxypyrimidines (21, pp.200-201) compared with that of their respective benzene analogs. This is particularly evident when the functional group is in position 2, 4 or 6. Such functional groups in the 5 position are more nearly like their benzene counterparts.

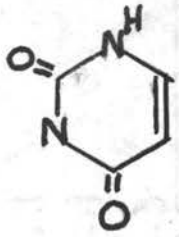
The activity of the chloropyrimidines has been accounted for by the electro-negativity of the ring nitrogen atoms (21, p.195). These produce a definite electron deficiency at positions 2, 4 and 6, and by way of induction a lesser deficiency at position 5. This makes replacement of the chlorine in substitution reactions easier. The same explanation does not account for the difficulty experienced in replacing hydroxy and amino hydrogen atoms in the types of reactions common to aromatic amines and phenols (21, pp.200-201). Electron deficiency at positions 2, 4 and 6

should increase the phenolic properties of the hydroxyl as in o-nitrophenol. These anomalies have caused considerable speculation about the actual structure of the hydroxy- and aminopyrimidines and it has been suggested (21, p.201) that they actually exist as the tautomeric keto- and imino-forms.

These theories have stimulated considerable interest in studies designed to determine more definitely the structure of various pyrimidine and purine compounds. Two of the methods which have proven most useful in this work are ultraviolet and infrared spectroscopy. Austin (3, pp.2141-2143) studied the ultraviolet spectra of uracil and methylated uracils and concluded that uracil assumed structure I in alcoholic solution. More recently Marshall and



I



II

Walker (23, p.1004) have used ultraviolet spectral data to demonstrate rather conclusively that uracil assumes structure II.

Further contributions to an understanding of the structure of these pyrimidine compounds have been made by the extensive infrared studies conducted by a number of

workers (11, pp.3062-3072; 31, pp.232-235; 2, pp.2911-2915; 29, pp.168-187; 18, pp.2939-2940; and 30, pp.274-281). From this work, Short and Thompson (29, p.180) conclude that the 2- and 4-hydroxypyrimidines and the 2,4-dihydroxypyrimidines probably exist in the ketonic form. The 4,6-dihydroxypyrimidines may have one -OH and one =O.

Ultraviolet spectral evidence for the structure of the aminopyrimidines is not nearly as conclusive, primarily because the 1- and 3-methylpyrimidines were not available. Marshall and Walker (23, p.1004) state that evidence indicates that 2-aminopyrimidines exist in the amino form, while amino groups in positions 4 or 6 exist in the imino form. A year later Short and Thompson (29, p.180) examined the infrared spectra of a number of substituted pyrimidines and concluded that the amino groups attached to the pyrimidine ring in positions 2, 4 and 6 all exist in the amino form. However, they point out that the $-NH_2$ groups have the absorption characteristics of an amide rather than those of an amine.

While the structures of the mono-amino pyrimidines have thus been fairly definitely worked out, the chemical reactivity of the 2-, 4- and 5-amino compounds is not as clear-cut. Some reactions have been run and conclusions have been drawn from these and from analogies to pyridine and nitrobenzene chemistry. The purpose of the work in this laboratory was to investigate the chemical

characteristics of these monoaminopyrimidines and to then extend the study into the field of aminopurine compounds by way of infrared spectroscopy.

The chemical reactivity of pyrimidine and substituted pyrimidines indicates that the electron density of the pyrimidine ring has been greatly reduced by the presence of two electro-negative nitrogen atoms in the ring. This results in a definite decrease in the ease of electrophilic substitution in the ring. Thus the chemistry of pyrimidine compounds more nearly parallels that of analagous pyridine compounds than it does that of similar benzene derivatives (21, pp.195-197).

In the case of the monoaminopyrimidines this decrease in electron density has resulted not only in a decrease in the activity of the pyrimidine ring toward electrophilic substitution, but also a decrease in the basicity of the amino groups attached to the ring. However, this decrease in reactivity is not particularly evident in the reactions of the three aminopyrimidines with vigorous reagents such as acetic anhydride or the sulfonyl chlorides. Whittaker (34, p.1566) has acylated 5-aminopyrimidine; Wheeler (33, p.291) has acylated 4-aminopyrimidine; and Phillips and Mentha (25, p.6202) have acylated 2-aminopyrimidine. In each case the reaction was carried out by heating the aminopyrimidine with acetic anhydride. While the same conditions were applied to all three of the

monoaminopyrimidines, one notes that the conditions were more vigorous than those needed to acylate aniline. Roblin, Winnek and English (27, p.570) prepared the sulfanilamide derivatives of the three monoaminopyrimidines using acetyl sulfanilyl chloride as the reagent.

Another fairly vigorous reagent is methyl iodide. Using this reagent, Overberger and Kogon (24, p.1067) were able to methylate 2-aminopyrimidine on the amino nitrogen. When Whittaker (34, p.1569) treated 5-aminopyrimidine with methyl iodide, he obtained 5-amino-1-methylpyrimidinium iodide, the methylation taking place on the ring and forming a quarternary salt. This reaction is a further indication of the electro-negativity of the ring-nitrogen atoms and of the drain of electrons from amino substituents on the pyrimidine ring.

The decrease in activity of the three aminopyrimidines becomes evident when milder reagents are used. Whittaker (34, p.1566) attempted to couple 5-aminopyrimidine with nitrosobenzene, a reagent which reacts quite readily with aniline to form azobenzene. Only when he heated the reaction in glacial acetic acid could he form the corresponding 5-phenylazopyrimidine. Similarly vigorous conditions were needed to form 5-p-nitrobenzylideneaminopyrimidine from p-nitrobenzaldehyde, a reaction that occurs quite easily with aniline. Diazotization, another common reaction with aromatic amines, does not give a stable diazonium

salt with 5-aminopyrimidine. Whittaker (34, p.1569), in a carefully controlled system, showed that the diazonium salt, if it acually formed, decomposed at once to release 77% of the amino nitrogen.

Further reactions with mild reagents have been attempted in this laboratory. 4-Chloroquinazoline, a reagent which is known to react with various aliphatic aromatic and heterocyclic amines (13, pp.1306-1308), gave uncharacterizable products with 5-aminopyrimidine and did not appear to react with the 2-amino-4-methylpyrimidine. No 5-(4-quinazolylamino)-pyrimidine was isolated, although the reaction was accompanied by formation of considerable highly-colored insoluble material. Whittaker (34, p.1566) attributes such dark material to oxidation of the starting material in the presence of mineral acid. This discoloration took place to some extent even when pyridine was added to absorb the hydrogen chloride formed in the reaction. The reaction product from 4-aminopyrimidine and 4-chloroquinazoline appeared almost entirely, as judged by carbon and hydrogen analysis to be the 4-aminopyrimidine.

