SYNTHESIS OF SOME AMINE STEROID DERIVATIVES

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SYNTHESIS OF SOME AMINE STEROID DERIVATIVES

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CHAPTER I

INTRODUCTION

In 1951, Z. M. Bacq (5) discovered that the compound, cysteamine (2-mercaptoethylamine) possessed antiradiation properties. When the hydrochloride of this compound was injected into mice shortly before x-irradiation, it was found to afford them protection from the harmful effects of radiation doses up to 1200 roentgen. Subsequent studies with compounds related to cysteamine revealed that for the most effective antiradiation properties, a drug must contain both a free amino group and a mercapto group which are not separated by more than three methylene groups (5).

Unfortunately, cysteamine was found to possess two undesirable properties. Z. M. Bacq (1) discovered that mice, irradiated one hour after the injection of cysteamine, behaved like the control mice. Therefore, mercaptoethylamine evidently had been metabolized rapidly in the body. Also, it was reported by J. R. Maisin and D. G. Doherty (5) that the toxicity of cysteamine probably would be too great for human protection at high radiation levels.

In 1961, L. Goodman and J. E. Christensen (3) proposed that if a mercaptoethylamine moiety were to be incorporated into some significant physiological molecule, the ratio of
the protective effect to toxicity might be considerably improved over that of cysteamine. Through their work, they have successfully incorporated the mercaptoethylamine group into a derivative of D-allose called 3-amino-1,6-anhydro-2, 3-dideoxy-2-mercapto-D-allopyranose but have not yet reported the effectiveness of the drug.

It was decided that if the mercaptoethylamine moiety could be successfully incorporated with a molecule of cholestane, both of the undesirable properties of cysteamine might be avoided or perhaps decreased enough to be negligible. Cholestane, being a derivative of cholesterol which is a normal constituent of the human body, might enable the proposed compound to display a lower toxicity than cysteamine. Also, the hydrocarbon nature of the cholestane molecule might slow its elimination through the urine and, therefore, afford greater periods of protection to the individual.

Initially, the preparation of 3β-(2-mercaptoethylamino)-cholestane was set as the goal of this thesis project. The
first step in the synthesis of 3β-(2-mercaptoethylamino)-cholestane was the catalytic hydrogenation of purified, commercial cholesterol to 3β-hydroxycholestane. The 3β-hydroxycholestane was oxidized by the action of dichromic acid to cholestane-3-one. In the structural formulas that follow, the 3β-cholestanyl and the 3—cholestanyl groups are represented by R.

\[
\text{R-OH} \xrightarrow{\text{sodium dichromate}} \xrightarrow{\text{acetic acid}} \text{R}=\text{O}
\]

The ketone was then converted to cholestane-3-one oxime by the action of hydroxylamine.

\[
\text{R}=\text{O} \xrightarrow{\text{hydroxylamine hydrochloride}} \xrightarrow{\text{sodium bicarbonate}} \text{R}=\text{NOH}
\]

The next step, the conversion of the oxime to 3β-amincholestane, had been carried out previously by R. D. Haworth and D. P. Dodgson (1). They allowed sodium and ethanol to react with the oxime compound to obtain an amine mixture which possessed a melting point range of 90 to 120°C. The crude mixture was acetylated by boiling acetic anhydride, and the

\[
\text{R}=\text{NOH} \xrightarrow{\text{sodium}} \text{R}=\text{NH}_2
\]

subsequent alcohol extract of the reaction mixture gave the 3β-acetamidecholestane with a melting point range of 245 to 246°C. The mother liquor gave an isomer in low yield which melted at 213°C.
C. W. Shoppee and his associates (6) had produced $3\alpha$-aminocholestane by the hydrogenation of cholestane-3-one with acetic acid, hydrogen, and platinum. The acetyl derivative of the $\alpha$ isomer possessed a melting point of 216°C. It was evident, by comparison of $\alpha$ isomer's melting point with that of the unknown isomer isolated by Bodgson and Haworth, that these compounds were identical. Therefore, the reaction of the oxime with sodium and ethanol gives predominantly the $\beta$ isomer, together with a very minute amount of the $\alpha$ isomer.

