Stability of 1-Substituted Adenines

Walter Frederick Floser
Union College - Schenectady, NY

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THE STABILITY OF 1-SUBSTITUTED ADENINES

A thesis presented to the Committee on Graduate Studies and the Department of Chemistry of Union College, Schenectady, New York, in partial fulfillment of the requirements for the degree of Master of Science.

by Walter Frederick Floser MS 1970

By Walter Frederick Floser (Student's signature)

Approved by Thesis advisor

Approved by Committee on Graduate Studies

Date 6/3/70
This thesis is dedicated to
Dr. John Sowa under whom I conducted
my research. I sincerely appreciate
his help and guidance.

I also wish to express my
appreciation to the faculty of the
Department of Chemistry.
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</table>
1-$\beta$-Hydroxyethyladenine (I) was prepared by the reaction of ethylene chlorohydrin with adenine at 76°C. It was separated from the reaction mixture by use of a Dowex 50W-X8 cation exchange chromatography column and a linear gradient elution system. I was placed in aqueous alkaline solution for two weeks and checked at different intervals to determine its stability. I was also refluxed in an aqueous alkaline solution for 70 min. and its stability was checked. I proved to be stable under all conditions.
INTRODUCTION

Although the toxicity of alkylating agents was observed in 1898, it was not until 1942 that investigation of alkylating agents was intensified. In 1946 Gilman and Philips (22) published data which indicated the potential use of nitrogen mustards as alkylating agents in cancer chemotherapy. The general term mustard originally was used for the war gas compound bis-β-chloroethyl sulfide, but now refers to compounds containing at least one β-chloroethyl group attached to a sulfur or nitrogen atom. Alkylations (40) have been shown to occur through a cyclic 'onium ion intermediate.

Of all the alkylating agents, nitrogen mustards have exhibited the greatest diversity of pharmacological activity. Nitrogen mustards (46) (6) (43) have been shown to inhibit respiration and glycolysis. There is thought to be some degree of correlation between the extent of such inhibition and carcinogenic action (7), cell division (antimitotic effects) (27), and carcinostatic action of the agent. (47) Other experiments have shown that mustards deactivate viruses and directly alkylate nucleic acids.

There are numerous possible sites for in vivo alkylations. One of the most difficult problems has been to determine how each of these reactions is responsible for the physiological and biological effects. In vitro alkylations of purines have
been studied in hope that such studies would solve the above problem. The alkylation of adenine has been studied most extensively and similar mechanisms described regardless of the alkylation agent. In one case (21) different products were obtained. Based on the results obtained by Sowa (44) in the alkylation of hypoxanthine and the diversity of pharmacological activity of mustards it was considered that one facet of the alkylations had been overlooked, that is, the differences in charge distribution in the activated complex for mustards versus other alkylation agents.

Gaucher (21) postulates that the 6-product he found in the alkylation of adenine by N,N-diethyl-β-chloroethylamine was formed by rearrangement of the 1-product. The 6-product might have been formed directly and one piece of evidence might be the study of the stability of the 1-β-N,N-diethylaminoethyladenine. This study was begun in this thesis.
DETECTION SYSTEMS

I. Means of Separating Reaction Mixtures

The method of ion exchange chromatography with a linear gradient system was used to separate adenine from its alkylated derivatives. The column was a Lab Crest F/P glass column with a diameter of 2 cm and a height of 50 cm. Dowex 50W-X8 cation exchange resin, Baker Chemical Company, Phillipsburg, N. J., 50-100 mesh in the hydrogen form was used as a resin.

The resin was washed with 1 N sodium hydroxide, several aliquots of distilled water until a pH of 7 was obtained, 7 N hydrochloric acid, distilled water again until the wash reached pH 7, and finally with the starting elutriator used in the chromatography. The column was washed to remove anything which was bound to the resin. The resin, as a slurry in the starting elutriator, was poured into the column already filled with the starting elutriator. The elutriator was slowly removed at the bottom of the column while more resin was being added. When the desired amount of resin had been added usually to a height of 37 cm the column was stopped. The resin was not permitted to become dry. While the resin was being added the column was continuously tapped to ensure uniform packing.

Before a sample was put on the column, the column was washed with the starting elutriator until an absorbency of .05 or
less was observed for the ultraviolet absorption at 250 m\textmu m, 
260 m\textmu m, and 280 m\textmu m.

