REACTIONS OF KETOSTEROIDS WITH SULPHURIC ACID

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TO

DR. J. H. LINFORD

FOR HIS EXCELLENT ADVICE AND KINDLY GUIDANCE

IN THIS FIRST VENTURE INTO THE REALM

OF RESEARCH, THE AUTHOR IS DEEPLY GRATEFUL

ABSTRACT

A STUDY OF KETOSTEROIDS IN SULPHURIC ACID

Steroids produce an intense ultraviolet and visible absorption spectrum when dissolved in sulphuric acid. This property has proved to be a most useful tool in their detection and assay in minute quantities. Hence it is desirable to have a good understanding of the reactions that occur and of the species that give rise to the spectra. A family of steroids, emphasizing the C_3 keto group, was studied by its ultraviolet absorption in concentrated commercial sulphuric acid, 100% acid, and fuming sulphuric acid. The absorption spectra were simple curves with a single maximum. The optical density of the maximum increased with time, both at room temperature and at 100°C. The magnitude of the absorption peak, and the rate at which the equilibrium value was attained, were found to be affected by the group at the C_{17} position, and/or the presence of unsaturation in the steroid nucleus. The results are attributed to protonation of the C_3 keto oxygen in 98% and 100% acid, and the removal of the C_3 keto group in fuming acid.

Cryoscopic measurements, and tests for the detection of SO_2, a product of oxidation, were also carried out. The cryoscopic data gave no conclusive evidence as to the number of particles present in 100% H_2SO_4, due to the solution of a steroid. A very large number were found. This was attributed to the ability of the large steroid molecule to disrupt the structure of the solvent surrounding it, rather than to the solvent's ability to fragmentize the steroid. The tests for SO_2 indicated its absence

in the steroid solutions of all three acids.

It is concluded that the absorption spectra arise from protonation of the C_3 keto group, and that no fragmentation of the steroid molecule occurs in sulphuric acid.

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INTRODUCTION

"The generic term 'steroid' is employed to indicate all those substances that are structurally related to the sterols and bile acids, to the extent of possessing the characteristic perhydro 1, 2, cyclopentano phenanthrene ring system" (1). The steroid nucleus plays a major role in a large number of processes. Many of the essential materials of both plants and animals have this ring as the fundamental structure. These compounds, many of which have been isolated and synthesized, differ in their functions as a result of the different groups appended to various positions on the nucleus. Not all the steroids are biologically active. Often the only difference between an active and inactive form lies in the steric configuration of a single hydrogen atom.

Despite the vast amount of research that has been done in all phases of steroid chemistry --- degradation, synthesis, stereochemistry, biological activity ---- no theory has been advanced as to the basic function of the obviously essential perhydrocyclopentanophenanthrene ring. No theory elucidates the relation between configuration and activity. At the present time, many empirical data are being collected, and it is hoped that the near future will see a solution of this problem.

When treated with strong acids, steroids often give a display of varied colours. This has proved to be an excellent method for their detection in microscopic quantities. Particularly intensively studied is the reaction with sulphuric acid. Characteristic absorption spectra

can be produced under different conditions at both ultraviolet and visible wavelengths. This reaction forms the basis of the present investigation which is, in essence, the examination of a series of keto steroids in sulphuric acids of varied strengths with a view to deriving a better understanding of the reaction occurring, of the role of the steroid nucleus, and of the effect of acid strength on the reaction. Because anhydrous sulphuric acid has such a convenient freezing point it was decided, prior to examining the keto steroids spectroscopically, to observe their cryoscopic effects in the acid. From the freezing point depression, the number of particles present in solution can be calculated. A knowledge of this fact helps immeasurably in postulating a reaction mechanism. Section A is devoted to this phase of the work.

In section B, a series of keto steroids was examined in sulphuric acid of three strengths --- Analar which is approximately 98% H_2SO_4 by by weight, 100% H_2SO_4, and 15% fuming sulphuric acid. In a given acid, there was a striking similarity in the spectra of all the steroids. Though the spectra in Analar and 100% were the same, there was a major shift of peak in fuming sulphuric acid.

It is suggested here, that in Analar and 100% acid the absorption spectra arise from the protonated C_3 ketone group. The shift in peak in fuming acid is due to a nucleophilic attack on the C_3 by $HS_2O_7^-$ ion resulting in an irreversible displacement reaction, which involves the loss of water. In all three acids, the optical density increased with time. This is attributed to a time-dependent protonation reaction.

To support the premise of protonation, another strong protonating reagent, 70% perchloric acid was used. Here also, those steroids

tested, gave spectra which were identical in shape and peak position with the Analar spectra.

Since the H_2SO_4 is known to oxidize many organic compounds, it was suggested that this possibility be examined. One of the products of oxidation will be SO_2. Grant (2) describes a very sensitive test for this compound --- the limit of detection being 1 µgm/ml. No SO_2 was found in the acid solution of the steroids; therefore it is not likely that oxidation is occurring.

In general it has been shown that

(a) steroids do not lend themselves to accurate cryoscopic study in H_2SO_4 and a reason for this has been advanced.

(b) the ultraviolet spectra give a quantitative measure of the extent of reaction, and for different acids, the nature of the reaction occurring.

concentration of the solute if this does not dissociate.

The Clapeyron Clausius equation states that

$$\ln \frac{P_s}{P_o} = \frac{\Delta H_v (T_o - T)}{R T_o T}$$

(1) where ΔH_v = heat of vapourization of pure solvent.

R = the gas constant

This equation holds true because P_s and P_o are on the same vapour pressure curve, CD.

Similarly $\ln \frac{P_s}{P} = \frac{\Delta H_s (T_o - T)}{R T_o T}$ (2) where ΔH_s is the heat of sublimation of the pure solid solvent.

from (1) and (2) $\ln \frac{P}{P_o} = \frac{(\Delta H_s - \Delta H_v)(T_o - T)}{R T_o T} = \frac{\Delta H_f \Delta T_f}{R T_o T}$ (3)

where ΔH_f = heat of fusion of solvent.

(3) relates the vapour pressure of pure solid solvent, and pure liquid solvent at temperature T. However from the diagram it can be seen that at temperature T, the vapour pressure of the solution equals that of the solid and hence equation (3) must also relate the vapour pressure of the solution to that of pure solvent at the temperature T.

If Raoult's law is applicable then

$\frac{P}{P_o} = N_1 = 1 - N_2$ where N_1, N_2 = mole fractions of solvent and solute respectively

$$\ln (1 - N_2) = - \frac{\Delta H_f \Delta T_f}{R T_o T}$$

where N_2 is small, $\ln (1 - N_2)$ approximately equals $- N_2$ and

$T_o T \simeq T_o^2$

$$-N_2 = -\frac{\Delta H_f \, \Delta T_f}{R T_o^2}$$

$$\Delta T_f = \frac{R T_o^2}{\Delta H_f} N_2$$

finally expressing in molalities

$$\Delta T_f = \frac{R T_o^2 \, m}{\Delta H_f \, \frac{1000}{M_s}}$$

m = molality of solute

1000 = refers to 1000 gms. solvent

M_s = molecular weight of the solvent

The Quantity $\frac{R T_o^2}{\Delta H_f \, \frac{1000}{M_s}}$ is called the cryoscopic constant of the solvent and is designated by K_f. It is characteristic of the solvent only. K_f is equal to the observed freezing point depression resulting from the solution of 1 mole of an ideal solute in the solvent.

Not all solutes are ideal. A measure of the departure from ideality is given by the ratio of the effect produced by a concentration m of non-ideal solute to the effect produced by an equal concentration of ideal solute. This ratio is designated by the letter i and is called the van't Hoff factor.

$$i = \frac{\text{observed}}{\text{ideal solute}} = \frac{M_f}{M \text{ experimental}}$$

where M_f = molecular weight of the solute calculated from the formula

M_e = experimentally observed molecular weight of solute

The definition of the van't Hoff factor requires that i be the measure of all deviations from non-ideality, no matter what the

origin. Yet, for a long time following Arrhenius, i was generally accepted as equal to the number of kinetically separate particles in solution whether existing as molecules or ions. The error of this interpretation was indicated by Lewis and Randall (3). Today, it is generally accepted that i is the result of the total deviation from ideality, arising from both the number of different particles, and the forces they exert. The difficulty of resolving i into these components, has required a separate method of calculating the actual number of particles present in solution. This number is called the ν factor. Though there is a fundamental theoretical difference between i and ν , the numerical difference becomes of consequence only in very accurate calculations. Where high accuracy is not demanded, i.e. in a case where it is desired to distinguish whether a high molecular weight solute breaks up into 2 or 3 particles, the van't Hoff factor is a sufficiently good guide.

Gillespie et al (4) have developed an equation for calculating ν - the number of particles in solution in sulphuric acid.

$$\frac{\Delta\theta}{\Delta m_2} = \nu_2 K_f \left[1 - .0035\bar{e} + \frac{\{(2s_2 - \nu_2)\nu_2 m_2 + \nu_3 m_2 + \nu_2 s_3 - \nu_2\nu_3) m_3\}}{\nu_2 m_1} \right]$$

where m_1 = solvent m_2 = solute A m_3 = solute B

S_2 = number of molecules of solvent which react with 1 molecule of A to give total of ν_2 particles.

S_3 = number of molecules of solvent which react with 1 molecule of B to give total of ν_3 particles.

m values refer to molal quantities.

$\overline{\Theta}$ = mean freezing point depression

$\overline{m}_2$ = mean molality of the solute A

K_f = cryoscopic constant of sulphuric acid

$\frac{\Delta\Theta}{\Delta m_2}$ = is the equation for the small but finite change in the freezing point depression caused by a small but finite change Δm_2 in the molality of solute A.

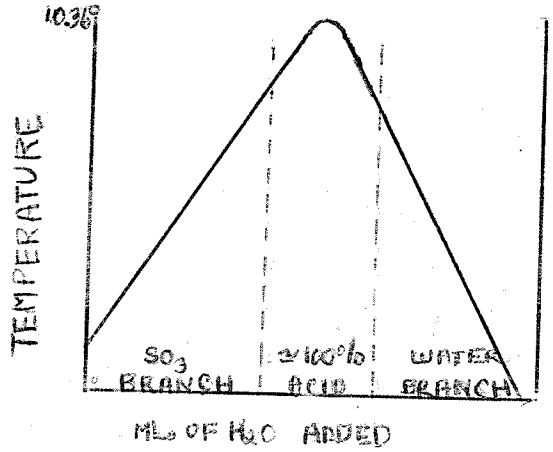
Where high accuracy is not required

$$\gamma_2 \simeq i = \frac{M_{formula}}{M_{experimental}}$$

THE SOLVENT - SULPHURIC ACID

As early as 1907, Hantzsh published research investigations related to the cryoscopic properties of sulphuric acid. The freezing point of sulphuric acid, is, in his opinion, the best criterion of its purity. More recently, Gillespie, Ingold and Hughes (4) reviewed much of the earlier work, redetermined and published new values for the freezing point of H_2SO_4 (10.36°C. and the cryoscopic constant 5.98° /mole) developed a good theoretical discussion, and experimental support, for many reactions in sulphuric acid.