In addition to undergoing diazotization, aromatic amines can be coupled with other diazonium salts to form either a diazoamino compound or an ortho- or para-azoderivative of aniline. While the monoaminopyrimidines do not appear to form stable diazonium salts, they will couple in cold solution with p-chlorodiazonium chloride.

The products in the case of 2-amino-4-methylpyrimidine[1] and 4-aminopyrimidine are probably 2-amino-5-(4'-chlorophenylazo)-4-methylpyrimidine[2] and 4-amino-5-(4'-chlorophenylazopyrimidine. Lithgoe, Todd and Topham (22, p.315) found that the 2,4- and 4,6-diaminopyrimidines coupled with p-nitro- and p-chlorobenzenediazonium chlorides to form the corresponding 5-phenylazopyrimidine derivatives. In the case of the 5-aminopyrimidine the product is probably the 5-(4'-chlorophenyldiazoamino)-pyrimidine.

Aromatic amines react quite readily with potassium cyanate in acid solution to give the corresponding phenyl ureas (20, p.8). Similar reactions tried with 5-aminopyrimidine and 2-amino-4-methylpyrimidine give no isolable pyrimidyl urea. In similar reactions with cyanamide in alcoholic solution the starting material was recovered and cyanoguanidine, the dimer of cyanamide, was isolated.

Fischer, Neumann and Roch (17, p.758) found that 5-uriedouracil could be made in 60% yield by fusing uracil and urea. This reaction was tried on 2-amino-4-methylpyrimidine but no 4-methyl-2-uriedopyrimidine could be isolated. A similar reaction with 4-aminouracil gave a

---

[1]2-Amino-4-methylpyrimidine was used because it was readily available. The methyl group should contribute very little to the activity of the amino group in position 2 (23, p.1006).

[2]The product, however, could not be purified sufficiently to obtain satisfactory analytical data.

63.4% yield of the corresponding 4-uriedouracil. Other work reviewed by Lythgoe (21, pp.194-207) indicates that the presence of the hydroxyl groups on the pyrimidine ring has considerable activating effect on the amino group. The pyrimidines with a single amino group in position 2, 4 or 5 would be expected to be less active than 4-aminouracil.

In one further reaction diazomethane was used in an effort to methylate the amino groups in positions 2, 4 and 5. This reagent has been used to effect methylation of uracil (10, p.215) and of 9-methylxanthine (4, p.232). The respective aminopyrimidines were treated with a solution of diazomethane in ether but there was no reaction even when methanol was added as a catalyst. In each case the starting material was recovered.

In general the mild or weakly electrophilic reagents have not found the electron density on the amino nitrogen atoms of the aminopyrimidines high enough to effect the sort of reactions which aromatic amines normally undergo. While the electro-negative nitrogen atoms cause an overall lowering of the activity of the amino groups on the pyrimidine ring, they do not affect all amino groups to the same extent. The amino substituent in position 5 can be made to undergo a number of the reactions common to aromatic amines if the severity of the conditions is increased. The amino group in position 4 requires either more severe conditions or a more vigorous reagent and the amino group in

position 2 requires the use of the strongest reagents available.

The chemical reactivity of the aminopyrimidines is in good agreement with the infrared data (29, p.180) which indicated that the 2-, 4- and 5-aminopyrimidines all exist in the amino form in the solid state but that the 2-aminopyrimidine has more the character of an amide, and that the 4-aminopyrimidine has some of the same characteristics but to a lesser extent.

An investigation of the infrared spectra of 2- and 6-aminopurine was undertaken to determine whether these compounds had the same general structural characteristics as the analogous pyrimidines. The infrared spectra of seven purines have been published by Blout and Fields (6, pp.479-484). These compounds, guanine, adenine, xanthine, hypoxanthine, theophylline, theobromine and caffeine, are all obtained from natural sources. Adenine, 6-aminopurine, is one of the aminopurines whose infrared spectrum was of particular interest in this study. However, three of these compounds, theobromine, theophylline and caffeine, are N-methylated xanthine derivatives. Thus there were only four types of purine compounds whose spectra were known. In order to assign infrared absorption frequencies to particular types of structure or particular functional groups, broader knowledge of the infrared spectra of purine

compounds was needed. Accordingly the infrared spectra of eleven additional compounds including the purine-free-base were determined. These spectra are reproduced as infrared spectral curves in Figures 1 to 4. In addition the observed peaks are recorded in Table I.

The study of the infrared spectrum of purine-free base itself should be most fruitful in the search for absorptions characteristic of this ring system. The infrared spectra are composed of vibrations due to the component parts of the molecule as well as to those of the molecule as a whole; many of the latter are to be found in the region below 1350 cm.$^{-1}$.

Examination of the data in Table I indicates several regions of intense absorption which will be designated as group I (2500-3500 cm.$^{-1}$), group II (1550-1700 cm.$^{-1}$), group III (1000-1550 cm.$^{-1}$) and group IV (below 1000 cm.$^{-1}$) for purposes of discussion.

The region in which group I lies, 2500-3500 cm.$^{-1}$, contains the hydrogen stretching vibrations along with possible overtones and combinations of lower frequencies. The most conspicuous feature of this region is the absence of strong absorption peaks above 3000 cm.$^{-1}$ in purine itself. When an amino group is substituted in position 2 or 6, well-resolved peaks appear between 3100 and 3400 cm.$^{-1}$. In all the compounds (with the possible exception of 2-N-diethylaminopurine) there is a broad absorption peak

