SENIOR PROJECT

THE SYNTHESIS OF 3@-CHOLESTENE-Δ^5-CARBOXYLIC ACID

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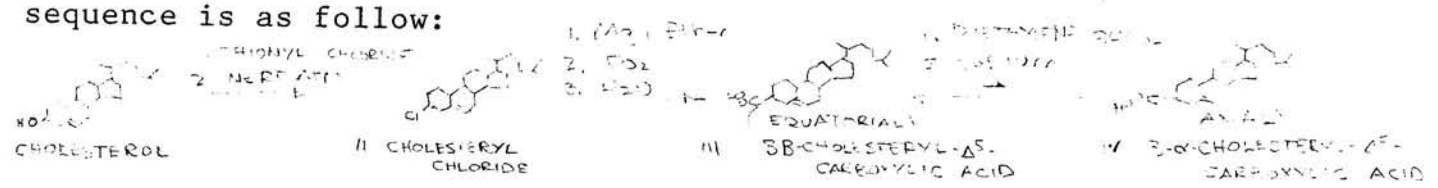
ABSTRACT

3@-cholestene-Δ^5-carboxylic acid was synthesized using a three step procedure starting with cholesterol. The first step, the synthesis of cholesteryl chloride, yielded 92% of the product; melting point of 97°. The second step, the synthesis of 3β-cholestene-Δ^5-carboxylic acid, gave a product of a melting point of 218°-220° in 44% yield. The final step, the epimerization of 3β-cholestene-Δ^5-carboxylic acid (carboxylic group equatorial) to 3@-cholestene-Δ^5-carboxylic acid (carboxylic group axial) was unsuccessful in numerous attempts. A yellow-tannish product, which was thought to be the @-acid, was examined; melting point analysis indicated there was no yield of the @-acid.

The failure for epimerization can be atttributed to many parameters, among which included; poor drying techniques for the solvent diethylene glycol, poor laboratory work-up of the β-acid and improper reaction conditions for the epimerization. This report will outline and describe the procedure used for the synthesis and speculate at possible alternatives should 3@-cholestene-Δ^5-carboxylic acid be synthesised in the future.

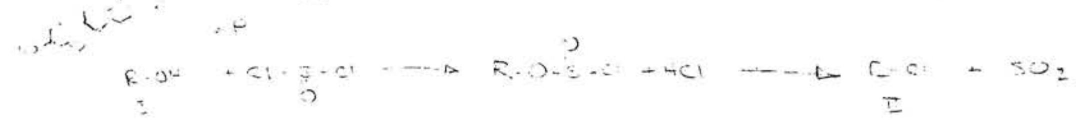
INTRODUCTION

The synthesis of 3@-cholestene-Δ^5-carboxylic acid involved a three step synthesis starting with cholesterol. The overall sequence is as follow:



In this experiment, Peter Wintersdorff master's thesis, "Acid Hydrolysis of Some Steriod Esters",[1] was followed with modifications suggested by Gary Stoddard.[2]

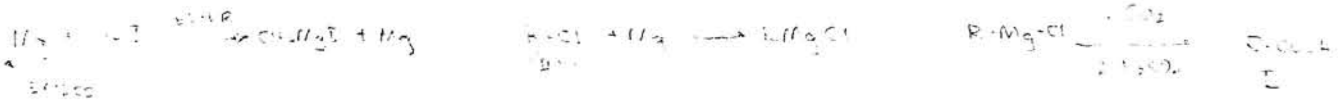
The first step was the synthesis of cholesteryl-chloride(II), which involved an alcohol-chloride conversion. The following mechanism is suggested:[3]



$$\underset{I}{R\text{-}OH} + Cl\text{-}\underset{O}{S}\text{-}Cl \longrightarrow R\text{-}O\text{-}\overset{O}{S}\text{-}Cl + HCl \longrightarrow \underset{II}{R\text{-}Cl} + SO_2$$

The yield in this step was approximately 92% (Wintersdorff reported 88%).[4]