The freezing point curve of sulphuric acid is qualitatively indicated below.



The freezing point curve of sulphuric acid should be discussed in three parts, since in effect, there exist three distinct regions in it (a) the H_2O branch (b) the central part (c) the SO_3branch.

THE WATER BRANCH

Beyond a concentration.05 molal the water branch of the curve is linear, indicating the existence of a proportional relation between temperature decrease and increasing concentration. The reaction occurring is

$$H_2O + H_2SO_4 \longrightarrow H_3O^+ + HSO_4^-$$

The value of the cryoscopic constant would indicate that the slope should be 11.96. In fact, Gillespie (4) measured it to be 11.21. The discrepancy is attributed to the incomplete ability of all water molecules to accept a proton from H_2SO_4, or its incomplete transformation into the conjugate acid. As the concentration of water decreases further, it is expected that the slope rise to its maximum possible value. Instead it falls to zero. This is due to two reactions which the solvent undergoes when it is in a nearly pure state (a) auto protolysis (b) self dehydration.

THE CENTRAL PART

The central part of the freezing point curve is well rounded

and at one point its slope is zero. It is generally accepted that the rounded part of the curve is due to the following two reactions

(1) autoprotolysis

$$2\,H_2SO_4 \rightleftharpoons H_3SO_4^+ + HSO_4^- \quad K_{AP} = 1.7\times10^{-4}\ GM.MOL^2/KGM^2$$

(2) self dehydration

$$2\,H_2SO_4 \rightleftharpoons H_2S_2O_7 + H_2O$$

$$H_2S_2O_7 + H_2SO_4 \rightleftharpoons H_3SO_4^+ + HS_2O_7^-$$

$$H_2O + H_2SO_4 \rightleftharpoons H_3O^+ + HSO_4^-$$

Summary $2\,H_2SO_4 \rightleftharpoons H_3O^+ + HS_2O_7^-$ $K_{id} = .7\times10^{-4}\ GM.MOL^2/KGM^2$

From these reactions it is obvious why both H_2O and SO_3 cause a rounding off. Water gives rise to this reaction

$$H_2O + H_2SO_4 \rightleftharpoons H_3O^+ + HSO_4^-$$

The ions produced repress both dissociation reactions, decreasing the number of particles present, and raising the freezing point. This rise, however, is offset by the fact that foreign species are being instroduced, causing a lowering of the freezing point. What is observed, is a balance between the two opposing effects.

Similarly for SO_3 solute ---

$$SO_3 + H_2SO_4 \rightleftharpoons H_2S_2O_7$$

$$H_2S_2O_7 + H_2SO_4 \rightleftharpoons H_3SO_4^+ + HS_2O_7^-$$

These ions also suppress both dissociation processes. The rounding off on the SO_3 side of the cryoscopic peak is here again a balance between the decrease in particles due to repression of the dissociative reactions, and the increase in particles due to the presence

of the solute SO_3.

Gillespie et al (4) have calculated the value of the freezing point of theoretically pure, undissociated sulphuric acid --- 10.62°C. Their best experimental value is 10.36°C. From the difference between the two, the actual concentration of ions present in 100% sulphuric acid, has been calculated. It is .043 molal. This corresponds to a concentration .0215 molal of univalent ions.

It is to be emphasized that this is the minimum ionic concentration that will be present in sulphuric acid.

THE SO_3 BRANCH

When SO_3 dissolves in H_2SO_4 a reaction occurs:

$$H_2SO_4 + SO_3 \rightleftharpoons H_2S_2O_7$$

Even for very concentrated solutions this reaction is almost complete. (4) Hence it is expected that this would be all the more so, in relatively dilute solutions. Disulphuric acid is a somewhat weak acid in sulphuric acid. Its ionization constant is $K_{A(H_2S_2O_7)} = .028$ gm mol^2/kgm , and the reaction is

$$H_2S_2O_7 + H_2SO_4 \rightleftharpoons H_3SO_4^+ + HS_2O_7^-$$

The partial ionization results in a slope of 7.40 deg.gm.- mol.$^{-1}$kg. In dilute solutions the slope should rise to the limiting value of 11.96 deg. gm.- mol^{-1} kg. However this expected rise is offset by the increased self-dissociative processes of the solvent. The observed slope near the maximum is a balance between these two reactions. No 'true' theoretical character can be attributed to its numerical value.

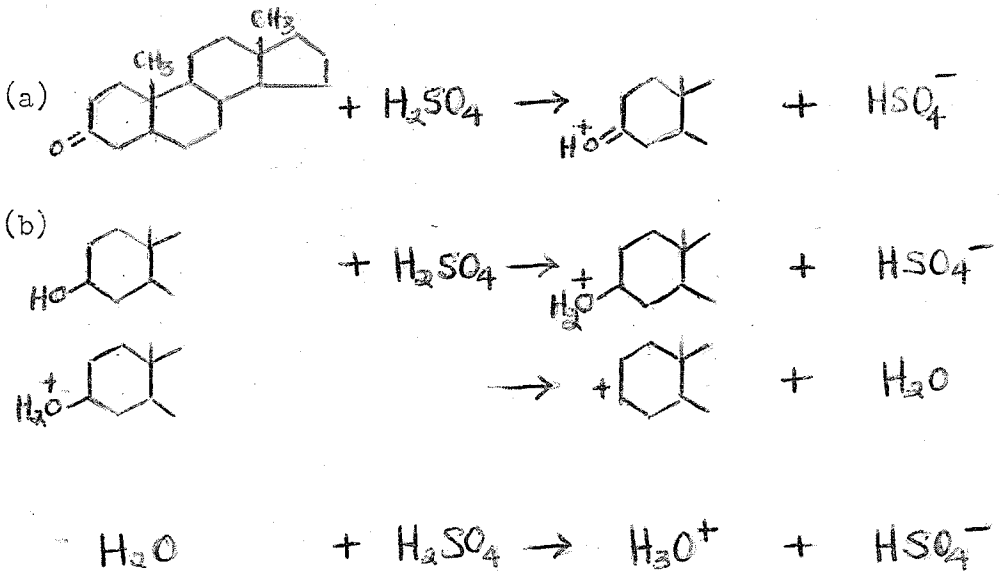
In discussing the nature of the reaction of a compound with sulphuric acid one must consider the nature of the sulphuric acid. If the reaction is dependent on the ionic species present, then the results

will be affected substantially when the reactant, sulphuric acid, is varied from fuming to analar or 98% acid.

A CRYOSCOPIC STUDY OF SOME STEROIDS IN SULPHURIC ACID

Introduction

J. H. Linford (5), reports that the reaction between H_2SO_4 and a steroid containing hydroxyl or ketonic groups, and/or double bonds is either protonation, or dehydration, with consequent carbonium ion formation.



A study of the freezing point depressions caused by steroids dissolved in approximately 100% H_2SO_4 should reveal the number of particles present in the solution. The cryoscopic work was guided by the earlier research of Gillespie, et al (4). A part of their experi-

ments was repeated in order to ascertain the accuracy of the present technique and apparatus. The value of the cryoscopic constant used in the present calculations was that determined by Gillespie ---- 5.98°/mole.

The equations used for the calculation of the number of particles is the simple formula

$$M_{exp.} = \frac{1000\ K_f\ g}{G\ \Delta T}$$

$$\frac{M\ \text{formula}}{M\ \text{exp.}} = \text{no. of particles} = i$$

K_f -- cryoscopic constant for sulphuric acid

g -- weight in grams of solute

G -- weight in grams of solvent

ΔT -- freezing point depression

$M_{formula}$ -- formula molecular weight

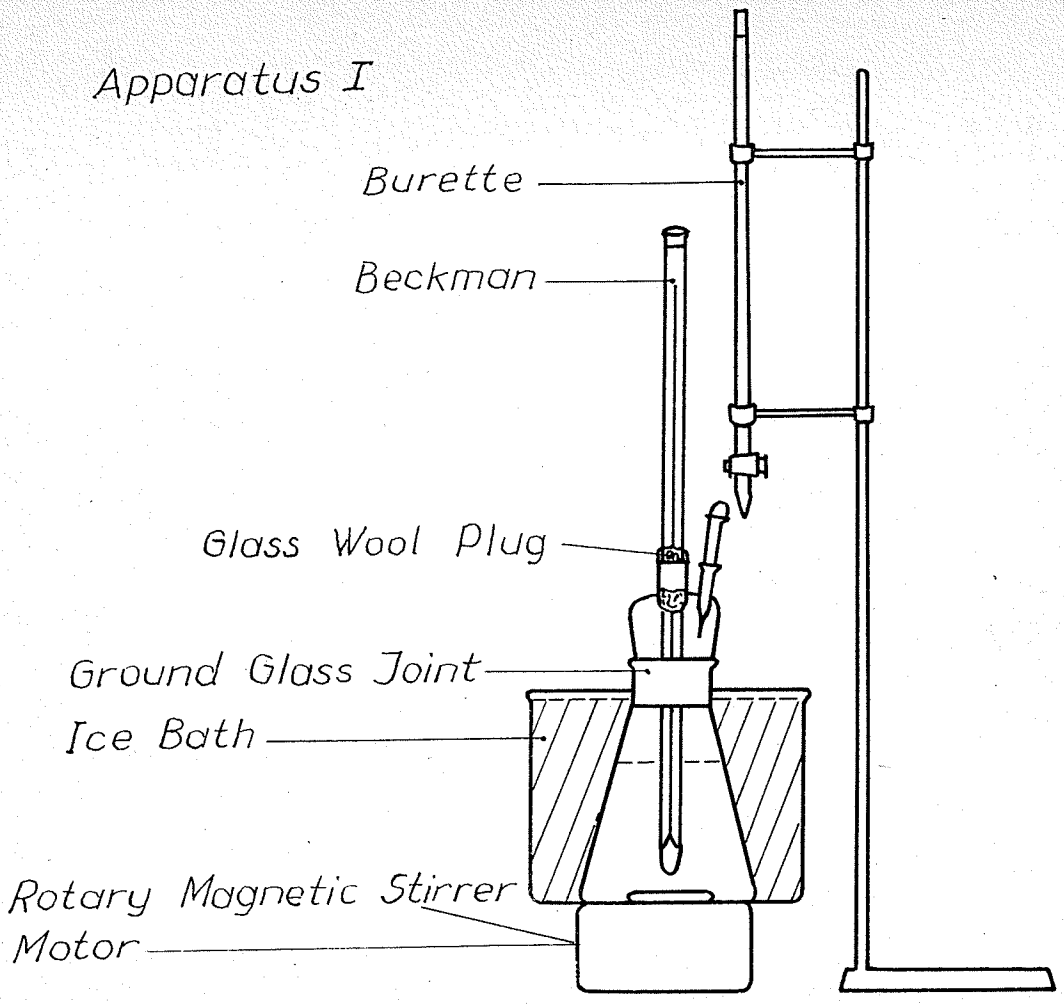
$M_{experimental}$ -- experimal molecular weight.