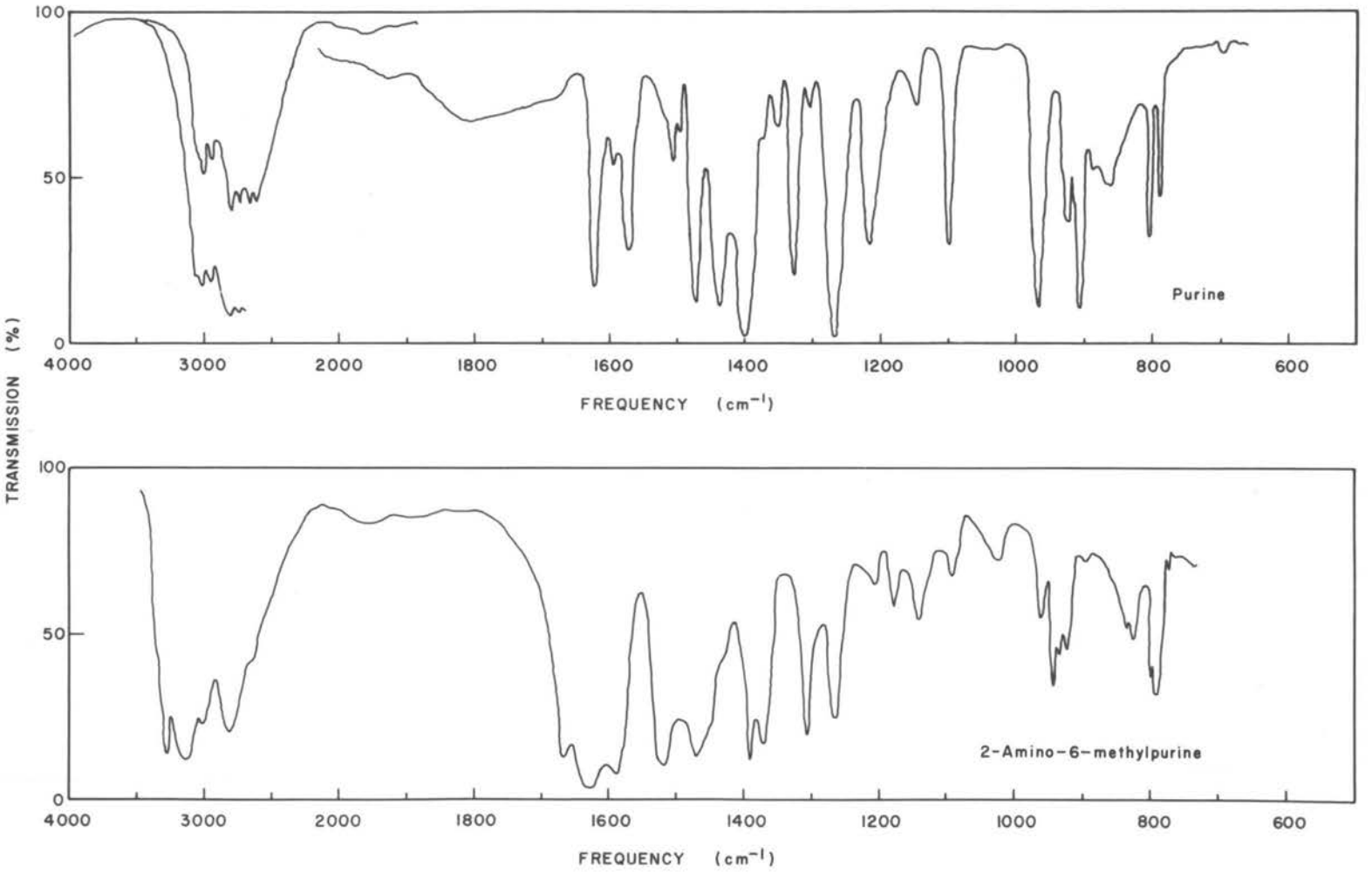

100
50
0
TRANSMISSION (%)
4000
3000
2000
1800
1600
1400
1200
1000
800
600
FREQUENCY (cm⁻¹)
Purine
2-Amino-6-methylpurine

FIGURE 1

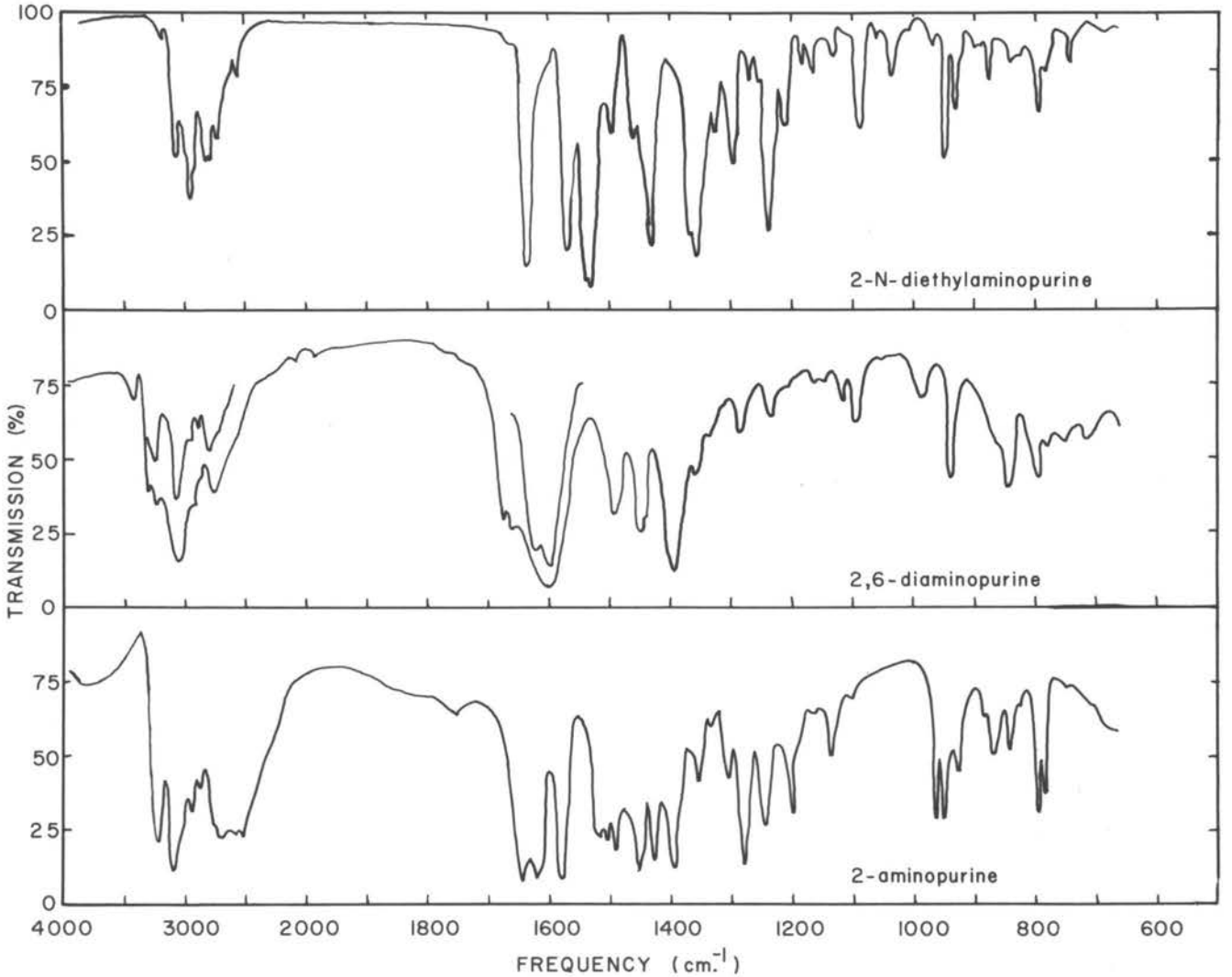

100
75
50
25
0
2-N-diethylaminopurine
75
50
25
0
2,6-diaminopurine
TRANSMISSION (%)
75
50
25
0
2-aminopurine
4000
3000
2000
1800
1600
1400
1200
1000
800
600
FREQUENCY (cm.-1)