The purification of the $3\beta$-aminocholestane was a problem which Robert Havranek and Norman J. Doorenbos (4) described as an arduous and lengthy procedure involving recrystallization and chromatographic techniques. Pure samples were obtainable only in a small yield. R. Havranek and N. Doorenbos (4) stated that these amines probably formed intermolecular complexes with steroid impurities which are difficult to separate. This type of complexing has been observed frequently with steroid alcohols.

The next two steps in the synthesis of the mercaptoamine had been carried out by Robert Havranek and Norman J. Doorenbos (4). They reacted the impure $3\beta$-aminocholestane with 2-chloroethanol in benzene to prepare $3\beta$-(2-hydroxyethylamino)-cholestane with a melting point range of 172.5 to 173.5°C.

\[
\begin{align*}
R-NH_2 & \quad 2\text{-chloroethanol} \\
\text{benzene} & \quad \text{sodium carbonate}
\end{align*}
\Rightarrow R-NHCH_2CH_2-OH
\]

\[
\begin{align*}
R-NH_2 & \quad 2\text{-chloroethanol} \\
\text{benzene} & \quad \text{sodium carbonate}
\end{align*}
\Rightarrow R-NHCH_2CH_2-OH
\]
The 3β-(2-hydroxyethylamino)cholestane was treated with thionyl chloride to produce 3β-(2-chloroethylamino)cholestane hydrochloride, possessing a melting point of above 300°C with decomposition.

\[
\begin{align*}
R-NHCH_2-CH_2-OH & \xrightarrow{\text{thionyl chloride, benzene}} R-NHCH_2CH_2-Cl \\
\end{align*}
\]

The final step in the synthesis was that of reacting the chloroamine with sodium hydrosulfide to produce 3β-(2-mercaptoethylamino)cholestane.

\[
\begin{align*}
R-NHCH_2CH_2-Cl & \xrightarrow{\text{sodium hydrosulfide, ethanol, sodium bicarbonate}} R-NHCH_2CH_2-SH \\
\end{align*}
\]

There is, however, another hypothetical way of producing the mercaptoamine. The 3β-aminocholestane could, upon reacting with 2-mercaptoacetic acid, give N-(3β-cholestanyl)-mercaptoacetamide. This compound then could be reduced with lithium aluminum hydride to give 3β-(2-mercaptoethylamino)cholestane.

\[
\begin{align*}
R-NH_2 & \xrightarrow{\text{mercaptoacetic acid, benzene}} R-NHCOCH_2-SH \\
R-NHCOCH_2-SH & \xrightarrow{\text{lithium aluminum hydride, tetrahydrofuran}} R-NHCH_2CH_2-SH \\
\end{align*}
\]
CHAPTER I BIBLIOGRAPHY


CHAPTER II

EXPERIMENTAL

Preparation of 3β-Hydroxycholestane

In a 500 milliliter Phillips beaker were placed 19.3 grams (0.05 mole) of commercial cholesterol (recrystallized from pure ethyl acetate) and 260 milliliters of ethyl acetate. The mixture was heated on a steam bath to effect solution and then was transferred to a hydrogenation bottle. To the hot solution was added one drop of perchloric acid (60 per cent Mallinckrodt) as a promoter and 0.4 gram of platinum oxide as a catalyst. The hydrogenation bottle was then set in a Parr hydrogenation apparatus (model-ca) and the solution placed under a pressure of twenty pounds per square inch. The apparatus was allowed to run until the hydrogen pressure had dropped five pounds per square inch. This took approximately one hour. The bottle was removed from the Parr apparatus, and two drops of a 50 per cent solution of sodium hydroxide was added to neutralize the perchloric acid. The solution was filtered, and subsequent refrigeration of the filtrate for twelve hours caused the first crop of 3β-hydroxycholestane to appear. These crystals were separated from the filtrate by suction filtration. The remaining filtrate was then
evaporated to dryness and the resulting residue recrystallized from hot methanol to give the second crop of \( 3\beta \)-hydroxycholestane. This product was dried in a vacuum desiccator over calcium chloride for twelve hours.

