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THE ELECTROCHEMICAL OXIDATION OF
CHOLESTERYL ACETATE DIBROMIDE.

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THE ELECTROCHEMICAL OXIDATION
OF
CHOLESTERYL ACETATE DIBROMIDE

BY

ABRAHAM COOPER

A THESIS
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ABSTRACT

Cholesteryl Acetate Dibromide was dissolved in carbon tetrachloride, suspended with mixing in 4.5 molar solution of sulfuric acid and electrolyzed at a lead dioxide anode. The anodically prepared film of lead dioxide, when maintained at potentials (relative to a calomel electrode) above 1.5 volts, was a catalyst for the oxidation. A new and mild oxidation took place at the catalytic electrode surface that resulted in attack on the tertiary hydrogen atom at the 25 carbon. The mildness of the oxidation prevented the decomposition of the unconverted raw material and permitted the recovery of up to 96% of the cholesteryl acetate dibromide and derivatives. Conversion of starting material ranged from 30 to 55%. The products recovered after debromination were 25-Hydroxy cholesteryl acetate and 24-Dehydrocholesteryl acetate mixed with 25-Dehydrocholesteryl acetate. The molar yield of products ranged from 85 to 93%.

This work is a contribution to the technique of degrading cholesterol in a manner that makes available a method of synthesis of more valuable steroids in high yield.

APPROVAL OF THESIS

FOR

DEPARTMENT OF CHEMICAL ENGINEERING
NEWARK COLLEGE OF ENGINEERING

BY

FACULTY COMMITTEE

APPROVED: ______________________

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PREFACE

Since the mid-nineteenth century, the cholesterol molecule has been assaulted by oxidizing agents in attempts to produce useful products. To date, attack on the aliphatic side chain has yielded only 10% of the starting material in useful form.

The subject of the present thesis is the conversion of cholesterol with the agency of the electric current. Electricity as a reagent for oxidation of organic compounds has been in use for more than 100 years.

Others have reported on such studies as recently as 1949, but the result of the present work is believed to be the first achievement of useful derivatives in appreciable amount.

ACKNOWLEDGMENTS

Thanks are due to Dr. Charles L. Mantell, who opened the door into electro organic chemistry in his graduate course of Electrochemical Engineering. His guidance throughout the development of the thesis problem has been an inspiration in this newly growing field.

The helpfulness of other members of the Department of Chemical Engineering, who pointed the way to the proof of the problem, is also truly appreciated.

Raw materials for the work were kindly supplied by Armour Pharmaceutical Company of Kankakee, Illinois. The Schering Corporation of Bloomfield, New Jersey, furnished samples of steroids that helped in proving the identity of products made in the research.

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I. INTRODUCTION

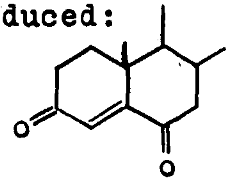
For more than one hundred years Cholesterol has been prominent as a starting material for many series of functional derivatives. Many of the early workers in steroid chemistry sought to convert this inexpensive, readily available animal product to useful compounds by oxidation of the functional groups on the nucleus, as well as by degradation of the side chain.

In the early 1900's, Windaus(77) oxidized cholesterol with alkaline permanganate to the cis-triol in 5% yield. More than thirty years later it was reported from Russia, Germany and Switzerland, that the same result had been achieved with osmium tetroxide with yields ranging from 60-88%. In 1908 Pickard and Yates (54) reported the preparation of the trans-triol. They heated cholesteryl acetate with acetic acid and hydrogen peroxide. Cholesterol will give (91% yield) the same trans isomer when treated with performic acid.

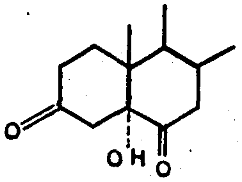
When N-bromosuccinimide is used in aqueous acetone containing a small amount of acetic acid, cholesterol is oxidized to the 6-keto derivative of the trans-triol in 49% yield. Westphalen(74) was able to oxidize cholesterol and its acetate to a mixture of 5,6-oxides with perbenzoic acid.

Mauthner and Suida(48) reviewed the oxidation of cholesterol in the years 1846-1896. Their paper also reported on a new oxidizing agent, chromic acid.

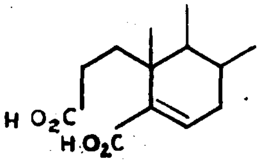
They produced:



(40%) Δ4-cholestene-3,6-dione

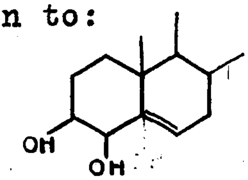


2% cholestane-3,6-dione-5α-ol



(2.9%) Diels acid

The dione has also been produced with sodium dichromate in benzene-acetic acid at 0°C. in two days, in the same 40% yield.(25) Selenium dioxide was used by several different investigators to convert cholesterol by allylic oxidation to:



Δ5-cholestene-3β,4β-diol

When cholesterol is colloidally dispersed at pH 8.5 in aqueous alcohol containing sodium stearate and aerated for five hours at 85°C., 40% is converted to 7-keto cholesterol, as well as small amounts of 7α and 7β -hydroxycholesterol which may be biological precursors of provitamin D_3(78). Cornforth et al, (18) report successful reaction of ozone with cholesterol. This was achieved by passing the gas into a dry hexane solution until precipitation was complete. The yield of ozonide was 80%.

Early in the 1930's, it was recognized by many workers in steroid chemistry that androstenolone was a compound of importance because of its relation to cholesterol and the likelihood that it was a biogenetic starting material of both androgens and estrogens. It was at this point that serious attempts were made in the oxidative degradation of a protected cholesterol derivative as a path to androstenolone.

According to Fieser and Fieser,(28) preparation of androstenolone from cholesterol had been reported in 1935 by the German branch of Schering and by at least two other European investigators. The starting material was cholesteryl acetate dibromide. This was oxidized with chromic acid in acetic acid solution, debrominated and after removal of a large acidic fraction, the small neutral extract was treated with semicarbazide hydrochloride and sodium acetate in ethanol. The yield of usable material was very small.

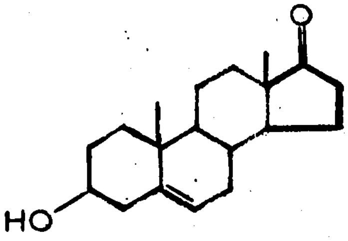
Wallis and Fernholz(70) reported an overall yield of semicarbazone acetate of about 1%. Butenandt(16) in Danzig studied variations in the procedure. He found that addition of sulfuric acid and temperatures in the range of 50°C. favored this product to the extent of 2.5% yield. After World War II, the officers of the American Military Government in Germany disclosed in a FIAT Report No. 996, that in Berlin, Schering A.G. had used mixtures of ethylene dichloride and acetic acid at 10-20°C. to carry out this oxidation. After improvements were made by Schering in New Jersey, yields from 7-8% resulted.

Patent 164,549 issued in 1949 in Austria to G. Kereszty and E. Wolf, disclosed a substantial improvement when cholesteryl acetate dibromide is isomerized by heating to a dextrorotatory mixture prior to oxidation. Maas and deHeus(44) studied the oxidation of the two pure dibromides as well as other halogenated derivatives and determined that a 7.7% yield could be achieved by oxidation at 0°C. from the $5\alpha,6\beta$-dibromide and from the $5\beta,6\alpha$-isomer 15.3%.

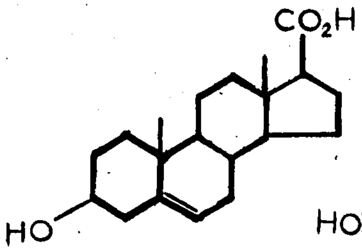
Table 1. lists all the oxidation products isolated after debromination and hydrolysis of the reaction mass. The major product androstenolone (1) is formed together with a considerable quantity of pregnenolone, a precursor in hormone syntheses. The acid (3) 3β-hydroxy-Δ^5-cholenic acid is formed by splitting at the 24-carbon

TABLE 1.

OXIDATION PRODUCTS OF CHOLESTERYL ACETATE DIBROMIDE



(1)



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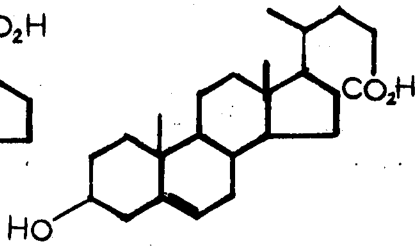

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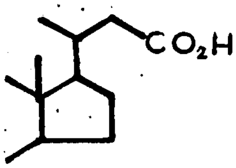

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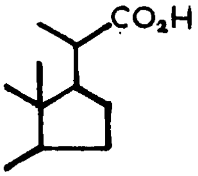

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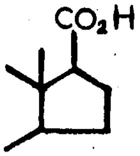

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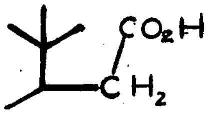

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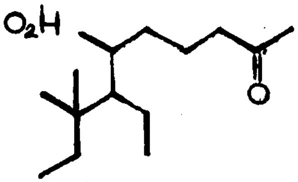

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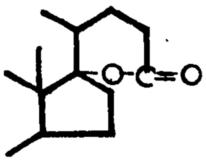

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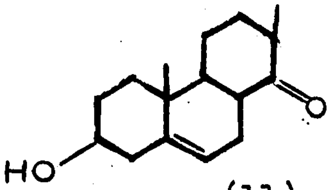

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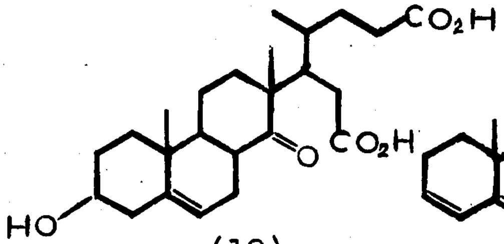

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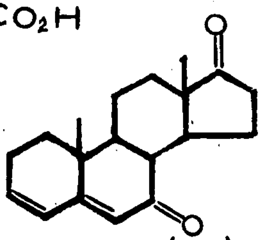


(13)

atom with the production of acetone. The acid fraction has also yielded smaller amounts of 3β-hydroxy-Δ^5-norcholenic acid (4), the bisnor acid (5), the 3β-hydroxy-Δ^5-etienic acid (6), bilianic acid (7) and the C_{27} acid 3β-hydroxy-Δ^5-cholestenic-26-acid (8). The ketone (9) has been identified by Wieland and Meischer.(75) Researchers at Ciba isolated the neutral 24→27 lactone (10). The ketone (11) with D ring and side chain completely lost, was characterized by Koster and Logemann(40) at Schering.

When the acid fraction in benzene was extracted with dilute soda ash solution, a ketonic dibasic acid proved to have the structure shown in (12). In some cases, elimination reactions have occurred on some intermediates as (13) which is $\Delta^{3,5}$-androstadiene-7, 17-dione; the end product of acetic acid removal from 3β-acetoxy-Δ^5-7-one. While oxidative attack may occur in several ways, the lactone (10) as well as (1), (2), (3), (6), (7), and (9) all appear to have resulted in the formation of a tertiary alcohol as a phase in the process. The same mechanism has been proved in the reaction described in the main body of the present work where the 25-hydroxy cholesteryl acetate, as well as the dehydro products, were isolated and identified.

In addition to all these methods, cholesterol has been subjected to air in the presence of ultraviolet radiation with various acids produced by oxidative ring opening. The cholesterol molecule was exposed to X-radiation in the absence of oxygen, to give in aqueous or acetic acid solutions, the 17-keto cholesterol and the cholestane $3\beta,5\alpha,6\beta$ - triol. All these latter reactions appear to have been free radical initiated with primary attack at the 7-position to give the 7-hydroperoxide. This loses water and results in the 7-keto derivative. It can react with more cholesterol to yield a 7-hydroxyl derivative. The oxygen atom that is released is taken up to form an epoxide. Subsequent dehydration or oxidation processes would account for the formation of the other compounds that are isolated. It is possible that with irradiation by X-rays (where the presence of oxygen is not necessary), attack of hydroxyl radicals (derived from the solvent) on the 5,6-double bond takes place. Little or no oxidation of the 3-hydroxy group occurs.(17)

The proposed 7-hydroperoxide intermediate has not been isolated, although in the similar case of the aerial oxidation of cholest-5-en-3-one, a 6 hydroperoxide has been obtained in crystalline form. Recently, Gollnick and Neumuller(32) have obtained a Δ^6-5-hydroperoxide by photochemical oxidation of cholesterol.

It is difficult to decide to what extent some oxidation products are derived from the working up process, since the conditions that prevail are ideal for their formation, unless air is rigorously excluded. Even in the absence of biological material, oxidation can occur; e.g. in the recovery of cholesterol from the dibromide. Working in a nitrogen atmosphere is a precaution which can be applied to most sterol work, and samples (particularly noncrystalline ones) are stored at low temperature, in the dark (or in amber glass bottles), and in a nitrogen atmosphere. Fieser, et al(30), noted the susceptibility to air oxidation of the 25 carbon atom of cholesterol. The 25 alcohol was isolated from samples that ranged in age from 4 to 24 years.

Cholesterol labelled with C^{14} is particularly labile in the presence of air (Dauben and Payot)[20]. It is converted into the usual mixture of oxidation products.

The only work uncovered by the literature survey that dealt directly with the use of the electrolytic cell in oxidative change of cholesterol and derivatives, was authored by Andras Kramli(41) of the Hungarian Biological Research Institute at Tihany, Lake Balaton, Hungary. The work was reported in the publication of that organization in the early part of 1947.

Seven years earlier there were two Hungarian Patents issued (61)(62) that concerned the same study. Dr. Kramli appears to have been connected with both patents at the time of his association with the pharmaceutical firm of Gideon Richter & Co.

In all three papers, electrolyses are described which indicate that very small yields of cholesterol derivatives were made. The raw material was dissolved in glacial acetic acid containing potassium acetate and oxidized at a platinum anode. Dr. Kramli proposes that "nascent oxygen, produced anodically" is the active oxidizing agent.

The conditions specified in the paper(41) were followed and then varied in attempts to improve the results, with no success. From that point on, a different approach to oxidation of cholesteryl acetate dibromide was followed in order to degrade the aliphatic side chain. A report of this work is the subject of the present thesis.

II. ELECTROCHEMISTRY OF ORGANIC COMPOUNDS

The electric current under the right conditions will act as a reducing or oxidizing agent. The liberation of hydrogen at the cathode and oxygen at the anode in an aqueous solution, that results from the passage of electricity through the solution, is evidence of appropriate conditions. Many early workers from 1800 and later recognized this fact and recorded their observations on electrolytic phenomena. Michael Faraday(25) was one of the first to see the possibilities of using electricity for synthetic chemical manufacture. He forecast in 1834 that,". . .Much information regarding the composition. . . of such bodies as the vegetable acids and alkalies, and organic compounds generally, will probably be obtained by submitting them to the action of nascent oxygen, hydrogen, chlorine, etc., at the electrodes; and the action seems the more promising because of the thorough command which we possess over the attendant circumstances, such as the strength of the current, the size of the electrodes, the nature of the decomposing conductor, its strength, etc., all of which may be expected to have their corresponding influence upon this final result."

Following Faraday's empirical contributions, Kolbe(39) made some important discoveries which laid the foundation of electrolytic oxidation and reduction. His most important contribution was the systematic investigation of the electrolysis of salts of monobasic aliphatic acids for the preparation of hydrocarbons.

Electrolytic methods naturally attracted interest from the industrial standpoint, since electricity makes a clean and controllable method available for oxidation, reduction, halogenation and substitution reaction for the introduction of various groups into organic molecules. Technologists quickly realized, that with no necessity for special chemical reagents to be removed later from the reaction mass, and the wide range in varying the operating conditions to conduct the process, the electrolytic method would have advantages. With the many points in favor of electrolytic oxidation and reduction, some fundamental disadvantages must not be overlooked. These reactions are slow and require a large amount of space on a commercial scale. More importantly, one should consider the continued action of the cell on reaction products.

In any electrochemical cell, passage of an electric current displaces the potentials of the electrodes. During electrolytic oxidation or reduction the electrodes are not in a state of equilibrium. The electrodes are considered to be polarized. Any pair of electrodes which comprise a cell, and are connected to an outside source of voltage so as to permit the passage of current, are considered to be polarized. Materials which reverse this tendency are called depolarizers. In the electrolytic oxidation or reduction of a compound which is susceptible to oxidation or reduction, that compound tends to return the electrode to its state of equilibrium.

As reduction involves the increase in negative charge or the gain of electrons, and oxidation the decrease in negative charge or the loss of electrons, a system may be devised where a state of equilibrium exists between the oxidized and the reduced forms represented by

$$\text{Red.} \rightleftharpoons n_e + \text{Ox.}$$

where Red. and Ox. represent the reduced and the oxidized form and n_e the number of electrons involved in this change of state.

In order to have a reversible oxidation-reduction process it is necessary that both forms be present, and also that minute changes in potential result in the change of one form to another, depending on the extent of the potential change.

If the potential be made more positive, then oxidation with loss of electrons tends to compensate for this positive change in potential. A more negative potential is compensated for by addition of electrons to the oxidized form. Therefore, the equilibrium potential for any reversible system as indicated by the above equation, can be given by the following expression:

$$E = E_o - \frac{RT}{nF} \text{Ln} \frac{(Ox.)}{(Red.)}$$

where E_o is the standard oxidation-reduction potential, Ox. and Red. the concentrations of oxidized and reduced forms, and n the number of electrons involved in the transformation.

Most processes involving the electrolytic oxidation or reduction or organic compounds are irreversible. There are, however, some exceptions such as quinone $\rightleftharpoons$ hydroquinone. An irreversible system has no definite potential that changes according to the concentration of oxidized and reduced form in the system, such as derived by application of the thermodynamic expression for the reversible system. The potential of irreversible systems depend more on the nature of the medium, its degree of acidity or alkalinity, the electrode and its history, than on the concentrations of oxidized and reduced forms. The simple principles which hold for reversible electrode processes are not applicable to such systems. There is a difference between chemical reversibility and thermodynamic reversibility. A ketone $\rightleftharpoons$ secondary alcohol may be chemically reversible, but does not always give a definite thermodynamically reversible potential. When the ketone has been reduced to the alcohol it may also be oxidized electrolytically back to the ketone. This is possible, but different conditions of potential, medium, pH and electrode material are required.

Acidity or alkalinity of medium, concentration of depolarizer, electrode material at which electrolysis is performed, define the electrode potential. A discussion of electrode potential and each of the various factors which control and contribute to this potential at the electrode surface follows:

Electrode Potential

When tin and hydrochloric acid are used to reduce a ketone, an alcohol is formed. A two electron change has taken place with the addition of a proton to form the alcohol. This reaction has a definite E.M.F. If the reaction were carried out in a cell at the same E.M.F. in a suitable medium, the electrode would contribute the two electrons and the medium, the hydrogen necessary for the reduction.

The E.M.F. of this reaction is called the electrode potential or a reference potential v. a standard reference electrode (e.g. saturated calomel electrode). Two methods are available to attain the desired electrode potential. First, is in the use of electrode material of proper overpotential. Overpotential means the difference between potential at which gas evolution is first observed, and the reversible potential of the same electrode in the same solution. The higher the hydrogen overpotential of the electrode generally, the more powerful its reducing ability, or the greater the energy content of the proton liberated. The oxidizing ability and the energy content of the oxygen liberated at the anode similarly depends on the oxygen overpotential. This potential may be considered as a measure of the potential energy of the hydrogen or oxygen liberated.

Aqueous solutions of acids and bases, in general, have a "decomposition potential" in the vicinity of 1.7 volts at a smooth platinum electrode (Table 2).(1) "Decomposition potential" means the potential at which appreciable current begins to flow through the solution, accompanied by hydrogen evolution at the cathode and oxygen evolution at the anode. "Decomposition potential" is the same in different solutions since the hydrolysis of water is common to all cases, with the discharge of hydrogen ions at the cathode and hydroxyl ions at the anode, the hydroxyl ions forming oxygen according to the following scheme:

$$2OH^- \longrightarrow 2\cdot OH + 2e$$
$$2\cdot OH \longrightarrow H_2O + 1/2O_2$$

Aqueous solutions of halogen acids have lower decomposition potentials (Table 3)(1), since the discharge potential of the halogen is lower than that required for hydroxyl ion discharge.

The relative ability of a metal to reduce or oxidize can be obtained from Table 4(1). Mercury, zinc, lead and tin should be the most desirable electrodes to use for difficult reductions, and nickel, platinum and copper for compounds easily reduced.

In acid media, the reduction of nitro compounds at a high overpotential electrode such as lead, mercury, tin or zinc yields the amine; low overpotential metals such as nickel or platinum give intermediate reduction products such as phenylhydroxylamines or azoxy- and hydrazo- compounds derived from phenylhydroxylamines.(23)

Electrodes (used) for electrolytic oxidations are limited since it is difficult to achieve a stable anode potential with most electrodes in the presence of depolarizer. The potential generally rises rapidly from the low value, at which the anode dissolves, to the high value for passivity and oxygen evolution. Platinum and

TABLE 2.