The much more elaborate formula used by Gillespie was not employed here since it was only desired to know the experimental molecular weight approximately -- within 10 or 15%. The large value of the formula weight would easily reveal whether i was 2, 3, 4 or 5.

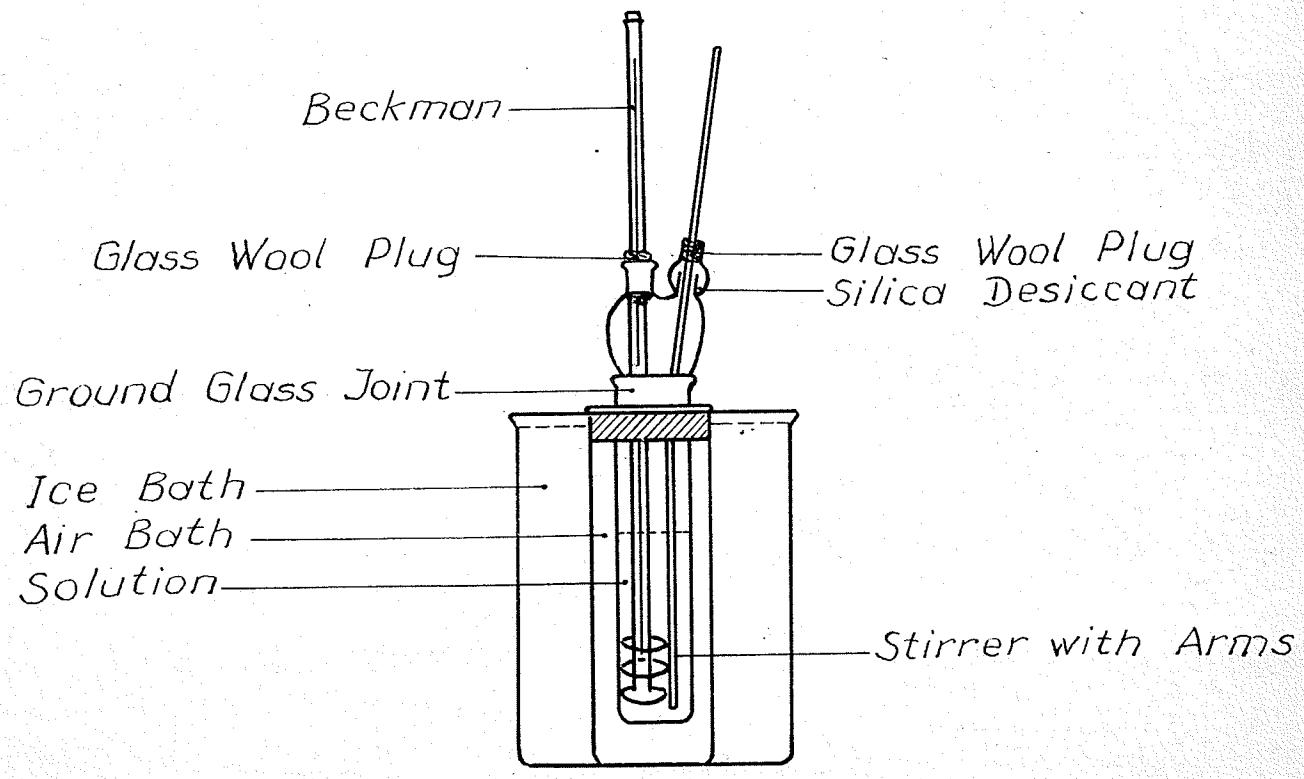
Apparatus and Chemicals

Two separate sets of apparatus were used --- Fig. 1 -- for the preparation of the approximately 100% H_2SO_4, Fig. 2 -- for the determination of the depression of the freezing point of the solvent occasioned by the presence of the solute.

Apparatus I



Apparatus II



98% by weight acid, henceforth referred to as AnalaR sulphuric, and fuming (15%) sulphuric acid were acquired from British Drug Houses. The titrant for the fuming acid was 37% H_2SO_4 prepared by adding 150 ccs. Analar to 68 ccs. double distilled water and diluting the resultant to twice its volume. Ethanol was purchased from Commercial Alcohols Limited and distilled once in an all glass apparatus. Sulphuryl chloride was obtained from British Drug Houses and used directly. Ammonium sulphate used was that from Baker Chemicals, and was dried to constant weight. Cholesterol came from British Drug Houses, cholestanol from Delta Chemical Works, N.Y., cholic acid from the Matheson Company Incorporated, Joliet Ill. All the steroids were recrystallized twice from ethanol, or a suitable alcohol-water mixture. They were dried to constant weight by heating for 15 hours under the reduced pressure of a vacuum pump.

Preparation of approximately 100% acid

100% H_2SO_4 was taken as that acid which gave the maximum freezing point on the Beckman thermometer.

Approximately 150 ml. of fuming H_2SO_4 were put into the erlenmeyer of Fig. 1. 37% H_2SO_4 was added from a burette. 0.5 ml. were added at a time and the freezing point determined. Toward the end, or maximum, 5 drops were added at a time. When the maximum had been reached, 0.3 ml. of 37% was added in excess. This was to insure that the acid was now of a composition which would give a freezing depression proportional to the concentration of the solute. Freezing of the acid, in those instances where it was not spontaneous, was initiated by touching the sides of the erlenmeyer with dry ice. An air bath was not used because the large quantity of acid required too much time to cool in an air bath.

The acid thus prepared, was then transferred to a stock bottle, with a double ground glass top, and kept in a silica desiccator.

Procedures

Preparations and freezing point determinations of solutions of ethanol, sulphuryl chloride, and ammonium sulphate.

This work is a repeat of that done by Gillespie. (4) It was used to determine the limit of accuracy of the present apparatus and method. Gillespie showed that the compounds produced the following number of particles

ethanol $C_2H_5OH + H_2SO_4 \rightarrow C_2H_5HSO_4 + H_3O^+ + HSO_4^-$ -- 3 particles

ammonium sulphate

$(NH_4)_2SO_4 + H_2SO_4 \rightarrow 2NH_4^+ + 2HSO_4^-$ -- 4 particles

sulphuryl chloride

$SO_2Cl_2 + H_2SO_4 \rightarrow$ NO REACTION -- 1 particle

Since all these solutes dissolve readily in H_2SO_4, solution could be effected and the consequent depression measured immediately. The weight of the solvent, and that of the solutes C_2H_5OH and SO_2Cl_2 was calculated from the density and the volumes used.

The solvent was put into apparatus of fig. 2. Its freezing point was determined thus: the ice bath cooled the air bath, this caused the temperature of the acid to fall. When it was believed that the acid was super-cooled, it was lifted slightly from the air bath, and the sides touched with dry ice to initiate crystallization. It was immediately put back into the air bath. The temperature when freezing

takes place rises -- that value which after this rise, remains constant for 2 minutes is taken as the freezing point. The Beckman is tapped before each reading to remove the possibility of the mercury sticking to the glass. The freezing point is determined three times -- the second and third times the amount of supercooling is made a minimum by touching the dry ice to the tube immediately after the temperature has fallen below the freezing point observed the first time. Throughout the whole determination, the solvent is vigorously hand-stirred.

After the freezing point of the solvent was determined, the apparatus was opened at the ground glass joint and the solute quickly added. The apparatus was immediately closed again. Ethanol and sulphuryl chloride were dropped in from a pipette, ammonium sulphate from a tared bottle. Vigorous stirring insured the complete solution and uniform concentration. The freezing point was then determined in the same manner as that for the solvent. Here also three determinations of each freezing point were made. Three additions of solute were usually made in one experiment.

A blank was conducted to determine the depression arising from moisture entering the apparatus during the addition of solute. Exactly the same procedure was used as in the determination for solute. This correction -- .04°C -- arises every time the apparatus is opened. This value is quite constant for several trials on different days. The freezing point of the acid in the apparatus changed .08°/hour. It takes approximately one hour to run an experiment.

The data for these experiments are tabulated in Table I the freezing point depressions are corrected for the moisture entering the apparatus when it is opened. They are not corrected for the temperature drop in the solvent itself during the time of the experiment.

Evaluation of Results

Since we are only attempting to distinguish between whole numbers of particles, rather than measuring an exact quantity like the cryoscopic constant, or the dissociation constant of the solute in the solvent, the accuracy and reproducibility achieved here, though inferior to that obtained by Gillespie, nevertheless distinguishes well between 1, 2, 3, and 4 particles. Hence for our purpose it is satisfactory.

Preparation and Freezing Point Determination for Steroids

Since the solubility of the steroids in sulphuric acid is low, it was deemed best to effect solution during a time period of approximately two hours. The steroid was weighed into small ground glass stoppered bottles and a known quantity of solvent added. The bottles were kept during the two hour period in a silica dessicator. The solution was then transferred to the freezing apparatus of Fig. 2. The freezing point was determined in the same manner as for the solvent described in the previous section.

For the initial pure solvent value, a blank was carried through exactly the same procedure as the solutions, and its freezing point was used.

One isolated experiment was conducted using benzene as the solvent. It has a freezing point near that of H_2SO_4 --- 5.51° and a cryoscopic constant of 4.90° $gm.mol.^{-1}$ $kg.^{-1}$ Refer Table II line 7.