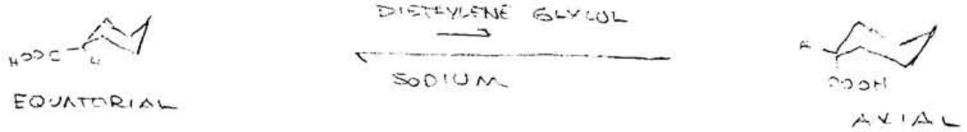
The second step, a Grignard reaction of the alkyl chloride, gave approximately 44% yield (Wintersdorff claimed 47%). The following reactions are suggested:[5]



Iodomethane was introduced inorder to free magnesium from impurities such that the activated magnesium can react much more efficiently in the Grignard.

The final step involved the epimerization of the β-acid(III) (carboxylic group in the equatorial position), to the @-acid(IV) (carboxylic group in the axial position). This step had a reported yield of 10%.[6] In this experiment, however, no @-acid was produced.

It was first thought that in the final recrystalization, @-acid was obtained, upon further analysis it was proven otherwise.[7] The following equilibrium is suggested.[8]



The reason for the unfavorable conversion may be attributed to several parameters as suggested in the abstract. The goal of this project was to synthesize a significant quantity of the 3@-acid(IV) for further use in subsequent experimental investigation. This project failed to carry out this objective, but perhaps the data provided in this experiment and the mistakes learned, can better enhance the synthesis of 3@-acid in the future.

PROCEDURE

The experimental procedure as mentioned previously, was directly taken from Peter Wintersdorff master's thesis,[1] with alternations provided by Gary Stoddard.[2]

Preparation of cholesteryl chloride; The purification of cholesterol was bypassed owing to the ample purity of the cholesterol from the stock.[9]

Simple distillation was used to purify the necessary solvents for this synthesis. A calcium chloride drying tube was affixed to the bent adapter to minimize moisture. The recovery of aniline was in the range of 91°-93° with the resulting product light yellow in color. As for the purification of thionyl cholride, dipentene was used in a 1:4 mixture with the feed for the distillation.[10] The distillate was colorless and recovery was between 76°-78°.

Cholesterol (100 g) was dissolved in 200 ml of chloroform and 40 ml of freshly distilled NN'-dimethylaniline contained in a 500 ml, three-neck-round-bottom flask. The flask was equipped with a thermometer, an inlet tube for argon and an addition funnel, Fig. 1. The addition funnel contained 24 ml of freshly distilled thionyl chloride in 80 ml of chloroform. After the cholesterol had completely dissolved and a very slow flow of argon was circulating through the vessel, with constant stirring, the reaction temperature was slowly lowered to 5° with an ice-water bath. In earlier trials, Dry Ice was used to speed up the process; this technique, however, solidified the reaction mixture, causing the magnetic stirrer to be ineffective and thus the reaction temperature never reached 5°. This problem was circumvented by exercising patience in lowering the reaction temperature. Once at 5°, the thionyl chloride solution was added dropwise to the cholesterol solution. The initial drop caused thick fumes to form and turned the cholesterol solution to a yellow purplish color. After two-three ml addition, the solution turned to a purple color which then yielded a light yellow color after more thionyl chloride was added. During the two hour process of dropwise addition of the thionyl chloride the temperature fluctuated between 4°-9°. The reaction mixture was stirred overnight after the thionyl chloride had been exhausted.

The following day, it was observed that the cholesteryl chloride solution had turned to a deep, brown color. This solution was thus poured into a liter of 95% ethanol and kept at room temperature for 15-30 minutes. The solution was then placed in an ice bath for one hour where tannish crystals started to form. The crystals were collected via vacuum filtration and were recrystalized in a minimum amount of acetone.[11] The cholesteryl chloride crystals were collected and the mother liquor saved for recycling purpose. The total yield including that amount from the recycled mother liquor was 96.5 g or 92.3%. The crystals had a melting point of 95°.[12]

Preparation of 3β-cholestene-Δ^5-carboxylic acid; Simple distillation with a calcium chloride tube attached to a bent adapter was used to purify methyl iodide. The distillate was recovered between 41°-43°.