DECOMPOSITION POTENTIALS OF ACIDS AND BASES IN AQUEOUS SOLUTIONS

ACIDS	VOLTS	BASES	VOLTS
Nitric acid	1.69	Ammonium hydroxide	1.74
Sulphuric acid	1.67	Sodium hydroxide	1.69
Phosphoric acid	1.70	Trimethylammonium hydroxide	1.74
Trichloracetic acid	1.66		
Perchloric acid	1.65	Diethylammonium hydroxide	1.62
		Potassium hydroxide	1.67

TABLE 3.

DECOMPOSITION POTENTIALS OF AQUEOUS HALOGEN ACID SOLUTIONS

Hydriodic acid	0.52 volts
Hydrobromic acid	0.94 volts
Hydrochloric acid	1.31 volts

TABLE 4.

CATHODIC AND ANODIC ELECTRODE OVERPOTENTIALS

	Hydrogen Overpotentials (In N.H_2SO_4)	Oxygen Overpotentials (In N.KOH)
Palladium	0.00	0.43
Gold	0.02	0.53
Iron	0.08	0.25
Smooth Platinum	0.09	0.45
Silver	0.15	0.41
Nickel	0.21	0.06
Copper	0.23	-
Cadmium	0.48	0.43
Tin	0.53	-
Lead	0.64	0.31
Zinc	0.70	-
Mercury	0.78	-

sometimes gold are nearly always passive, and may be used to obtain graded potentials.

When the oxidative process is caused by the presence of oxygen in an active form, a high anode potential indicates a more effective oxidizing power. This is not always true. There are processes which appear to be independent of the anode potential. With these cases it may be that the oxidizing agent may not be oxygen, but hydrogen peroxide. For a metal to be used as an anode it must be passive in the electrolytes used. In the absence of halogens, metals such as platinum or gold are usually not attacked. Where high current densities are used, this is so for anodes of cobalt and nickel. At higher current densities, iron can be used. Once iron has become passive it is possible to use the electrode at low current densities. Lead can also be used in acid solutions of moderate concentration. In a sulphuric acid medium, lead forms a sulphate coating which is converted into an oxide coating. In alkaline medium it is necessary to use metals whose oxides are insoluble. Lead acts as an insoluble electrode at high current densities in this medium, but at low current densities it will go into a solution. Platinum is as suitable in alkaline, as it is in acid medium.

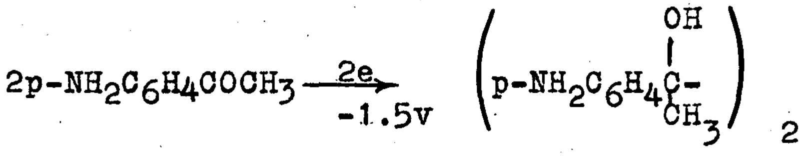
To establish the desired electrode potential at the working electrode, a high hydrogen overpotential metal (mercury) is used for reductions, or a high oxygen overpotential metal (platinum) for oxidations, while the applied voltage across the cell is controlled to obtain the required electrode potential.

With the same electrode, medium and temperature, it is possible to make different products from the same raw material by variation of the electrode potential. Haber(34) showed this in his studies on the reduction of nitrobenzene. The first step is the formation of nitrosobenzene, followed by the hydroxylamine and finally aniline:

$$C_6H_5NO_2 \xrightarrow{2e} C_6H_5NO \xrightarrow{2e} C_6H_5NHOH \xrightarrow{2e} C_6H_5NH_2$$

Each stage is controlled by the potential at the cathode surface. p-Aminoacetophenone can be reduced at a mercury cathode to either the hydrol or pinacol, depending on the potential at the mercury cathode:

$$p\text{-}NH_2C_6H_4COCH_3 \xrightarrow[-1.1v]{2e} p - NH_2C_6H_4CH\ (OH)\ CH_3$$



$$2p\text{-}NH_2C_6H_4COCH_3 \xrightarrow[-1.5v]{2e} \left(p\text{-}NH_2C_6H_4\overset{OH}{\underset{CH_3}{C}}-\right)_2$$

Electrode potential is one of the most important variables in electrolytic reduction. In a reduction at a constant current density, the initial potential increases as the reduction proceeds.

TABLE 5.

REDUCTION IN AQUEOUS METHANOLIC POTASSIUM ACETATE

p-Methoxyacetophenone ⟶ Pinacol

Reference Electrode: Calomel

Current Density = 0.288 Amp/cm^2

Time in Minutes	0	3	7	11	14.6
Cathode Potential in Volts	2.40	2.48	2.58	2.79	3.00

Table 5 summarizes the reduction of p-methoxyacetophenone under such conditions. If the plan is to attack the more easily reduced center in a compound having two reducible functions, under constant current density conditions, an electrode of proper overpotential must be selected to prevent possible reduction of both centers. Electrolysis at a specific potential is equivalent to using one reducing or oxidizing reagent, whereas constant current density electrolysis is comparable to using a number of reducing or oxidizing reagents in the same reaction vessel.

Constant current density electrolysis is also of great value. If a compound has one or more reducible centers and complete reduction is desired, electrode potential is not important so long as it is greater than the minimum potential required.

When an increasing potential is impressed on a cathode in a cell with no depolarizer, the flow of current is small (a), and is attributed to diffusion of hydrogen from the electrode, or to a trace of reducible material in solution. (See Fig 1). When the potential reaches a voltage (b), the evolution of hydrogen gas bubbles are observed at the cathode, and current increases rapidly, (Curve A).

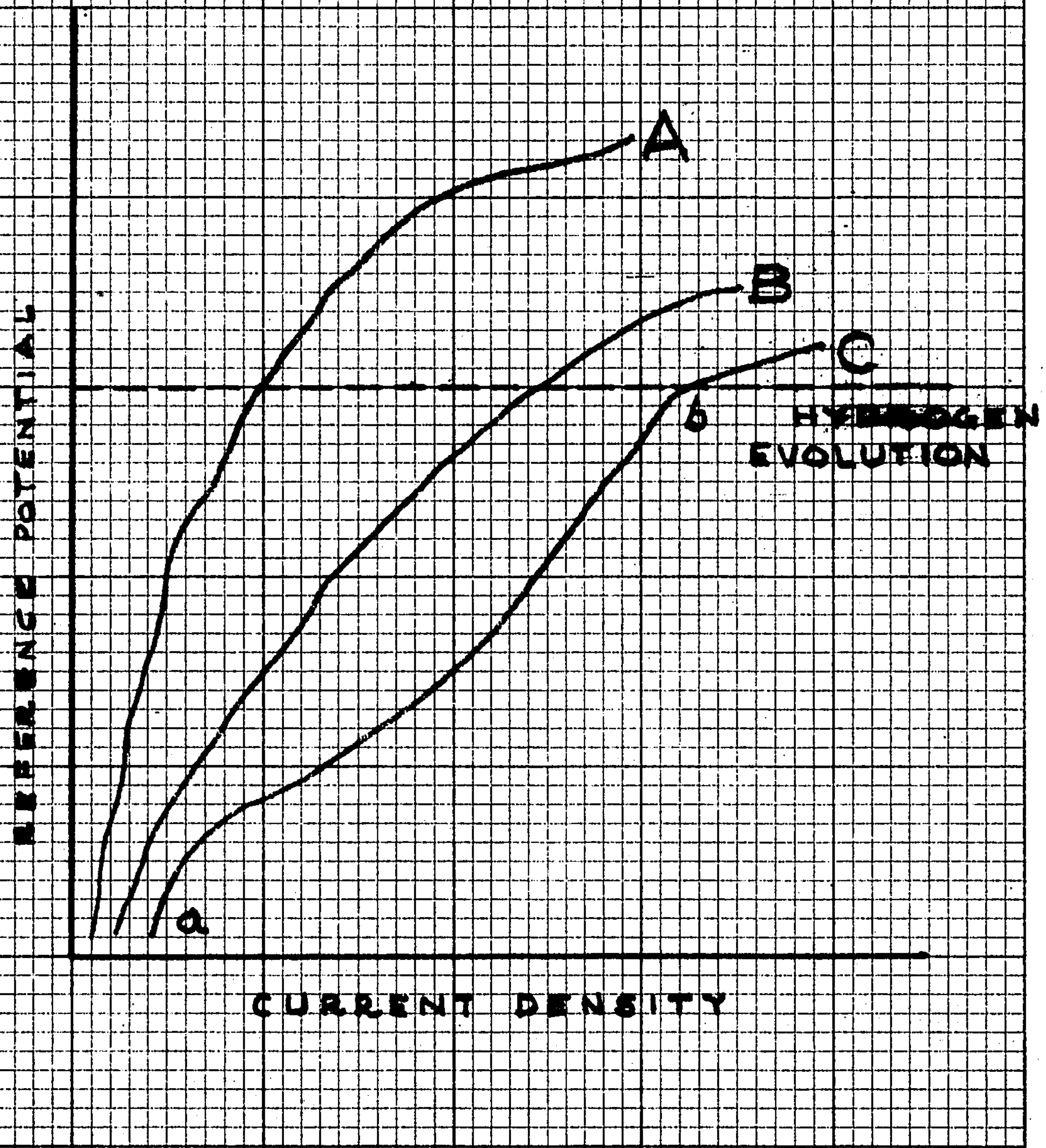
With a depolarizer which reduces relatively slowly, a curve such as B will be obtained. A compound which is quickly reduced will give a curve C. Curves B and C show that by regulating the electrode potential, the reducing power of the electrode may be controlled. It does not suffice to set the current density at a particular value, for, removal of depolarizer due to its reaction with hydrogen will cause the potential to increase, with resultant hydrogen evolution. Current density can be regulated to maintain a constant electrode potential.

Catalytic Effect of Electrodes

The overpotential of the electrode is a measure of its reducing ability. There are times when the type of

FIGURE 1

REFERENCE POTENTIALS IN CATHODIC REDUCTIONS



electrode material has a catalytic effect on the reaction. In these instances a low overpotential electrode may be as effective as, or more effective than a higher overpotential electrode. In the cathodic reduction of nitric acid to ammonia, if a relatively high overpotential cathode such as amalgamated lead is used, a small percentage of ammonia is obtained together with a large amount of hydroxylamine, while spongy copper, which has a low overpotential, gives high yields of ammonia.

When ketones are reduced at cathodes of relatively high overpotentials, the pinacol is generally obtained. With a cadmium electrode of lower potential the hydrocarbon or the alcohol is obtained. The ability of high overpotential electrodes to reduce resistant compounds can also be destroyed by the presence of catalytic quantities of other metals such as copper, platinum or silver in the medium or electrode. The electrode must be pure. For this reason mercury is usually suitable for reductions and platinum for oxidations, since they are available commercially in high purity.

The catalytic effect of the anode metal is sometimes greater than the consideration of potential. In the oxidation of iodate in alkaline medium at a smooth platinum electrode, increase in electrode potential results in increased yield of periodate. While the use of a lead dioxide electrode results in still better yield, even though the anode potential of this electrode is slightly less. Methyl alcohol, subjected to anodic oxidation in dilute sulphuric acid at a smooth platinum anode, gives formaldehyde with an 80 per cent efficiency. With platinized platinum or lead dioxide anodes, the decrease in yield of formaldehyde is accompanied by increase of the more highly oxidized products, formic acid and carbon dioxide. In many oxidations, better results can be obtained with platinized platinum or lead dioxide.

Catalysts

The addition of various salts to the electrolyte, have an effect on the products obtained from an electrolytic reduction, in addition to the catalytic effect of a particular electrode material or an alloy electrode on the reaction. Addition of salts of lead and mercury to low overpotential electrodes, such as nickel, increase the efficiency of the electrode. Some metals can plate out on the cathode, react with the depolarizer, and then be redeposited from solution. Reduction of nitro-compounds to their hydrazo-derivatives in a basic medium is catalyzed by zinc, lead or tin hydroxide in the catholyte. Lead increases the overpotential of the cathode, but with the zinc or tin salts the effects may be partly due to the chemical action of the metal. Indigo is reduced to indigo-white at a zinc cathode. The same reduction can be accomplished chemically with zinc. In the synthesis of methyl anthranilate from the methyl ester of o-nitrobenzoic acid, a 40% yield is obtained using a lead cathode. A catalytic amount of stannous chloride in the electrolysis medium increases the yield to 90%.

Electrolytic reductions at a mercury cathode in an alkaline medium, or in the presence of an alkali salt, may result from chemical reduction by the amalgam. Benzoic acid in an alkaline medium can be reduced to the tetrahydro compound. The same reaction is accomplished chemically with sodium amalgam. In experiments by two German chemists in 1904, on the controlled potential reduction of nitrobenzene in an alkaline medium, it was reported that copper powder increased the efficiency for the formation of aniline from 33.2 to 52.6%. It is doubtful if chemical action can account for the reduction even though phenylhydroxylamine can be reduced to aniline with copper powder. The addition of powdered metals, particularly copper, when used with a copper cathode, have caused nitro-compounds to be reduced to their respective amines. It has been possible in this way, to reduce some azoxy compounds such as o-azoxychlorobenzene to their respective amines.

Increased efficiency is also obtained by the addition of ions which may exist in two stages of oxidation; e.g. titanium, vanadium, chromium, iron, and cerium. These are referred to as "hydrogen carriers". Ti^{+4}, is reduced at the cathode to Ti^{+3}, and being a strong reducing agent, reacts with the substance present in the solution and is reoxidized to Ti^{+4}. The resultant ions are reduced cathodically, and the process continues.

The reduction of nitro compounds to the amines is assisted by the addition of a tin salt. Catalytic amounts of antimonic or arsenious oxides in the sulphuric acid medium used in the reduction of N, N-dimethylphenylacetamide results in an increase in yield of the phenyl ethyl amine. The yield at a lead cathode increased from 41 to 80 % as a result of using the oxide catalysts. The addition of iodine increases the speed of reduction of certain nitro compounds to the amine.

Electrodes may also be poisoned. Use of a lead cathode will result in an excellent yield of aniline from nitrobenzene. If one wishes to stop the reduction at p-aminophenol, the efficiency of the lead cathode can be diminished by the addition of arsenic or bismuth powder or copper sulphate to the catholyte.

Ions which are capable of existing in two valence states are also used to carry out many electrolytic oxidations. The action of these "oxygen carriers" is comparable to that of the "hydrogen carriers" already mentioned. Potassium permanganate has been used as a catalyst for the manufacture of saccharin by the electrolysis of toluene sulphonamide. Anthracene has been oxidized to anthraquinone in an acetone-sulphuric acid solution at a platinum anode with a 55% efficiency. Addition of cerium, chromium or manganese salt results in an increase to 80% efficiency. Acetic acid has been made by oxidation of paraldehyde in dilute sulphuric acid, in the presence of salts of cerium, manganese, molybdenum, or vanadium.

The effectiveness of catalysts varies. In the oxidation of naphthalene to naphthoquinone, catalytic effect increases in the following order: manganous sulphate, dichromate and ferrocyanide, potassium chlorate, vanadic acid, ceric nitrate. Different catalysts have also resulted in different oxidation products. A chromium salt in catalytic quantities, will cause toluene suspended in a sulfuric acid anolyte to be oxidized to benzoic acid, while a manganous salt will result in the manufacture of benzaldehyde. (See U.S.P. 867,575; 1907).

Fluorides in the electrolyte have the effect of increasing the overpotential at platinum, gold, silver and carbon anodes. Their use has a pronounced effect on the reaction process. Skirrow(67) obtained a more efficient oxidation of naphthalene and benzene in an aqueous hydrofluoric acid solution than in a medium containing the same equivalent of sulphuric acid. Perchlorates have been found to have the same effect as fluorides.

The Electrolyte

Products which result from anodic or cathodic electrolysis are to a large degree influenced by the nature of the electrolyte. In the reduction of p-dimethylamino-acetophenone, an acid medium favors the lower melting pinacol. When benzophenone is reduced in aqueous-acid, the pinacone, a rearrangement product of the benzophenone pinacol, is obtained. In a basic medium the pinacol is obtained. In acid solution, acetaldehyde is oxidized to acetic acid; in alkaline medium formic results. Aniline in dilute acid is oxidized to aniline black or quinone, while in alkali, azobenzene is formed. Concentrated sulphuric acid results in the production of p-aminophenol.

Generally, the number of solvents that may be used for anodic or cathodic processes are limited. Water cannot be used alone, because of poor solubility of the organic compound in this medium. Satisfactory concentration of depolarizer is achieved by mixing water with other solvents. Oxidations can be performed in aqueous methanol or acetic acid, absolute methanol, glacial acetic acid, acetic acid-benzene mixtures, dimethylformamide and dioxane. Water in the solvent system will lower the resistance of the solution and effect economy in the power required to run the reaction and in the heat to be removed from the cell. If the heat removal is excessive, it may cause undesirable side reactions. Where non-aqueous media must be used for an oxidation or reduction, it is possible to reduce the overall cell resistance by separating the anolyte from the catholyte with a porous divider, and to use either an aqueous catholyte or anolyte respectively.

The number and kind of electrolytes which can be used in an aqueous medium, is almost unlimited, but in nonaqueous media, salts such as lithium chloride, lithium nitrate, potassium acetate have been used. Acids are usually as satisfactory in nonaqueous media as they are in aqueous media. Organic bases, such as tetramethlammonium hydroxide, are suitable for basic electrolysis in non-aqueous media. McKee and Brockman(49) use a novel method to increase the solubility of organic compounds.

The desired solubility is achieved by salting in the organic compound in saturated solutions of sodium p-toluene-sulphonate or sodium cymenesulphonate.

Wetting agents have been found useful for solubilizing the organic compound in an aqueous medium. However, when vigorous gassing occurs, foaming in the cell may be difficult to control. Non-aqueous solvents have been avoided by utilizing a well agitated suspension of the organic compound. Good results can be obtained in many instances, but the efficiency of this method is considerably lower than employing a solution of the substance.

Reactant Concentration

The concentration of the depolarizer does not affect the nature of the products obtained; but the formation of bimolecularly reduced compounds is favored by high concentration, as the probability of collision between the reactants is increased. Reaction rate is affected by increasing the concentration of depolarizer, thus permitting higher current density to be maintained with good process efficiency. For a reversible process, the "limiting current density" at which one attains 100% efficiency is directly proportional to the concentration of the depolarizer. But in an irreversible process, this is an approximation, a guide in deciding on the concentration of depolarizer to use for a given current density, to try to achieve best efficiency. The concentration ~~of~~ reactant being oxidized or reduced at the electrode surface differs from that in the bulk of the medium. A concentration gradient exists from the electrode to the electrolyte. The diffusion layer, is the surface layer in which most of the concentration change takes place.

It is possible to obtain an approximate limiting current density for irreversible reactions if certain assumptions are made. The first is that the thickness of the diffusion layer is 0.05 cm. thick(33). This will decrease with increased temperature and agitation. The second assumption is that the diffusion coefficient of the depolarizer is 0.6 to 0.8 cm.^{2} day^{-1}, which is a value for nonelectrolytes capable of reversible oxidation or reduction, e.g. quinone $\rightleftharpoons$ hydroquinone. Fick's Law where δ is the thickness of the diffusion layer gives:

$$\delta = \frac{A\ DCn}{0.895\ i}$$

A=the area of the electrode; D=the Diffusion coefficient; i=the current; C=concentration of depolarizer and n=difference in charge between the oxidized and reduced form. Substitution of 0.05 cm. for δ, and expression of i/A per sq. cm. as the current density (c.d), results in:

$$\text{c.d.}(\text{amp./cm.}^2) = 0.0223\ \text{DCn}$$

with concentration given as gram-moles/litre. (This equation is for 20°C.) Limiting current density will increase at approximately 2.5 per cent per degree.

The optimum limiting current density is for a specific concentration of depolarizer, and therefore, the depolarizer should be replenished as it reacts, or the efficiency will diminish. Reduction at a controlled potential accomplishes essentially the same purpose. As reactant concentration is depleted, the current drops to a plateau, indicating a completed reaction.

An illustration of limiting current density in preparative work was reported(2) on the reduction of N,N-dimethylaminoethyl tetrachlorophthalimide to the isoindoline. Comparison was made between the optimum current efficiency obtained using constant current density and at a controlled electrode potential. Under constant current density 45% current efficiency was obtained. The use of controlled potential increased the efficiency to 77%.

THE EFFECT OF MIXING

To maintain a high concentration of depolarizer at the electrode surface proper mixing of the electrolyte is needed. The more efficient the agitation, the higher is the limiting current density which can be used for maximum reduction or oxidation efficiency with depolarizers which react rapidly. The rate of agitation will not have as much effect on the maximum current for best efficiency with materials which react more slowly. Agitation is necessary in order to bring unreacted starting material to the electrode surface.

