This investigation is concerned with the synthesis of a series of N-(6-purinyl) amino acid ethyl esters and N-6-(2,4-dichloropyrimidinyl) amino acid ethyl esters. The aminolysis procedure of Daly and Christensen was used for this investigation. The starting materials were either (1) 6-chloropurine or (2) 2,4,6-trichloropyrimidine together with certain acid ethyl esters. The procedure consisted of refluxing a 1 to 2.5 molar ratio of the purine or pyrimidine with the desired amine in some inert solvent (absolute ethyl alcohol or n-butanol). The products were isolated either by (1) crystallization from the reaction mixture, or (2)
crystallization from the reaction mixture after the addition of water, or (3) removing the solvent by steam distillation. Crystallizing the product from water. Procedures for the synthesis of the following compounds are described:

1. N-(6-purinyl) DL-β-phenylalanine ethyl ester 
   \( \text{(C}_{16}\text{H}_{17}\text{N}_{5}\text{O}_{2}) \) 
   Calculated: C=61.7, H=5.50; Found: C=61.9, H=5.81; M.P. 197°-198°.

2. N-(6-purinyl) Glycine ethyl ester 
   \( \text{(C}_{9}\text{H}_{11}\text{N}_{5}\text{O}_{2}) \) 
   Calculated: C=48.9, H=5.01; Found: C=48.50, H=5.10; M.P. 272°-274°.

3. N-(6-purinyl) DL-alanine ethyl ester 
   \( \text{(C}_{10}\text{H}_{13}\text{N}_{5}\text{O}_{2}) \) 
   Calculated: C=51.1, H=5.57; Found: C=51.5, H=5.93; M.P. 198°-199°.

4. N-(6-purinyl) L-tyrosine ethyl ester 
   \( \text{(C}_{16}\text{H}_{17}\text{N}_{5}\text{O}_{3}) \) 
   Calculated: C=58.7, H=5.24; Found C=58.3, H=5.67; M.P. 150°-152°.

5. N-(6-purinyl) 2-amino-4-methyl-pyrimidine 
   \( \text{(C}_{10}\text{H}_{8}\text{N}_{7}) \) 
   Calculated: C=50.8, H=4.27; Found: C=50.8, H=4.32; M.P. 264°-265°.

6. N-(6-purinyl) tyramine 
   \( \text{(C}_{13}\text{H}_{13}\text{N}_{5}\text{O}) \) 
   Calculated: C=61.2, H=5.13; Found: C=60.8, H=5.18; M.P. 254°-255°.

7. N-(6-purinyl) DL-leucine ethyl ester 
   \( \text{(C}_{13}\text{H}_{19}\text{N}_{5}\text{O}_{2}) \) 
   Calculated: C=56.30, H=6.91; Found: C=55.9, H=7.19; M.P. 210°-211°.

8. N-[6-(2,4-dichloropyrimidinyl)]-DL-β-phenylalanine ethyl ester 
   \( \text{(C}_{13}\text{H}_{15}\text{N}_{3}\text{O}_{2}\text{Cl}_{2}) \) 
   Calculated: C=52.9, H=4.41; Found: C=52.7, H=4.37; M.P. 99°-100°.
APPROVED:

[Signature]
Professor of Chemistry
In Charge of Major

[Signature]
Chairman of Department of Chemistry

[Signature]
Chairman of School Graduate Committee

[Signature]
Dean of Graduate School

Date thesis is presented [Feb. 9, 1960]
Typed by Penny A. Self
To my wife
ACKNOWLEDGMENT

The author wishes to acknowledge his appreciation to Dr. B.E. Christensen for his assistance in the preparation of this thesis.
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THE SYNTHESIS OF CERTAIN N-(6-PURINYL) SUBSTITUTED AMINO ACID ESTERS

The presence of a cell growth factor in coconut milk had been known for several years. In 1944 the Van Overbeck group (34, p. 219-224) succeeded in increasing the concentration of this factor 170 fold but were unable to isolate or characterize the compound. Nevertheless at this concentration the factor could still be diluted 1/19,000 and show a maximum activity on the assaying tissue. Strong and co-workers (23, p. 1392) concentrated the factor 4,350 times but were unable to obtain enough pure material to characterize the compound.