The elution system was a linear gradient system (3) which delivered hydrochloric acid to the top of the column. The elution system consisted of two Erlenmeyer flasks of the same size connected by a glass tube siphon and a glass pressure-equalizing tube. Each flask was filled to the same height with their respective normality of hydrochloric acid. The flask containing the lowest concentration of hydrochloric acid was equipped with a magnetic stirrer which dispersed the acid being siphoned over from the other flask before it reached a second siphon leading to the top of the column. The heights of the liquid in the flasks were adjusted to be equal by use of a ringstand and a metal ring, thus allowing the liquid levels in the two flasks to recede at the same rate thereby maintaining a linear gradient. This gradient elution system tends to reduce the tailing of chromatographic peaks and produces sharper resolution. (Diag. I)

The reaction mixture was dissolved in the starting elutriator and added to the top of the column with a pipette. When all of the sample was on the column it was washed with ten 5-ml increments of starting elutriator. This moved the mixture down the column and protected it against any loss of resolution due to any disturbance at the top. The column was then connected to the gradient by means of a siphon, and the
LINEAR GRADIENT ELUTION SYSTEM

4 N HCl

1.5 N HCl

Magnetic Stirrer

Diag. I
chromatography was begun.

Best results were obtained when 5 percent or less of the column capacity was used. The capacity of the column was found by the following method. By using the formula \( V = \pi r^2 h \), where \( V \) = volume of cylinder, \( r \) = radius of the column, and \( h \) = height of the column, the volume of the column was determined. This multiplied by 1.8 meq/ml, the capacity factor given by Baker Company, gave the capacity of the column in milliequivalents. By using the equivalent weight of adenine as the equivalent weight of the mixture an approximate capacity of the column used could be calculated.

The fractions were collected in 16 x 150 mm test tubes using a fraction collector (Rinco Instrument Company, Greenville, Illinois) consisting of a 300 tube capacity turntable, and a volume system with collecting intervals of 25 ml. Approximately 200-400 fractions of 25 ml were collected depending on the size of the gradient elution system used. A drop time of one drop every five seconds, 1/10 ml per drop, was used for best resolution. Since the resin volume shrunk during the chromatography, due to the increasing concentration of acid, the flow rate decreased making it necessary to adjust the drop time periodically.

The test tubes were numbered and the fractions checked for ultraviolet absorption at 250, 260, 280, and 290 \( \mu \text{m} \). Appropriate dilutions were made with hydrochloric acid. The
spectral data were obtained on the Beckman D. U. Quartz Spectrophotometer using a matched set of Beckman quartz cuvettes of 10 mm light path. Spectral curves, when required, were recorded automatically by using a Perkin-Elmer 202 Ultraviolet and Visible Spectrophotometer. Every second tube was read until an absorption above background was obtained. The absorbency ratios at A250/A260, A280/A260, and A290/A260 were recorded. Since these ratios are independent of the concentration of a substance, a chromatographic peak containing only one compound will have constant ratios which give only a straight line if several successive fractions have only one compound.

The linearity of the gradient was checked by titrating various fractions using standardized sodium hydroxide with phenolphthalein as the indicator. A complete chromatograph consisted of a plot of absorbance at 260 μ/ against fraction number, the acid gradient and absorbance ratios.

II. Isolation of Compounds

All reaction mixtures and chromatographic fractions were concentrated with a Buchler Model FE-2 Flash Evaporator connected to an oil pump via two dry ice—acetone traps and a manometer. The reaction mixtures were generally evaporated at a pressure of 10-50 mm. An I²R infrared lamp was used to apply heat to the flask containing the reaction mixture. An
alternative method used was freeze-drying the products on a Virtus Model 10-135 Freeze-Drying Apparatus.

III. Thin Layer Chromatography

Thin layer chromatography was found to be invaluable in substantiating and observing reactions. All thin layer chromatography was run in ascending fashion on Eastman Alumina Chromatogram Sheet 6063. Strips 7.7 cm in length and 1.7 cm in width were cut from the alumina sheet. The strips were placed in an oven at 105°C for 30 min. to activate them, and then placed in a desiccator until used. A 1:1 ratio of 95 percent alcohol and acetone was found to be the best solvent system. The reaction mixture was applied to the alumina with a 10 lambda pipette (1λ = .001 ml), and a I2R infrared lamp used to dry the solvent more quickly. The spots applied in this manner were kept small allowing for better resolution.

A 150-ml salt jar with its walls lined with filter paper was used as the developing chamber. Four ml of the above eluent was placed in the bottom of the jar and allowed to saturate the filter paper forming an atmosphere saturated with solvent. The alumina strip was placed in the developing chamber and after the solvent moved three-quarters of the way to the top of the alumina, the thin layer strip was removed from the jar. The solvent front was marked with
pencil and the plate dried. The plate was then placed into another 150-ml salt jar saturated with iodine vapor. The stained spots from the reaction were easily seen and circled with pencil. This method was the simplest way to detect spots.