The Effect of Temperature

The temperature of the catholyte or anolyte influences a number of factors controlling the process during a cathodic reduction or anodic oxidation. An increase in temperature decreases the overpotential of the electrode. The decrease depends on the electrode material, but is of the order of 0.02 to 0.03 volt for each 10°C. rise. Platinum, silver, nickel and copper, whose hydrogen overpotential values are about 0.15 volt at 20°C., show almost no action and negligible overpotential when used at 100°C. A high overpotential electrode, at elevated temperatures has high enough overpotential to accomplish many difficult reductions. This temperature effect on overpotential has been used to stop a reaction at an intermediate point. If absolute alcohol is oxidized in dilute sulphuric acid at elevated temperatures, a satisfactory yield of acetaldehyde is obtained. These results are due to the increase of overpotential with the decrease in temperature, and partly to the lower volatility of the acetaldehyde at this temperature which permits its further oxidation. It is also possible to use higher current densities at elevated temperatures. This permits shorter electrolysis periods with better yields, where additional exposure of the reaction product causes its destruction. The rate at which the raw material comes in contact with the electrode surface increases as the temperature rises, so that a slow process may be considerably improved by raising the temperature. The efficiency of oxidation of an alkaline formate solution at an iron anode is doubled when the temperature of the medium is maintained at 75°C., as compared to 16°C. On the other hand, the reduction of a ketone to a pinacol proceeds quite satisfactorily at the lower temperatures in an acidic medium; but at elevated temperatures, the desired pinacol may rearrange to the pinacone. Instability of raw material may require the use of low temperatures. If a phthalimide is subjected to cathodic reduction in an acid medium at much above room temperature, hydrolysis occurs first, so that the yield of the isoindoline is reduced.

THE EFFECT OF TIME

When an electrolysis is conducted at controlled electrode potential, prolonged exposure of the product to the current is not too serious. Under this condition, the current will drop until a plateau is reached, at which time the electrolysis is completed.

Under fixed current density conditions, the electrolysis time should be kept as short as possible, when the products of electrolysis are chemically unstable. An increase in electrode potential at the working electrode with decreasing depolarizer concentration could result in further oxidation or reduction. A too lengthy electrolysis may result in partial destruction of the reaction product due to its migration to the opposite electrode. When a new reaction is to be studied, as a first trial, it should proceed for its theoretical time (T), as computed from the equation based on Faraday's Law:

$$T_{(\mathrm{min})} = \frac{MW}{g} \quad \frac{96{,}500n}{60\ i}$$

Where MW is the molecular weight of the depolarizer, g, the weight of reacting substance in grams, n, the number of electrons involved in the process, and i, the current in amperes.

The results of initial runs may then be used as a guide to later work. In cases where the electrolysis is performed using a suspension, prolonged electrolysis may be necessary to attain satisfactory yields because of low efficiency.

III. EXPERIMENTAL WORK

General Approach to the Problem

The many investigators whose works described the oxidation of cholesterol or its derivatives were divided, in the introduction, into two groups. Those who worked with the whole molecule and others who confined their studies to oxidation of the side chain.

The cholesterol molecule has been a useful starting material for many classes of steroids and was the subject of these investigations to find inexpensive methods for preparing androgens, estrogens and pregnanes from the more readily available starting steroid. The best route to these major classes of useful products is through shortening of the eight carbon atom aliphatic side chain attached to the 17-carbon. This required that the steroid nucleus be protected at the 3 alcohol by esterification and at the Δ^5 unsaturation by bromination. This same method has been used successfully before.

The paper by Kramli(41) on electrochemical oxidation of cholesterol derivatives was studied to find a general approach to the present work. The first plan was to improve on the scant yields of Kramli's work by application of Haber's principle of control of anode potential.

Duplication of Kramli's work gave no useful product. Cholesteryl acetate dibromide (5 grams) and 25 grams of anhydrous potassium acetate were dissolved in 200 ml of glacial acetic acid. The solution was poured into a 400 ml porous alundum diaphragm. The anode in the first experiment was graphite that was immersed in the solution. This anolyte compartment was put into the catholyte.

The catholyte was a 1:1 mixture of 50% aqueous sodium acetate and glacial acetic acid. The cathode was a lead cylinder. Current density was 90 milliamperes per square centimeter of anode surface. The anolyte was maintained at 16 Amps through the cell for thirty minutes.

At the end of the run the anolyte was separated and debrominated with 3 grams of zinc dust. No material was isolated from the cell mass. Three additional trials under the same conditions, all resulted in failure to produce any product at all.

The next series of four experiments were the same as the first except that a perforated platinum anode was used. Again no product was made.

A similar set of experiments was carried out with a calomel electrode and bridge in contact with the anode. In Table 6 the data shows the conditions.

TABLE 6

CONSTANT POTENTIAL ELECTROLYSIS WITH PLATINUM ANODE

Anolyte: 200 ml of glacial acetic acid containing 5 g of cholesterol acetate dibromide and 25 g of potassium acetate.

Anode-Platinum:
Reference electrode - saturated calomel.

Diaphragm:
Alundum.

Catholyte:
200 ml mixture of 1:1 50% aqueous sodium acetate and glacial acetic acid.

Cathode: Lead.

Temperature:
80 - 90°C.

Open Circuit Anode Potential:
0.23 volts.

Time of Electrolysis:
60 Minutes.

EXP.	ANODE POTENTIAL VOLTS	AVERAGE CELL CURRENT AMPS	CELL V.
1	1.25	4.7	6.2
2	1.75	5.2	6.5
3	2.50	5.8	6.7
4	2.90	6.5	7.0

This last set of experiments produced no product. After those runs, it was decided to proceed at lower temperature to prevent destruction of the starting material. At 30°C. about half of the starting material was recovered, but no additional product was made. Thin layer chromatographs were used to check the progress of the reaction.

It was then decided that a lead anode might produce better results. In acetic acid solution, the lead was coated with the white acetate and no current flowed through the cell because of the high resistance of the film.

From this point, trials were made with other anolytes to prevent formation of a non-conductive coating on the anode. But now solubility of the starting steroid became a problem since the best anolytes, the mineral acids, seemed useless as solvents.

The cholesteryl acetate dibromide was added to 5%, 10% and 15% solutions of dodecyl-benzene sulfonic acid and suspended by mixing. The suspensions were not stable. The steroid was then dissolved in carbon tetrachloride and successfully suspended in the three different concentrations of sulfonic acid.

In three experiments, 5 grams of cholesteryl acetate dibromide was dissolved in 50 ml of carbon tetrachloride and the sample was suspended successively in 350 ml of 5%, 10% and 15% aqueous solutions of dodecylbenzene sulfonic acid. The suspensions were stable as long as they were mixed.

Electrolysis of these suspensions at a carbon anode caused severe oxidation of the anode and was interrupted by excessive foaming of the anolyte within one minute. With platinum and lead anodes the same foaming caused the anolyte to climb out of the cell so badly that the electrolysis was stopped.

The decision was then made to suspend the carbon tetrachloride solution in 4.5 molar sulfuric acid. With a carbon anode severe destruction was encountered in the anolyte. A run made with a platinum anode did not show any new product on a thin layer chromatogram, but 80% of the starting material was recovered. When the first experiment was made with a lead anode that had previously been anodized to form an oxide, a new product appeared as a second spot on the chromatogram with a third material that did not elute from the starting point. Furthermore, the weight of products recovered from the cell mass appeared to remain constant.

A new attempt was made to repeat this last experiment while the anode potential was maintained at a constant value. The conditions for the trial were:

Anolyte: 2.5 grams of cholesteryl acetate dibromide dissolved in 25 ml of carbontetrachloride was suspended by mixing at 30°C in 250 ml of 4.5 molar sulfuric acid.

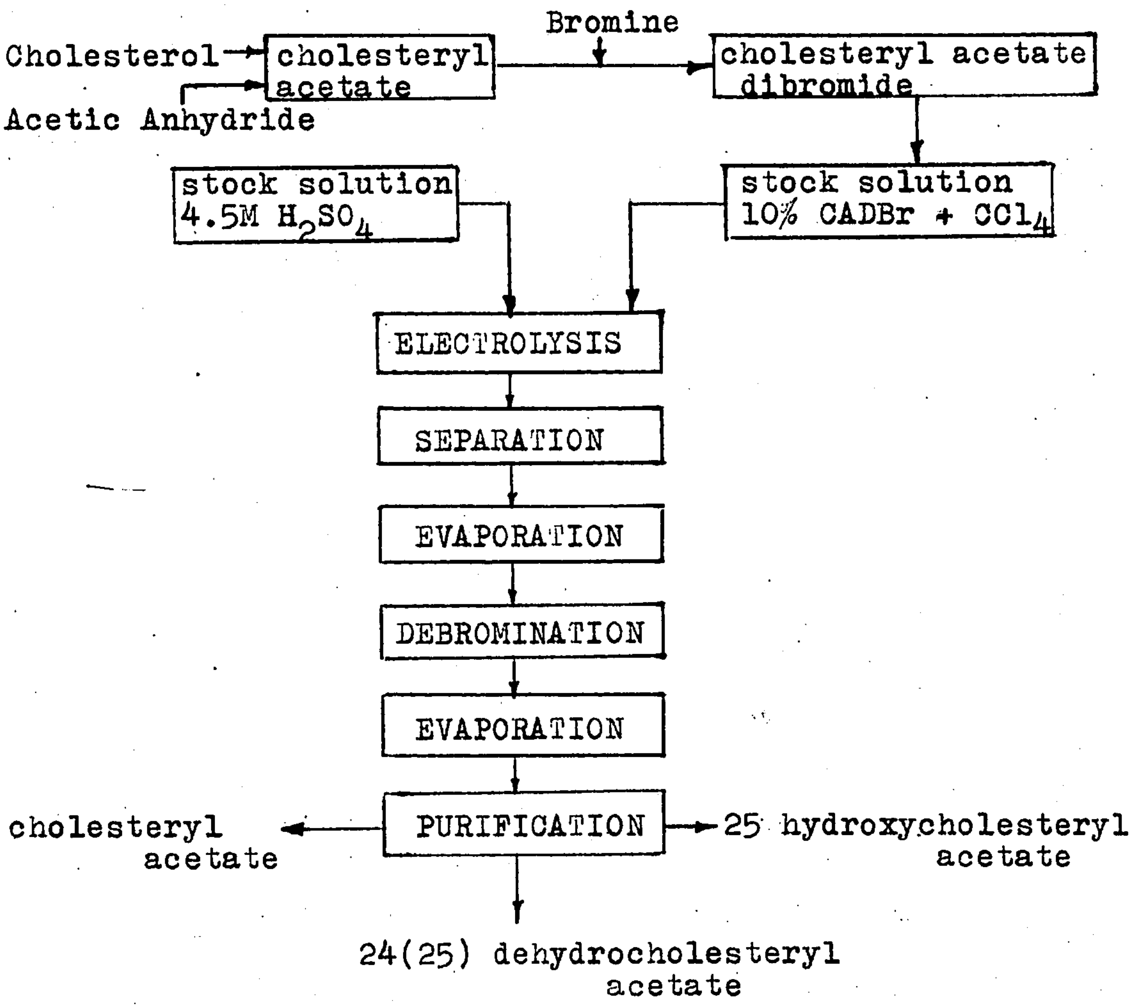
Anode:	Lead dioxide on lead - 80 cm^2.
Catholyte:	200 ml of 4.5 molar sulfuric acid.
Cathode:	1/8" diameter lead wire.
Diaphragm:	400 ml porous alundum cup.
Reference electrode:	Saturated calomel.

The experiment was begun by turning on the power to the cell with the current set at 10 amperes. The initial anode potential was 2.5 volts. As electrolysis proceeded, it was necessary to reduce the cell current to maintain the anode potential at 2.5 volts. After ten minutes the cell current had been reduced to 2 amperes and production of gas in the cell had almost stopped. Several attempts were made to run at constant anode potential by raising the current to 25 amperes and the voltage to 35 volts, but in each case the anode potential could only be held stable by reducing the current to zero. Since under these conditions it would take too long to achieve any conversion, it was decided to run the future trials at constant current density.

The success of the first constant current density experiment with a lead dioxide anode in a sulfuric acid anolyte led to the development of a program for study that appeared to have promise.

Flow Sheet

From the results of the preliminary experiments, the following process flow for the experimental program was developed:



Raw Material Preparation

The raw material, cholesteryl acetate dibromide, was prepared from commercially available cholesterol derived from animal tissue, as follows:(48)

100 grams of cholesterol was refluxed with 200 ml of acetic anhydride for one hour. On cooling, the crystalline acetate separated. The mass was filtered and washed with glacial acetic acid.

The wet crystals were dissolved in 1000 ml of diethyl ether. To this was added a solution of 16 ml of bromine in 500 ml of glacial acetic acid. After a few minutes of stirring, crystals precipitated. The entire mass was permitted to stand overnight. The crystal slurry was filtered and washed once with cold glacial acetic acid and then three times with petroleum ether (39°C. to 49°C. boiling range). The crystals were air dried at room temperature. Weight of the dry material was 130 grams. Melting point was 115-117°C. Fieser(28) reports 115.4°C. and 117.6°C. for two crystal forms.

It was found that this material was stable to light and heat for periods up to six months in carbon tetrachloride solution. If stored as a dry powder it decomposed in less than a month. In ethylene dichloride or chloroform, the brominated ester decomposed in a few days to form dark red solutions.

Oxidation

The oxidation was conducted by loading 250 ml of 4.5 sulfuric acid in a 400 ml porous alundum beaker, placing a lead anode of 80 sq. cm. in the electrolyte and surrounding this with a cathode chamber containing 200 ml of 4.5M sulfuric acid with a lead wire cathode, and then pre-electrolyzing the anode at low current density (1.5 ma per sq. cm.) to form the lead dioxide film. After this film had been formed, the depolarizer, a solution of 2.5 g. of cholesterol acetate dibromide in 25 ml. of carbon tetrachloride, was poured into the anode compartment. The anode compartment contained a glass cooling coil and a 0° - 100°C. thermometer. A calomel electrode was positioned in contact with the anode surface.

Reactions were carried out at current densities ranging from 62.5 to 250 milliamps per square centimeter. Temperature varied from 30°C. to 40°C. The range of total time of electrolysis was varied from 30 minutes to 240 minutes. The data in Table 7 show the variables studied.

Debromination

At the end of a run the anode compartment was removed from the cell and the entire mixture was poured into a separatory funnel. The anode compartment was rinsed with

TABLE 7

TABLES OF ELECTROLYSIS DATA

Constant Conditions

Anode: Pb/PbO_2 80 cm^2.

Anolyte: 250 ml of 4.5 M H_2SO_4 with 25 ml. of carbon tetrachloride that contained 2.5 gm cholesteryl acetate dibromide.

Diaphragm: Alundum beaker - 400 ml.

Cathode: Lead wire - 1/8" diam.

Catholyte: 200 ml. 4.5 M H_2SO_4

Magnetic Mixer: 4 cm x 0.5 cm. dia. bar (approximately 50 rpm)

E_A = Anode Potential in Volts (vs.S.C.E.);

θ = Time from start in minutes.

Run 1. 30°C 5 Amps (63 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+4.80	+4.00	+3.25	+2.70	+2.40	+1.80	+1.75

Cholesteryl Acetate Recovered: 0.82 gm

Total Dehydrocholesteryl Acetate Recovered: 0.90 gm

Conversion: 55.0%

Yield: 90.4%

Run 2. 40°C. 5 Amps (63 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+5.0	+4.00	+3.30	+2.80	+2.50	+2.00	+1.75

Cholesteryl Acetate Recovered: 0.88 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.87 gm.

Conversion: 51.5%

Yield: 93%

Run 3. 30°C. 10 Amps (126 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+4.25	+3.70	+2.70	+2.15	+1.85	+1.20	+1.15

Cholesteryl Acetate Recovered: 0.94 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.78 gm.

Conversion: 48.7%

Yield: 89.0%

Run 4. 40°C. 10 Amps (126 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+4.35	+3.70	+2.80	+2.20	+1.90	+1.30	+1.10

Cholesteryl Acetate Recovered: 0.91 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.82 gm.

Conversion: 50.0%

Yield: 90.0%

Run 5. 30°C. 15 Amps (189 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+4.00	+3.20	+2.30	+1.65	+1.50	+1.00	+0.80

Cholesteryl Acetate Recovered: 1.09 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.63 gm

Conversion: 40.0%

Yield: 86.5%

Run 6. 40°C 15 Amps (189 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+4.00	+3.30	+2.50	+1.75	+1.50	+1.10	+0.90

Cholesteryl Acetate Recovered: 1.05 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.65 gm.

Conversion: 42.0%

Yield: 85.0%

Run 7. 30°C. 20 Amps (252 ma/cm^2)

θ	0	10	30	50	60	100	120
E_A	+3.50	+2.60	+1.70	+1.00	+0.75	+0.45	+0.40

Cholesteryl Acetate Recovered: 1.27 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.47 gm.

Conversion: 30.0%

Yield: 85.0%

Run 8. 40°C. 20 Amps (252 ma/cm2)

θ	0	10	30	50	60	100	120
E_A	+3.60	+2.60	+1.85	+0.95	+0.80	+0.45	+0.45

Cholesteryl Acetate Recovered: 1.06 gm.

Total Dehydrocholesteryl Acetate Recovered: 0.48 gm

Conversion 36.0%

Yield: 87.0%

10 ml. of fresh carbon tetrachloride which was combined with the material in the separatory funnel. A lower organic layer was separated, transferred to an evaporating flask and held for further work. The sulfuric acid layer was washed twice with 10 ml. portions of carbon tetrachloride and all the organic layers were combined. The acid layer was discarded. It was found best to debrominate the reaction products as quickly as possible to avoid further decomposition. Carbon tetrachloride was removed by evaporation at room temperature at reduced pressure (100 torr). Residue was dried and then dissolved in a mixture of 20 ml. of ethyl ether and 10 ml. of glacial acetic acid. Two grams of zinc dust were added at room temperature while the solution was stirred. Mixing was continued for fifteen minutes. At the end of this time three drops of water were added to complete the reaction.

The solution was decanted from the solids into a 100 ml. separatory funnel. Solids were washed twice with 5 ml. of ethyl ether and this was combined with the decanted liquid.

The ether solution was washed four times with 50 ml. of water to remove all the acetic acid. The remaining ether solution was evaporated to dryness under vacuum (100 torr).

Product Purification

The separation and purification of the products of reaction were accomplished in one operation by the technique of preparative thin layer chromatography.(81) Routine technique was developed for separation of the mixed acetates isolated above, by the use of chromatoplates.

Chromatoplates were prepared, five at a time, from pyrex glass 400 mm x 200 mm x 3 mm. A mixture was prepared from 80 gm. of Merck (Darmstadt) silica gel G and 160 ml of tap water. This slurry was mixed for 60 seconds and then applied with a homemade plastic reservoir to the glass. Plates were vibrated to make the coating uniform. The plates were air dried for 30 minutes on a flat surface and then transferred to a carrier. Plates and carrier were activated for two hours in an electric oven at 90°C., removed from the oven and quickly placed in an airtight drying cabinet. The active plates were stored in this manner so that they would be ready for use when needed.

The technique for separating the components of the ether residue was determined by pilot tests on 0.25 mm thick silica gel G chromatographic plates. In this way the most useful solvent developing system was found to be a 50:50 mixture of petroleum ether and benzene. Iodine vapor was used to locate the separated steroids on the test plates.

The dry mixed acetates from the ether residue were dissolved in petroleum ether so that the concentration was 10% w/v. A 2 ml portion of this solution was applied across the 200 mm width of an activated chromatoplate that had been removed from the storage cabinet. This plate was then developed at room temperature in a pyrex tank that was saturated in the vapor space by pouring 400 ml of the petroleum ether-benzene solution over a filter paper liner. The development time for each plate was 180 minutes. The developer solution was allowed to run the full 400 mm length of the plate to achieve the maximum separation of the components of the acetate mixture. The plate was then removed from the developing tank and placed in a chamber that could be evacuated continuously by a water aspirator. In this way the solvent was evaporated at low temperature in order to prevent oxidation of the products on the plate.

The plate was then placed in a hood and covered with a piece of glass over its entire surface, except for 5 mm along one edge. This edge was developed with a pressurized spray of aqueous sulfuric acid containing formalin. The bands of silica that contained the three products were made visible by a color reaction. They were different shades of blue. The plate was removed from the hood and parallel scratches were made across the plate face where they had been located by the formalin solution. Each zone was then removed from the plate with a razor blade and collected separately in extraction flasks. Each of the three products collected were eluted from the silica with carbon tetrachloride. The material that had been collected from the front zone of the plate and later proved to be cholesteryl acetate, was recrystallized from a minimum quantity of acetone. The second band of material, which proved to contain a mixture of 24 and 25 dehydrocholesteryl acetate was charcoal treated first in benzene and then recrystallized from methanol.

The material which had been collected from the line of application on the chromatoplate was found to be 25 hydroxycholesteryl acetate, and was recrystallized from acetone after the carbon tetrachloride extract of the silica gel had been carbon treated and evaporated to dryness.

IV. EQUIPMENT DESCRIPTION

The apparatus that was used for the preparation of raw material as well as for purification of the reaction products consisted of the usual standard laboratory glassware.