Miller and co-workers (24, p. 2662-2663) in 1955 discovered another factor in extracts of brewers yeast. This factor, which they called kinetin, was found to be necessary for maximum growth in cultured tobacco wound callus tissue. In the absence of kinetin the tissue would attain limited growth but was incapable of cell division while in the presence of kinetin, maximum growth and cell division took place. Since kinetin seemed to be associated with high concentrations of nucleic acid this group tried to isolate the compound from other rich DNA sources i.e. herring sperm and calf thymus.
A four year specimen of herring sperm was found to contain this cell division factor but fresh sources were found to be inactive. When a watery slurry of either fresh herring sperm or calf thymus was autoclaved for thirty minutes the material was found to be very active in cell division cleavages.

The material resulting from the autoclaving of the DNA sources was dissolved in water and fractionated by ion exchange procedures. The resultant material was recrystallized several times and then characterized. The active principle was found to be 6-furfurylaminopurine (24, p. 2662-2663). This structure was confirmed by synthesizing this compound and comparing the physical and chemical properties with those of the natural materials.

In 1955 Hall and DeRopp (13, p. 6400) demonstrated that kinetin could not be present in natural DNA but was rather a product resulting from the autoclaving of DNA. When solutions of (1) deoxyadenosine or of (2) furfuryl alcohol and adenine were autoclaved for thirty minutes at pH 4.0 their products also gave considerable cell division activity. This leaves some doubt whether kinetin is the cell division factor or whether it is a compound similar to the cell division factor that has similar biological activity.
Ham and co-workers (14, p. 2648) observing that adenine retarded the growth of tentacles in decapitated hydra used this behavior to test various 6-(alkyl and arylsubstituted) amino purines. The assumption was made that the regeneration of hydra might well serve as a model for cell division in higher animals. Several 6-substituted amino and thio purines were synthesized and tested. Among these was kinetin which showed a relative inhibition twenty times that of adenine. The most potent inhibitor tested was 6-(7-phenylheptyl) aminopurine which had an activity 5,000 times that of adenine (31, p. 5100). A partial comparison of the results of the tests on hydra to those tests on tobacco callus tissue shows no correlation between the "kinetins" activity on plants and the "hydra retarders". This group found that some compounds that exhibit retardation of hydras had no effect for inducing cell division in tobacco wound callus tissue.

Although the mode of action of N-substituted-6-amino-purines is not known, it has been demonstrated that some of these compounds exhibit remarkable biological activity. It is possible that certain derivatives of these compounds will enhance cell division in animals as they do in plants or retard cell division in higher animals as has been demonstrated on
the hydra. Either of these properties would be of considerable interest to scientists in cancer research and related fields. It is for this reason that a series of compounds were synthesized resembling kinetin for the purpose of assaying their biological activity.

Purine is a nitrogen containing heterocycle with the following structural formula:

![Purine structural formula](image)

Purine compounds have long been known to be a constituent of nucleoproteins. Nucleoproteins are macro molecules composed of protein and nucleic acid. Nucleic acids are of two types; ribose nucleic acid (RNA), or the newer terminology pentose nucleic acid (PNA), associated with the cytoplasm of cells and deoxypentose nucleic acid (DNA) associated with the nucleus of cells. These nucleic acids are large polymeric molecules in which the monomeric units are nucleotides. Nucleotides are composed of a nitrogenous base, either
purine or pyrimidine, ribose and phosphoric acid units, and are the building blocks of nucleic acids much like amino acids are the building blocks of protein. Nucleoproteins are known to be constituents of all living cells both animal and plant, and also are found in the viruses (intracellular parasites). DNA is associated with and considered the major constituent of the genetic material chromosomes. Although the biochemistry of cellular reproduction is not known, DNA is thought to be the material responsible for the maintenance of heredity (10, p. 184). It has recently been established that the biochemical role of RNA is the synthesis of protein, and it is now thought that DNA is also essential and through some unknown means exerts control over this same process (16, p. 406).

The purine compounds contained in nucleic acid are the more common adenine (6-aminopurine) and guanine (2-amino-6-oxypurine) and the less common 6-methyl amino-purine. Other common naturally occurring purines are coenzyme A, xanthine (2,6-dioxypurine), hypoxanthine (6-oxypurine), and uric acid (2,6,8-trioxypurine). Many plant extracts contain purine compounds, e.g. caffeine

---

1. Any of a group of submicroscopic infective agents thought by some to be living organisms, and by others to be complex proteins capable of multiplication in living cells. Webster
and inosine (hypoxanthineriboside) (10, p. 203-206).