TABLE I

DEPRESSION OF FREEZING POINT OF H_2SO_4 BY SIMPLE SOLUTES

te	Weight solvent in gms.	Weight solute in gms.	Observed temp. change	Experimental Molecular Weight	Number of Particles	Calc. Exp. Molecular Weight	Formula Weight
$_2SO_4$	36.6 gms.	.4557	2.25°	33.2	4	132.8	
	29.3 gms.	.0817	.55	30.4	4	121.6	
					Average	127.7	132
)H	47.5	.0873	.838	13.3	3	39.9	
	40.26	.0873	.95	13.6	3	40.8	
	45.38	.1310	1.16	14.4	3	43.2	
	45.38	.2183	1.98	14.5	3	43.5	
					Average	39.9	46
$_2$	42.09	1.443	1.76	119.2	1	119.2	
	42.09	.48	.57	122.8	1	122.8	
	42.09	1.115	1.29	122.8	1	122.8	
	42.09	.24	.26	134.3	1	134.3	
					Average	124.9	134

TABLE II

DEPRESSIONS OF FREEZING POINT OF H_2SO_4 + C_6H_6 BY STEROIDS

oid	Weight solvent in gms.	Weight solute in gms.	Temp. change	Experimental Molecular Weight	Number of Particles i	i x Exp. Molecular Weight	Formula Weight
esterol	36.60	.0291	.42°	11.33	35 - 45	385 - 495	
	36.60	.0188	.33	9.26	"	315 - 405	
	43.92	.0195	.36	8.48	"	280 - 360	
	54.90	.0308	.328	10.23	"	350 - 450	
	54.90	.0448	.448	10.89	"	385 - 495	
enzene	17.58	.0673	.045	412	1	412	386.64
c Acid	40.26	.0456	.45	15.05	25 - 35	375 - 525	
	45.75	.0212	.15	18.04	"	450 - 650	
	45.75	.0333	.27	12.4	"	300 - 420	
							426.58
stanol	45.38	.0064	.04	17.47	25 - 35	425 - 595	
	36.60	.0155	.24	10.67	"	250 - 350	
	54.90	.0326	.23	15.7	"	375 - 525	
	54.90	.0373	.29	14.1	"	350 - 490	388.64

DISCUSSION

The cyroscopic data for the steroids is tabulated in Table II. In the case of the benzene solvent the presence of one particle is indicated. In sulphuric acid, the data suggest the presence of a very large number of particles --- between 35 and 45.

In attempting an explanation of these phenomena, these possibilities were considered.

I The depression is due not to the steroid but to occluded ethanol. This possibility was rejected because

(a) the steroid was dried to constant weight.

(b) a calculation reveals that if indeed the depression were caused by occluded solvent, then 100% of the solute dissolved would have to be solvent in order to produce the observed depression.

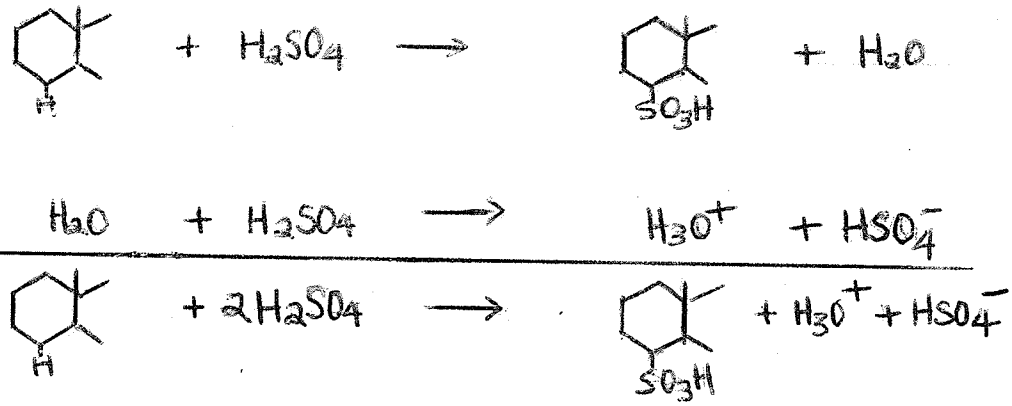
(c) the isolated case of cholesterol in benzene, which gives the predicted single particle, suggests the purity of the solute.

II The depression is due to an impurity in the solute other than solvent. This may be plausible since steroids are very difficult to get in pure form. However, the steroids sold by the given companies are of the highest purity attainable in their laboratories, and if an impurity remains, it must be difficult to separate. This suggests that very possibly it is a molecule whose structure and nature is similar to that of the steroid. Hence it would have a high molecular weight, -- yet only an impurity of low molecular weight, and only a substantial amount of it, would account for the observed results.

III The depression is due to contamination from the outside. This possibility was rejected due to the results observed on ethanol, sulphuryl chloride, and ammonium sulphate. Since the experimental molecular weights obtained for these compounds agree within 10% of the formula value -- it would indicate that there is little contamination from the outside.

IV Sulphonation occurs

If sulphonation were to occur, it would have to occur at several carbon atoms in order to produce the large number of particles. During sulphonation of one carbon atom -- three particles are produced



For a molecule like cholesterol which has 1OH group and 1 double bond sulphonation at 15 carbons gives 31 particles

the OH groups gives rise to 2 particles

the double bond gives " 1 particle

This would account for 34 particles. However it seems highly unlikely that such a reaction occurs.

V Fragmentation occurs

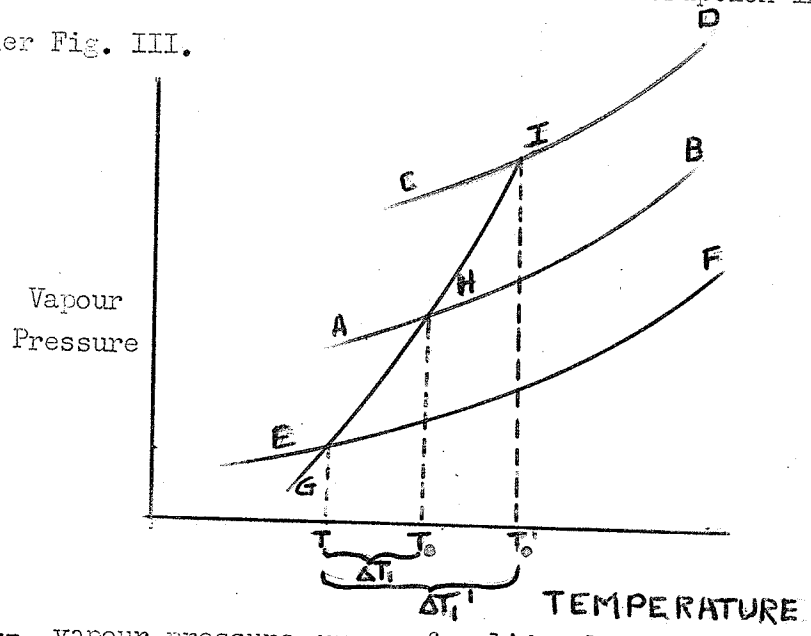
If sulphuric acid were to rupture the steroid molecule, one of the products of the oxidation would be sulphur dioxide. In

section B, it is shown that no SO_2 is present in the sulphuric acid solutions of steroids. Further, if fragmentation were to occur, it is highly unlikely that the resulting spectra of the steroid in acid, should be simple, with a single peak. Yet such is the nature of the spectra.

VI A Possible Explanation that was arrived at is this:

Since there is no evidence that any reactions besides protonation and double bond addition occur, one must conclude, that the abnormal cryoscopic behaviour of steroids in sulphuric acid must be due to a change in the nature of the solvent. Sulphuric acid is known to have pronounced structure in the liquid state. The vapour pressure curve represents an equilibrium between this liquid and vapour, at various temperatures. If the structure of the liquid were disrupted, partially or completely, the vapour pressure curve would be raised to an extent reflecting the amount of disruption in structure.

Consider Fig. III.



G H^I -- vapour pressure curve of solid sulphuric acid

AB -- vapour pressure curve of 'normal' liquid sulphuric acid

CD -- possible vapour pressure curve of liquid sulphuric acid whose structure has been partially disrupted.

EF -- vapour pressure curve of a solution of steroid in sulphuric acid

The concentrations of steroid used, should, through normal cryoscopic behaviour, have occasioned no freezing point depression of the solvent. Yet a substantial drop was observed. This drop is attributed to the ability of steroids to disrupt the internal structure of the solvent. The steroids are very large molecules. When, they become charged, through protonation, they are very soluble in sulphuric acid. Their size, plus their solubility, may be expected to disrupt the structure of the surrounding solvent. The cryoscopic effect can be seen from the diagram.

The vapour pressure curve CD is that of a theoretical pure sulphuric acid with the same amount of structure as that in the presence of steroid. The freezing point of this solvent would be T_o^1. The observed depression by steroid solute would be ΔT_1^1, and not the normal expected ΔT_1. The value of ΔT_1^1 will be a complex function of the concentration of steroid present. T_1 will decrease with increasing concentration. However T_o^1 will also change. At first, it may be expected to rise, but it must level off. The magnitude of the resulting depression will be difficult to predict. In view of the small concentration of steroid, the magnitude of this depression is rather surprising.

CONCLUSION

The present work indicates that cryoscopic studies of steroids in sulphuric acid cannot produce conclusive evidence of the nature of the reaction occurring. The reason for this has been attributed to the ability of the large steroid molecule to disrupt the structure of the solvent. The disruption of the internal structure of the sulphuric acid could be a "dis-association" caused by the field of the steroid, which acts as a large "electron cloud". This suggests the existence of an exciting field of study --- what is the minimum size of molecule which can produce this effect? Gold and Tye (6) mention that anthracene in sulphuric acid cannot be studied cryoscopically. In isolated experiments conducted here, it was found that both anthracene, and tetrahydronaphthalene gave unusually large depressions. However this line of research was not followed further.

THE ABSORPTION OF ULTRAVIOLET AND VISIBLE LIGHT BY ORGANIC COMPOUNDS

HISTORY

No attempts to relate the colour of an organic compound to its chemical constitution were made until Perkin in 1856, synthesized the dye known as Perkin's mauve. Graebe and Liegerman associated the colour of organic compounds with the presence of double bonds. Witt, in 1876, introduced the concept that formed the basis of all subsequent colour theories. He postulated the requirements of two conditions as prerequisites to the existence of colour: (a) the molecule must possess a potentiality for colour and (b) there must be present a salt-forming group to develop the colour potentiality. These two conditions are in fact the precursors of our modern chromophoric and auxochromic groups. No further theoretical advances were made until 1926 when Schroedinger published his historic paper, and quantum mechanics was born. A theory was sought that would relate colour to the energy levels. Bury, in 1934, suggested that the basis of the colour of an organic compound may lie in the quantum mechanical resonance phenomenon. The importance of the excited state of a molecule during the light absorption process was realized by A. L. Sklar, who proceeded, upon this basis, to calculate the absorption bands of a number of unsaturated hydrocarbons. Linus Pauling, by outlining a method of attack on the quantum mechanical problem of the colour of dyes, contributed greatly to our knowledge of the problem relating colour and constitution.