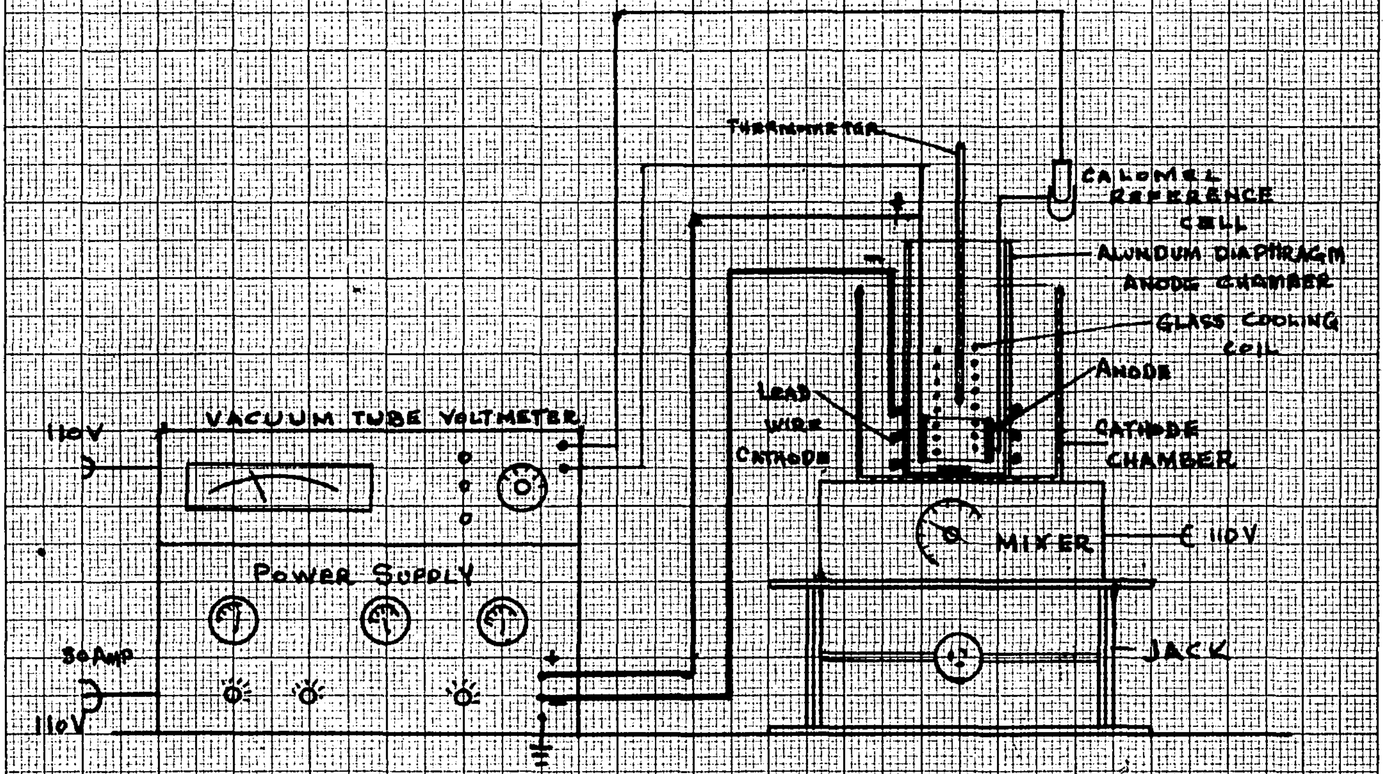
The electrolysis apparatus (See Figure 3) was composed of a power supply, the electrolytic cell, and a vacuum tube voltmeter for measuring anode potential.

The power supply was a Lambda solid state 1 KVA unit, Model LE-104, with current regulated output from 2.5 to 27 amperes; voltage regulation was possible over a 0 - 36 volt range. Connection to the cell was with No. 10 600 v. TFE insulated copper wire.

The electrolytic cell was made from a 600 ml beaker which contained the catholyte and 1/8" lead wire cathode surrounding a 400 ml porous alundum beaker. The anode chamber held a semicircular, perforated lead oxide anode of 80 cm^2 area. Inside the anode was a glass cooling coil made from 4 mm tubing with eight turns, 35 mm O.D. For agitation, the anode compartment contained a 40 mm x 4 mm bar magnet encapsulated in teflon. Temperature was measured by a 0 - 60°C. laboratory thermometer. The entire cell was supported by the stirrer mechanism.

FIGURE 3

ELECTROLYSIS APPARATUS



A standard calomel electrode Beckmann No. 34079 was held in direct contact with the anode and connected to a Hewlett-Packard Model 413A DC null vacuum tube voltmeter.

V. ELECTROCHEMICAL PREPARATION OF LEAD DIOXIDE

The preliminary success of the lead dioxide anode prompted a survey of the literature to reveal the properties of this material and the manner in which it should best be prepared.

Lead dioxide formed electrolytically, exists in orthorhombic α and tetragonal β crystal forms. Conditions for the formation of these two types have recently been the subject of a number of studies.(15)(59) Previous work in the U.S.S.R. concerning the behavior of α and β PbO_2 in sulfuric acid, has led Kiseleva and Kabanov(82) to propose that the β form is a consequence of H_2SO_4 chemisorption on the lead dioxide formed during anodic oxidation of lead. This explanation is challenged in a paper by R.A. Baker(4). He maintains that the Russian work implies that sulfate or bisulfate anions perform in part or whole a vital function in determining the modification of the PbO_2 formed, and challenges this implication with facts from his work as well as other recent support(3)(15) for the contention that it is hydrogen ion concentration which influences the nature of the lead dioxide deposit.

An analysis by Ruetschi and Angstadt(59) indicates that the following process takes place on anodization of lead in sulfuric acid:

Initially, a $PbSO_4$ film is formed. As this film grows to a critical thickness, it is accompanied by a critical ohmic drop, so that SO_4^{--} and H^+ ions are unable to penetrate the film easily. Any decrease in SO_4^{--} ions causes a corresponding equivalent loss of H^+ ions, because the system must remain electrically neutral. In the dense $PbSO_4$ layer, a high electric field is established with the consequent large voltage drop, and tends to repel H^+ ions from the microcavities of the inner part of the film and attracts SO_4^{--} ions and OH^- ions formed by dissociating water molecules. This causes the pH to stabilize at a high value in the interior of the corrosion film.

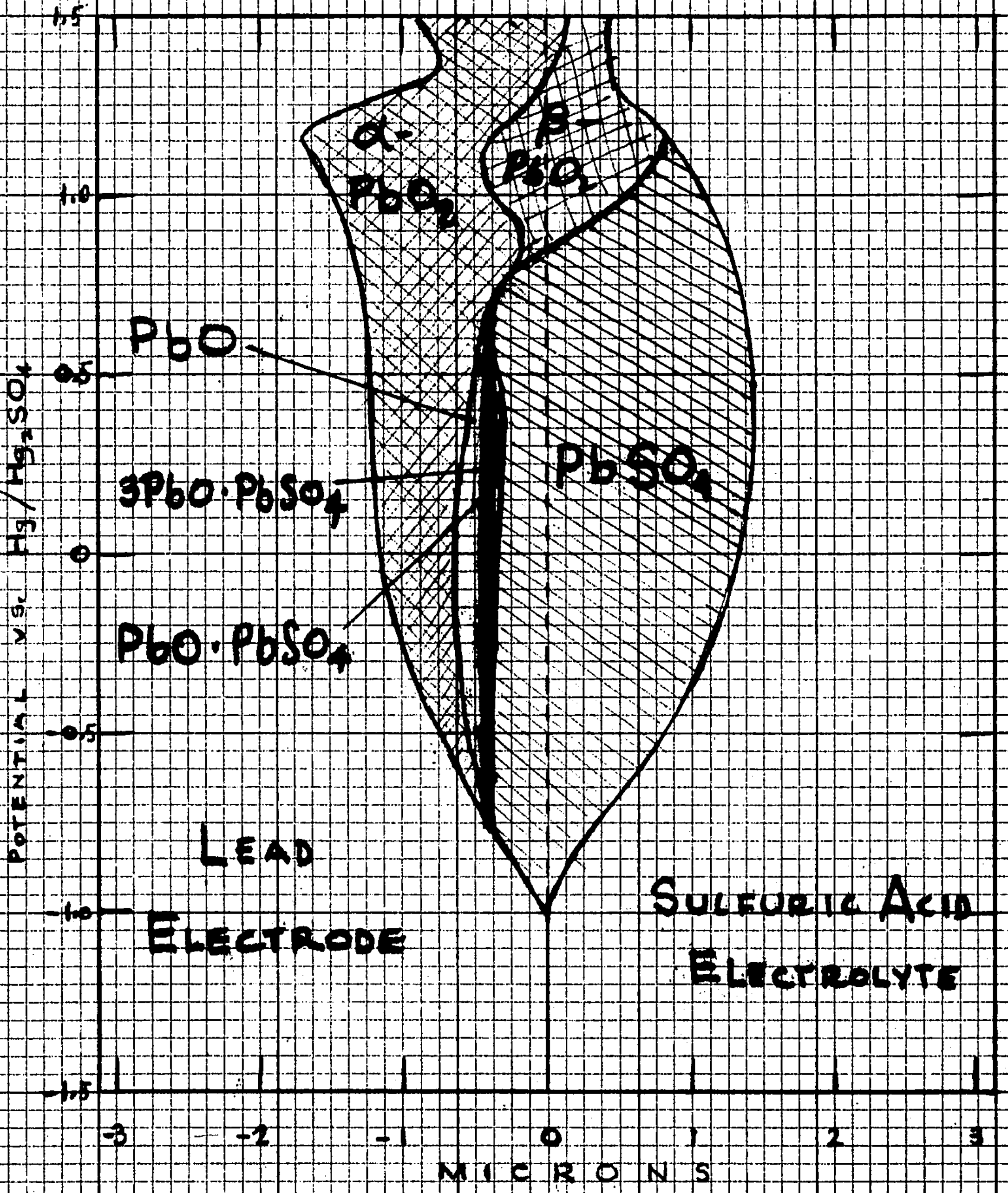
The pH gradient across the film is assumed to be abrupt after ionic diffusion has stopped and the reaction at the electrode limited by mass transport. The pH rises quickly to the point where OH^- ions will be available to oxidize lead to PbO or hydrated forms such as $5PbO \cdot 2H_2O$. These divalent lead compounds are rapidly oxidized to α PbO_2. This oxidation of PbO to α PbO_2 in the corrosion film takes place above -0.3v (vs STP H_2). As the electrode potential is raised, the outer sulfate layer and the PbO film increase in thickness as the ohmic drop increases. At high enough potentials, lead sulfate may be oxidized to PbO_2 in the more acidic outer part of the corrosion layer. At a pH of 2 in the interior of the film, $PbSO_4$ could be oxidized to PbO_2 at a potential of +0.7v (referred to standard Calomel electrode). At higher anodizing potential, β PbO_2 is present in the outer film. Ruetschi and Angstadt(59) were able to construct a schematic diagram of the composition of the corrosion film as a function of the potential at which it was formed. (See Figure 2). Their study concerned the variation of anode potential with time in a 24 hour period of anodic oxidation of a lead electrode. This analysis refers to films produced at constant potential and made from data collected at 30°C. in 4.2M. H_2SO_4 which corresponds almost precisely to the conditions used throughout the experimental work covered by the investigation of the anodic oxidation of cholesteryl acetate dibromide.

From the cross-section in Figure 3 several points uncovered in the present work are quickly explained. These points will be discussed below.

FIGURE 2

SCHEMATIC DIAGRAM OF THE COMPOSITION OF THE CORROSION FILM OR A LEAD ANODE AS A FUNCTION OF POTENTIAL

REF: ANODIC OXIDATION OF LEAD AT CONSTANT POTENTIAL
P. REUTSCHI & R.T. ANGSTADT
J. ELEC. CHEM. SOC. VOL 111 No. 12 p. 1329 (1964)



VI. ANALYTICAL TECHNIQUES

Present day techniques and devices, including gas chromatography, thin layer chromatography, infrared spectroscopy and devices for determining the physical properties of steroids, were employed for analysis and identification of the products of reaction.

Gas Chromatography

For gas chromatography, it was found necessary to use all glass contact parts from injection to exit to prevent decomposition. It was also important to have a heated injection section to assure complete vaporization of the sample. The column packing material was made from 80/100 mesh Diatoport S that had been acid washed and deactivated by two treatments with hexamethyl dichlorosilane before coating with a liquid phase of 3.8% silicone gum SE-30. The columns were used in the F & M. Scientific Corp. Model 400 and in Perkin Elmer's Model 811. Both machines were equipped with flame ionization detectors. The sample size in the case of the Model 400 was 2 micrograms per run and 6 micrograms per run for the 811. The column in the 400 was a 4 ft x 3 mm glass U-tube operated at 230°C. The injection port temperature was 275°C.; the hydrogen flame detector was at 235°C. Range was 10; attenuation was 32. Helium flow was 75 cc/min. The Perkin Elmer 811 contained a 4 ft x 3 mm coiled glass column at 240°C. Injection port temperature was 280°C. Detector temperature was 250°C. Helium flow was 70 cc/min. The column used in the Perkin-Elmer 811 machine made a slight separation of the 24 and 25 dehydrocholesteryl acetate. Both columns failed to show the presence of 25 hydroxycholesteryl acetate. This material was found by thin layer chromatography.

Thin Layer Chromatography

Thin layer chromatography is related to paper chromatography but produces much quicker results with better resolution. This technique was used according to the method of Van Daam(69) for qualitative identification of steroids on a 0.25 mm thick layer on silica gel G. For quantitative preparation of the products of reaction, the method of Korzun(81) was used on 1.5 mm thick silica gel G.

The use of the method of Van Daam(69) provided the first clue to the identities of the products of reaction. One of the products which eluted just behind cholesteryl acetate (chromatograms were run on debrominated reaction products) indicated that the two were closely related in molecular size and polarity. A sample of this material collected from the silica gel G melted at 90 - 92°C. Material left at the point of origin melted at 139°C. Careful study of the papers of Ryer(63), Idler(35) and

Fieser(30) together with the gas chromatographs and thin layer plates, made it easier to identify the main electrolysis products.

When the chloroform layer containing the cell products had been separated, and debrominated, as described above, the petroleum ether solution was spotted on 50 x 200 cm pyrex plates coated with 0.25 mm of activated silica gel G and developed with a 1:1 mixture of petroleum ether-benzene, dried and developed by iodine vapor.

In this way it was possible to see the debrominated acetate mixture contained three materials: the starting material which eluted first, a second material of similar polarity that ran 90% as fast as the cholesteryl acetate and a third material of much greater polarity which remained at the starting point. (This could have been more than one compound, but later proved to be the intermediate, 25-hydroxycholesteryl acetate).

Infrared Spectroscopy

The infrared spectrum can be used to characterize the oxygen containing functional groups of steroids and to determine configuration as well as locate centers of unsaturation.

If a compound has been previously prepared, its identity can be established by comparison with an authentic sample. Dobriner, et al(22) maintain that the specificity of such spectra has been confirmed by practical experience in thousands of cases.

Considerable use was made of this specificity to identify the products and to determine that some change of the raw material had occurred in the cell and not in subsequent workup. The infra red spectrograph was a Perkin Elmer-Model 21. Conditions of each analysis are noted on the spectra in figures 15 through 18.

Physical Properties

Physical properties used for identification of the steroids were melting points and optical rotation.

The melt points of all materials were made in a simple capillary tube. Thermometers were accurate Beckmann short range type. After a sample had been quickly melted to locate the proper range, a new sample was inserted when the heating bath was within 5°C. of the melt point. The bath was raised at 1°C. per minute till the sample melted.

Optical rotation data were obtained with a polarimeter made by Franz Schmidt & Haensch (Berlin) Serial No. 8348. In each case solvent and concentration are noted. A Sodium D lamp supplied illumination. Samples were cooled to 20°C.

Molecular weights were determined on a mass spectrometer, CEC Model 21-103.

Carbon and hydrogen analyses were run in a Model 185 F & M Scientific Corp. Carbon-Hydrogen-Nitrogen Analyzer. However, there were inaccuracies due to improper drying of the chromatographic column. Consequently these analyses were repeated by standard micro combustion technique.

VII. MATERIAL BALANCE

In all the experiments performed with a lead oxide anode, the organic and inorganic layers were separated, and the inorganic phase was washed twice with carbon tetrachloride. The carbon tetrachloride washes were combined with the first main body of extract and together treated to recover the products. In all cases the carbon tetrachloride had removed all organic material and a reasonably good balance of input and output was obtained.

The stoichiometry for preparation of cholesteryl acetate dibromide from cholesterol is:

Cholesterol + Acetic Anhydride + Bromine = Cholesteryl Acetate Dibromide

M.W. = 386.6 M.W. = 588.5

The theoretical yield of dibromide from 100 grams of cholesterol would be:

$$100 \times \frac{588.5}{386.6} = 152 \text{ grams.}$$

The actual yield was 130 grams.

$$\text{Molar yield} = \frac{130}{152} \times 100 = 85.5\%$$

In each run 2.5 g of cholesteryl acetate dibromide was used.

For run number two the total weight of dry debrominated products were shown to have a total weight of 1.75 g. Thin layer chromatogram preparative technique yielded: (Based on a 200 mg. aliquot)

	MW	g	Equal to g of Dibromide
Cholesteryl Acetate	428.7	0.88	1.21
24(25)-Dehydro Cholesteryl Acetate	426.7	0.79	1.09
25-Hydroxy Cholesteryl Acetate	444.7	0.08	0.10
		1.75	2.40

The 100 mg loss represents 4% of the charge.

Yield of Dehydro Derivatives

Since in practice it would be best to convert all of the 25-hydroxy compound to the dehydro derivatives, in order to further degrade the side chain to make useful products, let us consider the total amount of dehydro cholesteryl acetate to be equal to:

$$0.79\text{ g} + 0.08 \times \frac{426.7}{444.7} = 0.87\text{g}$$

2.5 grams of cholesteryl acetate dibromide is equivalent to: $2.5 \times \frac{428.7}{588.5} = 1.82$ g Cholesteryl Acetate

1.82 - 0.88 = 0.94 g of cholesteryl acetate equivalent reacted:

The yield was $\dfrac{0.87}{0.94 \times \dfrac{426.7}{428.7}} \times 100\% = \underline{93.0\%}$

Conversion

Consequently for this experiment the conversion was

$\frac{0.94}{1.82} = \underline{51.5\%.}$

Additional typical yields and conversions are listed in Table 7.

Yield of 25 Hydroxy Cholesteryl Acetate Recovered

$$\frac{0.08}{0.94 \times \frac{444.7}{428.7}} \times 100 = 8.2\%$$

Based on cholesteryl acetate equivalent converted.

VIII.RESULTS

After all conditions for electrolysis had been standardized by elimination of unworkable anodes, solvents and systems, experiments were conducted with PbO_2 anodes with the raw material in a saturated carbon tetrachloride solution suspended in 4.5 molar sulfuric acid. At first, attempts were made to conduct runs at different constant anode potentials. It was observed that in order to stabilize this potential, the cell current rapidly fell to zero, with no appreciable production of useful material.

In order to produce substantial oxidation, all future runs were made at constant current density. The data in table 7 show that a plot in figure 19 of anode potential varying with time at different current densities, is equivalent to the results obtained by Ruetschi and Angstadt(59) in the preparation of a lead oxide anode in 4.3 molar sulfuric acid at 30°C.

To determine if the reaction were truly electrochemical in nature, it was necessary to check the current efficiency for several runs. At current densities of 62.5 m.a./cm^2 (5 Amps with Electrode Area of 80 cm^2) a single run of one hour duration was equivalent to 5 ampere-hours or, since 26.8 ampere-hours equal one equivalent weight of reactant converted, we should have been able to produce (for a one electron reaction):

$\frac{5}{26.8} \times 444.7 = 83$ grams of 25-hydroxycholesteryl acetate which in turn would have dehydrated to form 80 grams of 24-dehydrocholesteryl acetate.

Since only about 1 gram was formed at most, the process would appear to have only a 1.2% efficiency. This is sufficient evidence to indicate that what was occurring was chemical and not electrolytic oxidation.

Yet, by examination of the results of several "blank" experiments, it is obvious that this oxidation cannot readily occur without the use of the electric current.

It was necessary to check the stability of the raw material to the conditions of the oxidation. Duplicate samples run in 4.5 molar sulfuric acid with no current, did not cause hydrolysis or degradation of the cholesteryl acetate dibromide. Thin layer chromatograms showed only cholesteryl acetate present after debromination of the reaction mass. This incidentally proved that debromination did not cause oxidation. Further proof of this is discussed below, under analysis of cell reaction mass.

It was found that no oxidation would occur in carbon tetrachloride solution that contained varying amounts of chemically produced lead dioxide.

However, in suspension in 4.5 M sulfuric acid, which contained chemically prepared lead dioxide, conversions in the range of 5% were obtained. When the lead anode was oxidized in sulfuric acid with the aid of the electric current and the current then shut off, injection of the raw material into the cell did not yield any product.

The explanation for all these observations may be found by examination of Figure 2 on Page 60. Ruetschi and Angstadt(59) have proved that the composition of the lead anode film in 4.3 M sulfuric acid is a function of the anode potential, and that when the potential is zero, the film on the electrolyte side is lead sulfate.

Since all the useful conversion was performed at anode potentials substantially above 2.5 volt, it appears from Figure 2 on Page 60 that the catalytic agent involved in the oxidation was the β form of lead dioxide.