The total amount of \( 3\beta \)-hydroxycholestane produced was sixteen grams, representing an 82.5 per cent yield. The melting point range of the product was 144° to 145°C.

**Preparation of Cholestane-3-one**

Twenty-six and one-tenth grams (0.0672 mole) of \( 3\beta \)-hydroxycholestane were suspended in 155 milliliters of glacial acetic acid. A hot solution of twenty-seven grams of sodium dichromate dihydrate, dissolved in 170 milliliters of glacial acetic acid, was slowly stirred into the suspension. The mixture was heated on a steam bath for five minutes and then allowed to set overnight. The next morning twenty milliliters of cold, distilled water was added to the mixture. The crude product was obtained by suction filtration and washed on filter paper four times, each time with 200 milliliters of cold, distilled water. The product was recrystallized from 200 milliliters of 4:1 hot absolute ethanol-acetone solvent, and the resulting crystals were dried in a vacuum desiccator over calcium chloride.

Seventeen and twenty-nine hundredths grams of cholestane-3-one, representing 66.7 per cent yield, was produced. The product's melting point range was 129° to 130°C.
Preparation of Cholestane-3-one Oxime

In a one-liter reaction flask, fitted with a reflux condenser, a magnetic stirrer, and a heating mantle, were placed five grams (0.0129 mole) of cholestane-3-one, 500 milliliters of absolute ethanol, five grams of hydroxylamine hydrochloride, and five grams of sodium bicarbonate. The reaction mixture was refluxed and stirred for two hours. The mixture was then filtered hot and the precipitate washed with 100 milliliters of boiling, absolute ethanol. To the hot filtrate was slowly added, while stirring, 250 milliliters of boiling water. The suspension was allowed to cool slowly to room temperature and then was placed in an ice bath. The cold mixture was filtered by suction filtration, and the precipitate was dried in a vacuum desiccator containing calcium chloride.

Five and eight-hundredths grams of cholestane-3-one oxime, representing a 98.1 per cent yield, was obtained. The compound's melting point range was 199.0°C to 200.5°C.

Preparation of 3β-Aminocholestan

To a two-liter flask, fitted with a reflux condenser, a heating mantle, and a magnetic stirrer, were added ten grams (0.0249 mole) of cholestane-3-one oxime and 700 milliliters of n-amyl alcohol. A slow stream of dry nitrogen was blown over this mixture for the total length of this reaction. The
flask was heated until the oxime dissolved in the amyl alcohol. The mixture was cooled at room temperature and placed in an ice bath. Then, sixty grams of sodium was immediately added to this mixture. As soon as the sodium-amyl alcohol reaction had decreased in intensity, the mixture was heated again and refluxed for one hour. Four hundred milliliters of hot water was added to the reaction mixture, and the n-amyl alcohol was distilled off. Since the water was also distilled with the n-amyl alcohol, and since the water suspension of the 3\(\beta\)-aminocholestane was desired, it was necessary to add 600 milliliters of hot water during the reaction. After distillation, the reaction flask, containing a nitrogen atmosphere, was sealed and refrigerated overnight. The next day the crude 3\(\beta\)-aminocholestane, insoluble in water, was removed easily by suction filtration. All attempts to purify the compound were of no avail.

Nine and fifty-five one hundredths grams of the crude product, amounting to a 99 per cent yield, was obtained. The crude material's melting point range was 75° to 110° C.