The first known purine was uric acid, discovered by Scheele in 1776, which opened the purine field to research. For the next 100 years most of the work done in this area involved the isolation and synthesis of these naturally occurring compounds. From 1882-1907, due to the work of Emil Fischer, new purine derivatives were synthesized and the chemistry of these compounds elucidated (20, p. 75-95).

Although the presence of the purines in nucleic acids had long been known, it was not until 1942 that attempts were made to synthesize purine analogs for the specific purpose of interfering with nucleic acid biosynthesis or function. Since this time many analogs of purine and pyrimidine have been synthesized and tested for antimetabolite and antimicrobial action. 6-Mercaptopurine is one such synthetic analog of purine that was found to be an inhibitor of the growth of both "lactobacillus casei" and experimental tumors. This compound has also been used for treatment of certain forms of leukemia in man. Thio guanine (2-amino-6-mercaptopurine) which was first prepared in 1948 showed marked inhibitory effects on "l casei" and other bacterial strains as well as inhibitory
effects on human leukemia but exhibited no advantage over 6-mercaptopurine. 2,6-Diaminopurine has also been synthesized and been shown to act as an inhibitor for many bacterial cultures; as well as sarcoma-180 in vitro. Other purines that have shown marked antimetabolite activity are: 6-chloropurine, 6-methylpurine, and 2-fluoroadenosine (15, p. 453-526).

A very recent and interesting purine derivative of considerable biochemical interest is puromycin. The structure of this compound was determined by Hutchings and co-workers who reported it to be 6-dimethylamino-9-[3'-deoxy-3'-(p-methoxyl-L-phenylalanylarnino)-D-ribofuranosyl]purine. Puromycin has a broad spectrum antibiotic which shows antibacterial activity to a variety of gram negative and gram positive bacteria "in vitro". Puromycin is effective against protozoa both "in vitro" and "invivo", and has been used clinically to treat human ameobiasis with good results (ninety eight percent effective). An unique property of puromycin is its effect against multicellular organisms. It was observed that puromycin was effective against tapeworms and oxyurids, a property that no other antibiotic has shown; in addition to the above mentioned properties puromycin shows inhibitory action to various malignant tumors. Structural modifications of the
puromycin molecule tend to modify the biological activity of the compound. The mode of action of puromycin is thought to be through an interference with purine metabolism (18, p. 177-187).

The theory of antimetabolite action that was almost universally accepted by workers in the antimetabolite field was the classical "Lock and Key" theory. According to this theory a particular enzyme fits like a key into the protein portion (apoenzyme) which then acts as a lock to form a coenzyme. This coenzyme then behaves as a biochemical catalyst in other metabolic reactions. This theory has proved to be useful; furthermore it explains the specificity and action of biological phenomena such as the antigen-antibody formation in man and other animals. This hypothesis implies that only relatively small changes within molecules were possible if the derivative were to remain biologically active.

This classical idea has recently been modified by Baker at Stanford Research Institute. It is Baker's hypothesis that a considerably smaller portion of the molecular configuration is important for the insertion into the apoenzyme. This then leaves a large portion of the molecule that can be altered rather drastically without destroying the fit (Lock and Key). This modification has definitely broadened the scope
of search for antimetabolites by allowing for greater molecular changes such as the addition of larger groups and even intermolecular variations (28, p. 38-39). At the present time Dr. Baker is trying to ascertain the area of a given molecule that is needed to form the apoenzyme. If this has been determined one would know exactly the parts of the molecule that can be altered in forming the antimetabolite. The next step would be to alter the molecule in a variety of ways and determine which groups show the greatest effect. From this type of approach it is hoped that a more systematic method for the synthesis of antimetabolites may be found.

Sutherland and Christensen synthesized twenty 6-alkylamino and 6-dialkylaminopurines with alkyl groups of from 6 to 18 carbon atoms (32, p. 2251-2252). These compounds were tested for biological activity and all showed varying degrees of kinetin type activity. Because of these and other results and observations stated above, the preparation of a series of N-(6-purinyl) amino acid esters looked very attractive.