FIGURE 2

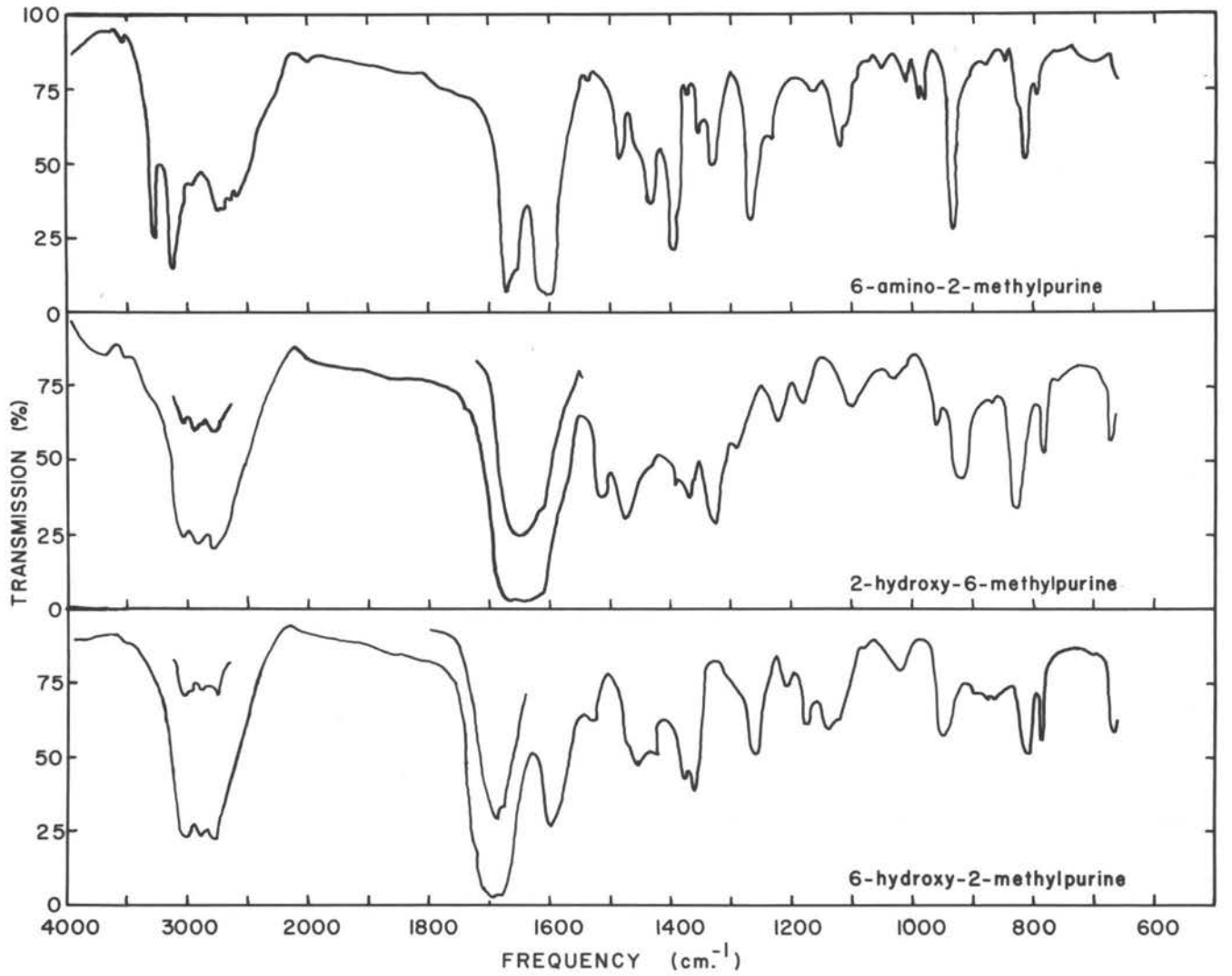

100
75
50
25
0
6-amino-2-methylpurine
2-hydroxy-6-methylpurine
6-hydroxy-2-methylpurine
TRANSMISSION (%)
4000
3000
2000
1800
1600
1400
1200
1000
800
600
FREQUENCY (cm.-1)