IV. Ultraviolet Spectrum and Its Use in Identifying the Alkylated Derivatives of Adenine

The ultraviolet spectra of adenine and its alkylated products are very similar and would be impossible to use for conclusive identification at one pH value. However, spectra are obtained in solutions of varying pH's with differences in spectra seen. To maintain the same conditions throughout the experiments and avoid the necessity of knowing the $pK_a$ values and concentrations of the various compounds, spectra was observed at pH values of 2 and 11 with a maximum absorbance of approximately 0.7. The solutions were adjusted to the pH values by use of a Leeds and Northrop pH meter with 1 N hydrochloric acid and 1 N sodium hydroxide. The blanks were made from distilled water and adjusted similarly to pH 2 and 11. The spectra were recorded automatically on a Perkin Elmer 202 ultraviolet and visible spectrometer. The spectral data obtained from the curves gave $\lambda_{\text{max.}}$ and $\lambda_{\text{min.}}$ at pH values of 2 and 11. Isoabsorptive and isobestic point data were obtained
from curves run on samples at pH 1, 2, 3, 10, 11 and 12. In order to assess the values of ultraviolet spectra in the identification of the alkylated adenines, a literature survey of available ultraviolet spectral data was compiled and is presented in Table I.

V. Proton Resonance Spectra of Alkylated Adenine Derivatives

All NMR spectral data were obtained by the following method. A .04 g sample was placed in 4 ml of D$_2$O and refluxed until the sample went into solution. A portion of this sample was placed in an NMR tube and the spectrum recorded by the Varian model A-60 NMR instrument. While the spectrum was being recorded the sample was crystalizing causing a broadening of the NMR peaks.
TABLE I

<table>
<thead>
<tr>
<th>Purine Derivative</th>
<th>( \lambda_{\text{max}}^a ) Acid</th>
<th>Base</th>
<th>Isoabsorptive Points(^a)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-NH(_2) (adenine)</td>
<td>262</td>
<td>267</td>
<td>282; 268; 235.5</td>
<td>35</td>
</tr>
<tr>
<td>6-NH(_2) (adenine)</td>
<td>262.5</td>
<td>269</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>6-CH(_3)NH(-</td>
<td>267</td>
<td>272</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>6-(HOCH(_2)CH(_2))(_2)NCH(_2)CH(_2)NH(-</td>
<td>274.5</td>
<td>273</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>6-(HOCH(_2)CH(_2))(_2)NCH(_2)CH(_2)CH(_2)NH(-</td>
<td>274.5</td>
<td>273</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>6-(CH(_3))^(_2)N(-</td>
<td>276</td>
<td>281</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>1-CH(_3)-6-NH(_2)</td>
<td>259</td>
<td>270</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>1-benzyl-6-NH(_2)</td>
<td>260.5</td>
<td>271.5</td>
<td>263.5; 237.5</td>
<td>41</td>
</tr>
<tr>
<td>1-CH(_3)-6-NH(_2)</td>
<td>258.1</td>
<td>269.5</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>1-CH(_3)-6-NH(_2)</td>
<td>259</td>
<td>270</td>
<td>263; 237</td>
<td>9, 29</td>
</tr>
<tr>
<td>3-CH(_3)-6-NH(_2)</td>
<td>274</td>
<td>272</td>
<td>281; 240</td>
<td>16, 9</td>
</tr>
<tr>
<td>3-CH(_3)-6-NH(_2)</td>
<td>274</td>
<td>273</td>
<td></td>
<td>25, 30</td>
</tr>
</tbody>
</table>

continued on page 12

\(^a\) \( \lambda_{\text{max}} \) and isoabsorptive points given in millimicrons
<table>
<thead>
<tr>
<th>Purine Derivative</th>
<th>$\lambda_{\text{max}}^a$</th>
<th>Isoabsorptive Points$^a$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-CH$_3$CH$_2$-6-NH$_2$-</td>
<td>275 273</td>
<td>286; 241</td>
<td>16</td>
</tr>
<tr>
<td>3-HOCH$_2$CH$_2$-6-NH$_2$-</td>
<td>275 275</td>
<td>285; 241</td>
<td>16</td>
</tr>
<tr>
<td>3-HOCH$_2$CHOHCH$_2$-6-NH$_2$-</td>
<td>275 275</td>
<td>286; 242</td>
<td>16</td>
</tr>
<tr>
<td>3-(CH$_3$)$_2$C=CHCH$_2$-6-NH$_2$-</td>
<td>274 273</td>
<td>282; 240</td>
<td>16</td>
</tr>
<tr>
<td>7-CH$_3$-6-NH$_2$-</td>
<td>272 270</td>
<td>251; 235; 221</td>
<td>16, 39, 30</td>
</tr>
<tr>
<td>7-(CH$_3$)$_2$C=CHCH$_2$-6-NH$_2$-</td>
<td>274 273</td>
<td>251; 235; 223</td>
<td>16</td>
</tr>
<tr>
<td>7-(CH$_3$)$_2$NCH$_2$CH$_2$-6-NH$_2$-</td>
<td>272 271</td>
<td>248; 236; 223</td>
<td>16</td>
</tr>
<tr>
<td>7-(CH$_3$)$_2$NCH$_2$CH$_2$-6-NH$_2$-</td>
<td>274 272</td>
<td>250; 236; 218</td>
<td>16</td>
</tr>
<tr>
<td>7-(CH$_3$CH$_2$)$_2$NCH$_2$CH$_2$-6-NH$_2$-</td>
<td>274 272</td>
<td>250; 236; 222</td>
<td>16</td>
</tr>
<tr>
<td>9-CH$_3$-6-NH$_2$-</td>
<td>261 262</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>9-CH$_3$-6-NH$_2$-</td>
<td>260 260</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>9-CH$_3$(CH$_3$)CHCH$_2$CH$_2$-6-NH$_2$-</td>
<td>252 264</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>9-CH$_3$CH$_2$-6-NH$_2$-</td>
<td>259 262</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>2-CH$_3$-6-NH$_2$-</td>
<td>266 271</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>8-CH$_3$-6-NH$_2$-</td>
<td>269 266</td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