Preparation of 3\(\beta\)-(2-Hydroxyethylamino)cholestan e

Three grams (approximately 0.00775 mole) of crude, 3\(\beta\)-aminocholestan e were dissolved in sixty milliliters of dried benzene. To this was added 7.95 grams (0.0075 mole) of anhydrous sodium carbonate. The reagents were mixed in a one-liter flask fitted with a reflux condenser, a heating
mantle, and a magnetic stirrer. The mixture was heated to refluxing with rapid stirring, and six grams (0.0075 mole) of 2-chloroethanol was added in one and one-half gram increments over the first three hours of refluxing. The reaction mixture was refluxed approximately twelve hours. Then, the mixture was cooled, and the sodium carbonate and sodium chloride precipitates were removed by gravity filtration. The filtrate was placed in a Borg revolving evaporator and the benzene removed under reduced pressure and slight heating. The resulting brown residue was mixed with approximately thirty milliliters of dry ethyl ether. The residue turned white in color. This was the crude, 3β-(2-hydroxyethylamino)cholestane. The mixture was suction-filtered, and the brown-colored ether filtrate was kept because it contained unreacted 3β-aminocholestane. However, this product was difficult to recover and was so slight in amount that it was discarded. The white precipitate was dissolved in hot, absolute ethanol and simultaneously treated with charcoal and potassium carbonate. Then, the hot solution was filtered by suction filtration, and the precipitate recrystallized from hot, 4:1 acetone-ethanol solvent.

After refrigeration and suction filtration, 0.85 gram of 3β-(2-hydroxyethylamino)cholestane was obtained. This amounted to a 25 per cent yield. The pure compound's melting point range was 171.0°C to 172.0°C. An infrared analysis was performed,
and pertinent absorption bands were found at 1050, 3050, 3300 and 1700 cm\(^{-1}\).

**Preparation of 3\(\beta\)-(2-Chloroethylamino)-cholestan
cetane Hydrochloride**

In a one-necked, 500 milliliter flask, fitted with a reflux condenser, a heating mantle, and a stirrer, were placed 0.81 gram (0.00168 mole) of slightly impure 3\(\beta\)-(2-hydroxy-
ethylamino) cholestane and forty milliliters of freshly distilled benzene. The mixture was heated to effect solution, and 0.81 gram (0.0068 mole) of thionyl chloride was added. A white precipitate immediately appeared which was probably the benzene-insoluble amine hydrochloride salt. The reaction mixture was refluxed for one and one-half hours. It was then cooled and placed in a Borg revolving vacuum evaporator which removed the benzene and excess thionyl chloride. The resulting brown-orange residue was dissolved in hot, absolute ethanol and treated with twelve grams of activated charcoal. The mixture was then filtered hot, and the resulting filtrate was a light orange-colored liquid. Overnight refrigeration caused the appearance of a crop of grayish-white, fine needles. Successive concentration of the filtrate produced more of the product. The crude product was dissolved once more in hot ethanol and was treated with three grams of charcoal in an attempt to purify the compound further. The resulting filtrate was again light orange in color, and the subsequent crystals again were grayish-white in color.
The total amount of crude 3β-(2-chloroethylamino)-cholestane hydrochloride produced was 0.08 gram, amounting to a 8.74 per cent yield. The compound did not melt at the reported temperature of above 300°C but melted over a range from 260° to 280°C. Subsequent infrared analysis revealed pertinent absorption bands at the following frequency ranges of 3200 to 3350, 2250 to 3000, 1400 to 1470, and 1560 to 1580 cm⁻¹.