FIGURE 3

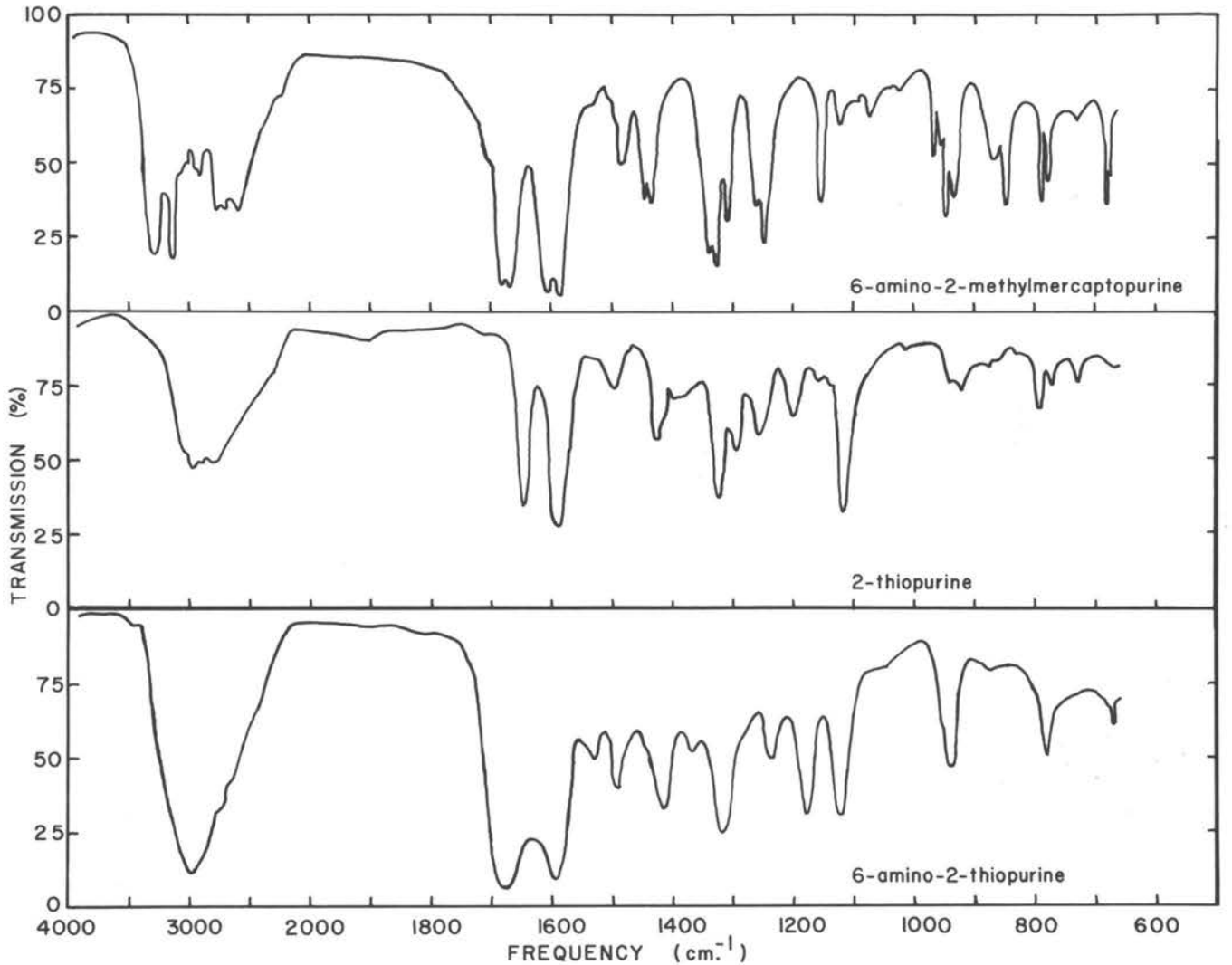

100
75
50
25
0
6-amino-2-methylmercaptopurine
2-thiopurine
6-amino-2-thiopurine
TRANSMISSION (%)
4000
3000
2000
1800
1600
1400
1200
1000
800
600
FREQUENCY (cm.⁻¹)

FIGURE 4

TABLE I

Infrared Absorption Spectra in Cm.$^{-1}$ of Purine Series

w, weak; m, medium; s, strong; vs, very strong; 1, Purine; 2, 2-N-Diethylaminopurine; 3, 2-Aminopurine; 4, 2,6-Diaminopurine; 5, 2-Amino-6-methylpurine; 6, 2-Methyl-6-aminopurine; 7, 2-Hydroxy-6-methylpurine; 8, 6-Hydroxy-2-methylpurine; 9, 6-Amino-2-methylmercaptopurine; 10, 2-Thiopurine; 11, 6-Amino-2-thiopurine.

1	2	3	4	5	6	7	8	9	10	11
3000vs	3076m	3240vs	3434m	3272vs	3271vs	3040vs	3040vs	3295vs	2974s	2987vs
2948vs	2953s	3110vs	3240s	3137vs	3120vs	2922vs	2885vs	3146vs	2905s	
2807vs	2822m	2957s	3080vs	3020s	2763s	2885vs	2785vs	2909s	2822s	
2748vs	2792m	2881s	2763s	2814vs		2792vs		2841s		
	2733m	2703vs						2763s		
	2564m	2595vs						2580s		
		2534vs								
1622vs	1632vs	1645vs	1673s	1667vs	1671vs	1649vs	1693vs	1681vs	1669vs	1679vs
1654m	1568vs	1620vs	1661s	1626vs	1601vs		1681vs	1667s	1591vs	1595vs
1571s		1579vs	1600vs	1587vs			1600s	1636s		
								1605vs		
								1586vs		

(Continued)

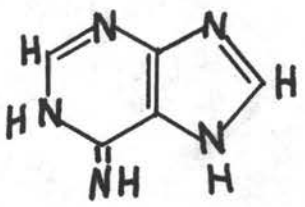
TABLE I (Continued)

1	2	3	4	5	6	7	8	9	10	11
1505m	1537vs	1516vs	1493s	1518vs	1535m	1512s	1535m	1485s	1497s	1534s
1497m	1532vs	1509vs	1450s	1470vs	1486m	1476s	1454s	1446s	1430s	1493s
1473vs	1493m	1493vs	1413s	1390vs	1432s	1393s	1429s	1435s	1405s	1415s
1437vs	1461m	1453vs	1408s	1371vs	1396vs	1369s	1378s	1339vs	1323vs	1367m
1400vs	1430vs	1429vs	1393s	1306vs	1374m	1329s	1363s	1327vs	1295s	1318s
1351m	1367vs	1408vs	1359s	1265vs	1353m	1291s	1261s	1309s	1258s	1237s
1329vs	1357vs	1395vs	1286m	1209m	1331s	1222m	1212m	1261s	1201s	1179s
1304vs	1327m	1357s	1234m	1178m	1266s	1182m	1178m	1248m	1160m	1123s
1269vs	1296s	1335m	1163w	1141m	1234m	1103m	1141m	1155s	1118vs	
1215s	1271m	1305s	1147w	1092m	1160m		1020w	1124m	1016m	
1146m	1239s	1280vs	1115m	1022m	1113m			1074m		
1099s	1211m	1245s	1096m		1052w			1025m		
	1184w	1204s			1012m			1025m		
	1165m	1172m								
	1130w	1139s								
	1088m									
	1037m									
966vs	967w	964s	983m	992w	987m	960m	948m	968m	944m	941s
922s	948m	952s	937m	961m	983m	917s	810m	956m	925m	782m
908vs	932m	929s	861m	942s	933s	868m	787m	947s	879m	
860s	880m	879m	802s	933s	848w	827s	666m	935s	794s	
804s	839w	871m	794m	922s	812m	782m		871m	774m	
789s	799m	844s	781m	895m	795m	673m		850s	730m	
	794m	797s	750m	837m				825m		
	785m	786s	711m	827m				788s		
	743w			799s				799s		
				793s				731m		
				774w				682s		
								677s		

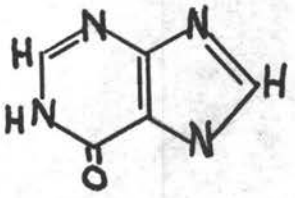
at about 2700 cm.$^{-1}$ and a gradually weakening edge extending on the low frequency side to below 2300 cm.$^{-1}$. Since X-ray studies (7, p.324; 14, p.81; and 8, p.92) have shown that related compounds form numerous intermolecular hydrogen bonds in the crystalline state, the infrared data are interpreted most readily by assuming the existence of hydrogen bonds of varying strength. In this connection it is interesting to note that Blout and Fields (6, p.483) observed in caffeine and theobromine the disappearance of the long wavelength tail centered at about 2700 cm.$^{-1}$ in the spectra of the other purines. This absorption is apparently due to an exceptionally strong hydrogen bond formed by the hydrogen atom at position 7.