$^a$ $\lambda$ max and isoabsorptive points given in millimicrons.
EXPERIMENTAL

I. Preparation of the N,N-Diethylaziridinium Chloride

The N,N-diethyl-β-chloroethylamine was prepared at Union College, mp 210-211°C (literature mp 210-211°C) (15). The N,N-diethylaziridinium chloride was prepared by adjusting an approximate 0.1 M solution of N,N-diethyl-β-chloroethylamine hydrochloride in distilled water to pH 12 with sodium hydroxide pellets and 2 N sodium hydroxide. The solution was allowed to stand at pH 12 for 10 min. and then lowered to pH 5 with 10 percent hydrochloric acid.

II. Alkylation of Adenine with Nitrogen Mustard at pH 5.0, 25°C (gradient 3.5 N-6 N Hydrochloric Acid in Two 2-Liter Flasks)

The adenine was obtained from Aldrich Company and N,N-diethyl-β-chloroethylamine hydrochloride was used as the alkylating agent. A solution of 0.127 g (0.074 moles) of mustard hydrochloride in 80 ml of water was changed to N,N-diethylaziridinium chloride as previously described. This solution was added to 0.5 g (.0037 moles) of adenine in 655 ml of distilled water resulting in a final reaction mixture which was $4.96 \times 10^{-3}$ M in adenine and $9.92 \times 10^{-3}$ M in alkylating agent. The solubility of adenine in water is $7 \times 10^{-3}$ M. (1)
The reaction was maintained at pH 5 in an oil bath held at 37°. The pH was checked at different intervals, and adjusted to pH 5 when needed. Because of the stability of the ethyleneimmonium ion, the reaction was not terminated until 91 hours had elapsed. During this time the highest pH recorded was 5.2. Thin layer chromatography was used to follow the reaction.

After the reaction was terminated the solution was flash evaporated. The mixture, 10.4 mg, was then placed on a Dowex 50W-X8 for separation. The ultraviolet data of the fractions, the pH gradient and the absorption ratios are given in (Fig. 1).

As was stated previously, thin layer chromatography of the reaction mixture was taken at frequent intervals. Thin layer plates indicated that there were at least four products besides adenine. (Fig. II)

**THIN LAYER CHROMATOGRAPH OF THE REACTION OF ADENINE WITH NITROGEN MUSTARD**

![Fig. II]
Absorbance Ratios  

Elution Gradient (Normality of HCl)

ION EXCHANGE CHROMATOGRAPH OF THE ALKYLATION OF ADENINE WITH NITROGEN MUSTARD

Absorbance—260 m

Fig. I
From this column one reaction product was separated enough to be identified through the use of the ultraviolet spectrophotometer as the 9-alkylated adenine. (Table II) In order to obtain better resolution of the reaction mixture a gradient system of 1.5 N-7 N hydrochloric acid in two 6-liter flasks was used. However due to mechanical difficulties with the fraction collector no significant data could be obtained despite numerous attempts.

III. Reaction of Adenine with Ethylene Chlorohydrin

The ethylene chlorohydrin, Eastman Kodak Chemical Co., was distilled before being used in the experiment. At first adenine was reacted with ethylene chlorohydrin at various temperatures: 0.1015 g (7.5 x 10^{-4} M) of adenine in 10 ml (1.5 x 10^{-1} M) of ethylene chlorohydrin at 95°C, 0.1025 g (7.6 x 10^{-4} M) of adenine in 10 ml of ethylene chlorohydrin at 131°C, and 0.1030 g (7.6 x 10^{-4} M) of adenine in 10 ml of ethylene chlorohydrin at 76°C. These reactions were terminated after two days and were followed at various intervals with thin layer chromatography. (Fig. III)

The reaction at 76°C was then run on a larger scale with 1.00 g (7.4 x 10^{-3} M) of adenine added to 100 ml (1.5 M) of ethylene chlorohydrin, the molar ratio of the reactants being 1:2000. This reaction was terminated after eighteen hours and the reaction solution was flash evaporated. The
# TABLE II