THEORY

"Absorption spectroscopy involves the dispersion of the incident or transmitted light, and the measurement of wave-lengths. The simplest means of effecting this, is to place the absorbing substance in the optical path of a spectroscope illumined by a constant source of light, and to observe the resulting absorption spectrum, in which regions of absorption appear as dark lines or bands." (7)

When a molecule absorbs, its energy is momentarily increased by an amount equal in energy to that of the inpinging photon. Mathematically, this may be expressed as

$$\Delta E = h\nu = \frac{hc}{\lambda}$$ where ν = frequency

ΔE = increase in energy

h = Planck's constant

c = velocity of light

λ = wave-length

The increase in energy is distributed through rotational, vibrational, and electronic energy levels. Relatively little energy is required to excite the first two. However only photons

of energy in ultraviolet region will, usually, excite the electronic levels. Thus, the region from 1000 -- 8000 A° represents electronic spectra.

The intensity of absorption is expressed by the Beer-Lambert Law

$$\log \frac{I_o}{I} = kcl$$

where I_o = intensity of incident light

I = intensity of emitted light

$\log \frac{I_o}{I}$ = optical density D

l = path length of absorption cell

c = concentration of solution in grams per liter.

k = specific extinction coefficient

When l = 1.00cm., and c = 1 molar, then $K = \epsilon$ = molecular extinction. The coefficient ϵ is a measure of the number of absorbing fragments, rather than of the differences in energy between the normal and excited states. It is a function of (a) the size of the molecule and (b) the probability of transition. For transition to occur, two processes must take place simultaneously: (i) the photon must strike the molecule (ii) the molecule must exist in a configuration that will permit excitation.

The latter brings in the factor of the probability of transition -- P. The maximum value that P may have is 1. High intensity absorption bands, are believed to have transitions of high probability -- such transitions are called 'allowed transitions'. Low intensity bands, less than 10^3, arise from transitions of low probability. When P = .01 or less, the transitions are called forbidden. P may also be called the transition moment.

For simple molecules, the curve relating λ, and ϵ consists of narrow groups of peaks, each of which corresponds to a transition from a particular combination of vibrational and rotational levels in the ground state to a particular combination in the excited state. Since the vibrational level corresponding to the equilibrium distance between nuclei is most populated, the transition from this level gives the most intense peak. On each side there will be smaller peaks arising from other less probable levels.

For complex molecules, the existence of a very large number of vibrational levels causes the individual peaks to accumulate under an envelope and these envelopes are observed as absorption bands. This effect is very pronounced in solution, where the fine structure character of a band, is seldom seen.

Several paths lie open to the excited molecule (a) the extra energy may be dissipated through vibration or rotation, and the molecule may return to the ground state (b) if before the molecule can dissipate its energy as in (a) it undergoes a downward electronic transition, the excess energy will be emitted as light -- the light will usually have

a frequency lower than that of the exciting light -- this phenomenon is known as fluorescence. (c) the molecule may disintegrate, either through photodissociation into free radicals, or through photo-ionization into ions.

Absorption in the ultraviolet or visible region invariably involves valency electrons. Since these are closely related to the chemical properties of a compound, the ultraviolet spectra provide basic information. It is proper that in dealing with the spectral properties of organic compounds in this region, the types of possible valency electrons be classified: (notation from molecular orbital theory)

1. σ electrons --- forming single bonds
2. π electrons --- forming multiple bonds
3. p electrons --- unshared electron pairs
4. 'odd' electrons --- present in free radicals
5. charge electrons --- present in ions.

Saturated hydrocarbons contain only σ electrons. Such compounds as methane and ethane exhibit absorption spectra in the far ultraviolet only. Their intense bands arise from two types of transition:
I N $\longrightarrow$ V transition. This involves a partial transfer of the electron forming a carbon-hydrogen, or a carbon-carbon bond, to one of the constituent atoms, and hence momentarily increasing the ionic character. Thus

$$-C-C \longrightarrow -\overset{+}{C}\ \overset{-}{C}- \quad \text{or} \quad -C-H \longrightarrow =C^{-}\ H^{+}$$

II N $\longrightarrow$ R transition. This is often called a Rydberg transition and represents the progressive removal of a σ electron away from both constituents, culminating in the complete ejection of the electron from the molecule with formation of a positive ion.

$$-\overset{|}{C}-\overset{|}{C}- \longrightarrow -\overset{|}{\overset{+}{C}}\cdot\overset{|}{C}- \quad \text{or} \quad -\overset{|}{C}-H \longrightarrow -\overset{|}{\overset{+}{C}}\cdot H$$

When in saturated compounds there are present elements other than carbon and hydrogen, i.e. nitrogen or oxygen, there are available p as well as σ electrons. The p electrons, because they do not form bonds, are held less firmly than σ electrons and consequently suffer Rydberg transitions at longer wavelengths.

Unsaturated, or π electron-containing compounds, undergo the same type of transition as σ electrons. However, owing to the less 'bonding' character of the π electrons and the concentration of the electron density laterally to the axis, rather than in the axis of the bond, the displacement of electrons occurs more readily than that of the σ electrons. The characteristic absorption of multiple bonds therefore lies at longer wavelengths than that of single bonds. Thus the carbon-carbon double bond in ethylene and the carbon-oxygen double bond in formaldehyde give rise to

(a) N $\longrightarrow$ R transition at 1200 - 1800 A^{o}

(b) N $\longrightarrow$ V transition at 1800 A^{o}. Both (a) and (b) have large ϵ values.

(c) weak bands in the near ultraviolet whose meaning is not too

clear. However, it is believed that they may be due to forbidden transitions N $\longrightarrow$ T for the carbon-carbon multiple bond; the band at 2800 A° for aldehydes and ketones has been ascribed to a forbidden transition in which 1 p electron is transferred from the oxygen to an antibonding orbital.

CHROMOPHORES AND AUXOCHROMES

The electronic spectra due to individual groups are modified by interaction with other groups present in the molecule. This interaction may be of three kinds --- vibration, electronic, or steric. The electronic interaction is the only one which results in marked displacements. These are very strong in those cases where the adjacent groups contain highly polarizable π or p electrons. The classical term for an arrangement of multiple bonds in adjacent positions i.e. separated by a single bond, and the consequent electronic interaction is "conjugation". In fact, it would more apt that it be termed ---π-π conjugation. Besides this, there is π-p conjugation which is very strong, and π-σ conjugation which is weak. Each type of conjugation results in a closing up of the ground and excited levels -- in fact, a decrease in transition energy and a resulting band displacement to longer wavelengths. Most organic compounds showing selective absorption in the accessible ultraviolet and visible region contain either π-π or π-p conjugation.

Witt's original nomenclature has been modified so that today, chromophore, and auxochrome, refer to π electron and p electron groups respectively.

In π-π conjugation, two chromophoric groups often produce

intense bands around 2300 A°. This is due to N ⟶ V transitions of this kind, $-C=C-C=O \longleftrightarrow {}^{+}C-C=C-\bar{O}$ and the resulting absorption bands are called K bands, (K for Konjugation). There are also present weak bands at longer wavelengths. These are probably due to the weak bands of the single chromophores, and are called R bands (R - for Radikal). The longer the conjugated system, the longer will be the wavelength at which the K and R bands appear. Burawoy (8) states that the addition of an acid molecule to the group will destroy the R band in all cases. However the K bands will upon addition of an acid molecule, be displaced towards longer wavelengths. The dielectric constant of the solvent also affects the K and R bands -- the K band will be displaced towards longer wavelengths, whereas the R band will be unaffected, or moved slightly towards shorter wavelengths. Since the R is not affected to any extent, it would seem, that it is the rotation levels of the excited state which are affected. The K band displacement would then mean that a particular rotation level is favoured.

π - p conjugation has been relatively recently recognized. The magnitude of the auxochromic effects depend upon the availability of the p electrons, and upon the position of the key atom in the periodic system. However it has been shown that there exists no relation between the latter and basicity, ionization potential or other simple parameters. G. N. Lewis and H. Calvin (9) describe an auxochrome, as a group which provides, through resonance, entirely new electronic paths, and hence, may not only shift old bands, but create new ones.

The absorption bands of p π conjugation are analogous to the K bands ascribed to N ⟶ V transition

$$-C=C-NR_2 \longleftrightarrow -\bar{C}-C=\overset{+}{N}R_2$$

Evidence that the p electrons are involved, exists in the fact that the auxochromic effect is almost completely absent in quaternary derivatives of the group V elements, where the p electron is used in bond formation. The effect of an auxochrome is considerably dependent upon the chromophoric system to which it is attached. Carbonyl groups behave very differently from ethylenic and benzenoid systems. In carboxylic acids, esters, and amides, no K bands due to $O = C - OR$ or $O = C - NR_2$ appear above 2000 A°, and at the same time the weak R bands of $C = O$ near 2600 A° disappear. These effects closely parallel the modification in the chemical properties of the carbonyl group, and the disappearance of ketonic character.

In general, the absorption due to isolated chromophores, i.e. separated by at least two CH_2 groups, is additive. This absorption, resulting from $\pi\sigma$ conjugation, has in a few isolated instances shown, however, that there may be appreciable spectral interaction between π or p electrons even across 2 or 3 single bonds. It is not known to what extent this effect can be attributed to induction or hyperconjugation.

The introduction of a permanent charge into a chromophoric system, effects a profound change in the absorption spectra. The charge may change a nonabsorbing species into an absorbing one - i.e.

colourless $(C_6H_5)_3CH$ and coloured $(C_6H_5)_3C^-$

The absorption of the latter is said to be due to the transition, in which the distribution of the resonating charge between the several available positions becomes momentarily altered under the influence of the light field. Such spectra are termed charge resonance spectra, and are dissimilar to the charge transfer spectra arising from $N \longrightarrow V$ transitions. The effect of auxochromes in such systems are large since

they provide seats for the resonating charge.

The solvent may have a marked effect on the absorption. Brode (10) divides solvents into two categories -- (a) nonpolar - which includes inactive compounds such as the saturated paraffins and (b) polar solvents which very often take part in an actual chemical combination with the absorbing molecule. In the latter cases, the molecule formed may have a charge, and now being a polar molecule, may give rise to a new sharp spectrum, such as is found in a non-polar solvent. Woodward (11), notes that a more polar solvent than alcohol will shift the strong absorption bands at 236 mμ of α β unsaturated ketones to lower wavelengths.