There are several known synthetic pathways for the preparation of 6-substituted aminopurines. One of these is the method used by Miller, et al. (24, p. 2662-2663) for
the first synthesis of kinetin; this was the method developed by Ellon and Hitchings (9, p. 3508-3510) in 1952. The starting material for this procedure is hypoxanthine which is reacted with $\text{F}_4S_{10}$ to form the 6-mercaptopurine, and in turn is converted to the key compound 6-methylmercaptopurine. The reaction of 6-methylmercaptopurine with the desired primary or secondary amine for from fifteen to eighteen hours in a sealed tube at temperatures of 110° to 180° yields the desired compound. This method has the disadvantage (1) that it does not always work, and (2) involves the use of sealed tube reactions. Furthermore mercapto compounds are usually difficult and unpleasant substances with which to work.

Another approach was developed by Baizer and co-workers (3, p. 1276-1277) to synthesize 6-substituted amino-purines from adenine. This method consists of reacting adenine with the acid anhydride or acid chloride and reducing the resulting amides with lithium aluminum hydride to the corresponding amines. This procedure is applicable to aliphatic, aromatic and heterocyclic amines but has the disadvantage that the necessary acyl chlorides and anhydrides are difficult to prepare. Furthermore, the necessary starting
materials for this investigation are not available.

During the course of this investigation a new synthetic procedure was developed and described by Whitehead and Traverso (37, p. 3971-3972) for the synthesis of 6-alkyl and 6-arylamiopurines. This novel synthesis involves transamination and utilizes as starting material adenine, the desired amine \cdot HCl, and the free amine. These workers synthesized 6-benzylaminopurine, 6-furfurylaminopurine and 6-phenylamiopurine in yields of from fifty to sixty percent. The procedure consists of heating an equal molar ratio of adenine and the hydrochloride salt of the amine and a two molar ratio of the free amine for eight hours at a temperature of 165° to 170°.

The aminolysis procedure that was developed by Daly and Christensen (7, p. 177-179) was used for the investigation described herein. Utilizing this procedure they were able to synthesize 6-morpholinopurine, 6-anilinopurine, 6-benzylaminopurine and 6-furfurylaminopurine. In the work of Sutherland and Christensen (32, p. 2251-2252) cited earlier, they used this method for the synthesis of twenty 6-alkylaminopurines and 6-dialkylaminopurines with yields of from 47 to 95 percent. Their procedure was to reflux 6-chloropurine with a
two and one half molar ratio of the desired amine in n-butyl alcohol for four hours. The products were isolated by either steam distillation or super heated steam distillation from an alkaline solution. Breshears and co-workers (5, p. 3789-3792) utilized this aminolysis procedure plus the observation that the chlorine activity in the 2-, 6- and 8- positions are quite different thus allowing stepwise reactions to synthesize a series of 2-chloro-6-amino substituted purines and 2,8-dichloro-6-amino substituted purines from 2,6-dichloropurine and 2,6,8-trichloropurine respectively. In addition to this 2,6-diamino-purines were synthesized with the same or different amines at the 2 and 6 positions by stepwise aminolysis of 2,6-dichloropurine, and finally 2,6,8-triaminopurines were prepared from 2,6,8-trichloropurine. In the 2,6,8-triaminopurine series no attempt was made to synthesize a trisubstituted aminopurine with different amines on each position, however a number of derivatives were made with the same amine at the 2 and 8 positions and with a different amine at the 6 position. This aminolysis procedure has been used with good results by many workers (5, p. 3790; 29, p. 449; 35, p. 5001; 6, p. 139) to synthesize 6-alkylamino- and 6-dialkylamino- purines, 6,8-alkylaminopurines and 2,6,8-alkyltriaminopurines;
this method was recently extended by Robins to the preparation of p-chlorophenyl, o-chlorophenyl and 3,4-dichlorophenyl-aminopurines (12, p. 1316).

One advantage not available to earlier workers in this area is the commercial availability of the essential starting material 6-chloropurine. The use of 6-chloropurine as a synthetic intermediate was made possible by Bendich who first synthesized this compound in 1954 (4, p. 6073-6077). Other workers had tried to chlorinate hypoxanthine with phosphorus oxychloride but were unsuccessful. Bendich found, however, that a complex formed which, when destroyed with base, permitted the extraction of 6-chloropurine from the reaction mixture with ether.