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>pH</th>
<th>( \lambda_{\text{max}} ) ( \Delta )</th>
<th>( \lambda_{\text{min}} ) ( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-( \beta )-diethy laminoethyladenine hydrochloride</td>
<td>2</td>
<td>259</td>
<td>258</td>
<td>.28</td>
</tr>
<tr>
<td>9-( \beta )-diethy laminoethyladenine hydrochloride</td>
<td>11</td>
<td>260</td>
<td>261</td>
<td>.18</td>
</tr>
<tr>
<td>Ref. 21</td>
<td>2</td>
<td>259</td>
<td>258</td>
<td>227</td>
</tr>
</tbody>
</table>

- These values are in millimicrons
- From a family of curves obtained at pH values of 1, 2, 3, 10, 11, and 12.
Fig. III

THIN-LAYER CHROMATOGRAPHS OF THE REACTION OF ADENINE WITH ETHYLENE CHLOROHYDRIN

Reaction at 131°C

After 1 1/2 hrs.  After 2 1/2 hrs.

Reaction at 95°C

After 1 1/2 hrs.  After 2 1/2 hrs.

Reaction at 76°C

After 1 1/2 hrs.  After 2 days
reaction mixture gave melting points at both 184°C and 235°C. Since adenine melts at a temperature greater than 300°C, this indicated that a reaction had taken place.

A small column of Dowex 50W-X8, 13 cm in length and 1/2 cm in diameter, was made to determine the gradient system to be used. A small sample was placed on the column and then the column was washed with increasing normality of hydrochloric acid. The effluent from the column was collected in tubes and checked for spectroscopic absorption at 260 nm. By this means it was determined that a gradient system run from 1.5 N to 4 N hydrochloric acid would be sufficient to separate the products.

A larger column was prepared as previously mentioned and a solution of the reaction mixture (1.4 g or approximately 1.3 percent column capacity) was placed on the column. The ultraviolet data for the fractions, the pH gradient, and the absorption ratios of the chromatogram are seen in Fig. IV. Details of the chromatographic run are given in Table III.

The ultraviolet spectra of peak A at pH 2 and 11, as well as the isoabsorption points obtained for a family of curves recorded at pH 1, 2, 3, 10, 11 and 12 compare favorably with those of other 3-substituted adenines. This is shown in Table IV. Further confirmation of the 3-product was obtained when the 3-β-hydroxyethyladenine was subjected to alkali conditions (1 N sodium hydroxide, 100°, 1 hr.) used by Elion (19) to convert 3-methyladenine to 3-methylhypoxanthine. The ultra-
<table>
<thead>
<tr>
<th>Peak</th>
<th>A260 at Max</th>
<th>Fraction Number at Max</th>
<th>Gradient Conc. of HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.0</td>
<td>45</td>
<td>1.7N→2.4N</td>
</tr>
<tr>
<td>B</td>
<td>37.27</td>
<td>77</td>
<td>2.4N→3.35N</td>
</tr>
<tr>
<td>C</td>
<td>2.15</td>
<td>151</td>
<td>3.8N</td>
</tr>
</tbody>
</table>
### TABLE IV

**Ultraviolet Spectral Identification of 3-β-hydroxyethyladenine**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>pH</th>
<th>A max</th>
<th>A min</th>
<th>280/260</th>
<th>Isoabsorptive Points a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-β-hydroxyethyladenine hydrochloride</td>
<td>Peak A</td>
<td>2</td>
<td>275</td>
<td>237</td>
<td>1.6</td>
<td>284; 242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>275</td>
<td>246</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>3-β-diethylaminocethyladenine hydrochloride</td>
<td>Ref. 21</td>
<td>1.8</td>
<td>276</td>
<td>236</td>
<td>1.5</td>
<td>287; 244</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.8</td>
<td>274</td>
<td>245.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>3-Ethyladenine</td>
<td>Ref. 16</td>
<td>1</td>
<td>275</td>
<td>---</td>
<td>---</td>
<td>286; 241</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>273</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

a—Values in millimicrons (mμ)
b—From a family of curves obtained at pH values of 1, 2, 3, 10, 11, and 12.
violet data obtained (pH 1, \( \lambda_{\text{max}} = 259 \text{ m}\mu \); pH 11, \( \lambda_{\text{max}} = 263 \text{ m}\mu \)) was similar to that given by Elion (19) for 3-methylhypoxanthine (pH 1, \( \lambda_{\text{max}} = 253 \text{ m}\mu \); pH 11, \( \lambda_{\text{max}} = 265 \text{ m}\mu \)).