To a two liter, three-neck-round-bottom flask, 36.0 g of magnesium turnings were added.[13] Argon gas was then ventilated through the flask slowly, taking care that the rate was not strong enough to evaporate ether out of the reaction vessel.[14] Cholesteryl chloride (100 g) was then dissolved in a liter of anhydrous diethyl ether in an addition funnel then affixed to the reaction vessel Fig. 2.

Reflux was initiated by adding five ml of methyl iodide dropwise to the magnesium turnings in the reaction vessel.[15] When the reflux came to steady state, five more ml of methyl iodide was added. Three-ten ml portion of methyl iodide was added later making the total amount of methyl iodide added to the flask 40 ml. After the addition of methyl iodide was comleted, cholesteryl chloride solution was introduced to the reaction vessel. The reaction mixture gradually turned to a greyish black due to the precipitate forming in the reaction vessel. The total addition took three hours with reflux (by a heating mantle), and with constant stirring conducted over the next two days.

At the end of the two day period, the dark greyish solution was cooled to room temperature then poured into a four liter beaker containing excess Dry Ice (CO_2). Care was taken that the mixture was decanted without the magnesium turnings.[16] Upon pouring the reaction mixture, there was a vigorous release of gas, causing the resulting mixture to froth. Eventually the mixture settled, with a greyish-like gel adhering to the bottom of the beaker.

The thick glue-like precipitate was then set at room temperature for an hour with occasional stirring after which 200 ml of 2 N sulfuric acid was added to hydrolyzed the solution mixture. After 30 minutes, the greyish precipitate turned to a milky like solution mixture. This mixture was tested for acidity with Congo Red test paper then transferred into a two liter separatory funnel. An ethereal layer formed on the top and a greenish gel precipitate settled on the bottom of the separatory funnel Fig.3. A few milligrams of sodium chloride crystals were added to the mixture inorder to clear the emulsion that formed.

The gel was then separated and the milkish ethereal layer worked-up.[17] A dilute solution of sodium thiosulfate was used to wash the ethereal layer once, the ethereal solution was then washed with water and filtered. The residue collected was then dissolved in anhydrous diethylether. The ether containing most of the soluble acid from the residue was removed by the rotary vacuum. This was repeated three times after which the residue left behind was then dissolved in 800 ml of benzene and stirred for 30 minutes. It was then placed in an ice bath to maximize the yield. The solution was filtered and the 3β-acid collected. After drying the crystals overnight, it was ground to a fine powder and further dried. 41.6 g of 3β-acid was recovered (m.p. 213°-224°).

The greenish aqueous gel was placed on a steam bath to evaporate the water. The gel was reduced to a brown oily solid after most of the water had been evaporated. This crude solid was then dissolved in 100 ml of n-pentane, upon which the solution of the mixture turned to a pinkish red and the crude solid on the bottom of the beaker turned to a deep rust. This solution mixture was filtered and the residue collected and dried. The dried residue was then dissolved in a minimum amount of concentrated sulfuric acid and heated in a steam bath for 20 minutes. The residue was then dissolved in benzene (200 ml) and filtered. The 3β-acid crystals collected amounted to 3.2 g (m.p. 210°-230°).

The two batches of 3β-acid was thus combined and recrystalized with benzene inorder to maximize the purity. A final yield of 41.1 g with a melting point of 218° resulted.

Preparation of 3@-cholestene-Δ^5-carboxylic acid; The success of the synthesis of the @-acid, may be speculated to be contingent upon the drying of the diethylene glycol. Numerous attempts were made to eliminate moisture from the solvent, the procedure finally employed is as follow:[18]

The solvent drying procedure called for approximately 10 grams of anhydrous magnesium sulfate to be place in a liter reagent bottle after which the bottle was filled with diethylene glycol. The bottle was stoppered with a calcium chloride drying tube and the content stirred with a magnetic stirrer for approximately two days. The glycol was vacuum filtered at the end of two days, and the method repeated using 10 grams of CaO as a drying reagent. The mixture was stirred for two days then filtered. The glycol was then placed with 10 grams of CaH_2 and the mixture stirred for another two days. The mixture was then filtered and the glycol poured into a liter round bottom flask with 10 grams of CaO. The mixture was heated (with a heating mantle) with constant stirring so that reflux may be initiated. See Fig. 4.