A method of synthesis of 6-chloropurine that is more of academic interest than synthetic value is the procedure of Giner-Sarolla and Bendich (11, p. 3932-3937). These workers found that 6-hydrazinopurine when reacted with ferric chloride in dilute acid solution could be converted in low yields to 6-chloropurine. This reaction obviously has no synthetic value because the hydrazino derivative must be synthesized initially from 6-chloropurine.

A new method of synthesizing 6-chloropurine was
recently developed by Hichens and Elion at Burroughs Welcome Company (17). This method consists of treating the mercaptopurine either 6-mercaptopurine or 2-amino-6-mercaptopurine with elemental chlorine at a temperature of less than thirty five degrees to obtain the corresponding chloro- or amino-chloropurine. It is this reaction that makes 6-chloropurine available commercially at this time. Robins has recently extended this reaction to the synthesis of mon-, di- and trichloropurines (29, p. 447-451).

The first synthesis of a compound related to the series proposed in this study was the synthesis by Carter (6, p. 139) of N-(6-purinyl) aspartic acid. This was done in order to prove the structure of the product of enzymatic reaction of adenosine-5-phosphate with fumaric acid. This method of synthesis was essentially an aminolysis reaction between the amino acid and 6-chloropurine in an aqueous solution of potassium hydroxide with a pH of 9.5. The yield was quite low, 175 milligrams of the dipotassium· 2 H₂O salt from two grams of 6-chloropurine. One of the difficulties was the purification in which it was necessary to utilize elution chromatography to obtain the product.

Shortly thereafter the work of Ward and co-workers
was published in which the synthesis of a relatively complete series of N-(6-purinyl) amino acids was described. This procedure for the reaction was quite similar to that of Carter, utilizing 6-chloropurine and the amino acid in a 1 to 2 molar ratio respectively. These materials were refluxed in an aqueous solution with the pH adjusted to 9 with sodium carbonate for three hours. After completion of the reflux the pH of the solution was adjusted to 3 with formic acid. Some of the products precipitated during this procedure and those that did were triturated with ether to remove the formic acid and purified by either recrystallization or reprecipitation from water. Those compounds that did not precipitate at pH 4.5 were isolated by elution chromatography on a Dowex-2-X8 column in the formate form utilizing formic acid as the eluent. By means of this procedure the group obtained eighteen N-(6-purinyl) amino acids in fairly good yields. Although the testing is incomplete at this time for antitumor activity one compound N-(6-purinyl)-L-glutamic acid has shown antitumor activity against carcinoma 755 in mice.

Inasmuch as the preparation of the purinyl amino acids by another procedure was published during this investigation, it was decided to extend this project to the synthesis of certain
N-pyrimidinyl amino acid ethyl esters. The procedure utilized by Nazeeri (27) for the monoaminolysis of 2,4,6-trichloropyrimidine with various amines seemed applicable to the current studies. This procedure consisted of mixing trichloropyrimidine and the desired amine in a one to one molar ratio, neutralizing the resultant hydrogen chloride with anhydrous ammonia and removing the ammonium chloride with water. When the trichloropyrimidine was reacted via the above procedure with various amino acid ethyl esters the only material that was isolated was 6-amino-2,4-dichloropyrimidine. Various modifications of the procedure were tried and in the process it was discovered that when 2,4,6-trichloropyrimidine and DL-β-phenylalanine, ethyl ester in a one to two molar ratio respectively, were refluxed for twelve hours in absolute alcohol the desired product, N-[6-(2,4-dichloropyridinyl)]-DL-β-phenylalanine ethyl ester could be isolated by crystallization from the concentrated reaction mixture.
EXPERIMENTAL

The aminolysis procedure of Daly and Christensen (7, p. 177-179) was adapted to the preparation of the compounds synthesized in this investigation. This procedure consists of refluxing 6-chloropurine and the desired free amine in a 1 to 2.5 ratio in some inert solvent; the solvents used were either N-butanol or absolute alcohol. The products were isolated either by (a) crystallization directly from the reaction mixture, (b) crystallization of the product from the reaction mixture which had been diluted with water, or (c) the addition of water to the reaction mixture and the removal of the solvent by steam distillation and the crystallization of the product from the aqueous solution. All of the products synthesized in this investigation were crystalline solids.