The ultraviolet spectra at pH 2 and pH 11 for peak B are believed to be those for the 1-\( \beta \)-hydroxyethyladenine and this is compared with literature values for other 1-alkylated adenines in Table V. The ultraviolet spectra at pH 2 and pH 11, and isoabsorption points for peak C indicate that the material is adenine. It should be noted that in all chromatograms from the Dowex 50W-X8 column adenine was observed to be eluted in the pH range of 3.1-3.8.

An attempt was made to prepare 1-\( \beta \)-chloroethyladenine from 1-\( \beta \)-hydroxyethyladenine hydrochloride with thionyl chloride (32) (34) at various temperatures without results. Peak B was flash evaporated and 0.3 g of the 1-\( \beta \)-hydroxyethyladenine hydrochloride was added to 25 ml of thionyl chloride at 75°C. The solution was observed at various intervals for three days. A sample was taken from the mixture. It was checked on the Beckman spectrometer at wave lengths of 250, 260, 280, and 290 m\( \mu \), and gave no absorption readings.

Another approach to chlorination was then tried. A sample of 0.1 g of 1-\( \beta \)-hydroxyethyladenine hydrochloride was added to 0.3 g of phosphorous pentachloride at 50°C in
# TABLE V

Ultraviolet Spectral Identification of 1-\(\beta\)-Hydroxyethyladenine

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>pH</th>
<th>(\lambda_{\text{max}}^a)</th>
<th>(\lambda_{\text{min}}^a)</th>
<th>(\frac{A_{280}}{260})</th>
<th>Isoabsorptive Points(^a) (\frac{A_{260}}{260})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(\beta)-Hydroxyethyladenine Hydrochloride</td>
<td>Peak B</td>
<td>2</td>
<td>260</td>
<td>233</td>
<td>.39</td>
<td>266; 241</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>268.5</td>
<td>238</td>
<td>.61</td>
<td></td>
</tr>
<tr>
<td>1-Methyladenine</td>
<td>Ref. 9, 29</td>
<td>acid</td>
<td>259</td>
<td>-</td>
<td>-</td>
<td>263; 237</td>
</tr>
<tr>
<td></td>
<td></td>
<td>basic</td>
<td>270</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1-Benzyladenine</td>
<td>Ref. 41</td>
<td>1</td>
<td>260.5</td>
<td>235</td>
<td>-</td>
<td>263.5; 237</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>271.5</td>
<td>241.5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\(a\)—Values in millimicrons  
\(b\)—From a family of curves obtained at pH values of 1, 2, 3, 10, 11, and 12.
20 ml of phosphorous oxychloride, and after four hours a sample was taken. Its ultraviolet spectrum was observed, and no appreciable absorption was recorded. Similar experiments at temperatures of 70°, 80°, and 105°C gave the same results.

The 1-β-hydroxyethyladenine was placed in a basic solution of pH 11 at room temperature and its ultraviolet spectrum was recorded after 2, 3, 7, and 14 days at both pH 11 and pH 2. At each time interval and value of pH, no change in the ultraviolet spectrum of 1-β-hydroxyethyladenine was recorded. The absorbency ratios at A250/A260, A280/A260, and A290/A260 were also recorded. These ratios were the same as those recorded for 1-β-hydroxyethyladenine.

A basic solution pH 12 of 1-β-hydroxyethyladenine was refluxed for 70 min., and the ultraviolet spectra was recorded at both pH 12, and pH 2. Both spectra were identical with the ones taken before the sample was refluxed. The absorbency ratios at A250/A260, A280/A260, and A290/A260 were also recorded. These ratios were the same as those for 1-β-hydroxyethyladenine. The method of thin layer chromatography previously described was used before and after reflux and in both cases one spot with an Rf value of .31 was found.

IV. NMR Data from the Ethylene Chlorohydrin Adenine Reaction Mixture

The NMR spectral data obtained are tabulated in Table VI, and the spectra obtained for adenine and the reaction mixture are given in Figs. V, VI and VII.
TABLE VI

Nuclear Magnetic Resonance Spectral Data
for Alkylated Adenine\(^{(a)}\)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Peak</th>
<th>Area</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>8.1 (broad singlet)</td>
<td></td>
<td>C(_2)-H, C(_8)-H</td>
</tr>
<tr>
<td>Ethylene chlorohydrin and adenine reaction mixture</td>
<td>8.73 (singlet)</td>
<td>1</td>
<td>C(_2)-H or C(_8)-H</td>
</tr>
<tr>
<td></td>
<td>8.64 (singlet)</td>
<td>1</td>
<td>C(_2)-H or C(_8)-H</td>
</tr>
<tr>
<td></td>
<td>8.38 (singlet)</td>
<td>2</td>
<td>C(_2)-H or C(_8)-H</td>
</tr>
<tr>
<td></td>
<td>4.1 (multiple)</td>
<td></td>
<td>CH(_2) (methylene)</td>
</tr>
<tr>
<td></td>
<td>4.65 (multiple)</td>
<td></td>
<td>CH(_2) (methylene)</td>
</tr>
<tr>
<td>Ethylene chlorohydrin and adenine reaction mixture(^{(b)})</td>
<td>9.6 (singlet)</td>
<td></td>
<td>C(_2)-H or C(_8)-H</td>
</tr>
<tr>
<td></td>
<td>8.95 (multiple)</td>
<td></td>
<td>C(_2)-H or C(_8)-H</td>
</tr>
<tr>
<td></td>
<td>4.8 (multiple)</td>
<td></td>
<td>CH(_2) (methylene)</td>
</tr>
<tr>
<td></td>
<td>4.25 (multiple)</td>
<td></td>
<td>CH(_2) (methylene)</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Solvent was D\(_2\)O and peaks are tabulated in parts per million (ppm, \(\delta\)) relative to tetramethylsilane.