From the data in Table 7, it is concluded that conversion is directly correlated with anode potential. The best conversions occurred when cell conditions permitted the highest anode potential.

Chemically derived lead dioxide, according to Burbank,(15) made at low temperature would consist mainly of the α configuration, but could be converted to the β form in air at approximately 300°C. This would account for the relatively small conversion with reagent grade lead dioxide.

Analysis of Cell Reaction Mass

As further proof that subsequent chemical treatment of the reaction mass was not responsible for oxidation of the raw material, an infrared spectrum for run number two is included in Figure 15. Here we see the spectrum (A) of the starting material with the spectrum of the reaction mass (B) superimposed. Fortunately, the carbon-bromine absorption occurs above 15 microns and does not interfere with the results.

The two peaks at 7.8 and 11.4 microns are identified as the 24-dehydro-vicinal double bond system. The major difference is seen in the two spectra where a new peak occurs at 7.8 microns in the reaction mass.

Physical Properties of Products

Proper identification of the products of reaction was made by collection of the melting points, optical rotations, molecular weights, infrared spectra and carbon and hydrogen content and comparison with known information. These properties are below in Table 8. It must be pointed out at this time that in the 1950 paper by Ryer(63), the author assumed that dehydration of 25-hydroxycholesteryl acetate proceeded completely to the 25-dehydro derivative. Dauben & Bradlow(20) in 1950, made the same assumption although they had used phosphorous tribromide in benzene in the dehydration.

But in 1957, Idler & Fagerlund(35) proved conclusively that the other workers mentioned above had produced mixtures of 24- and 25-dehydrocholesteryl acetate. Fair agreement is shown in comparison of the properties of the dehydrated steroids made in this research with the data of Ryer (Schering) since the milligram quantities of material used for identification were in fact supplied by Schering. Furthermore, the gas chromatographs obtained with the Perkin-Elmer 811 apparatus (Figures 4-9) on close examination show the two peaks of the 24 and 25 dienes.

TABLE 8

Properties of Reaction Products

	Cooper	Ryer(63)	Dauben(20)	Idler(35)‡
24(25) Dehydrocholesteryl Acetate				
M.P.°C.	91.-94.0	93.5-94.	92.5-93.5	112
α_D^{20*}	-43.4°	-43.6°	-42.8°	-44.4°

Analysis: Calculated for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87

Found:				
C =	80.80	81.39	81.64	81.60
H =	10.70	11.08	10.63	10.72

M.W.: Calculated 426.7
Found 426 From Mass Spectrum

	Cooper	Ryer(63)	Dauben(20)	Idler(35)‡
25-Hydroxycholesteryl Acetate				
M.P.°C.	140-141	140.2-141.2	138.5-140.0	-
α_D^{20}	-40.8	-40.4	-42.1	-

Analysis: Calculated for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88

Found:				
C =	78.25	78.15	78.80	-
H =	11.09	10.66	10.56	-

M.W.:
Calculated 444.7
Found 444 From Mass Spectrum

*2% Chloroform Solution
‡25-Dehydrocholesteryl Acetate

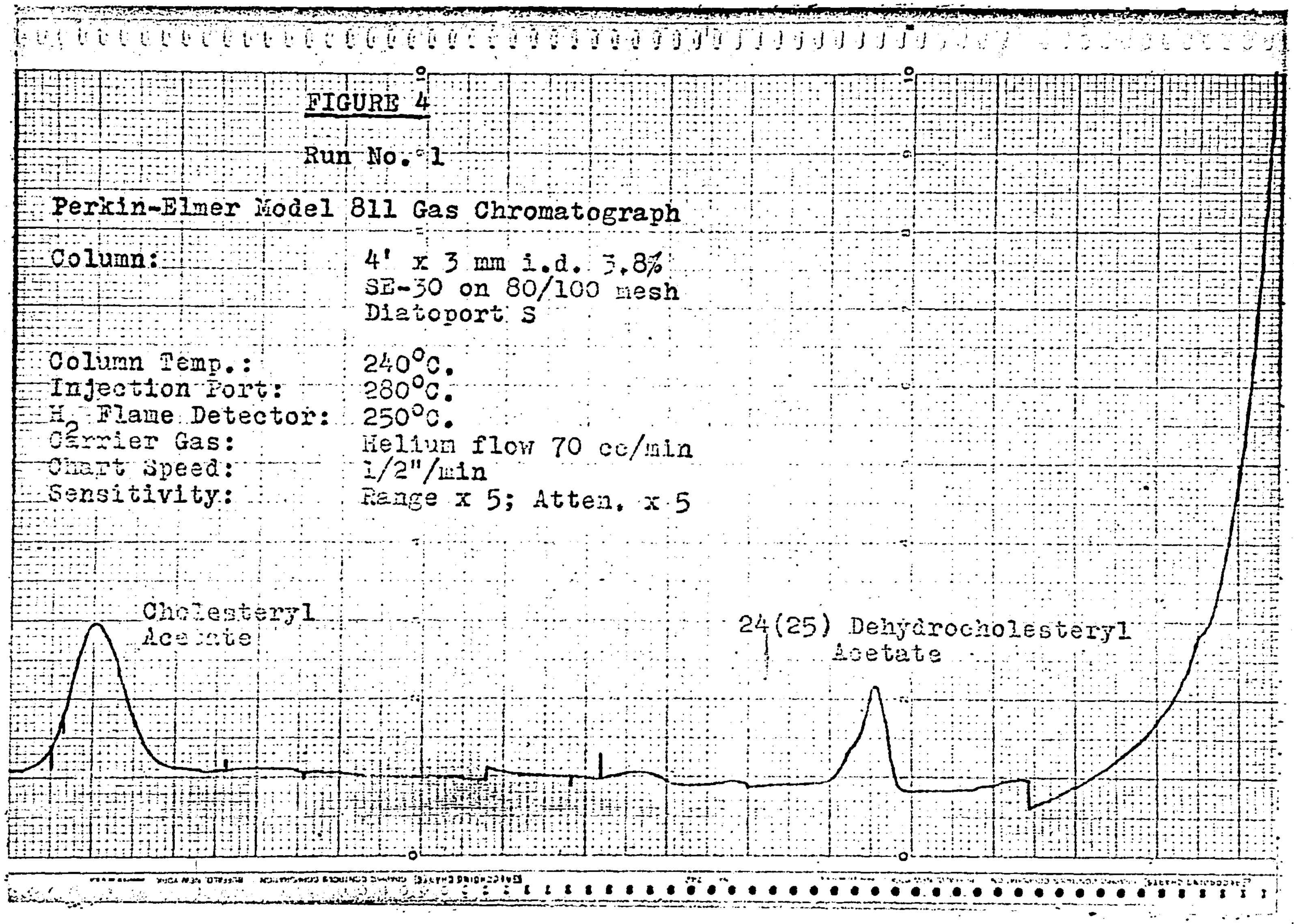


FIGURE 4

Run No. 1

Perkin-Elmer Model 811 Gas Chromatograph

Column: 4' x 3 mm i.d. 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 240°C.
Injection Port: 280°C.
H_2 Flame Detector: 250°C.
Carrier Gas: Helium flow 70 cc/min
Chart Speed: 1/2"/min
Sensitivity: Range x 5; Atten. x 5

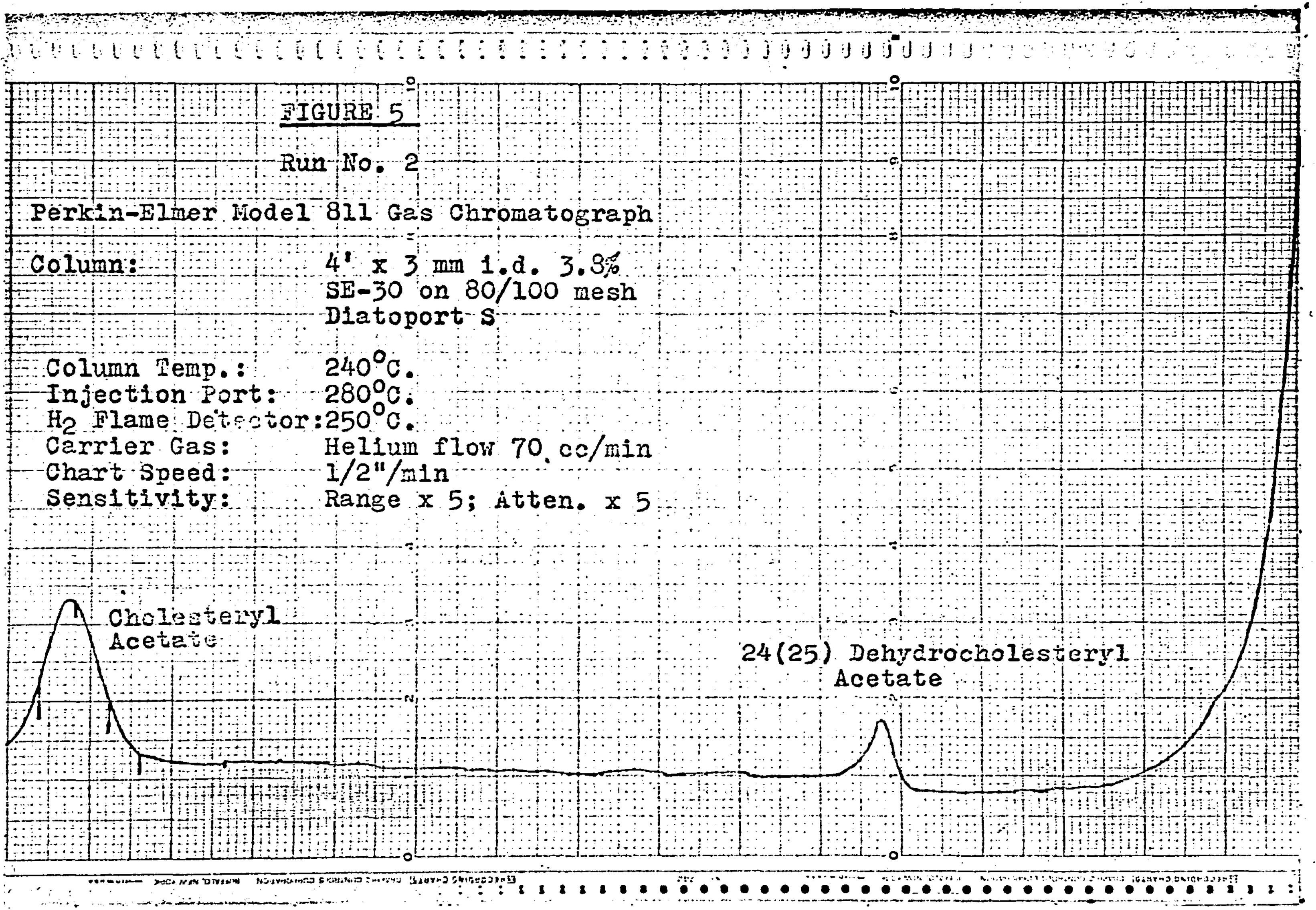


FIGURE 5

Run No. 2

Perkin-Elmer Model 811 Gas Chromatograph

Column: 4' x 3 mm i.d. 3.8%
SE-30 on 80/100 mesh
Diatoport S

Column Temp.: 240°C.
Injection Port: 280°C.
H_2 Flame Detector: 250°C.
Carrier Gas: Helium flow 70 cc/min
Chart Speed: 1/2"/min
Sensitivity: Range x 5; Atten. x 5

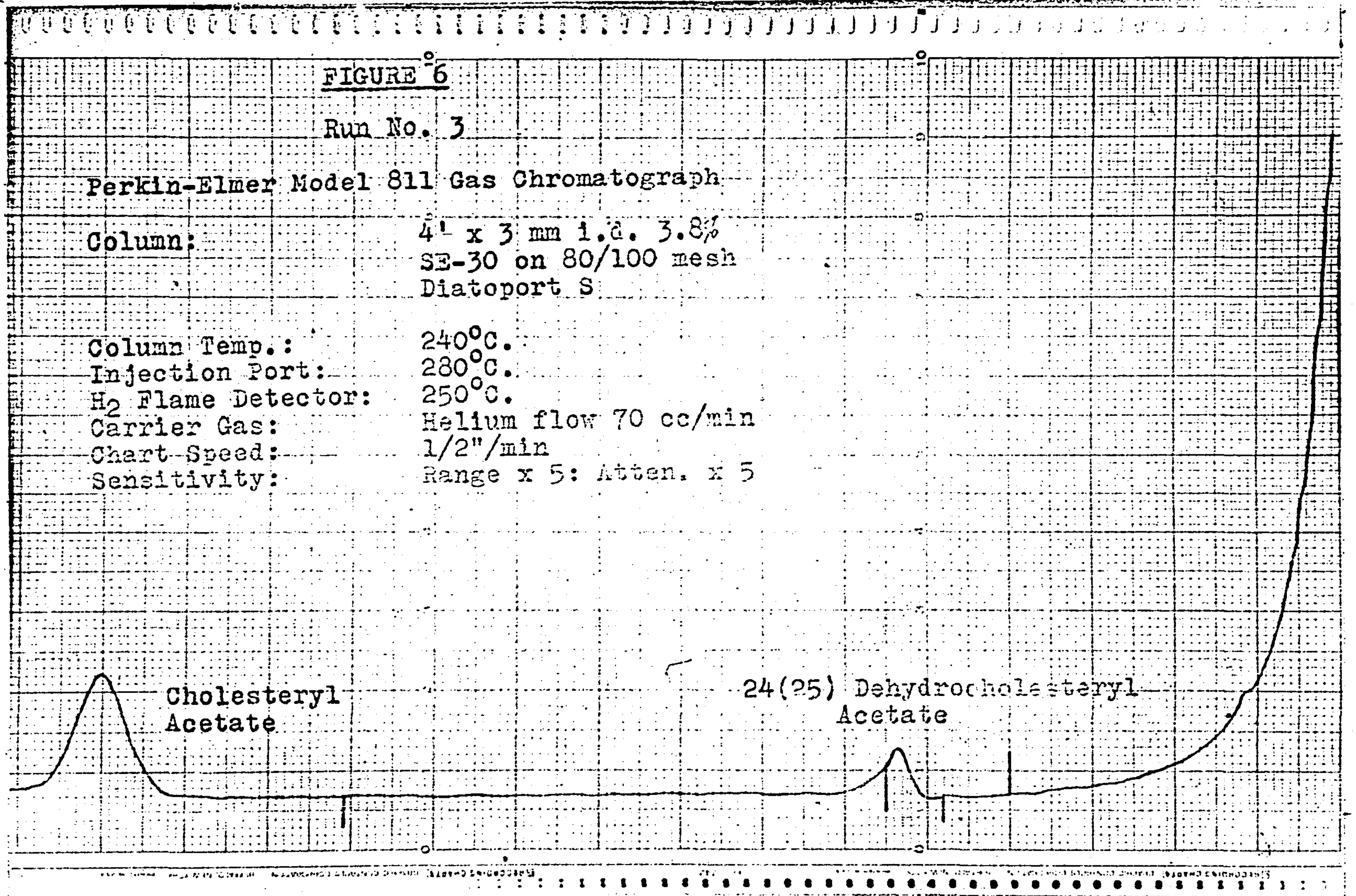


FIGURE 6

Run No. 3

Perkin-Elmer Model 811 Gas Chromatograph

Column:	4' x 3 mm i.d. 3.8% SE-30 on 80/100 mesh Diatoport S
Column Temp.:	240°C.
Injection Port:	280°C.
H_2 Flame Detector:	250°C.
Carrier Gas:	Helium flow 70 cc/min
Chart Speed:	1/2"/min
Sensitivity:	Range x 5: Atten. x 5

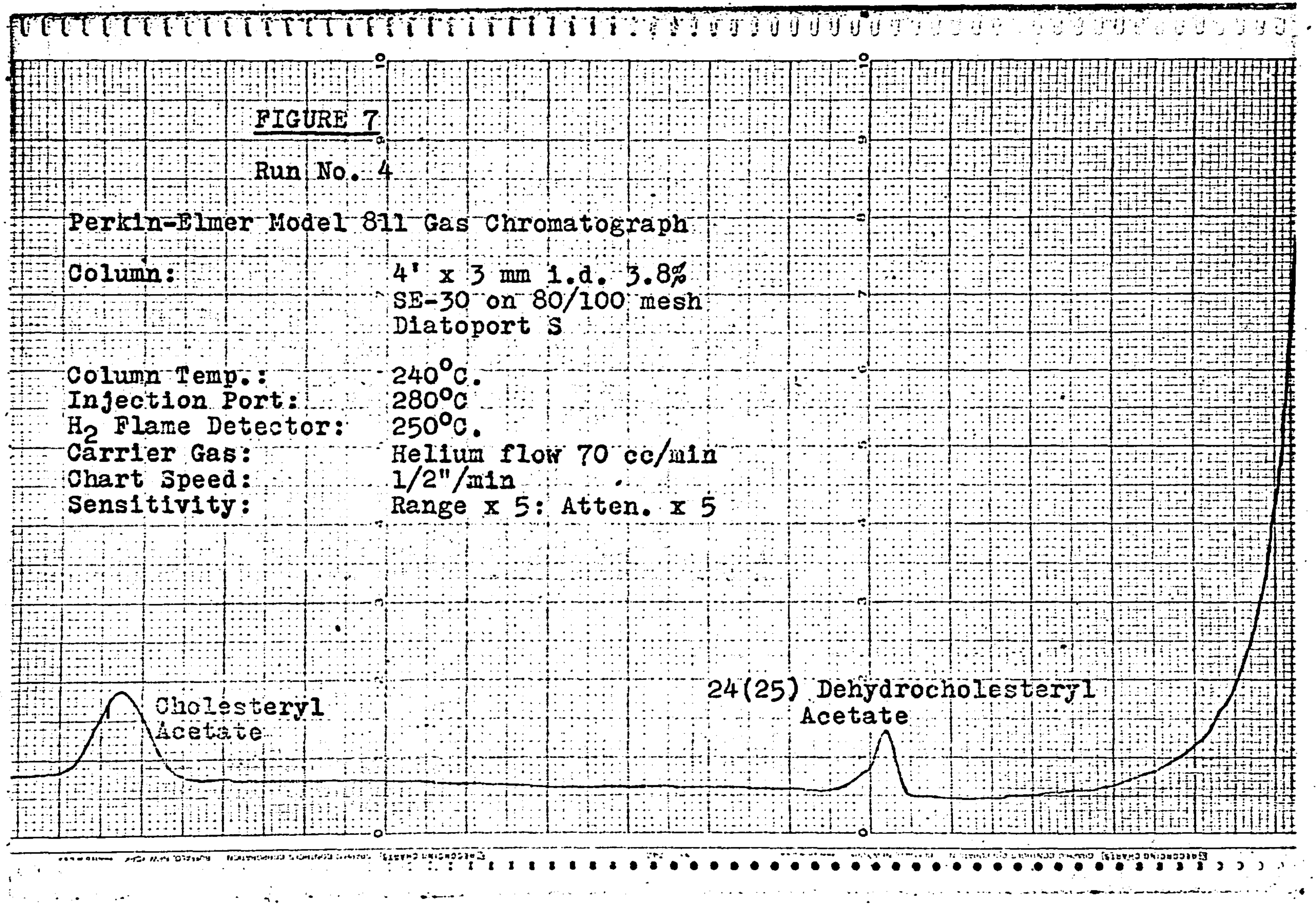
FIGURE 7

Run No. 4

Perkin-Elmer Model 811 Gas Chromatograph

Column: 4' x 3 mm i.d. 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 240°C.
Injection Port: 280°C
H_2 Flame Detector: 250°C.
Carrier Gas: Helium flow 70 cc/min
Chart Speed: 1/2"/min
Sensitivity: Range x 5: Atten. x 5