The position of the absorption peak in a nonpolar solvent is also dependent upon substitution at atoms which are part of the resonating system. Woodward (12) tabulated the empirical values that substituents on alicyclic dienes have upon the position of the absorption peak. These values were consequently used by Fieser (13) to calculate the peak position in alcohol of certain suitable steroids. The effects of stereochemistry on ultraviolet absorption spectra have been very aptly reviewed by E. A. Braude (14). In general, steric effects cause small shifts in the peak position due to steric interference of resonance. Seldom are sharp differences in position or the shape of the band observed.

This has been a brief summary attempting to elucidate why organic compounds absorb, the types of absorption spectra, and the main factors that affect absorption.

THE INSTRUMENT

The two basic laws which govern quantitative absorption spectrometry are:

I Lambert's Law which states that the fraction of light which is absorbed by a substance is independent of the intensity of the incident light

$$I = I_o \, 10^{-kl}$$

where I_o and I are the incident and emitted light respectively, l is the thickness of the cell, in centimeters and k, a constant called the extinction coefficient. K is the reciprocal of the thickness, measured in centimeters, through which the light must travel to reduce the intensity of the light to 1/10 its initial value.

II Beer's Law -- this states that the fraction of light which is absorbed is directly proportional to the number of absorbing molecules through which the light passes,

$$I = I_o \, 10^{-\epsilon l c}$$

where I, I_o, l are the same as above, c is the molar concentration and ϵ is the molecular extinction coefficient. ϵ is the value of $\log \frac{I_o}{I}$ when the concentration of the solution is 1 molar, and the cell length is 1 cm.

The term, optical density D, is used to designate

$$\log_{10} \frac{I_o}{I} = D = \epsilon l c$$

It is the logarithm of the ratio of the intensities of the incident to emitted light. In determining an absorption spectrum one measures the optical densities of the solution at various wavelengths.

FIGURE I

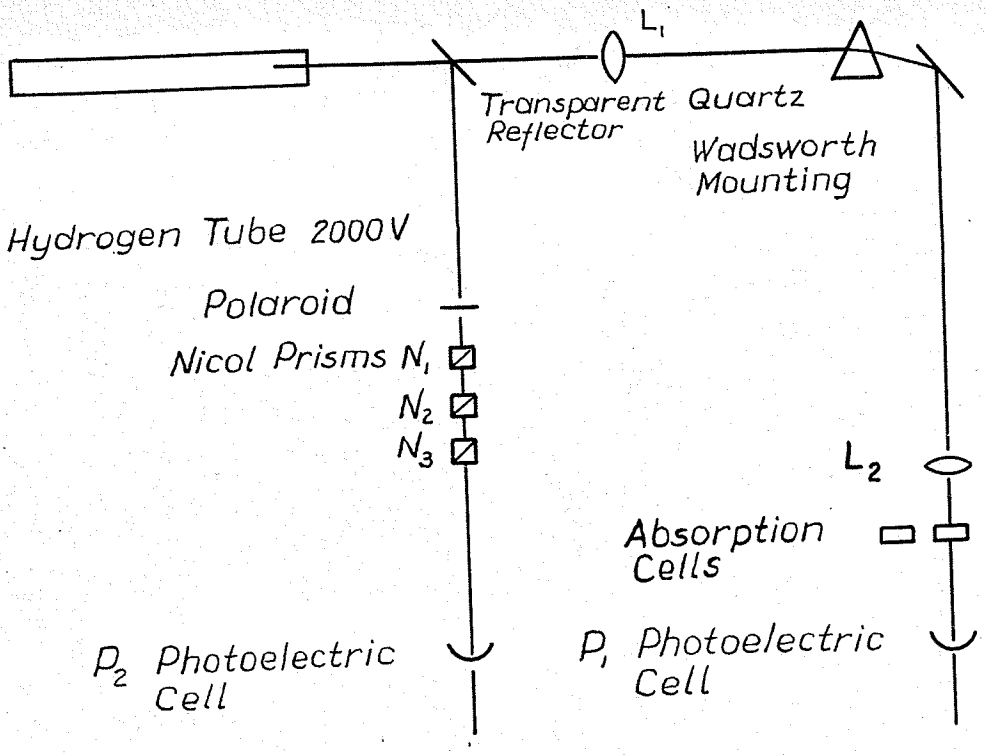
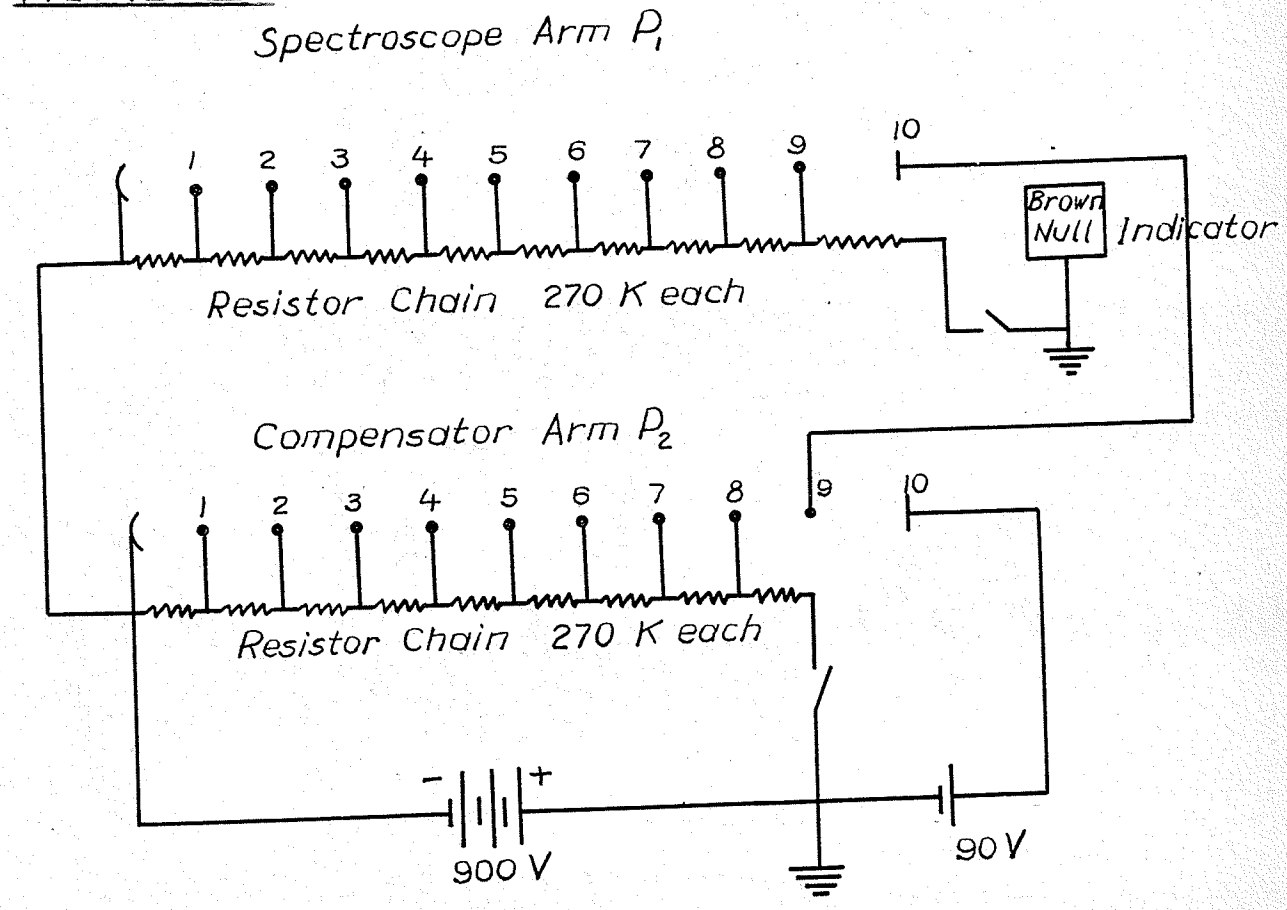


FIGURE II



For a given l, D is proportional to the concentration of the solution. A linear relation between c and D exists for most compounds at low concentrations -- absence of linearity is usually attributed to a change in the molecular species present.

For a known concentration of solute c, and a given length l, the optical densities measured over a range of wavelengths describe a curve characteristic of the solute. This curve, its shape and maximum intensity, is a constant of the solute, and is known as its absorption curve.

The instrument used is a Hilger Quartz Monochromator, of the constant deviation type. Fig. I gives a schematic diagram of the optics of the system; Fig. II that of the electric circuit of the photoelectric cells. The electric circuit was developed by E. Cartwright of the Manitoba Cancer Foundation. The slit widths were 3×10^{-3} inches. This allows the resolution of mercury lines differing by 6 A° at 3200 A° (31,250 cm^{-1}). The wave numbers are set by a calibrated drum, each wave number being focussed by lenses L_1 and L_2.

The instrument's calibration was checked by measuring a standard solution of potassium chromate. The peak positions, and the respective extinction coefficients were compared with those published by T. R. Hogness (15). The small difference of 3% may attributed to our use of a quartz plate compensator.

The procedure for measuring the optical density is this: With the nicol prisms set to maximum transmission, the light through

a cell of pure solvent, is balanced with the polaroid. A cell containing the solute is then substituted. Balance is restored by turning nicol prism N_2. The angle through which the prism is turned gives a measure of the decrease in intensity caused by the absorbance of the solution. The relation between the angle and the light intensities I_o and I for use in the equation $D = \log \frac{I_o}{I}$ is found as follows:

By balancing the solvent with the polaroid, I_o has been made equal to 1.

$$I = \cos^4 \theta$$

where θ is the angle of rotation

then

$$D = \log \frac{I_o}{I} = \log \frac{1}{\cos^4 \theta} = 4 \log \sec \theta$$

The procedure is repeated at regular small intervals of wave numbers over the whole range to be investigated. This is from 48000 cm^{-1} to 20000 cm^{-1}. At least twenty points are used to describe an absorption curve.

EXPERIMENTAL

The ketosteroids used for spectroscopy were donated by the Ciba Foundation and the Medical Research Council, England, Steroid Reference Collection. They were used as received without further purification. Their absorption spectra in ethanol agreed with literature values which attested their purity.

The sulphuric acids, Analar, fuming, and 100% are the same as those used in Section A. The 70% perchloric reagent was purchased from British Drug Houses.

The reagent used in testing for SO_2 was prepared and used according to the method of Grant (2).