\(^{(b)}\) Solvent was D\(_2\)O and HCl.

All samples were crystalizing while spectra were being recorded.
The C₂ and C₈ hydrogen of adenine fall in the region of 8.1—8.2. (14) These hydrogen shift upfield or downfield with different substituted adenines. The shifts in the C₂ and C₈ peaks of the reaction mixture indicate a substituted adenine. The single peak at 8.38 (Fig. VI) with an area equivalent to two protons could indicate that a 3-alkylated adenine was formed. (21) Jardetsky (23) stated that low pH causes a shift in the C₂ and C₈ protons of adenosine downfield. This was noted when the spectrum of the mixture was taken. (Table VI) The higher field multiples in Figs. VI and VII are attributed to the methylene protons. The two multiples are different than the single multiple obtained around 3.8 for that of ethylene chlorohydrin. (45)
DISCUSSION

The greatest number of 1-substituted adenines have been methylated adenines. The early studies dealt with the alkylation of adenosine in a basic solution with dimethyl sulfate, but no identification of products was given. (8) (31) In 1960 Brookes (9) was the first to synthesize and identify 1-methyladenine. He also observed its rearrangement to 6-methylaminopurine in base and its degradation to 5-aminoimidazole-4-N-methylcarboxamidine in acid. Jones (24) converted 2'-deoxy-1-methyladenosine hydroiodide to 1-methyladenine at pH 8.5 with aqueous ammonium hydroxide and found no evidence of rearrangement. However 1-methyladenosine was converted to the 6-methyladenosine when heated for 75 min. at 100°C in 0.025 N sodium hydroxide. Montgomery (38) made the 1-methyladenine from 1-methyl-9-propynyladenine under basic conditions and found no 6-substituted adenine. Other references (33) (41) dealing with the making of 1-methyladenine showed no rearrangement under basic conditions, although Leonard (29) did notice 1-benzyladenine rearranged to the 6-substituted adenine upon refluxing in 0.2 N sodium hydroxide for 70 min.

Kaplan (26) alkylated adenosine with ethylene oxide and obtained only one product, 1-β-hydroxyethyladenosine,
which was isolated as the perchlorate salt, and purified by recrystalization. He stated that at pH 9 and 0°C the 1-product slowly rearranged to the 6-substituted adenine. Gaucher, (21) by the alkylation of adenine with N,N-diethyl-β-chloroethylamine at pH 7, found the 6-substituted adenine. He postulated that this product was formed by the rearrangement of the 1-substituted adenine. It could equally well have been explained on the basis of direct alkylation at the 6-N position. If it could be established that the 1-product was stable under the reaction conditions, the direct alkylation concept would seem more likely and the sensitivity of the transition state to the charge on the nucleophile might seem to be important. Nevertheless, in summary of the above publications it can be seen that the 1-substituted adenine rearranged in alkaline solution to the 6-substituted adenine only under extreme conditions while rearrangement for the 1-substituted adenosine occurred at milder conditions.

The rearrangement of the 1-substituted adenine has been suggested to have a similar type rearrangement as seen by pyrimidines, (10) (11) (12) that is an opening of the pyrimidine ring from attack by hydroxide ion and a reclosing to form the 6-product.

For the 1-β-hydroxyethyladenosine Kaplan suggested the following rearrangement in alkaline solution. (26) (Reaction I)
Lawley, after finding that 1-methyladenine had pK\textsubscript{a} values of 7.2 and 11.0 with the former value assigned to the dissociation of a proton from the amino group and the latter from imidazole, formulated the following procedure for the compound in acid and base. (9) (Reaction II)
Gaucher, in his doctoral thesis, surmised the rearrangement of the 1-β-diethylaminoethyladenine to the 6-β-diethylaminoethyladenine by a similar procedure. (21)