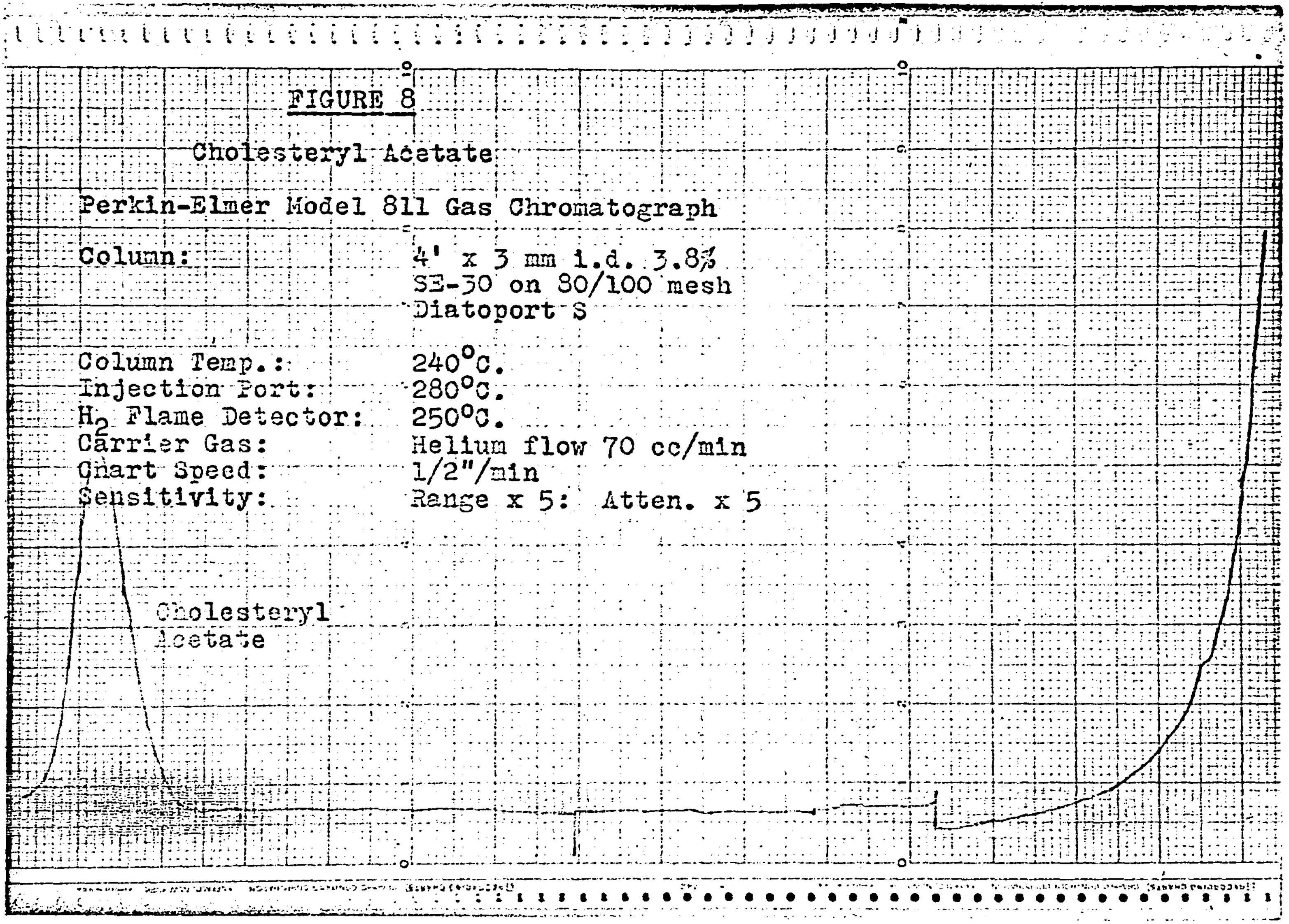


FIGURE 8

Cholesteryl Acetate

Perkin-Elmer Model 811 Gas Chromatograph

Column: 4' x 3 mm i.d. 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 240°C.
Injection Port: 280°C.
H_2 Flame Detector: 250°C.
Carrier Gas: Helium flow 70 cc/min
Chart Speed: 1/2"/min
Sensitivity: Range x 5: Atten. x 5

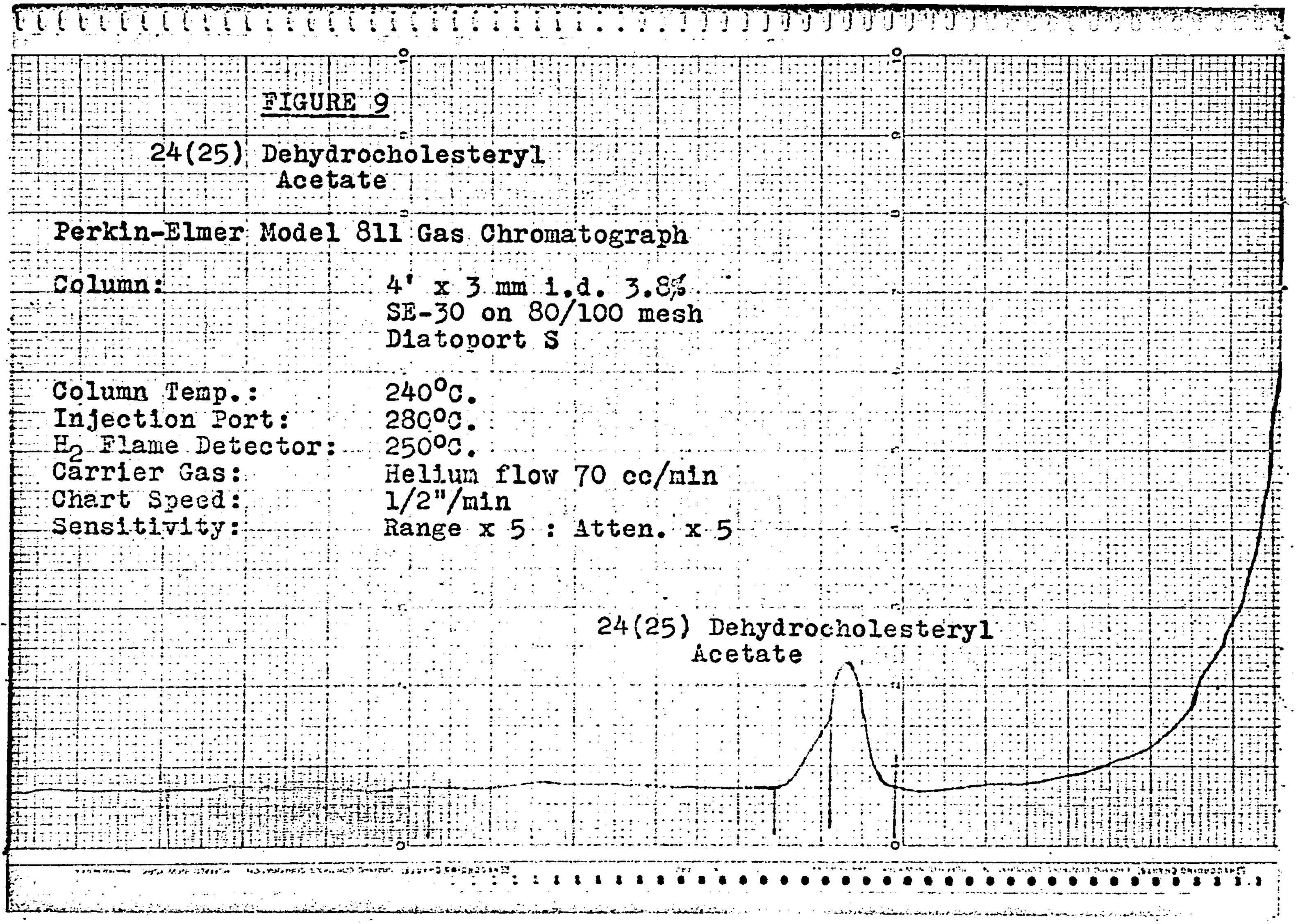


FIGURE 9

24(25) Dehydrocholesteryl Acetate

Perkin-Elmer Model 811 Gas Chromatograph

Column: 4' x 3 mm i.d. 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 240°C.
Injection Port: 280°C.
H_2 Flame Detector: 250°C.
Carrier Gas: Helium flow 70 cc/min
Chart Speed: 1/2"/min
Sensitivity: Range x 5 : Atten. x 5

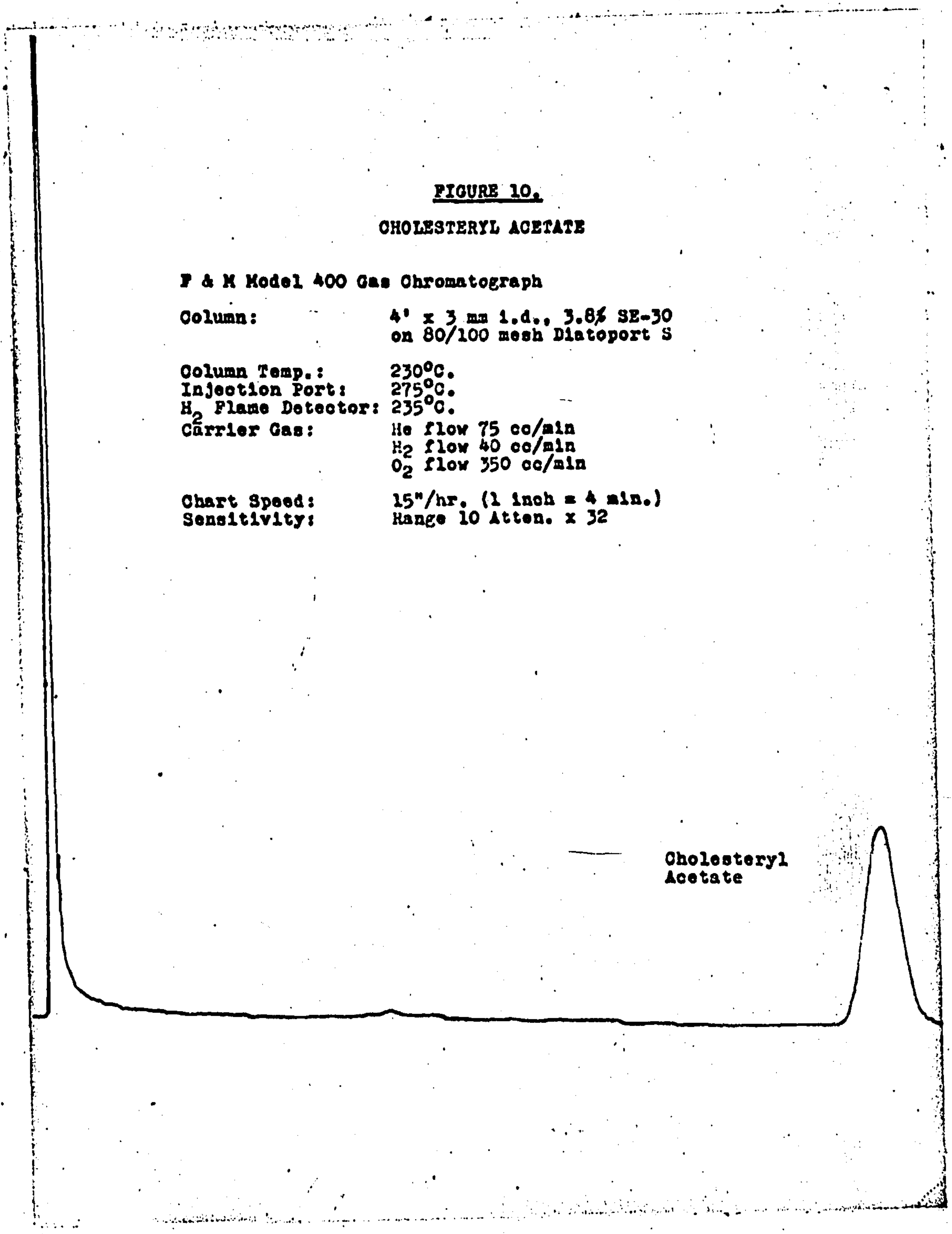


FIGURE 10.

CHOLESTERYL ACETATE

F & M Model 400 Gas Chromatograph

Column:	4' x 3 mm i.d., 3.8% SE-30 on 80/100 mesh Diatoport S
Column Temp.:	230°C.
Injection Port:	275°C.
H_2 Flame Detector:	235°C.
Carrier Gas:	He flow 75 cc/min H_2 flow 40 cc/min O_2 flow 350 cc/min
Chart Speed:	15"/hr. (1 inch = 4 min.)
Sensitivity:	Range 10 Atten. x 32

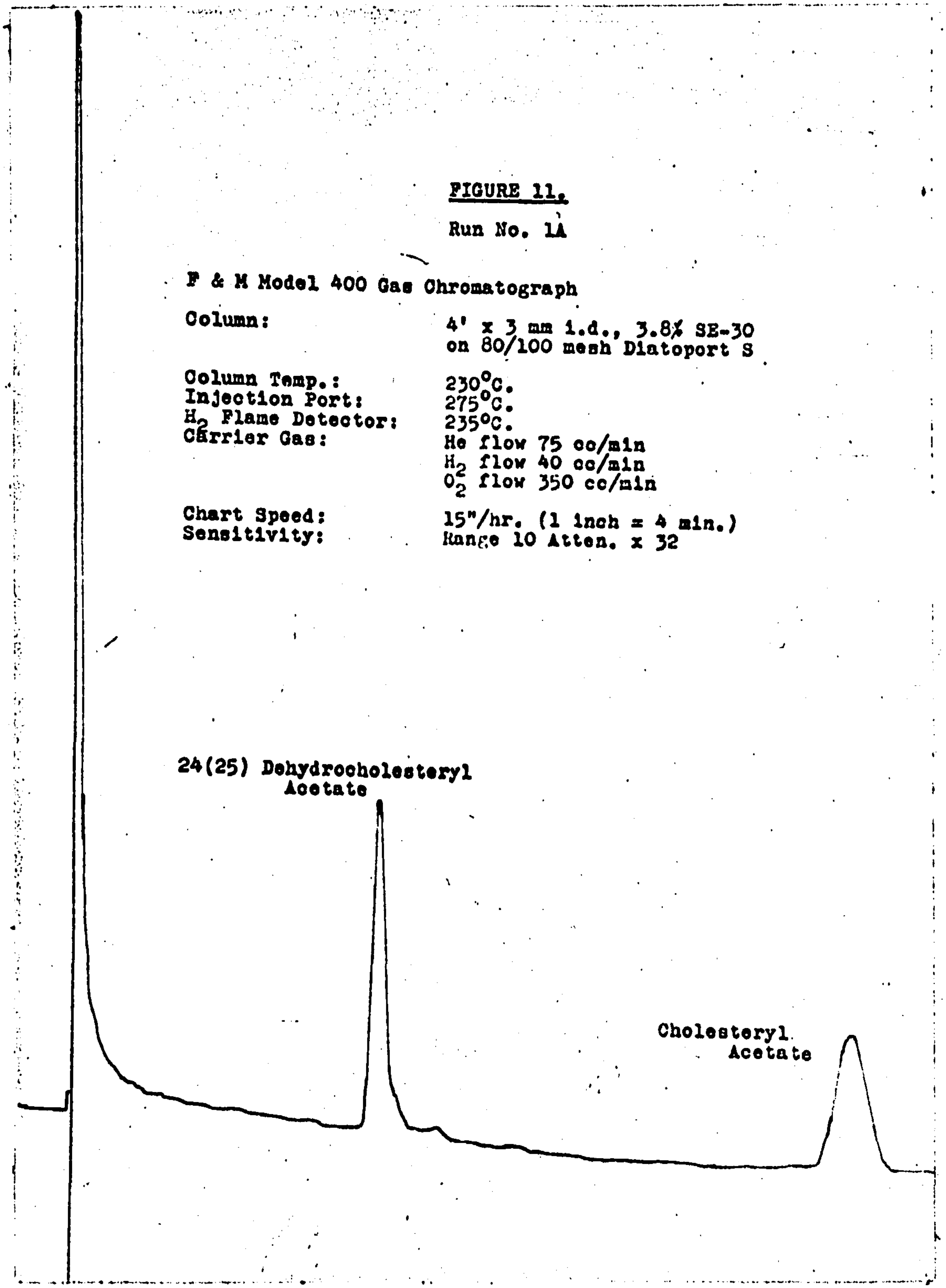


FIGURE 11.

Run No. 1A

F & M Model 400 Gas Chromatograph

Column:	4' x 3 mm i.d., 3.8% SE-30 on 80/100 mesh Diatoport S
Column Temp.:	230°C.
Injection Port:	275°C.
H_2 Flame Detector:	235°C.
Carrier Gas:	He flow 75 cc/min H_2 flow 40 cc/min O_2 flow 350 cc/min
Chart Speed:	15"/hr. (1 inch = 4 min.)
Sensitivity:	Range 10 Atten. x 32

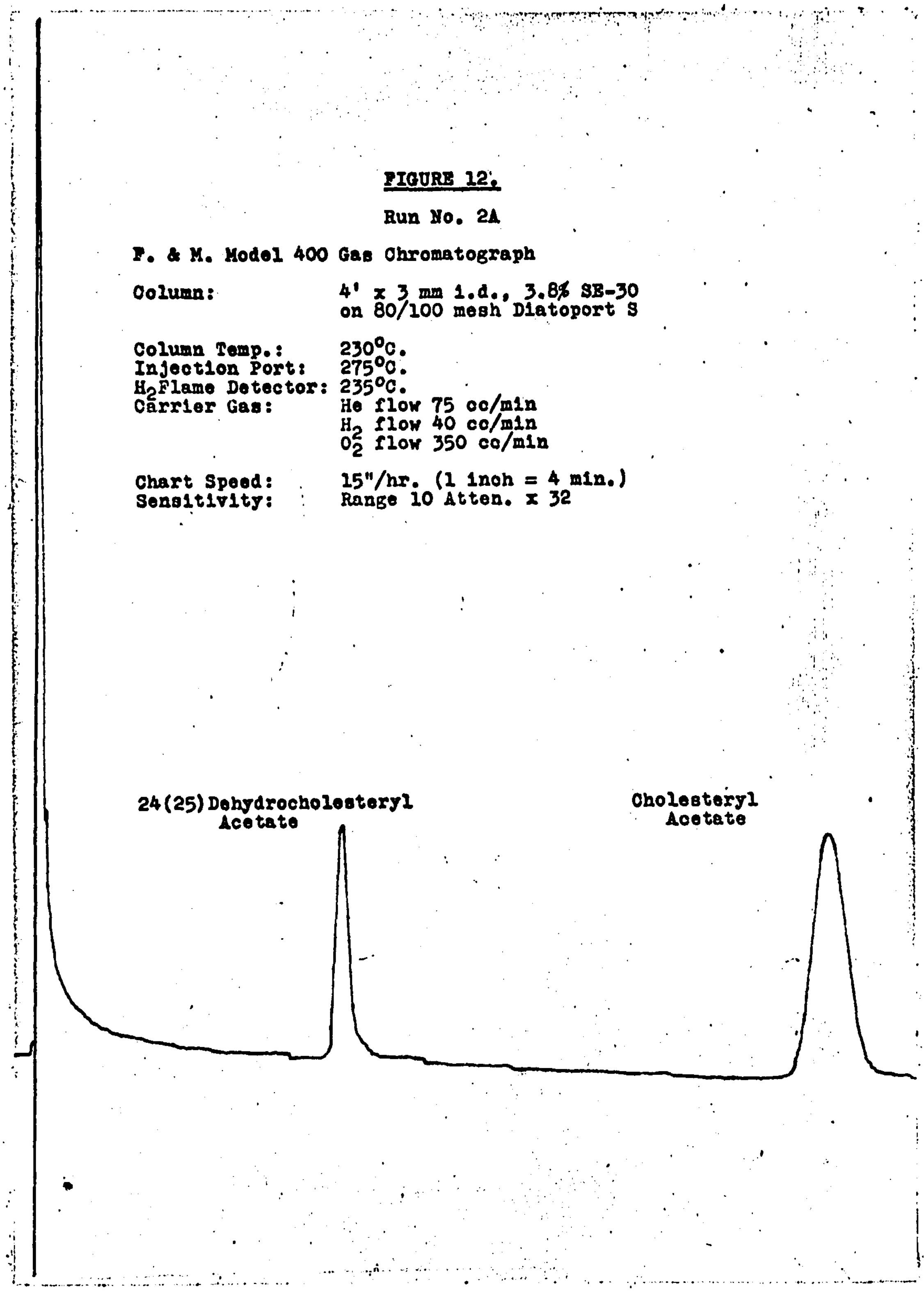


FIGURE 12.