In the area covered by group II, 1550-1700 cm.$^{-1}$, unsubstituted purine exhibits very strong absorption peaks at 1571 and 1622 cm.$^{-1}$. The corresponding frequencies in 2-N-diethylaminopurine occur at 1568 and 1632 cm.$^{-1}$. In some of the derivatives the apparently corresponding peaks are poorly resolved and lie closer to 1600 cm.$^{-1}$. Substitution of an amino group apparently produces a strong new band at about 1670 cm.$^{-1}$ and substitution of a hydroxyl group produces a corresponding maximum nearer 1700 cm.$^{-1}$. Blout and Fields (6, p.481) reported intense absorption bands in the regions 1670-1700 and 1558-1600 cm.$^{-1}$ which they attributed to C=C and C=N stretching modes in the purine ring system. The data obtained in the current work

indicate that the bond falling between 1670 and 1700 cm.$^{-1}$, observed by Blout and Fields (6, p.482), is probably not a double bond stretch in the ring but either a deformation of the NH_2 or OH group, or more likely the interaction of such a motion with the stretching of the external double bonds in structures A and B whose importance is attested by the



A



B

X-ray studies (7, p.324; 14, p.81; and 8, p.92). Considering the limited number of purine derivatives available to the previous workers, complete confirmation could hardly be expected.

Although numerous strong bonds occur in the region of group III, 1000-1550 cm.$^{-1}$, they vary too widely upon substitution to permit definite conclusions. However, in the case of the sulfur-containing purines the appearance of intense absorption in the region of 1323 cm.$^{-1}$ coupled with the failure to find absorption in the region of 2500 cm.$^{-1}$ is indicative of a thione rather than a thiol structure.

Again in the region of group IV, below 1000 cm.$^{-1}$, the spectra are relatively variable, but two rather similar regions of absorption seem to characterize the series. One

is a band falling between 925 and 975 cm.$^{-1}$, first observed by Blout and Fields (23, p.483), which is sometimes resolved into several components. The other is a doublet falling close to 800 cm.$^{-1}$, of which the higher frequency component is invariably the more intense.

The absorptions at or near 1622 and 1571 cm.$^{-1}$ in purine, according to this interpretation, are characteristic of the pyrimidine-like ring common to purine, pyrimidine and quinazoline. In pyrimidine itself strong or very strong peaks are found at 1610 and 1569 cm.$^{-1}$ (11, p.3062; 31, p.232; and 2, p.2911). In quinazoline strong absorptions, reasonably stable against substitutions, are found at 1622 and 1566 cm.$^{-1}$ (15, p.4834). Bellamy (5, p.237) has criticized the assignment of the higher of these two frequencies as a characteristic C=C and/or C=N ring vibration on the substantial grounds that such a frequency was observed previously in pyrimidines which in many cases involved amino-substitution, an objection which does not apply here. We are, however, at a loss to explain the disappearance of this band in the work of Short and Thompson (29, p.169).

The 2- and 6-aminopurines, whose spectra were of particular interest, have strong absorption peaks between 3100 and 3400 cm.$^{-1}$ where nitrogen-hydrogen bond stretching is found. They also have strong peaks between 1670 and 1700 cm.$^{-1}$. The latter are probably composed both of

deformation of the NH_2 group and of the stretching modes of the tautomeric =NH group. Thus the infrared data suggest that some tautomeric equilibrium exists in the monoaminopurines.

EXPERIMENTAL

5-(4'-Chlorophenyldiazoamino)-pyrimidine.--p-Chloroaniline (0.401 g., 0.00314 moles) was dissolved in a solution of 0.6 ml. concentrated hydrochloric acid in 2.9 ml. water. The solution was cooled to 5°C. and a solution of 0.235 g. sodium nitrite in 1.55 ml. water was added dropwise with stirring. A solution of 0.30 g. (0.00316 moles) 5-aminopyrimidine prepared according to the procedure of Smith (28, p.27) in 2.9 ml. water containing 0.27 ml. concentrated hydrochloric acid was cooled to 5°C. and added slowly to the diazotized solution. A solution of 1.118 g. anhydrous sodium acetate in 2 ml. water was added at one time. A bright red precipitate formed slowly. The suspension was stirred occasionally and held at 0°C. for 30 minutes. Fifteen ml. water was added and the suspension was filtered. The filter cake was allowed to dry. It gave 0.09 g. of product which melted at 92-130°C.

The filtrate was allowed to stand overnight and the product that formed was filtered and dried. It gave 0.27 g. of a light brown powder which melted at 170-180°C. A third fraction, 0.09 g., was recovered the third day. On analysis fraction 2 gave C. 52.04, 51.95; H. 3.68, 3.63. Calculated for $C_{10}H_8N_5Cl$: C. 51.39; H. 3.42.

4-Amino-5-(4'-chlorophenylazo)-pyrimidine.--A chilled solution of 0.235 g. sodium nitrite in 1.5 ml. water was

added dropwise with stirring to a solution of 0.401 g. (0.00316 moles) p-chloroaniline and 0.8 ml. concentrated hydrochloric acid in 3 ml. water. A solution of 0.30 g. (0.00316 moles) 4-aminopyrimidine, prepared according to the procedure of Brown (9, p.354), and 0.28 ml. concentrated hydrochloric acid in 3 ml. water was slowly stirred into the diazotized solution and a solution of 2.39 g. sodium carbonate monohydrate in 10 ml. water was added rapidly. A tan precipitate began to form as soon as the solution was made alkaline. The suspension was held at 0°C. for an hour and filtered. After two hours additional product had precipitated and this was filtered off. The combined products were recrystallized from benzene to give 0.282 g. yellowish crystals (38.5%) which melted at 204-206°C. Analysis: C. 51.73, 52.00; H. 3.77, 3.70. Calculated for $C_{10}H_8N_5Cl$: C. 51.39; H. 3.42.