5.5 ml. Analar sulphuric acid were added to 117 ml. of distilled water. To this were added 2 ml. of a 4% solution of fuchsine (from British Drug Houses) in ethanol. 4 ml. of this reagent are used with 1ml. of test solution in a determination.

Stock solutions of ketosteroids of known concentrations, ranging from 100 μgms. -- 200 μgms. per millileter were made up by dissolving a weighed quantity of steroid in 100 ml. ethanol. The solutions were kept in ground glass stoppered volumetric flasks.

To prepare the steroid for solution in acid, a quantity of ethanol solution was measured into a test tube with an 0.2 ml. pipette and dried in a boiling water bath for one hour. To ascertain that no steroid is lost in this process, two tests were performed:

(a) The alcohol solution was dried in an oven at 50°C over a period of two days. The optical densities of the spectra of the steroid, dissolved in Analar under identical conditions, but dried in the two

different ways, were identical.

(b) The steroid was dried in the boiling water bath and then redissolved in the original volume of ethanol. The optical densities of the initial and redissolved solutions were the same.

The reaction between sulphuric acid and the ketosteroids was observed at two temperatures (a) room temperature 24° - 25°C. and (b) the temperature of boiling water at atmospheric pressure - approximately 100°C. For the room temperature reactions, the steroids were dissolved in the acid, kept for the required length of time; 10 minutes, 1, 2, 3, 4, 7 days in a silica desiccator, put into the absorption cell, and the spectrum measured.

For the 100°C. reaction, the steroids were dissolved in the acid, the solution immersed in a boiling water bath for the required length of time, 3, 5, 10, 15 minutes, cooled in an ice bath, the absorption cells then filled, and the spectrum measured. The procedure was identical for all three acids - Analar, 100%, and fuming sulphuric acid.

In the study of the reaction between 70% perchloric acid and steroid it was only desired to know the peak position of absorption curve and not the equilibrium value of the optical density. Hence the spectrum was run one half hour after solution at room temperature.

To know whether the test developed by Grant (2) for SO_2 would be applicable in a medium as highly acidic as concentrated sulphuric acid, tests were carried out to estimate the limit of detection of SO_2. Sulphuric dioxide, prepared by adding dilute H_2SO_4 to Na_2SO_3, was bubbled into water, Analar, 100% H_2SO_4, and fuming H_2SO_4. The ultraviolet spectrum of the solutions was taken. From the molecular extinction coefficients for SO_2 in these media, published by V. Gold (16) the

III TABLE SHOWING RELATION WITH TIME, AT TWO TEMPERATURES, OF THE SPECTRAL POSITIONS AND OPTICAL DENSITIES OF THE ABSORPTION MAXIMA WHEN KETOSTEROIDS ARE DISSOLVED IN SULPHURIC ACID SOLVENTS A, 98% (ANALAR); B, 100%; C, 15% OLEUM. STEROID CONCENTRATION 10 μGM. PER ML. OF ACID SOLVENT.

Acid	U Max. ($cm^{-1} \times 10^{-2}$)	OPTICAL DENSITIES 24°C - 26°C. Time in Days 0	1	2	3	4	5	7	U. Max. ($cm^{-1} \times 10^{-2}$)	OPTICAL DENSITIES 98°C - 99°C. Time in Min. 0	3	5	10	15
5β ANDROSTANE - 3, 17 dione														
A	328	.245	.560	.556	.610			.616	328	.245		.546	.552	.628
B	330	.122	.658	.678	.695			.704	335			.631	.760	.816
C	350	.401	.706	.926	.926			.926	335 → 345	.401		.770	.884	.926
ANDROSTANE - 3, 17 dione														
A	328	.230	.520	.527	.544			.578	330	.230		.506	.532	.578
B	328	.275	.579	.594	.608			.604	335	.275		.662	.708	.734
C	345 → 350	.370	.631	.826	.878			.872	355	.370		.757	.793	.792
^{4}ANDROSTENE - 3, 17 dione														
A	335	.573	.904		.922			.924	335	.573		.956	.972	.932
B	335	.642	1.008	1.032		1.077		1.091	335	.642		1.081	1.042	1.011
C	335	.808							335	.808				
C	355		.761	.776			.824	.826	358			.856	.908	.964
TESTOSTERONE														
A	335	.836	.915		.934			.966	335	.836		1.040	1.059	1.077
B	335	1.030			1.252	1.264		1.161	335	1.031		1.145	1.104	1.024
C	335	.952							335	.952				
	350		.800	.862	.955			.962	355			.981	1.080	1.101
PREGNANEDIONE														
A	320	.00	.020		.055			.120	325			.241	.378	.449
B	320	.050	.312	.348	.411			.450	325			.525	.	
B									345				.648	.675
C	355	.190	.891	.980	.968			1.008	355	.190		.965	1.003	.998
PROGESTERONE														
A	345 → 335	.298	.488	.502	.568			.620	335	.298	.589	.596	.638	.648
B	345 → 335	.130	.508	.590	.610			.630	335	.130		.726	.776	.770
C	345 → 355	.330		.674	.683			.701	358	.330	.598	.700	.800	.802
DEHYDROCHOLIC ACID														
A	312	.00	.010		.027			.049	312	.00		.268	.372	.496
B	315	.00	.097	.166		.265		.381	325 → 315	.00		.840	.887	.957
C	352	.391	1.267		1.342			1.333	352	.391		1.410	1.414	1.475

An arrow - indicates a progressive shift of the absorption mzximum during the period under study.

TABLE III (b) ABSORPTION CHARACTERISTICS IN ETHANOL & PERCHLORIC ACID AT ROOM TEMPERATURE

Substance	Conc'n. in γm/cc	Solvent	Peak Posn. in ν x 10^2	Density
5β Androstanedione	209	Ethanol	-	-
Pregnanedione	189	"	-	-
Progesterone	9.69	"	416	.644
Testosterone	10	"	416	.662
Progesterone	11.6	Perchloric acid	345	.561
5β Androstanedione	10.9	" "	327	.335

TABLE IV ABSORPTION CHARACTERISTICS AT EQUILIBRIUM IN 98% ACID AT 100°C.

Substance		Peak Position in ν X 10^2	Optical Density 100γm/ml
5β Androstane 3, 17 dione	A	328	6.75
Androstane 3, 17 dione	B	325 → 328	6.12
Δ^4 Androstene 3, 17 dione	C	335	10.05
Testosterone	D	335	10.75
Progesterone	E	335	6.36
Pregnanedione	F	325	4.75
Dehydrocholic Acid	G	312	5.28

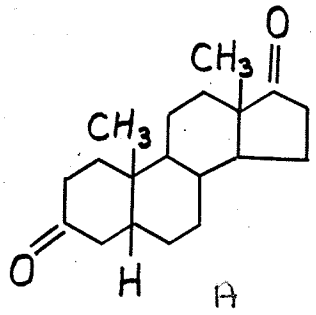
TABLE V ABSORPTION CHARACTERISTICS AT EQUILIBRIUM IN FUMING ACID AT 100°C.

Substance		Peak Position in ν x 10^2	Optical Density 100γm/ml
5β Androstane 3, 17 dione	A	355	10.00
Androstane 3, 17 dione	B	355	8.51
Δ^4 Androstene 3, 17 dione	C	360	11.02
Testosterone	D	360	12.08
Progesterone	E	360	9.40
Pregnanedione	F	355	10.40
Dehydrocholic Acid	G	350	15.31

concentrations of our solutions were calculated. They were subsequently diluted, till they contained approximately 1.5 μgms./ml. Then to 1 ml. of the solution, sitting in an ice bath, was added 4 ml. of the reagent, prepared as above. After 5 minutes, the visible spectrum was measured. In the presence of SO_2 the reagent turns deep red. Sometimes, this red colour may be hidden from the human eye by the yellowish brown colour of the reagent. However the sensitive photoelectric spectrophotometer can detect a concentration of 0.5 μ gm./cc.

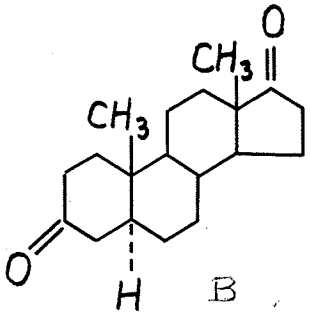
All data are tabulated in Table III.

FIGURE III



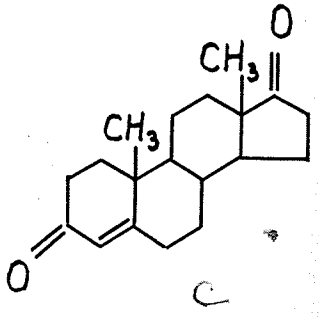
A

5β ANDROSTANE 3,17 DIONE



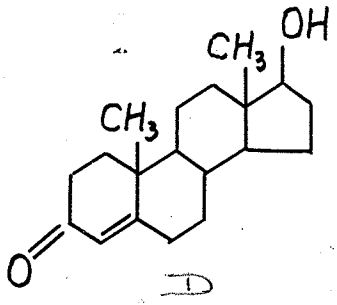
B

ANDROSTANE 3,17 DIONE



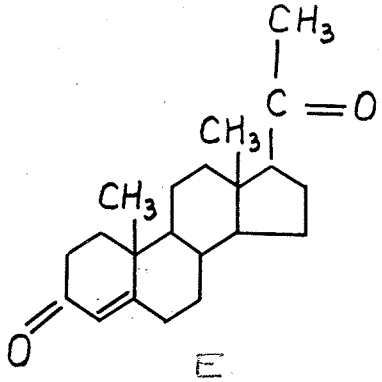
C

ANDROSTENE 3,17 DIONE



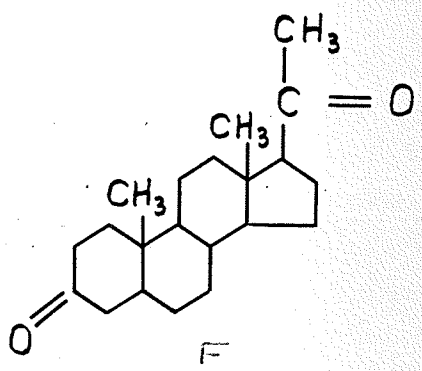
D

TESTOSTERONE



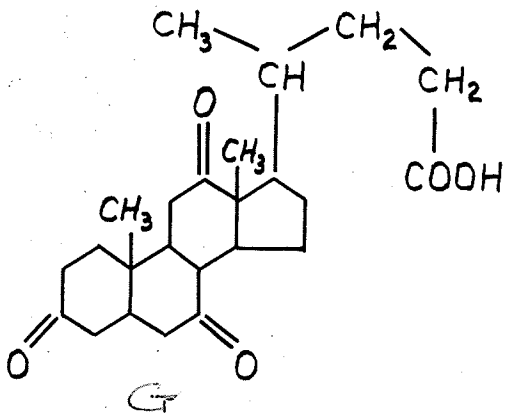
E

PROGESTERONE



F

PREGNANEDIONE



G

DEHYDROCHOLIC ACID

DISCUSSION

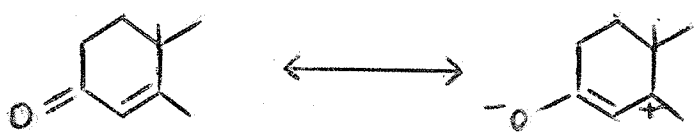
The absorption spectra of the ketosteroids shed considerable light not only on the reaction occurring in solution but also on the nature of the nucleus. By a systematic variation of structure in the steroid, one can relate changes in the absorption spectrum in a given solvent to the difference in structure. By a systematic change of the sulphuric acid solvent one can relate the change in spectrum to the ionic species present.