Run No. 2A

F. & M. Model 400 Gas Chromatograph

Column: 4' x 3 mm i.d., 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 230°C.
Injection Port: 275°C.
H_2 Flame Detector: 235°C.
Carrier Gas: He flow 75 cc/min
H_2 flow 40 cc/min
O_2 flow 350 cc/min

Chart Speed: 15"/hr. (1 inch = 4 min.)
Sensitivity: Range 10 Atten. x 32

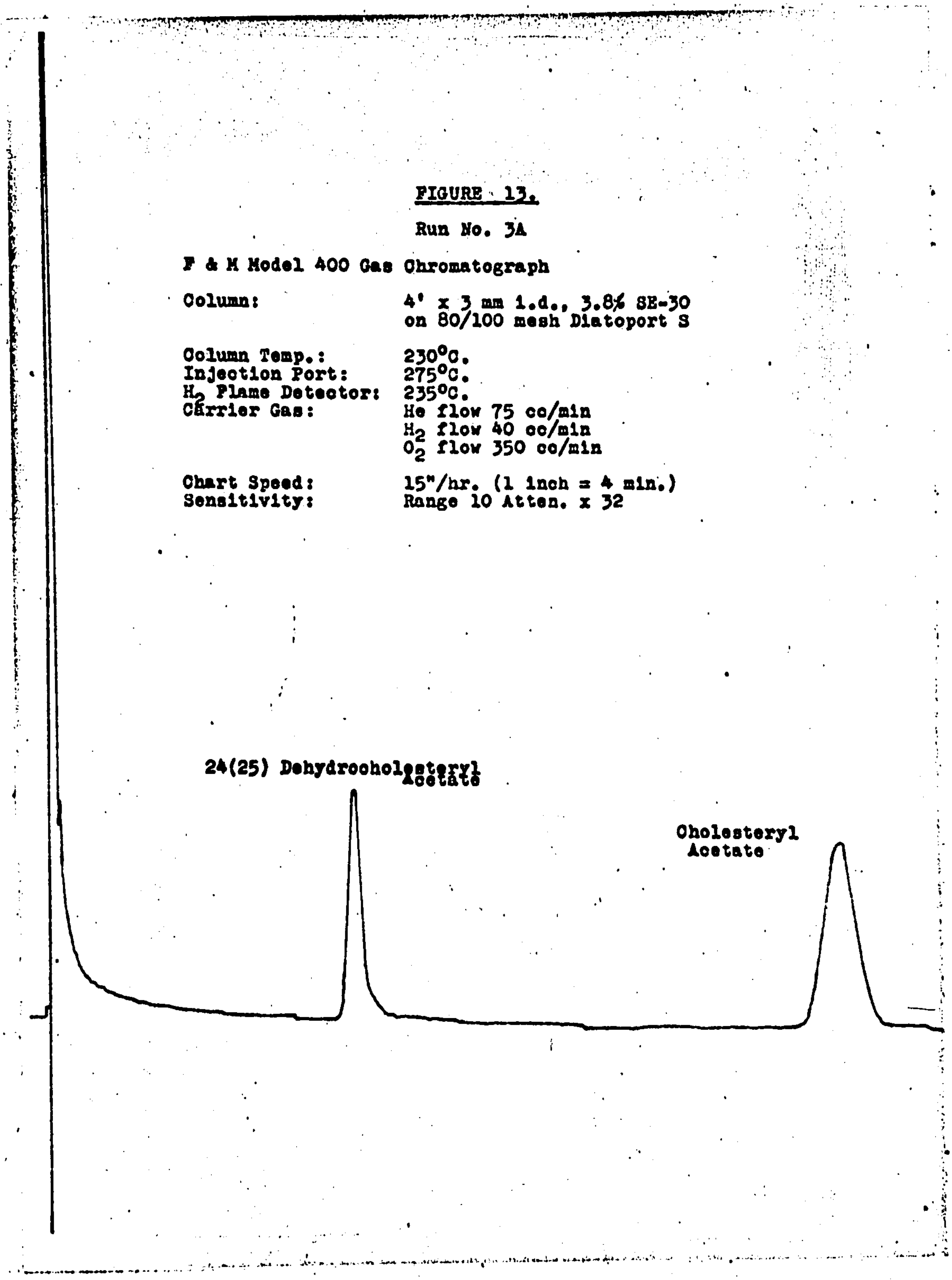


FIGURE 13.

Run No. 3A

F & M Model 400 Gas Chromatograph

Column: 4' x 3 mm i.d., 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 230°C.
Injection Port: 275°C.
H_2 Flame Detector: 235°C.
Carrier Gas: He flow 75 cc/min
H_2 flow 40 cc/min
O_2 flow 350 cc/min

Chart Speed: 15"/hr. (1 inch = 4 min.)
Sensitivity: Range 10 Atten. x 32

FIGURE 14.

Run No. 4A

F & M Model 400 Gas Chromatograph

Column: 4' x 3 mm i.d., 3.8% SE-30 on 80/100 mesh Diatoport S

Column Temp.: 230°C.
Injection Port: 275°C.
H_2 Flame Detector: 235°C.
Carrier Gas: He flow 75 cc/min
H_2 flow 40 cc/min
O_2 flow 350 cc/min

Chart Speed: 15"/hr. (1 inch = 4 min.)
Sensitivity: Range 10 Atten. x 32

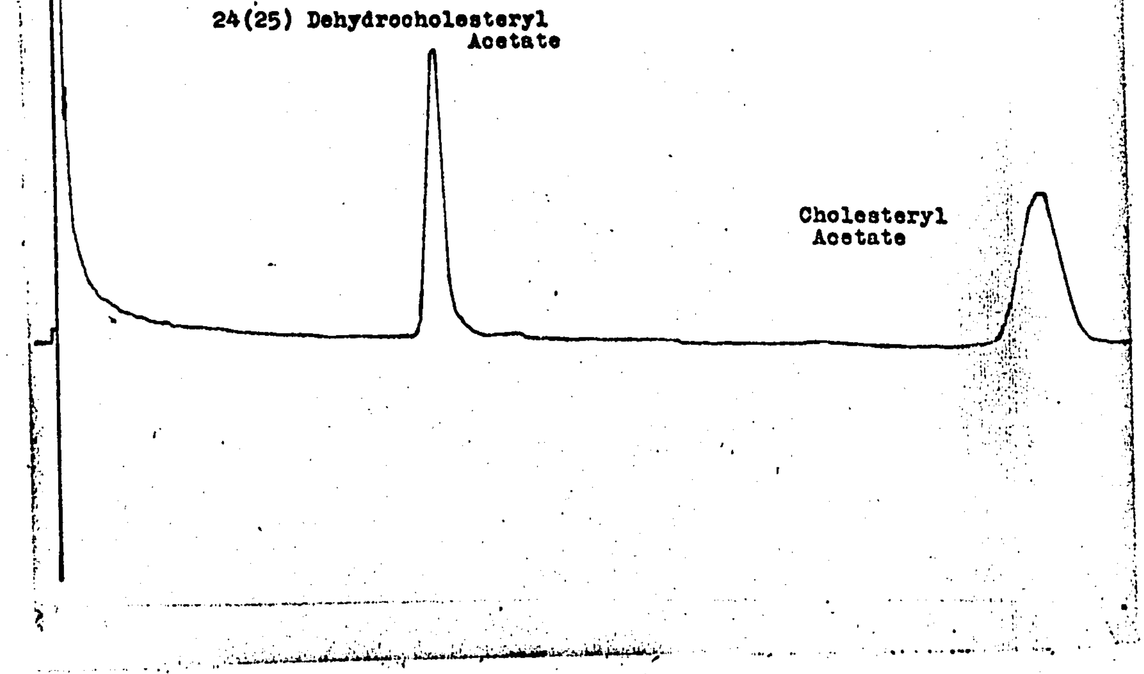


Figure 15

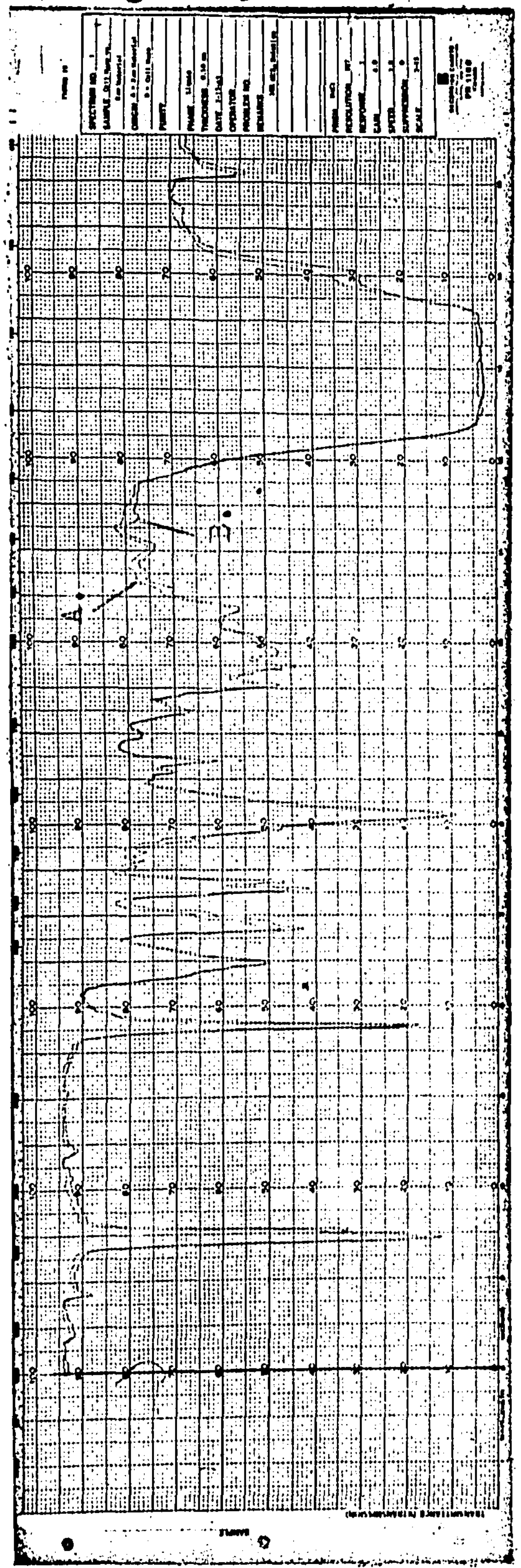


Figure 16

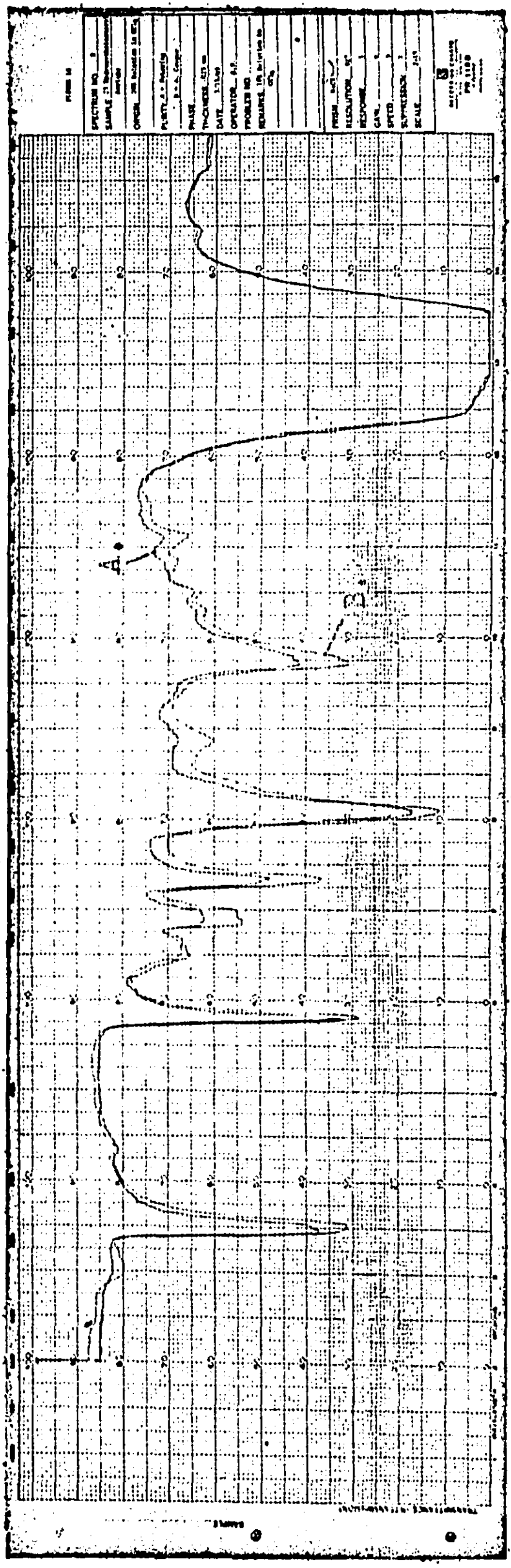


FIGURE 17.

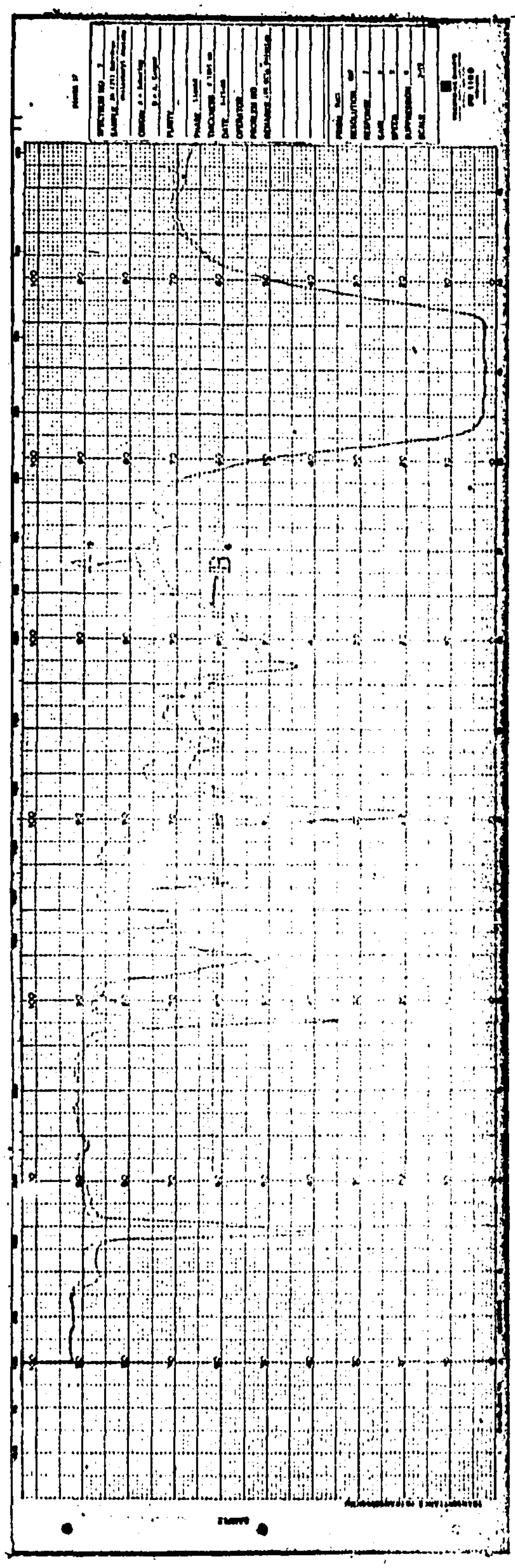


FIGURE 18.

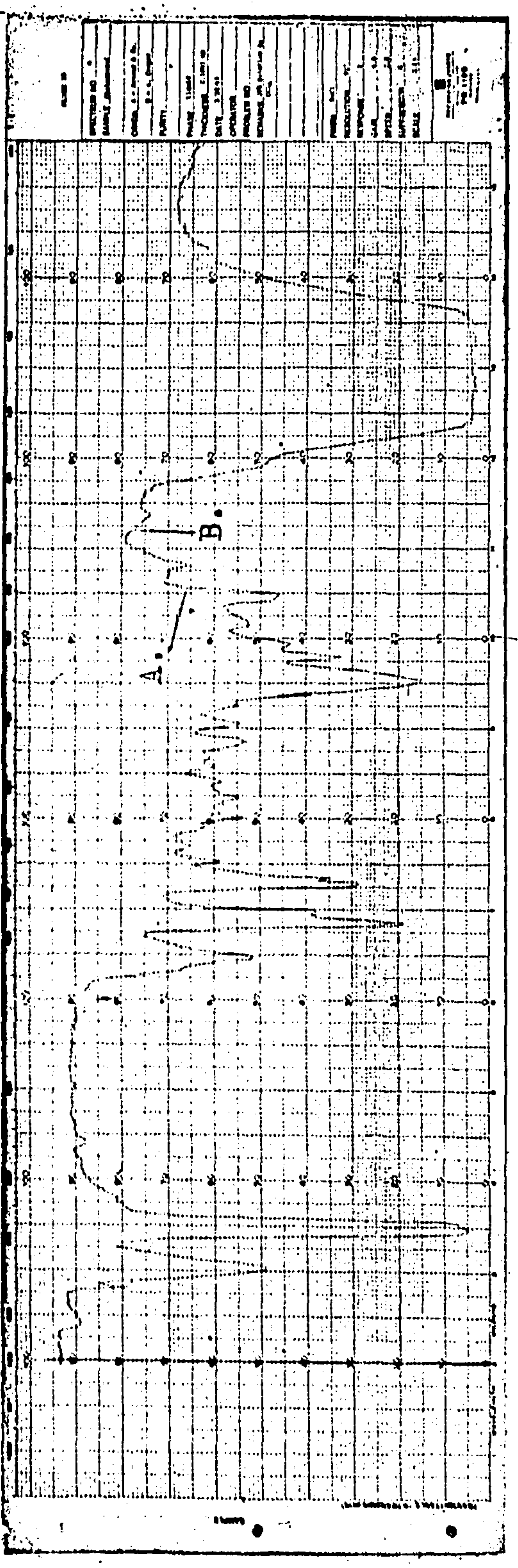


FIGURE 19

PLOT OF ANODE POTENTIAL VS. TIME OF ELECTROLYSIS

at current densities from 62.5 to 250 ma/cm^2

lead/lead dioxide anode - 80 cm^2

Anodic Oxidation of Cholesteryl Acetate Dibromide

Anolyte: 4.5M H_2SO_4 with a suspended solution of CCl_4 containing cholesteryl acetate dibromide

Temp.: 30 to 40°C.

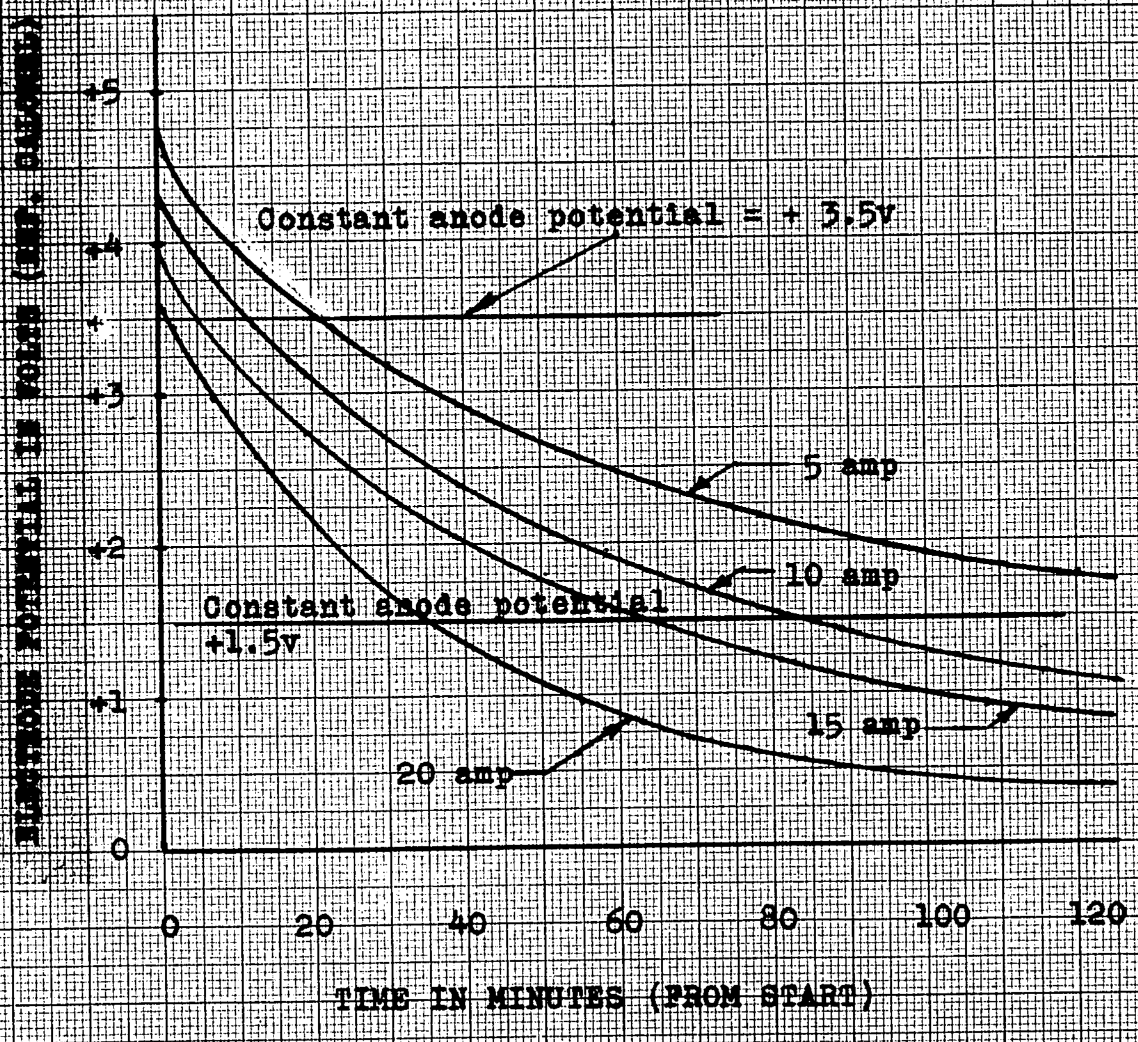
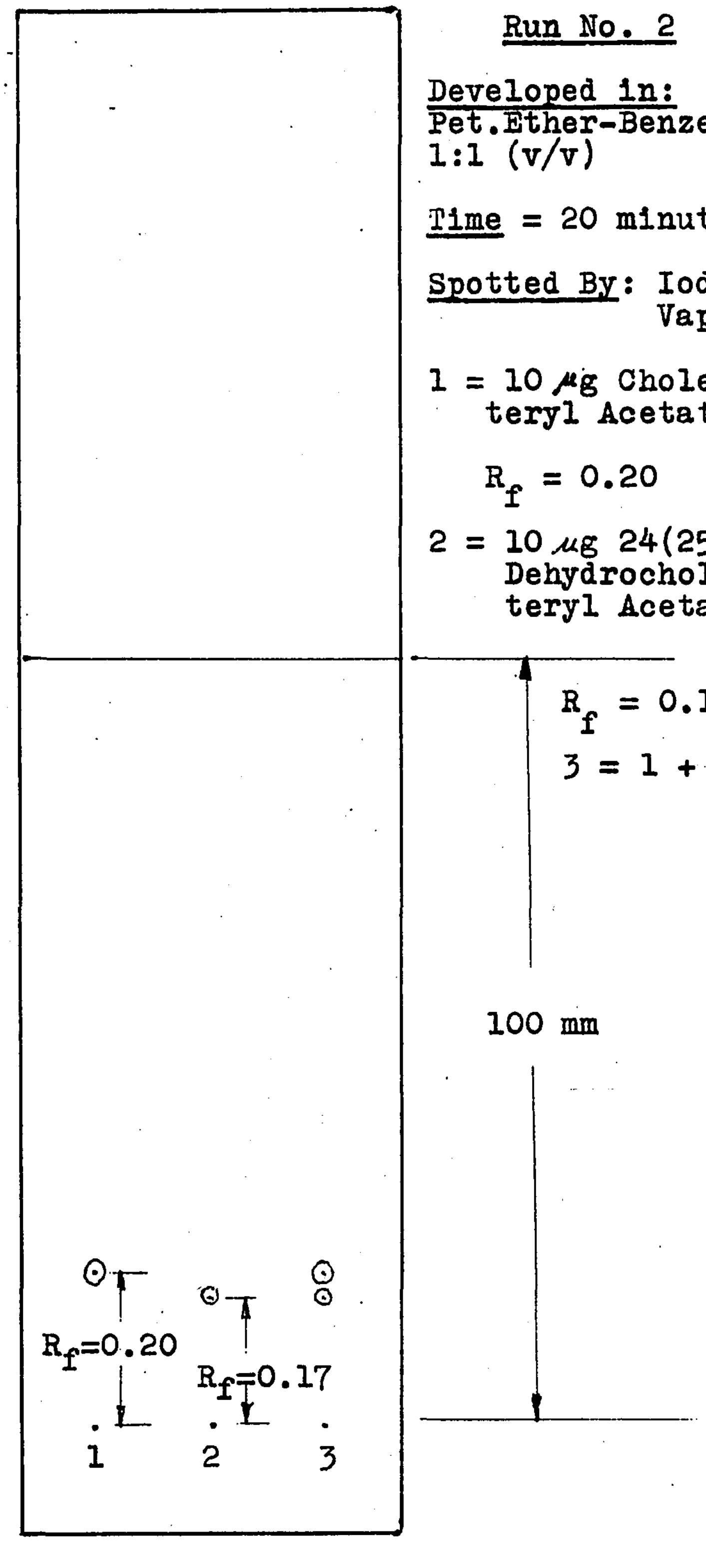