2-Amino-5-(4'-chlorophenylazo)-4-methylpyrimidine.--A solution of 0.401 g. (0.00316) moles) p-chloroaniline and 0.79 ml. concentrated hydrochloric acid in 3 ml. water was cooled to 5°C. and diazotized by adding dropwise with stirring a solution of 0.235 g. sodium nitrite in 1.5 ml. water. A solution of 0.344 g. (0.00316 moles) 2-amino-4-methylpyrimidine, obtained through the courtesy of Victor Smith, and 0.28 ml. concentrated hydrochloric acid in 3 ml. water was added slowly with stirring. The solution was made alkaline by the addition of 2.18 g. sodium carbonate

monohydrate in 6 ml. water. A light colored precipitate formed. It was allowed to stand for an hour and then was filtered to yield 0.95 g. of yellowish crystals which melted at 164-178°C. After 2 1/2 hours the filtrate was filtered again and 0.287 g. of tan crystals which melted at 167-183°C. with decomposition were recovered. After standing overnight a third crop of crystals, 0.196 g., m. 170-186°C., was recovered. The second crop of crystals was recrystallized from hot benzene--petroleum ether (30-60°C., 1-1)--to give 0.138 g. tan crystals which melted at 180-182°C. with sublimation.

Reaction of 5-aminopyrimidine with 4-chloroquinazoline.--5-Aminopyrimidine (0.250 g., 0.00263 moles) was dissolved in 50 ml. absolute alcohol in a three-necked flask equipped with reflux condenser and gas inlet tube. The air was flushed out with nitrogen and 0.432 g. 4-chloroquinazoline, prepared according to the procedure of Endicott et al. (16, p.1300), was added. Nitrogen was bubbled through the solution for 36 hours, during which time the solution gradually darkened and a precipitate slowly formed. The dark-brown precipitated material (0.167 g.) was filtered off, digested with hot absolute alcohol for 5 hours, filtered and cooled. The dark-brown alcohol-insoluble material (0.108 g.) melted above 300°C. and gave an analysis C. 48.23, 48.43; H. 4.86, 4.70.

Reaction of 4-aminopyrimidine with 4-chloroquinazoline.--4-Aminopyrimidine (0.500 g., 0.00526 moles) and 4-chloroquinazoline (0.788 g., 0.00478 moles) were dissolved in 25 ml. absolute alcohol and 0.1 ml. concentrated hydrochloric acid was added. The solution was allowed to stand 16 hours and was then made alkaline with 10 drops 6 N. sodium hydroxide. The solvent was evaporated *in vacuo*, the residue dissolved in alcohol-water (1-1) and filtered from a small amount of insoluble material. The filtrate was evaporated to dryness. The residue was boiled with ethyl acetate which removed some gummy material. The ethyl acetate-insoluble material was extracted with 100 ml. hot alcohol, leaving 0.08 g. of white crystals of sodium chloride. The alcoholic solution was treated with Norite and diluted with 50 ml. benzene to induce crystallization. The cream colored powder which separated was found on analysis to be mostly 4-aminopyrimidine.

Reaction of 2-amino-4-methylpyrimidine with 4-chloroquinazoline.--When this reaction was attempted, the starting material was recovered.

Methylation.--5-Aminopyrimidine (0.00458 moles) and an ethereal solution of diazomethane prepared from 2.5 g. N-nitrosomethylurea were allowed to stand for 48 hours. The pyrimidine did not all dissolve. Methanol (5 ml.) was then added to catalyze the reaction. The pyrimidine gradually

went into solution over a period of 24 hours. Evaporation of the solvent left the starting material as the residue. The starting material was recovered in similar experiments with 4-aminopyrimidine and with 2-amino-4-methylpyrimidine.

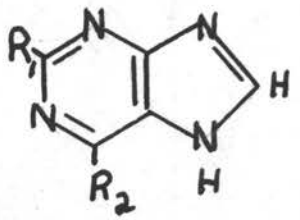
Preparation of purines.--The mono- and di-substituted purines were prepared previously in this laboratory (26, pp.264-265). The salts were converted to the free bases for the purpose of this study. Purine was prepared by the procedure of Albert and Brown (1, p.2067). All samples were tested for homogeneity by paper chromatography according to the procedure of Vischer and Chargaff (32, p.781).

Preparation of samples.--The free bases of all compounds were examined as solids sublimed on rock salt crystals at 10^{-5} mm. pressure in a manner similar to that of Blout and Fields (6, p.483). Sublimation data are included in Table II.

Instrumentation and measurement of infrared spectra.--The instrument used was a Perkin-Elmer model 12C spectrophotometer modified as described by Culbertson et al. (15, p.4835). The spectra were obtained by single beam operation of a NaCl or LiF prism followed by a point by point comparison of the spectrum of the sample with that of a blank. The single beam method was preferable because of the high resolution obtainable. Such resolution was desirable since several of the films, notably that of

TABLE II

Molecular Distillation of Purine Series



Compound R_1	Compound R_2	Sublimation Temp., °C.	Compound R_1	Compound R_2	Sublimation Temp., °C.
H	H	140	NH_2	NH	150
$(C_2H_5)_2N$	H	133	SH	H	220
NH_2	CH_3	170	CH_3	OH	220
CH_3	NH_2	180	OH	CH_3	220
CH_3S	NH_2	160	SH	NH_2	230
NH_2	NH_2	170			

purine itself (see Figure 1) yielded numerous very sharp absorption peaks with a width of the order of 10 $cm.^{-1}$.

SUMMARY

The published ultraviolet and infrared data indicate that 2-, 4- and 5-aminopyrimidines exist in the $-NH_2$ form. The chemical reactivity of these compounds reported in the literature indicates that they are much less reactive than the corresponding benzene analogs but that they will react with the more vigorous reagents such as acetic anhydride.

The present work has shown that these compounds will react with such less vigorous reagents as certain diazonium salts. 4-Chloroquinazoline gave uncharacterizable products with 5-aminopyrimidine and did not appear to react with either 2-amino-4-methylpyrimidine or 4-aminopyrimidine. It was further found that diazomethane, cyanic acid and cyanamide did not react with the monoaminopyrimidines. Thus they act more like amides than like aromatic amines.