Attempted Preparation of N-(3β-Cholestanyl)-mercaptoacetamide

In a one-liter reaction flask, fitted with a reflux condenser, a Dean-Stark trap, a heating mantle, and a calcium chloride drying tube, were placed 500 milliliters of dried benzene and 3.16 grams of Matheson's 70 per cent grade mercaptoacetic acid (actually containing 2.21 grams of mercaptoacetic acid or 0.024 mole). This solution was heated to refluxing, and the 0.95 milliliter of water was caught in the Dean-Stark trap. The water was removed from the trap, and 9.29 grams (0.024 mole) of impure 3β-aminocholestane was added to the reaction flask. A white precipitate immediately formed. The mixture was refluxed for five days. The total amount of water caught in the Dean-Stark trap was 0.1 milliliter. The mixture was filtered by suction filtration, and the precipitate was dried over calcium chloride in a vacuum desiccator. The white precipitate burned with a yellow, smoky flame and was also found to be insoluble in water. This solid
weighed 7.85 grams and melted above 220°C with the evolution of gas. Infrared spectra analysis revealed pertinent absorption bands at 2350 to 3600, 1630 to 1680, 1550 to 1600, and 1300 to 1430 cm$^{-1}$.

The benzene filtrate was evaporated to dryness, and an attempt to recrystallize the white residue from hot, ethyl acetate proved to be fruitless. The residue was removed from the ethyl acetate by suction filtration and dried in a vacuum desiccator over calcium chloride. The weight of the dry compound was 0.54 gram. The compound started melting at 151°C, evolved gas around 230°C, and decomposed at 275°C. Subsequent infrared data showed the compound to have absorption bands at 2450 to 3500, 1300 to 1430, and 1480 to 1680 cm$^{-1}$.

**Attempted Preparation of 3β-(2-Mercaptoethylamino)cholestane**

In a two-necked, 300-milliliter flask, fitted with a calcium chloride drying tube, a magnetic stirrer, and a glass tube for bubbling through hydrogen sulfide, were placed seventy milliliters of dry, absolute ethanol and 2.3 grams (0.1 gram atom) of sodium. After the sodium had dissolved in the alcohol, hydrogen sulfide was bubbled through the solution for about one hour. Then, 0.08 gram (0.00016 mole) of impure, 3β-(2-chloroethylamino)cholestane hydrochloride was dissolved in the mixture. The flakes of the chloroamine turned black upon entering the solution and then dissolved. The mixture was stirred rapidly for about one hour. During this time,
the solution became a light-brown color. The solution then was stirred for approximately twelve hours more. At the end of this time, 100 milliliters of water was added to the solution. The solution became a translucent-green color, except for a few small, black particles which were removed by suction filtration. These particles could not be analyzed because only a very minute amount of them were present. To the filtrate was added 100 milliliters of benzene and one gram of sodium bicarbonate. A small number of bubbles were produced. At the interface, between the water solution and the benzene solution, appeared some small, black particles. There were enough present to undergo infrared analysis. The infrared spectrum showed absorption peaks at frequencies of 3200, 1400, and 1690 cm\(^{-1}\). This compound was labeled A.

The benzene layer was removed and was evaporated to dryness by the use of a Borg vacuum evaporator. The residue was a green, viscous material. Attempts to dry this substance failed. The compound was subjected to infrared analysis, using a Nujol mull. The resulting spectrum revealed absorption of 3300, 3000, 2900, to 2600, 1610, 1450, 1375, 1250, 1020, and 790 cm\(^{-1}\). This compound was labeled B.
Methods of Analysis

Infrared

All the infrared spectra, with the exception of one Nujol mull, were obtained from KBr discs containing the solid samples. The sample discs were prepared by grinding one milligram of the product with 300 milligrams of KBr and subjecting them to 20,000 pounds of pressure per square inch in a hydraulic press. A Perkins-Elmer Model 21 Infrared Spectrophotometer was used to make the spectrograms.

Melting Points

Melting points were determined by the use of a mineral oil-capillary melting point apparatus.
CHAPTER III

RESULTS AND CONCLUSIONS

There were numerous and varied difficulties discovered in this investigation. The most prominent problems were associated with the sodium-ethanol reduction of cholestan-3-one oxime to \(3\beta\)-aminocholestan. The reaction very seldom proceeded in the same manner, and the products were usually varied in physical and chemical properties. Along with the steroid-amine impurities mentioned by Havrenek and Doorenbos (3) and the contaminating presence of the \(\alpha\) isomer (2), other major contaminants were also in evidence.