FIGURE 20

Thin Layer Chromatogram on Silica Gel G



Run No. 2

Developed in:
Pet.Ether-Benzene
1:1 (v/v)

Time = 20 minutes

Spotted By: Iodine Vapor

1 = 10 µg Cholesteryl Acetate

$R_f = 0.20$

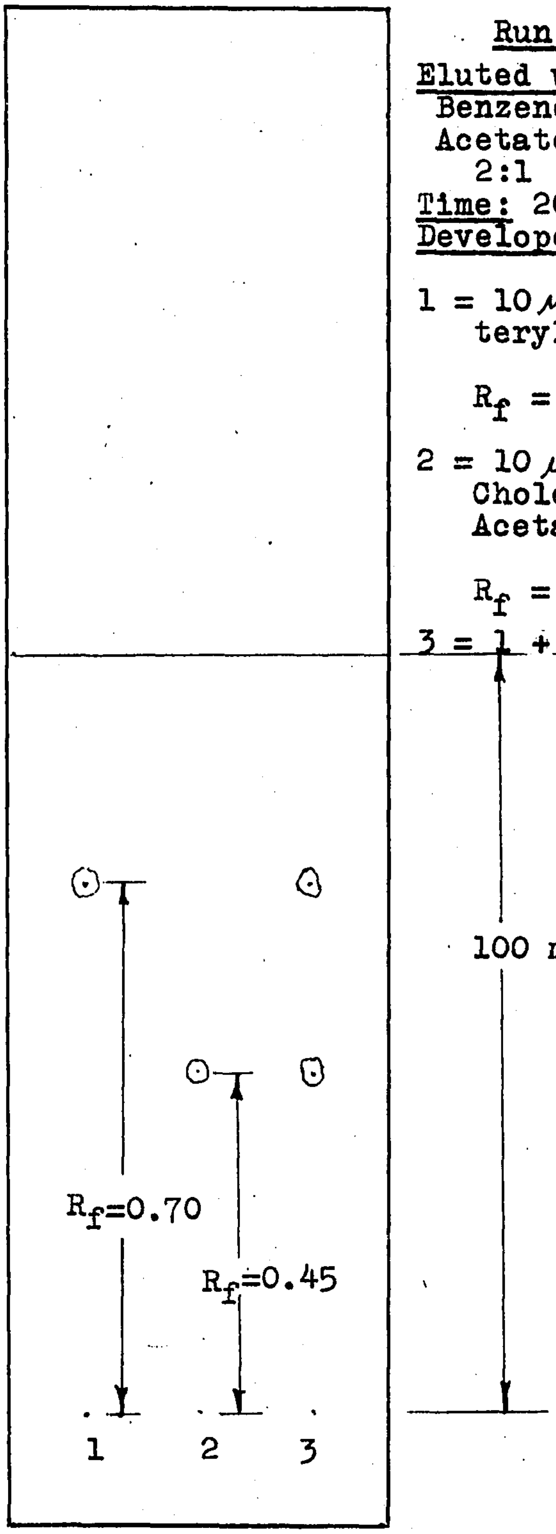
2 = 10 µg 24(25) Dehydrocholesteryl Acetate

$R_f = 0.17$

3 = 1 + 2

FIGURE 21

Thin Layer Chromatogram on Silica Gel G



Run No. 2

Eluted with:
Benzene-Ethyl Acetate
2:1 (v/v)
Time: 20 minutes
Developer: Iodine Vapor

1 = 10 μg Cholesteryl Acetate

$R_f = 0.70$

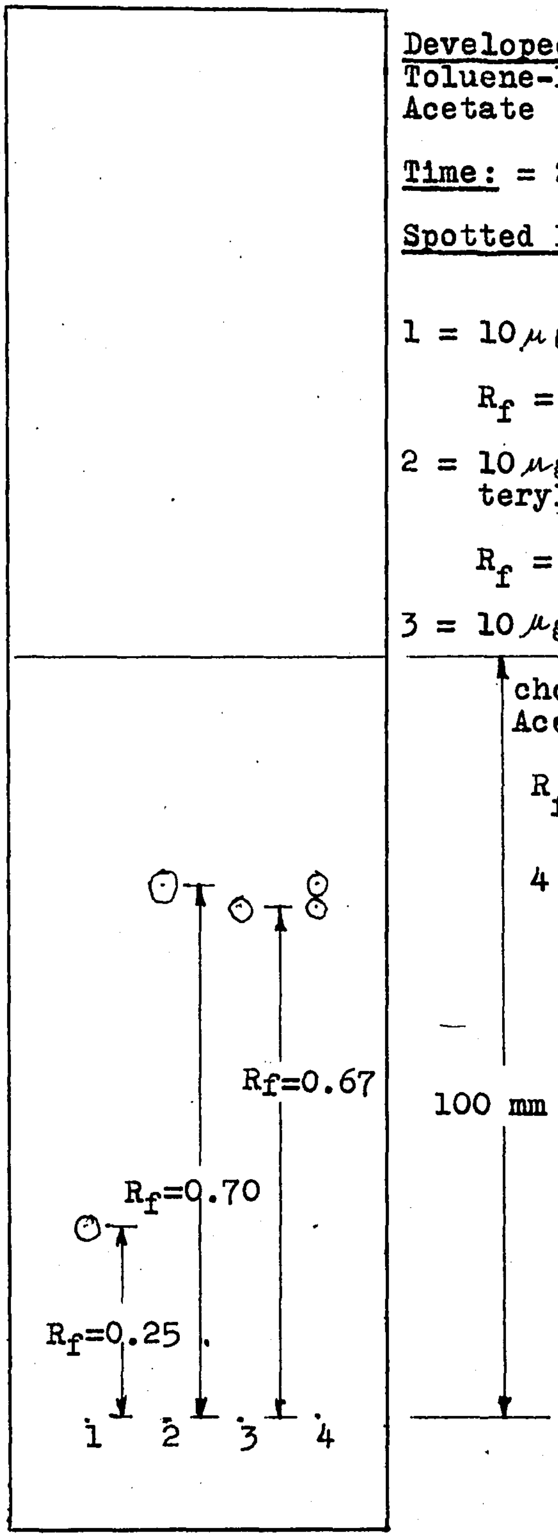
2 = 10 μg 25-Hydroxy Cholesteryl Acetate

$R_f = 0.45$

3 = 1 + 2

FIGURE 22

Thin Layer Chromatogram on Silica Gel-G



Developed in:
Toluene-Ethyl
Acetate 9:1 (v/v)

Time: = 20 minutes

Spotted By: Iodine Vapor.

1 = 10 μg Cholesterol
R_f = 0.25

2 = 10 μg Cholesteryl Acetate
R_f = 0.70

3 = 10 μg 24(25) Dehydrocholesteryl Acetate
R_f = 0.67

4 = 1+2+3

R_f & R_t Values of Products vs. Known Compounds

In chromatography where materials elute together, there is similarity in polarity of the compounds and also in molecular weight(52). Characteristic constants for materials of similar structure have been correlated with R_f and R_t values. The constants are based on interatomic distances measured on molecular models.

These relative elution distances (R_f) in thin layer chromatography are a reproducible and accurate means of identification and their use has today become an extremely powerful analytical tool in every phase of chemistry and chemical manufacture. With gas chromatography these same properties result in precisely repeatable retention times (R_t).

$$R_f = \frac{\text{Distance Material Runs}}{\text{Distance Solvent Runs}} .$$

In Figure 20 is a diagram of a thin layer chromatogram on 0.25 mm thick silica gel G that has been eluted with petroleum ether - benzene (1:1). Iodine vapor development was used to locate the eluted steroids. Elution time was 20 minutes. 1 = 10 μg cholesteryl acetate. 2 = 10 μg 24 (25) dehydro cholesteryl acetate. 3 = mixture of 1 and 2.

In Figure 21 is a diagram of a thin layer chromatogram that shows cholesteryl acetate separated from 25 - hydroxycholesteryl acetate. The substrate was silica gel G (0.25 mm); solvent system-benzene-ethyl acetate (2:1); elution time 20 minutes. 1 = 10 μg cholesteryl acetate; 2 = 10 μg 25-hydroxycholesteryl acetate; 3 = mixture of 1 and 2. For this system, Van Daam(69) gives $R_f = 0.96$ for cholesteryl acetate, and $R_f = 0.41$ for 25-hydroxycholesteryl acetate.

Cholesterol, prepared by the reduction and saponification of 24 (25) dehydrocholesteryl acetate was spotted on a TLC plate and eluted as in Figure 22. solvent- toluene-ethyl acetate (9:1); development time - 20 minutes; 1 = cholesterol; 2 = cholesteryl acetate; 3 = 24 (25) dehydrocholesteryl acetate; 4 = mixture of 1, 2 and 3.

In Figures 4 - 14, gas chromatographs show the characteristic, reproducible retention times for cholesteryl acetate and 24 (25) dehydrocholesteryl acetate. The last run made on a P.E.-811 shows the result of injecting a mixture of known material (Schering) with 24 (25) steroid acetate produced from the debrominated reaction mass by preparatory TLC. R_t - cholesteryl acetate = 29.5 min. (measured from start to center of maximum peak). R_t - 24 (25) dehydrocholesteryl acetate = 11.5 min.

This information supports the conclusion that the oxidation has resulted in attack of the hydrogen attached to the 25 carbon to produce 25-hydroxycholesteryl acetate which then dehydrates to the mixture of 24 and 25 dehydrocholesteryl acetates.

Infrared Spectra of Products vs. Known Compounds

Further corroboration of the conclusion that 25-hydroxycholesteryl acetate has been manufactured in the oxidation under study, is shown by the infrared spectrum of material prepared from the debrominated reaction mass compared (in spectrum 2 - Figure 16) with the sample of known material (Schering). Here the comparison is close, with minor differences at 1530 cm^{-1} due possibly to slight impurity that was a result of the method of preparation. Otherwise, the two samples match peak for peak, intensity for intensity.

The small differences exhibited by spectrum 3 (Figure 17), which compares a known (15 years old) sample of the mixtures 24 and 25 dehydrocholesteryl acetate may be due to differences in the ratios of the two isomers as well as age of sample and method of manufacture.

These "fingerprints" are conclusive proof of product identity.

Conversion of Products to Cholesterol

In order to definitely prove the identities of the products of this investigation, the 25 - hydroxycholesteryl acetate that had been recovered from several runs was converted by the method of Koechlin and Reichstein(37) to 25-dehydrocholesteryl acetate.

The hydroxy acetate (255 mg) was refluxed for one-half hour with 7.50 ml of dry pyridine and 0.25 ml of phosphorous oxychloride. The mass was cooled to 0°C. and slowly poured with mixing into ice water containing 0.25 ml of hydrochloric acid. The mass was permitted to crystallize overnight at -20°C. The crystals were filtered on a microfunnel, washed to neutrality with ice water and dried in air. Dry crystals weighed 230 mg. This material was dissolved in 2 ml of benzene and treated with 30 mg of Nuchar C-190-N, filtered and the benzene was evaporated. The solids were recrystallized from a minimum amount of methanol, filtered and washed with methanol. The air dried material weighed 200 mg. The melting point was 91.0 - 94.0°C. In 2% chloroform the material showed an optical rotation of Sodium D light at 20°C. of -43.4°. These values compared well with the data of Ryer, et al(63).

The 200 mg. of product was reduced with hydrogen in 20 ml. of absolute ethyl alcohol with 50 mg. of 10% palladium on charcoal at 20°C. and one atmosphere. The solution was filtered and concentrated to 3 ml. Potassium hydroxide (100 mg) was added and the mass refluxed for 1 hour. The solution was cooled to 0°C. and slowly poured into ice water with mixing. Crystallization occurred in 24 hours at -20°C. The product was filtered, washed and air dried to give 180 mg. of crude product. Two recrystallizations from acetone yielded 110 mg. of dry cholesterol, M.P. 149.5 - 150.5; α^{20}_{D} -35.5° (2% in dioxane). The infrared spectra compared well with the raw material. (See Figure 18).

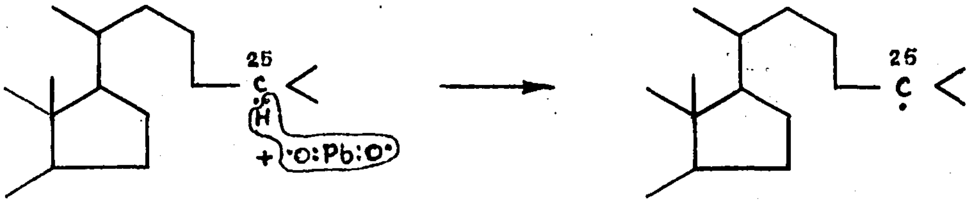
Attack at the 20 tertiary carbon atom could also give products which would be reducible to cholesterol. However, the work of Petrow(80) has shown that a C-20 alcohol has physical properties that are different from the C-25 material prepared in this work. The C-25 alcohol was prepared by saponification of the acetate.

The melting points and optical rotations are compared below:

Compound	Diol		3-Acetate	
	M.P.°C	α^{N}_{D}	M.P.°C	α^{N}_{D}
C-20(80)	136°	-53°	156°	-58°
C-25	180°	-39°	142°	-40°

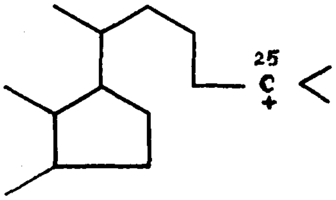
Mechanism of Oxidation

The oxidation of the cholesteryl acetate dibromide has most probably occurred by attack on the hydrogen on the 25 carbon by the oxygen atom of lead dioxide with formation of the free radical (II).



(I) (II)

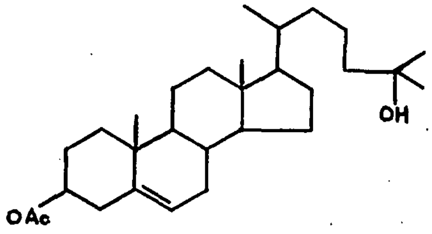
The free radical then loses an additional electron to a second oxygen atom to form the carbonium ion (III).



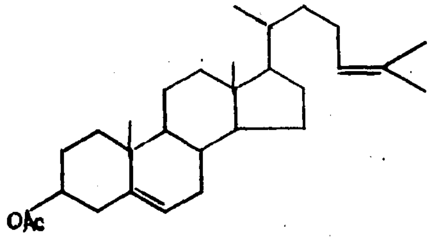
(III)

Carbonium ion (III) then reacts with water to form the protonated alcohol which loses a proton to yield a 25 alcohol. The carbonium ion by unimolecular first order elimination of a proton will also result in formation of the 24 or 25 olefin, depending on where the proton is lost. Products of the reaction after debromination are:

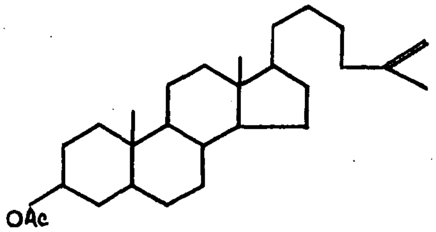
<u>25-Hydroxycholesteryl Acetate</u>



24-Dehydro Cholesteryl Acetate



25-Dehydro Cholesteryl Acetate



General Conclusions

Generally, it may be concluded that by proper selection of the conditions for electrolytic oxidation of cholesteryl acetate dibromide, the products of the reaction after debromination were 24 and 25 dehydrocholesteryl acetate, the intermediate 25 hydroxycholesteryl acetate and unreacted cholesteryl acetate.

This investigation included the elimination of constant potential electrolysis; carbon and platinum as anodes; and the use of wetting agents to solubilize the raw material.

The technique that was evolved included oxidation of cholesteryl acetate dibromide in saturated (10%)

solution in carbon tetrachloride, suspended by mixing in a 4.5 molar sulfuric acid at 30°C. and 40°C. over a range of current density from 62.5 to 250 amperes per square centimeter, at an electrochemically prepared lead dioxide anode in an alundum diaphragm cell. The specific condition of anode potential maintained electrically above 2.5 v (relative to a saturated calomel electrode) resulted in the lead oxide phase of β configuration (tetragonal).

Over this range of conditions it was possible to recover products and starting material that were equivalent to 96% of the starting dibromide. Conversion of raw material ranged from 30 to 55%.

The molar yield of products based on cholesteryl acetate dibromide ranged from 85 to 93%.

IX. RECOMMENDATIONS

Steroids per se are not the most stable of compounds. But someplace between the almost gentle oxidizing conditions uncovered here, and the sledgehammer techniques described in the literature in the past hundred years, there is a middle range.

That range could perhaps be found if conditions (resistance) could be adjusted so that cell potentials of the order of 100 to 200 volts could be impressed. This should be studied. It is possible also that temperatures from below 0°C. to 30°C. and from 40°C. to 100°C. may prove of value in the electrolysis, although a higher boiling solvent would have to be used.

There were some indications from a few side experiments that by greatly extending the time of electrolysis, different products would be formed. This was not pursued because the overall yields and recoveries were quite low. Perhaps these yields may be improved by more work.

Constant anode potential was elusive in many attempts to use this method that is so useful in electrolytic reduction. It may yet be possible to achieve such control by proper choice of electrolyte and electrode. Although my own attempts to find a homogeneous system to electrolyze were not successful, this could well be the reason that such control was not possible.

While it is true that some slight oxidation was achieved with the use of chemically prepared lead dioxide with no current (and no anode), the dioxide was converted to the sulfate in sulfuric acid solution and so could not act for long. If the α or β form were to be used in some other medium that would not react with the lead dioxide, results similar to the present achievement may be possible.

It is also recommended that the technique developed in this work be generally applied to a series of related steroids and further that it be studied as a useful method for the oxidation of compounds containing tertiary carbon atoms.

It is also suggested that a study be made of standard chemical methods or further electrolytic procedures that may be used to degrade the now unsaturated side chain to more useful derivatives.

Finally, the assignment could be made for an engineering study that would result in a plant design and economic study of the production of steroids from cholesterol by the process developed here.

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ADDENDA

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Since 1944 he has been a practicing chemical engineer engaged in the design, development and operation of chemical processing plants. He is presently employed as Manager of Development for the Universal Oil Products Chemical Company at East Rutherford, New Jersey.

A period of four years was required to complete the academic credits and accomplish this research and dissertation.