**Synthesis of amino acid ethyl ester hydrochlorides**

Most of the amines used in the aminolysis procedure during this investigation were amino acid ethyl esters. The reason for utilizing the esters was to insure the presence of a free base and avoid the effects of a "zwitter" ion. The amino acid ethyl ester hydrochlorides were synthesized from
the corresponding amino acids utilizing a Fischer esterification procedure as outlined by Werbin and Palm (36, p. 1382) for the synthesis of lysine ethyl ester hydrochloride.

This esterification procedure was carried out in a 200 ml round bottomed flask containing 10 grams of the amino acid and 100 ml of absolute ethyl alcohol which was saturated with dry hydrogen chloride gas at room temperature and which required occasional cooling in an ice bath. Upon saturation of the solution the addition of hydrogen chloride gas was discontinued, the flask was then fitted with a calcium chloride protected condenser and the material was refluxed for three hours on a steam bath. During this period all of the acid dissolved in the acidic alcoholic solution. The acidic alcohol solution was thereupon removed by vacuum distillation. The amino acid ethyl ester hydrochloride was redissolved in absolute ethyl alcohol and ethyl ether was added until the solution was just at the point of precipitation. This solution was then placed in the deep freeze to crystallize overnight and the resultant crystals of the amino acid ethyl ester hydrochloride were removed by filtration and air dried.

The amino acid ethyl ester hydrochloride salts of the following amino acids were prepared by this procedure:
Amino Acid | Per cent yield
--- | ---
1. DL-alanine | 81.0
2. DL-leucine | 72.4
3. DL-phenylalanine | 84.5
4. L-tyrosine | 93.0

These compounds were pure enough upon isolation to be used directly in the aminolysis procedure.

**Synthesis of glycine ethyl ester hydrochloride**

Glycine ethyl ester hydrochloride was prepared from the procedure outlined in Organic Synthesis Collective Volume 2 (21, p. 310-312). This procedure consisted of refluxing on a steam bath a mixture of: absolute ethyl alcohol (167 ml) which had been saturated in the cold with dry hydrogen chloride gas, ninety six per cent ethyl alcohol (290 ml) and methyleneaminoacetonitrile (23 grams) for a period of three hours. During the course of this reaction ammonium chloride crystallized out of the reaction mixture and upon completion of the reaction the ammonium chloride was removed by filtration of the hot solution. The glycine ethyl ester hydrochloride was obtained in an overall yield of 65.2 per cent from the cooled
reaction mixture and air dried. The methyleneaminoacetoneitrile used for this reaction was prepared in this laboratory following the procedure in Organic Synthesis Collective Volume 1 (I, p. 355-357).

**Preparation of the free bases of the amino acid ethyl ester hydrochloride salts**

Before the amino acid ethyl ester hydrochlorides could be used in the aminolysis procedure it was necessary to convert these salts to the free amines; the following procedure was used in this investigation: The calculated amount of the amino acid ethyl ester hydrochloride was dissolved in a minimum of absolute ethyl alcohol. Then an equivalent molar ratio of freshly cut sodium was dissolved in a second portion of ethyl alcohol to form the calculated amount of sodium ethoxide. The two solutions were combined and the resultant insoluble sodium chloride was removed by filtration. For those reactions in which ethanol was the solvent the 6-chloropurine was added directly to the filtrate. For the reactions in which N-butanol was the solvent the measured volume of N-butanol was added to the filtrate and the ethanol was removed by distillation.
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<thead>
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<th>Compound</th>
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<th>pH 12.0 (NaOH)</th>
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<td>λ_max, μ</td>
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* These results were obtained on a Beckman Model DB Spectrophotometer with a narrow slit width setting.
### TABLE II