The first attempted sodium-ethanol reduction of the oxime resulted in an impure \(3\beta\)-aminocholestan which possessed a melting point range of \(80^\circ\) to \(104^\circ\)C. The impure compound successfully reacted with 2-chloroethanol to produce pure, \(3\beta\)-(2-hydroxyamino)cholestan. Unfortunately, however, a majority of the subsequent attempts to produce even the impure \(3\beta\)-aminocholestan were rather fruitless. In some cases, a yellow, viscous material was obtained which could not be purified by benzene, ethanol, or water extractions. At other times, the reaction product possessed an opaque melting point which was indicative of inorganic contaminates. Subsequent extractions with benzene were to no avail.
An infrared analysis was performed on a sample of 3β-(2-hydroxyethylamino)cholestane, possessing a melting point range of 166° to 171°C. The resulting spectrum revealed a medium absorption band at 1050 cm⁻¹ which indicated the presence of a C-O bond. The appearance of a weak absorption band at 3300 cm⁻¹ could have been due to N-H of a secondary amine (1, p. 251). There was no indication of any primary amines. All other absorption bands were due to the cholestane part of the molecule, with the exception of weak bands at 1780 and 3050 cm⁻¹ which could not be assigned. The conclusions drawn from the infrared spectra were that the hydroxyethylamine had been produced and that it was not contaminated by the 3β-amincholestane but by some other unknown substance.

A third area of difficulty was associated with the preparation of 3β-(2-chloroethylamino)cholestane hydrochloride from the reaction of the impure 3β-(2-hydroxyethylamino)cholestane with thionyl chloride. Once again, a procedure of Havranek and Doorenbos (3) was followed exactly. The results, however, were far from promising. Difficulty was encountered in the decolorization of the ethanol extract of the reaction residue. Repeated treatment of the hot, ethanol extract with excessive amounts of activated charcoal managed to reduce the deep-orange color of the solution to a light-orange color but no further. Concentration of the ethanol extract resulted in a grayish-white, solid residue which possessed a
melting point range of 260° to 280°C. This was far from identical to the product obtained by N. Doorenbos and R. Havranek (3), who obtained colorless, fine-white needles which melted above 300°C with decomposition.

An infrared analysis was performed on the crude product, which was obtained by the reaction of thionyl chloride with \(3\beta-(2\text{-hydroxyethylamino})\)cholestane. Medium absorption bands at frequencies around 2600 cm\(^{-1}\) and a weak band at 1575 cm\(^{-1}\) indicated the presence of an ammonium-type ion. A weak band at 3300 cm\(^{-1}\) suggested the possibility of a secondary amine. There was no indication of a hydroxyl group. The most important aspect of the spectra was the total lack of any band indicating a carbon-chlorine absorption. If the carbon-chlorine bond had been present, a strong absorption should have occurred in the 800 to 600 cm\(^{-1}\) region (1, p. 329).

Since there was no evidence of the carbon-chlorine bond or indication of alkyl sulfites, which often result from the action of thionyl chloride on primary alcohols (4, p. 71), it must be concluded that the desired reaction did not take place, and \(3\beta-(2\text{-chloroethylamino})\)cholestane was not produced.

Even though infrared analysis did not verify the existence of \(3\beta-(2\text{-chloroethylamino})\)cholestane in the crude, reaction product, the material was allowed to react with sodium hydro-
sulfide in a rather ineffectual attempt to produce \(3\beta-(2\text{-mercaptoethylamino})\)cholestane. The results, as might have been expected, were rather ambiguous.
Infrared analysis of the black precipitate (compound A), which appeared at the interface between the benzene and water layers, resulted in a simple spectrum possessing only three prominent absorption peaks. A rather broad absorption peak in the range from 3320 to 2750 cm\(^{-1}\) could have been partially indicative of a secondary amine. A broad absorption around 1400 cm\(^{-1}\) could have been partly due to absorption by C-CH\(_3\) and \(-\text{CH}_2-\). There was no definite indication of a mercapto group. An absorption band at 1690 cm\(^{-1}\) could not be assigned.