The infrared spectra of aminopurines indicate that these compounds exist primarily in the $-NH_2$ form but strong bands between 1670 and 1700 $cm.^{-1}$ suggest that there may be some tautomeric $=NH$ groups present.

BIBLIOGRAPHY

1. Albert, Adrian and D. J. Brown. Purine studies. I. Stability to acid and alkali. Solubility. Ionization. Comparison with pteridines. Journal of the chemical society 1954:2060-2071.

2. Angyal, C. L. and R. L. Werner. The tautomerism of N-heterocyclic amines. II. Infrared spectroscopic evidence. Journal of the chemical society 1952:2911-2915.

3. Austin, Janet Evans. The ultraviolet absorption spectra of some derivatives of uracil. Journal of the American chemical society 56:2141-2143. 1934.

4. Baxter, R. A. and F. S. Spring. Application of the Hofmann reaction to the synthesis of heterocyclic compounds. II. Synthesis of xanthine from glyoxaline-4:5-dicarboxyamide and of 9-methylxanthine from 1-methylglyoxaline-4:5-dicarboxyamide. Journal of the chemical society 1945: 232-234.

5. Bellamy, L. J. The infra-red spectra of complex molecules. New York, Wiley, 1954. 323p.

6. Blout, Elkan R. and Melvin Fields. Absorption spectra. VIII. The infrared spectra of some purines and pyrimidines. Journal of the American chemical society 72:479-484. 1950.

7. Broomhead, June M. The structures of pyrimidines and purines. II. A determination of the structure of adenine hydrochloride by X-ray methods. Acta crystallographica 1:324-329. 1948.

8. _____. The structures of pyrimidines and purines. IV. The crystal structure of guanine hydrochloride and its relation to that of adenine hydrochloride. Acta crystallographica 4:92-100. 1951.

9. Brown, D. J. Improved syntheses in the pyrimidine series. I. The use of pyrimidine-thiols. Journal of the society of chemical industry 69: 353-356. 1950.

10. Brown, D. J., Earl Hoerger and S. F. Mason. Simple pyrimidines. II. 1:2-dihydro-1-methylpyrimidines and the configuration of N-methyl uracils. Journal of the chemical society 1955:211-217.

11. Brownlie, I. A. Infra-red spectroscopic measurements of substituted pyrimidines. II. The absorption spectra of di-, tri- and tetra-substituted pyrimidines. Journal of the chemical society 1950:3062-3072.

12. Burchenal, Joseph H. et al. Effects of 6-mercaptopurine in man. Proceedings of the American association for cancer research 1:7-8. 1953.

13. Christensen, B. E., Bruce Graham and A. J. Tomisek. Quinazolines. III. Synthesis of 4-alkylaminoquinazolines. Journal of the American chemical society 68:1306-1308. 1946.

14. Cochran, W. The structures of pyrimidines and purines. V. The electron distribution in adenine hydrochloride. Acta crystallographica 4:81-92. 1951.

15. Culbertson, Harry, J. C. Decius and Bert E. Christensen. Quinazolines. XII. A study of the infrared spectra of certain quinazoline derivatives. Journal of the American chemical society 74:4834-4838. 1952.

16. Endicott, Margaret M. et al. Quinazoline derivatives. I. The synthesis of 4-(4'-diethylamino-1'-methylbutylamino)-quinazoline (SN 11,534) and the corresponding 2-phenylquinazoline (SN 11,535). Journal of the American chemical society 68: 1299-1301. 1946.

17. Fischer, F. Gottwalt, Wilhelm Paul Neumann and Josef Roch. Eine neue Synthese der Harnsaure und des Xanthins. Chemische Berichte 85:752-760. 1952.

18. Goulden, J. D. S. The structure of the aminopyridines. Journal of the chemical society 1952: 2939-2940.

19. Hitchings, George H. et al. Studies on analogs of purines and pyrimidines. Annals of the New York academy of sciences 52:1318-1334. 1950.

20. Kurzer, Frederick. Arylureas. In Schreiber, R. S. (ed.) Organic synthesis. Vol. 31. New York, Wiley, 1951. 122p.

21. Lythgoe, B. Some aspects of pyrimidine and purine chemistry. Quarterly reviews (London) 3:181-207. 1949.

22. Lythgoe, B., A. R. Todd and A. Topham. Experiments on the synthesis of purine nucleosides. V. The coupling of pyrimidine derivatives with diazonium salts. A method for the preparation of 5-amino-pyrimidines. Journal of the chemical society 1944:315-317.

23. Marshall, J. and James Walker. An experimental study of some potentially tautomeric 2- and 4(6)-substituted pyrimidines. Journal of the chemical society 1951:1004-1017.

24. Overberger, C. G. and Irving C. Kogon. Monomer synthesis. Methylation of 2-aminopyrimidine. Journal of the American chemical society 76: 1065-1068. 1954.

25. Phillips, Arthur P. and John Mentha. Acylation of some aminopyrimidines. Journal of the American chemical society 76:6200-6202. 1954.

26. Robins, Roland K. et al. Purines. II. The synthesis of certain purines and the cyclization of several substituted 4,5-diaminopyrimidines. Journal of the American chemical society 75:263-266. 1953.

27. Roblin, Richard O., Jr., Philip S. Winnek and Jackson P. English. Studies in chemotherapy. IV. Sulfanilamidopyrimidines. Journal of the American chemical society 64:567-570. 1942.

28. Smith, Victor Herbert. The preparation of some tetrahydropyrimidines and related compounds of possible biological interest. Ph.D. thesis. Corvallis, Oregon state college, 1955. 44 numb. leaves.

29. Short, L. N. and H. W. Thompson. Infra-red spectra of derivatives of pyrimidine. Journal of the chemical society 1952:168-187.

30. Sutherland, G. B. B. M. Some problems in the interpretation of the infra-red spectra of large molecules. Discussions of the Faraday society 1950 (9):274-281.

31. Thompson, H. W., D. L. Nicholson and L. N. Short. The infra-red spectra of complex molecules. Discussions of the Faraday society 1950(9): 222-235.

32. Vischer, Ernst and Erwin Chargaff. The separation and characterization of purines in minute amounts of nucleic acid hydrolysates. Journal of biological chemistry 168:781-782. 1947.

33. Wheeler, Henry L. Researches on pyrimidins:on some salts of cytosin, isocytosin, 6-aminopyrimidin and 6-oxypyrimidin. Journal of biological chemistry 3:285-297. 1908.

34. Whittaker, N. A new synthesis and the chemical properties of 5-aminopyrimidine. Journal of the chemical society 1951:1565-1570.