**Analytical Data**

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<th>Name of Compound</th>
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<th>Value %H</th>
<th>Found %C</th>
<th>Value %H</th>
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<td>5.50</td>
<td>61.9</td>
<td>5.81</td>
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<td>48.50</td>
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<td>(\text{C}<em>{13}\text{H}</em>{13}\text{N}_{5}O)</td>
<td>61.2</td>
<td>5.13</td>
<td>60.8</td>
<td>5.18</td>
</tr>
<tr>
<td>N-(6-purinyl)DL-leucine ethyl ester</td>
<td>(\text{C}<em>{13}\text{H}</em>{19}\text{N}<em>{5}\text{O}</em>{2})</td>
<td>56.30</td>
<td>6.91</td>
<td>55.9</td>
<td>7.19</td>
</tr>
<tr>
<td>N-[6-(2,4-dichloropyrimidinyl)]-DL-(\beta)-phenylalanine ethyl ester</td>
<td>(\text{C}<em>{13}\text{H}</em>{15}\text{N}<em>{3}\text{O}</em>{2}\text{Cl}_{2})</td>
<td>52.9</td>
<td>4.41</td>
<td>52.7</td>
<td>4.37</td>
</tr>
</tbody>
</table>

* Calculated for \((\text{C}_{10}\text{H}_{9}\text{N}_{7})_2 \cdot \text{H}_{2}\text{O}\)
**N-(6-purinyl) glycine ethyl ester**

Glycine ethyl ester hydrochloride (4.48 grams 3.20 x 10^-2 mole) was dissolved in 25 ml of absolute ethanol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.736 grams 3.20 x 10^-2 mole) in 25 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was placed in a 100 ml round bottomed flask and 6-chloropurine (2.00 grams 1.28 x 10^-2 mole) was added. The flask was fitted with a condenser protected by a calcium chloride drying tube and refluxed on the steam bath for twelve hours. The resultant light amber solution was then placed in the deep freeze. A cream colored crystalline product was obtained from this mixture by filtration. A second crop of crystals was obtained by adding water, removing the ethanol by distillation, and crystallizing the material from the aqueous solution; yield 2.44 g. (86%), M.P. 272°-274°.

**N-(6-purinyl) DL-β-phenylalanine ethyl ester**

DL-β-phenylalanine ethyl ester hydrochloride (7.35 grams 3.20 x 10^-2 mole) was dissolved in 25 ml of absolute
ethyl alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.736 gram 3.20 x 10^{-2} mole) in 25 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was placed in a 100 ml round bottomed flask and 6-chloropurine (2.00 grams 1.28 x 10^{-2} mole) was added. The flask was fitted with a calcium chloride protected condenser and the material was refluxed for twelve hours on a steam bath. The resultant light amber solution was placed in the freezer to crystallize. One crop of crystals was obtained by filtration of this mixture and a second crop of crystals was obtained by distilling off half the volume of alcohol and adding an equal volume of water and crystallizing the solution in the refrigerator; yield 2.3 g. (58%), M.P. 197°-198°.

N-(6-purinyl) tyramine

Tyramine hydrochloride (0.700 gram 4.00 x 10^{-3} mole) was dissolved in 10 ml of absolute alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.0920 gram 4.00 x 10^{-3} mole) in 10 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and to the filtrate was added 10 ml
of n-butyl alcohol and the ethyl alcohol was removed by distillation. Next 6-chloropurine (0.250 gram 1.6 x 10^{-3} mole) was added to the butanol solution. The flask was fitted with a reflux condenser and the material was refluxed using a Glasco heating mantle for six hours. After the reaction was complete 50 ml of water was added to the amber solution and the N-butyl alcohol was removed by steam distillation. The white crystalline product was obtained by crystallization from the aqueous solution and was removed by filtration; yield 0.311 grams (76%), M.P. 254°-255°.

N-(purinyl) DL-alanine ethyl ester

DL-alanine ethyl ester hydrochloride (4.90 grams 3.20 x 10^{-2} mole) was dissolved in 25 ml of absolute ethyl alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.736 gram 3.20 x 10^{-2} mole) in 25 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was placed in a 100 ml round bottomed flask and 6-chloropurine (2.00 grams 1.28 x 10^{-2} mole) was added. The flask was fitted with a condenser protected by a calcium chloride drying tube and refluxed on the steam bath for twelve hours. The
resultant light yellow solution was placed in the deep freeze. A white crystalline product was obtained from this mixture by filtration. A second crop of crystals were obtained by adding an equal volume of water to the alcohol and cooling; yield 2.18 g. (71%), M.P. 198°-199°.