Since the mercapto group was not indicated, it was evident that compound A was not 3/β-(2-mercaptoethylamino)cholestan. Instead, the compound was probably a mixture of unknown hydrocarbon impurities.

Another reaction product (compound B) was isolated from the reaction mixture by benzene extraction. Subsequent infrared analysis of compound B produced a complex spectrum which was somewhat difficult to analyze.

Unfortunately, the Nujol absorption peak probably blocked out or overlapped more significant peaks. Absorption, indicative of the mercapto group, could not have been determined positively because a Nujol absorption band at 2900 cm\(^{-1}\) was near enough to overlap the weaker peak created by S-H in the 2600 to 2550 cm\(^{-1}\) range. A medium absorption peak at 3300 cm\(^{-1}\) could have been representative of a secondary amine. Also, a very weak absorption peak at 1610 cm\(^{-1}\) was indicative of a
secondary amine. Whether or not the NH$_2^+$ ion was present was somewhat difficult to determine because of overlapping Nujol absorption bands.

The infrared spectrum of compound B was quite complex and contained many peaks which could not be assigned. A definite identification of compound B was not possible. Even if compound B had contained 3/β-(2-mercaptoethylamino)-cholestane, there were so many varied organic impurities present that it is doubtful that purification techniques could have separated the pure mercapto compound.

Because the 3/β-(2-mercaptoethylamino)cholestane could not be produced via 3/β-(2-chloroethylamino)cholestane, another synthetic route was developed. It was thought that perhaps 3/β-aminoocholestane could be allowed to react with 2-mercaptoacetic acid to produce N-(3/β-cholestanyl)mercaptoacetamide which in turn could be reduced to 3/β-(2-mercaptoethylamino)-cholestane by the action of lithium aluminum hydride.

When the impure 3/β-aminocholestane was added to the 2-mercaptoacetic acid-benzene solution, an abundant, white precipitate immediately formed. Subsequent infrared analysis revealed strong absorption peaks at 1560 and 1380 cm$^{-1}$. It was known that the carboxylate ion gives rise to two absorption bands between 1610 and 1550 cm$^{-1}$ and between 1400 and 1300 cm$^{-1}$ (1, p. 174). For this reason, it was apparent that the benzene insoluble precipitate was a salt containing the carboxylate ion.
Further study of the spectrum revealed a strong, absorption peak at 1575 cm\(^{-1}\) which could have been due to NH\(_2\)^+. However, this is an area in which data could easily be misinterpreted because the secondary amide group, as well as the carboxylate ion, absorbs in this region. The only other absorption peak of interest was a weak one at 2550 cm\(^{-1}\) which probably was caused by the presence of a mercapto group.

From the infrared data gathered on the benzene-insoluble precipitate, it can be assumed that, instead of N-(3β-cholestan-1-yl)mercaptoacetamide, the ammonium carboxylate salt was formed.

After the ammonium carboxylate salt had been removed from the reaction mixture, the benzene solution was evaporated to dryness. The resulting brown-colored, solid residue, which weighed only 0.54 gram, was subjected to infrared analysis. The spectra revealed the presence of a weak band at 2550 cm\(^{-1}\) which could have been due to a mercapto group. Strong bands at 1570 and 1380 cm\(^{-1}\) were indicative of the carboxylate ion. Also, a peak at 1620 cm\(^{-1}\) was detected, which probably was caused by the NH\(_2\)^+ ion.

Spectra of the benzene soluble and benzene insoluble substances were quite similar. If the amide compound was produced, after five days of refluxing the reaction mixture, it was only in a very small yield. The major product of the reaction appears to have been the salt. Evidently, 3β-(2-mercaptoethyl-amino)cholestane cannot be produced in this manner.
CHAPTER III BIBLIOGRAPHY


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