The flask was cooled and converted to a distillation apparatus[19] at the end of two days of reflux. See Fig. 5. The apparatus was constructed in such a fashion that internal pressure provided from argon gas, minimized the moisture in the vessel.

The glycol distillate was collected between 239°-242° after which 10 grams of sodium metal was added.[20]

For the epimerization step of the synthesis, 10.5 grams of 3β-acid was added to 400 ml[21] of glycol and refluxed Fig. 6. During reflux, a crust-like residue had formed at the interface of the 3β-acid-glycol mixture.[22] The mixture was then cooled and poured into 500 ml of water, thus turning the mixture to a mirky brown. Hydrochloric acid (2 N) was then added dropwise to the mixture until acidity was confirmed by Congo Red test paper.[23]

The dried residue was then placed in a flask with 100 ml of n-pentane. After shaking the content, the mixture was filtered and the mother liquor saved. This washing with pentane was repeated three times, each time the mother liquor collected and saved. The pentane filtrate was placed in an evaporating dish and the pentane evaporated by natural convection.

After all the pentane had evaporated, hair-like crystals were left behind (m.p. 190°-210°), which was dissloved in 20 ml of acetone and refluxed for seven minutes. The hot mixture was then filtered and the precipitate collected.[24] Approximately two grams of the crude residue was recovered, melting point of 190°-218°.

The acetone filtrate that was collected was evaporated in the same manner as the pentane procedure. After all the acetone had evaporated, there was a tannish residue left behind which was then dissolved in 20 ml of 95% acetic acid. This mixture was heated for several minutes and then filtered. Approximatedly 100 mg of tannish-yellow crystals was collected (m.p. 180°-220°). Further recrystalization with acetic acid caused the melting point range to decrease to 175°-190°.

The two sample finally yield in this project (m.p. 190°-218° and 175°-190°) was saved in a vial and stored.

CONCLUSION AND DISCUSSION

In view of the fact that the experiment was an utter failure, the experienced gained has more than compensate this fruitless effort. It is believed that the lack of experience may have inhibited the progress in this experiment. Take for example the step to synthesize cholesteryl chloride; the reaction mixture had to be cooled to 5° such that optimum conditions may be achieve when thionyl chloride reacts with cholesterol. In the attempt to speed up the reaction, Dry Ice was used to bring the reaction mixture to a rapid cool; this rapid cooling, however, froze the mixtue thus inhibiting the reaction. This step was repeated several times before it was realized that an ice-water bath, although slow, accomplished the job quite well. The most tedious step in this project, however, came about when working with the sticky-like residue of the 3β-acid. It was frustrating to work in that 80% of the process was spent cleaning glassware. The residue adhered to sepratory funnels, beakers and flask which also resulted in a significant loss of 3β-acid.

If the synthesis of 3β-acid was frustrating, the attempted epimerization of 3@-acid was a heartbreaker. After eight attempts the only product recovered was a crude yellow-tannish crust with a melting point of 175°-220°. It is believed that the epimerization did not take place because of moisture contamination on the glycol. It was first believed, however, that @-acid was produced and because of considerable impurities the melting point was not crisp. It should be noted however, that if @-acid was present with β-acid then the range should be 145°-218°, but since the range was much narrower and did not encompassed the melting point of @-acid (155°), then it may be assume that there was no @-acid.with considerable impurities.

It was mentioned that a significant factor for the unsuccessful attempt may be attributed to the moisture of the solvent, ethylene glycol. Other factors that may also play a part includes; improper refluxs condition of the epimerization, contamination on the sodium metal and or poor work-up after the reflux of the β-acid. There are endless possibilities or combinations thereof.