The experimental data obtained from the 1-β-hydroxyethyladenine in basic solution indicated that the compound was quite stable to rearrangement. This fact, along with Kaplan's (26) report of the instability of the 1-β-hydroxyethyladenosine, suggested that the hydrogen in the 9-position of adenine might have an important function in the stability of the 1-substituted adenine. This was further substantiated by the fact that Jones (24) noticed the rearrangement of 1-methyladenosine in alkaline conditions and Montgomery (38) noticed that no rearrangement occurred in alkaline conditions for 1-methyladenine. The loss of the proton at the 9-position on 1-methyladenine could stabilize the positive charge at the 1-position while for 1-methyladenosine with the 9-position substituted this could not occur. (Reaction III)

\[
\begin{align*}
\text{R} & = \text{Ribose} \\
\text{Me} & = \text{Methyl}
\end{align*}
\]

Reaction III

\[
\begin{align*}
\text{R} & = \text{Ribose} \\
\text{Me} & = \text{Methyl}
\end{align*}
\]
Also since the 1-\(\beta\) -hydroxyethyladenine was quite stable, there is strong indication that the activation energy may play an important role in the substitution of adenine.

Further work, such as obtaining an elemental analysis, and isolating products obtained under strong acid conditions, could be helpful in substantiating to a greater degree the 1-\(\beta\) -hydroxyethyladenine. Also from the 1-\(\beta\) -hydroxyethyladenine one could make the 1-\(\beta\) -diethylaminoadenine. A possible means would be by tosylating the alcohol (\(^{17}\)) and then reacting this product with diethylamine.

In the alkylation of adenine with nitrogen mustard it was assumed that the nitrogen mustard reacts through a cyclic three-membered ring intermediate. The proof for this intermediate was observed through proton nuclear magnetic resonance spectrum of N,N-diethyl-\(\beta\) -chloroethylamine. \(^{(44)}\) Superimposed on the quartet assigned to the two methylene groups from the two ethyl groups in the three-membered aziridinium ring was a sharp singlet indicative of four equivalent protons. This singlet was subsequently assigned to the four protons of the two methylene groups found in the aziridinium ring. It has also been found, through kinetic studies of the N,N-diethylnitrogen mustard, that the cyclic ethyleneimmonium ion was unusually stable.

The experimental work with the alkylation of adenine with N,N-diethyl-\(\beta\) -chloroethylamine was carried out at pH 5.
The purpose of the acid solution was to hinder any rearrangement. Note that the rearrangement was presumably a cleavage of the pyrimidine ring and subsequent ring enclosure depending upon the basicity of the free amino group. Thus in an acid media with the amino group protonated rearrangement would be hindered. If the pyrimidine ring should be broken the ring closure would be impossible. The linear gradient system from pH 1.5—pH 7 should be able to separate the products from the alkylation of adenine at pH 5. It would indicate whether the 1-substituted adenine, 6-substituted adenine, side products or any combination of all three were formed. If the 1-substituted adenine was formed it could be isolated and further reactions carried out at different pH ranges to determine its stability.

The different ultraviolet spectra obtained at various pH's are invaluable in determining the different substituted adenines. These differences arose from the fact that protons were added or lost at various pH's on the different ionic species. These species depended upon the pKₐ value of the molecule and the pH of the solution. The shift in maximum absorbency due to different species formed at different pH's was useful in determining the exact substituted adenine. Also if the spectral curves of two species overlap, this point may be called "isoabsorptive point" or point of equal absorption. (21) However, if the two species were inter-
convertible so that the total quantity was constant and an isoabsorptive point would have an absorbency that depended only on the total number of equivalents of the two species, and then it is called an isobestic point. (20) (36) Furthermore, the spectra of the two species, taken over the pH range in which they occur, would have the same isobestic points. For example: adenine has pK\textsubscript{a} values of 4.3 and 9.8; (2) only neutral and cationic species are present between pH 1 and 7, while only neutral and anionic species exist between pH 7 and 13. Thus the crossover points from the spectra of the experimental pH's 2 and 11 are isoabsorptive points and not isobestic points.
SUMMARY

The 1-β-hydroxyethyladenine was prepared from the reaction between ethylene chlorohydrin and adenine at 76°C. The 1-substituted adenine was separated from the reaction mixture by use of a linear gradient ion exchange chromatography. The stability of the 1-β-hydroxyethyladenine was checked under alkaline conditions and it was found to be stable.

The stability of the 1-substituted adenine indicated the improbability that the 6-substituted adenine could be formed from the rearrangement of the 1-substituted adenine. It could also indicate that adenine could be alkylated by direct alkylation and the sensitivity of the transition state to the charge on the nucleophile might be important. Thus a guideline could be established predicting the results that could be obtained from the alkylation of adenine and guanine with different alkylating agents.
2. Ibid; p. 441.
3. Alm, R.S., R.J.P. Williams, and A. Tiselius; Acta Chem. Scand., 6, 826 (1952).


45. Varian Assoc.; Nuclear Magnetic Resonance at Work, Series, Number 15.