\[ \text{N-}(6\text{-purinyl}) \text{ L-tyrosine ethyl ester} \]

L-tyrosine ethyl ester hydrochloride (0.982 gram \(4.00 \times 10^{-3}\) mole) was dissolved in 10 ml of absolute ethyl alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.0920 gram \(4.00 \times 10^{-3}\) mole) in 10 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was placed in a 40 ml round bottomed flask and 6-chloropurine (0.250 gram \(1.60 \times 10^{-3}\) mole) was added. The flask was fitted with a condenser protected by a calcium chloride drying tube and refluxed for twelve hours. The resultant light yellow solution was placed in the deep freeze. A white crystalline product was obtained from this mixture by filtration. A second crop of crystals was obtained by adding water, distilling off the alcohol, and crystallizing the material from the aqueous solution; yield 0.313 g. (60%), M.P. 150°-152°.
**N-(6-purinyl)-2-amino-4-methylpyrimidine**

To 10 ml of n-butanol in a 20 ml round bottomed flask was added 2-amino-4-methylpyrimidine (0.780 gram 8.00 x 10^-3 mole) and 6-chloropurine (0.500 gram 3.20 x 10^-3 mole). The flask was equipped with a condenser and refluxed using a Glasco heating mantle for ten hours. During the period of reflux the solution changed from a yellow color to a dark brown. To the brownish solution was added 225 ml of water and a total of 75 ml of solution was steam distilled. The distillate contained all of the n-butanol and the white crystalline product was obtained via crystallization from the residual aqueous solution; yield 0.270 g. (37%), M.P. 264°-265°.

**N-(6-purinyl) DL-leucine ethyl ester**

DL-leucine ethyl ester hydrochloride (6.26 grams 3.20 x 10^-2 mole) was dissolved in 20 ml of absolute ethyl alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium (0.736 gram 3.20 x 10^-2 mole) in 25 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was
placed in a 100 ml round bottomed flask and 6-chloropurine (2.00 grams 1.28 x 10^{-2} mole) was added. The flask was fitted with a calcium chloride protected condenser and refluxed on the steam bath for twelve hours. The resultant light amber solution was placed in the deep freeze to crystallize. One crop of crystals was obtained by filtration of this mixture and a second crop was obtained by adding an equal volume of water and crystallizing the product from an aqueous alcoholic solution; yield 3.134 g. (89%), M.P. 210°-211°.

2,4,6-trichloropyrimidine

The synthetic intermediate 2,4,6-trichloropyrimidine was synthesized in this laboratory by the procedure of Baddiley and Topham (2, p. 678-679). The procedure consists of the addition of 52 grams of barbituric acid in small portions over a period of fifteen minutes to a mixture of 88.5 ml dimethylanaline and 156 ml of phosphorus oxychloride. During the addition the mixture turned darkish brown in color and considerable heat was evolved. The resultant mixture was refluxed for fifteen minutes and cooled and poured over 800 grams of cracked ice. The trichloropyrimidine was extracted from the aqueous mixture with ether. The ether was
evaporated off and the 2,4,6-trichloropyrimidine was purified by distillation. Trichloropyrimidine 35 grams (36%) was recovered distilling at 102° at 18 mm Hg.

\[ \text{N-6-(2,4-dichloropyrimidinyl)-DL-} \beta \text{-phenylalanine ethyl ester} \]

\[ \text{DL-} \beta \text{-phenylalanine ethyl ester hydrochloride (2.50 grams} \times 1.09 \times 10^{-2} \text{ mole) was dissolved in 25 ml of absolute ethyl alcohol. This solution was added to a solution of sodium ethoxide made by dissolving sodium 0.246 grams in 20 ml of absolute ethyl alcohol. The resultant sodium chloride was removed by filtration and the filtrate was in a 100 ml round bottomed flask and 2,4,6-trichloropyrimidine (1 gram} \times 5.45 \times 10^{-3} \text{ mole) was added. The flask was fitted with a calcium chloride protected condenser and the material was refluxed for twelve hours with a Glasco heating mantle. The resultant solution was concentrated to 30 ml and one crop of white crystalline material was obtained. A second crop of crystals was obtained by further concentration of the filtrate; yield 0.314 g. (13%), M.P. 99°-100°.} \]
BIBLIOGRAPHY