Should this experiment be repeated, it is suggested that an investigation of a more efficient method of drying glycol be employed. If this is not possible perhaps a less hygroscopic solvent be used. The synthesis of 3β-acid and and cholesteryl chloride imposes no problem; it is the epimerization of the @-acid that poses the problem. In summary the project was a learning experience. It gave the sensation of an independent investigation that can not be learned in the classroom.

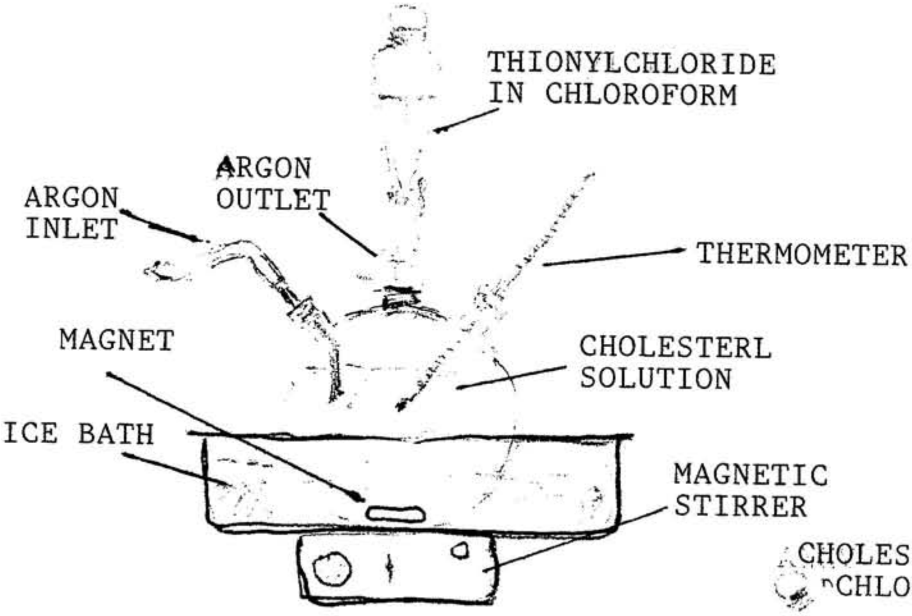


FIGURE 1

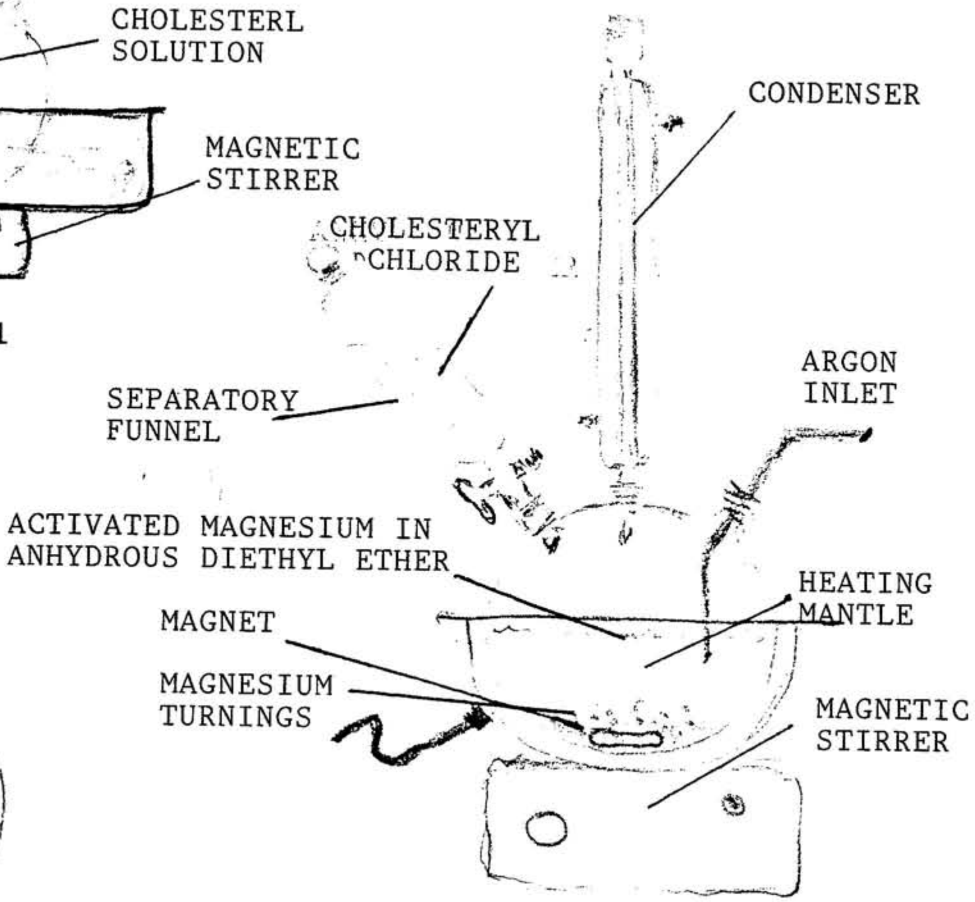


FIGURE 2

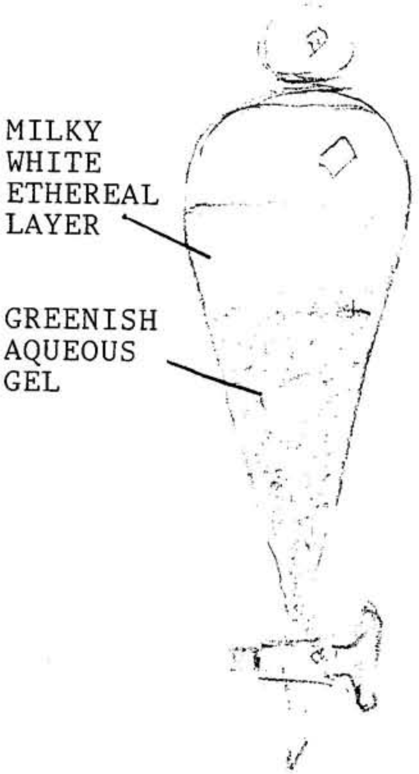


FIGURE 3

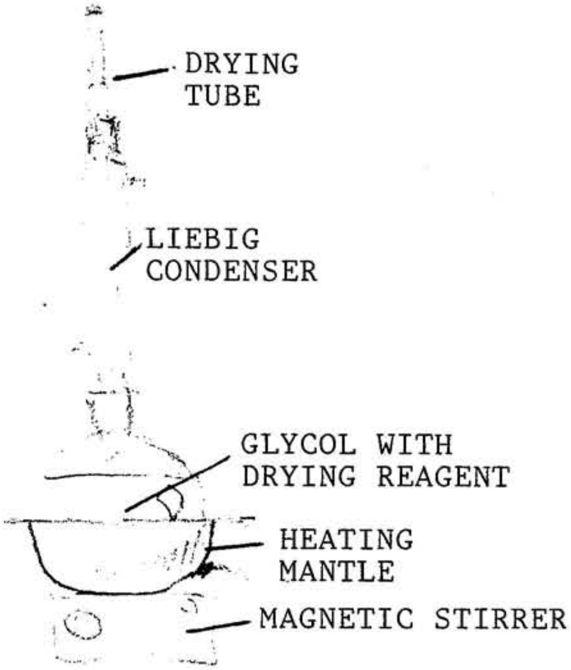


FIGURE 4

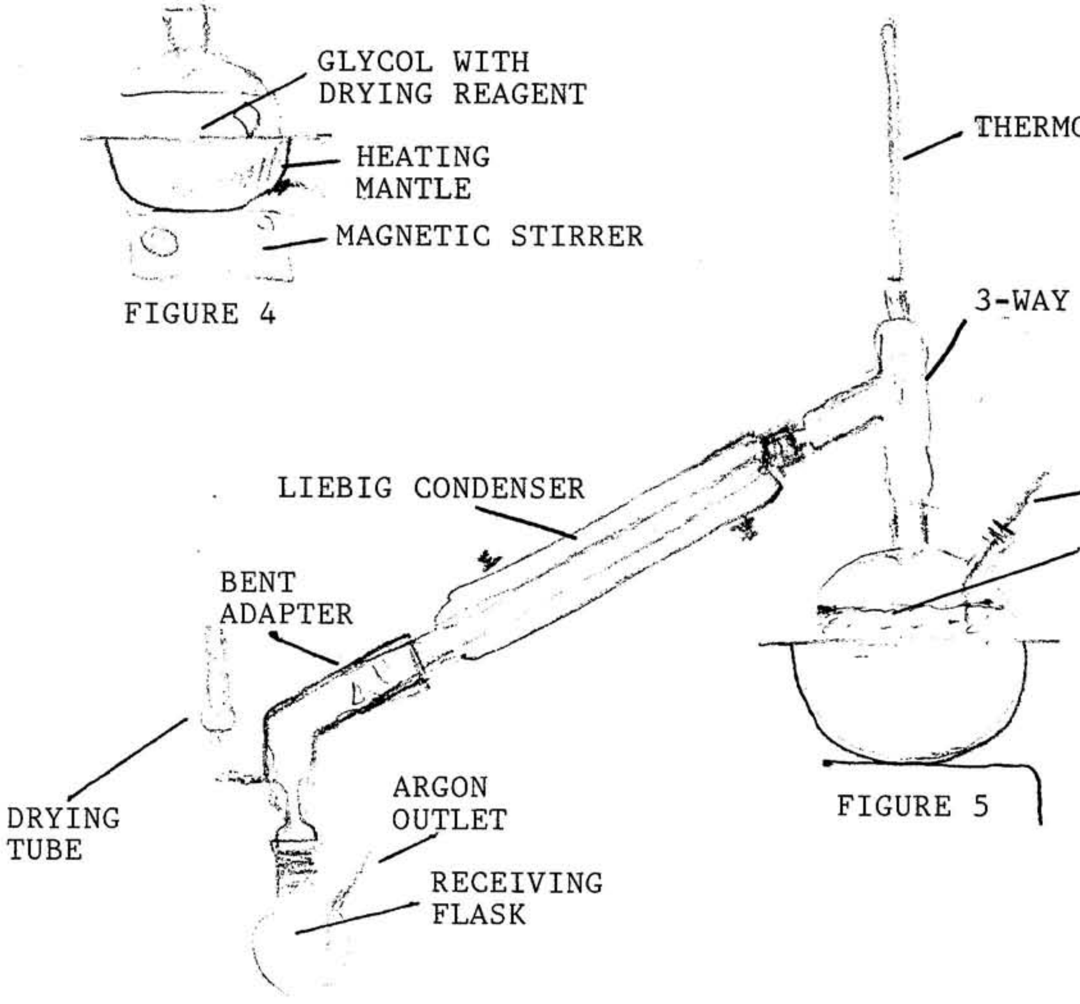


FIGURE 5

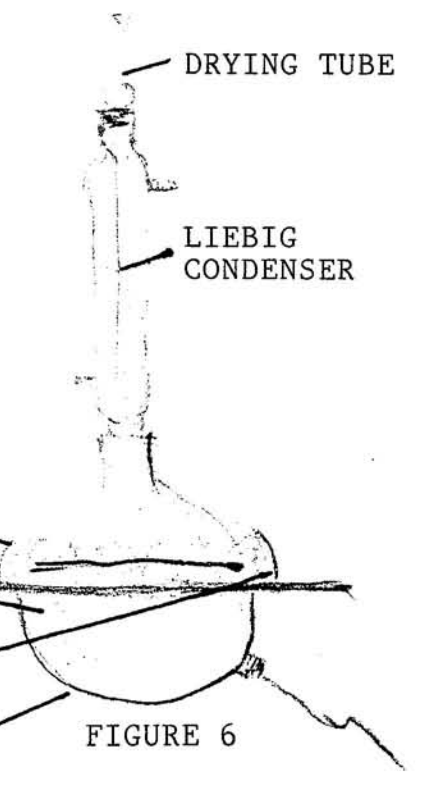


FIGURE 6

Footnotes

1 The procedures for the synthesis can be found between pages 49 and 54 of Peter Wintersdorff's thesis.

2 The modifications suggested by Gary Stoddard were from personal communications with him throughout this experiment.

3 This outline was obtaine from Morrison & Boyd's Organic Chemistry, 3rd Edition, page 601.

4 Wintersdorff cites 46.1 gram yield; page 50 of his thesis.

5 This method of improving the Grignard, was obtained from an article given to me by Dr. Hellberg. The idea is to use methyliodide to clean the magnesium for the Grignard.

6 According to Wintersdorff, product was not obtained until epimerization was carried out under complete anhydrous conditions p.54 and 55.

7 In a melting point analysis of this product, it was confirmed that the product gave physical properties to that of β-acid. See page 44 of the laboratory notes.

8 Page 20 of Peter Wintersdorff thesis.

9 Wintersdorff outlines the purification procedure in page 49.

10 Upon personal communication with Dr. Hellberg, it was suggested that another student (Sigfried Reich) had purified thionylchloride by mixing dipentene in a set amount. The resulting distillate was very pure as indicated by the colorless solution; the procedure was introduced here.

11 Due to the extremely high solubility of the cholesterylchloride to the acetone, a minimum amount of solvent was used to maximize the yield of crystals.

12 The literature value for the melting point of cholesterylchloride is 96°. See reference # 3.

13 To minimize moisture on the magnesium turnings, the turnings were baked in the vacuum oven for several hours prior to use.

14 On one occasion the argon flow was such that after an overnight reflux, all the solvent had evaporated from the reaction vessel causing the reaction mixture to harden.

15 In three cases, there were losses of material because addition of substantial amount of methyliodide. The reaction became so exothermic upon addition of the methyliodide, that the mixture boiled over and out of the reaction vessel.

16 On an occasion the magnesium turnings were poured together with the reaction mixture into the Dry Ice which resulted in an impossible separation between the turnings and the glue-like precipitate.

17 At this point, this greenish gel was left on the side and work-up was performed on the ethereal layer.

18 This procedure combines several techniques from previous failures. The product yield by this procedure was discovered to be 3β-acid.

19 The reflux column used for the reflux of diethylene glycol was converted to a condenser column for the distillation of diethylene glycol.

20 It was pointed out later that the sides of the sodium be shaved so that moisture clinging to the surface of the sodium may be eliminated.

21 It should be noted that the original glycol had been stored for a week. Gary Stoddard, however, pointed out that the glycol should never be stored because of its hygroscopicness. He suggested that perhaps distilling the glycol a second time would lead to a dryer solvent. It was this solvent that was used.

22 It was suggested by Gary Stoddard that the interface between the floating 3β-acid and the glycol solution is where epimerization occurs, thus the region upon which glycol boils (242°) and 3β-acid melts (218°) should be well insulated for optimum conditions. The round bottom flask with its content was thus insulated with glass wool wrapped by aluminum foil.

23 Upon acidification the mixture turned from its murky brown to a light tannish color, with the residue precipitate floating on the surface of the mixture.

24 On a previous occasion, the residue was submerged in water for the wash, this process however, caused the residue to be less acidic thus forming an emulsion. This residue-water mixture could not readily be separated by vacuum filtration. The problem was solved by acidification of the residue.

REFERENCES

1 Peter Witersdroff, "Acidic Hydrolysis of Some Steriod Esters", M.S.Thesis, San Diego State University, San Diego, CA, 1968.

2 G. Roberts, C.W. Shoppee and R.J. Stephensen, J. Chem. Soc., 2705, 1954.

3 Handbook of Chemistry and Physic, 57th edition, 1977.

4 Morrison, Boyd, "Organic Chemistry", 3rd Edition, 1978.