The ketosteroids studied and their structures are shown in FigureIII. They were so chosen that they had in common the following features: (a) the steroid nucleus and (b) the C_3 keto group. Since the nucleus itself does not absorb in this region of the U.V. the similarity between the ketosteroids must be attributed to the common C_3 keto group. Any differences between the spectra in a given sulphuric acid will probably be due to structure differences i.e. the occurrence of a double bond, and the group attached at the C_{17} position.

THE ALCOHOL SPECTRA

FigureIV indicates the typical spectra of the ketosteroids in alcohol. Testosterone is characteristic of the conjugated ketones, pregnanedione, of the saturated ones.

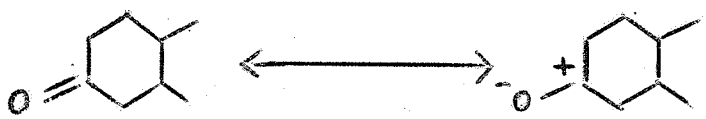
The absorption peak at 416 x 10^2 cm^{-1} is probably a K band resulting from the resonance.



The weak R band which Braude (7) suggests exists for conjugated

ketones at 340 x 10^2 cm^{-1} has not been observed, possibly because of the low concentration used.

Pregnanedione, being saturated, is not expected to produce a K band and none is observed. The R band, despite the greater concentration, and the fact that there is a C_{20} keto group also present, still has not appeared. If at higher concentrations it were to appear, it would be due to an N ⟶ V transition such as;



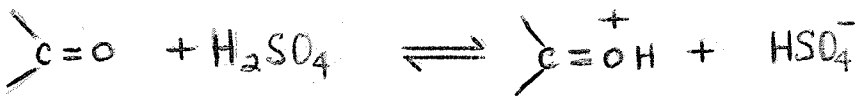
The experimental value for the optical density of 100 µgm/ml. testosterone in ethanol is 6.31. The value published in the literature is 5.94 (5). The difference may be attributed to the error in weighing the 20 mgm. sample for the stock solution, and use of a quartz compensator.

ACETIC ACID SPECTRA

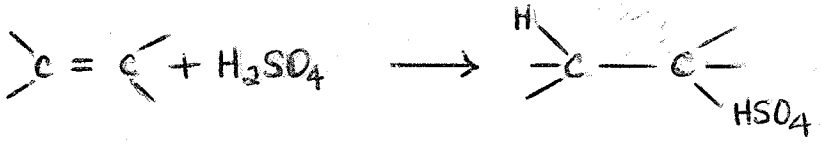
It was thought desirable to measure the absorption spectra of the ketosteroids in acetic acid. However, because of the considerable absorption of this solvent beyond 400 x 10^2 cm^{-1}, it was impossible to do so. No peaks existed for any steroids below 400 x 10^2 cm^{-1}. This indicates that, probably, the steroids in alcohol and in acetic acid exist in the same form.

THE ANALAR SPECTRA

Extensive work has been conducted on the reactions of ketones and sulphuric acid, (17), (18), (20), (21). It is generally accepted that the interaction is one of simple protonation.

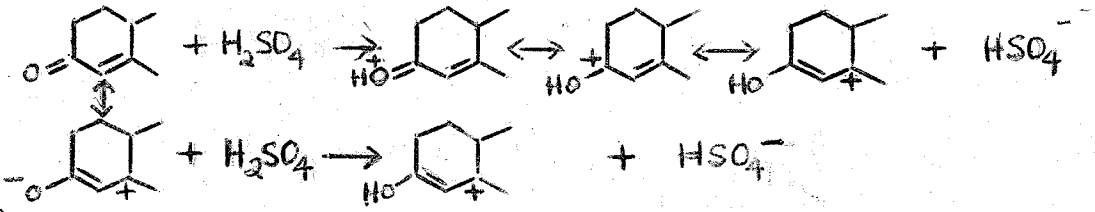


Similarly, exhaustive study on the interaction of olefins with sulphuric acid has been reviewed by Gillespie (17), and indicates that the fundamental reaction is one of alkylbisulphate formation



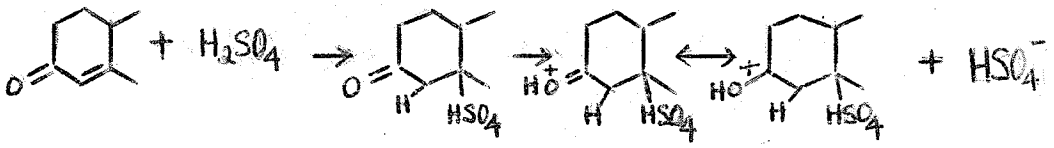
However there is no reference in the literature on the reactions of policyclic non-aromatic conjugated ketones in sulphuric acid, nor the products that are likely to be formed. The possibilities are these:

(a) simple protonation of the ketone



(b) simple addition to the double bond

(c) addition to the double bond and protonation



(d) protonation and then addition to the double bond

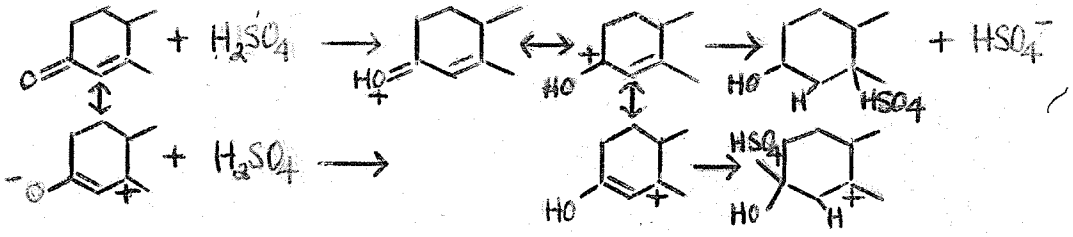


FIGURE VI

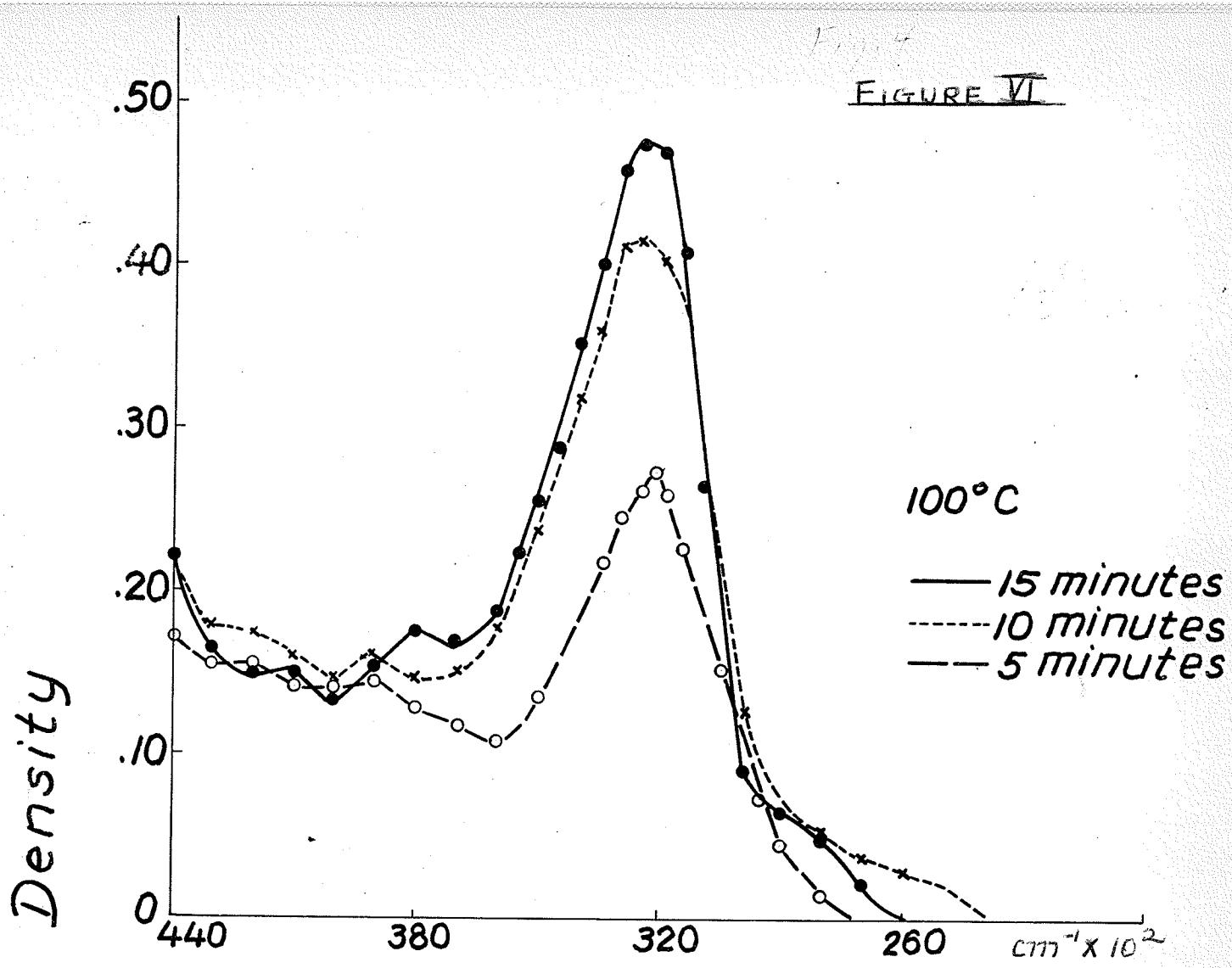
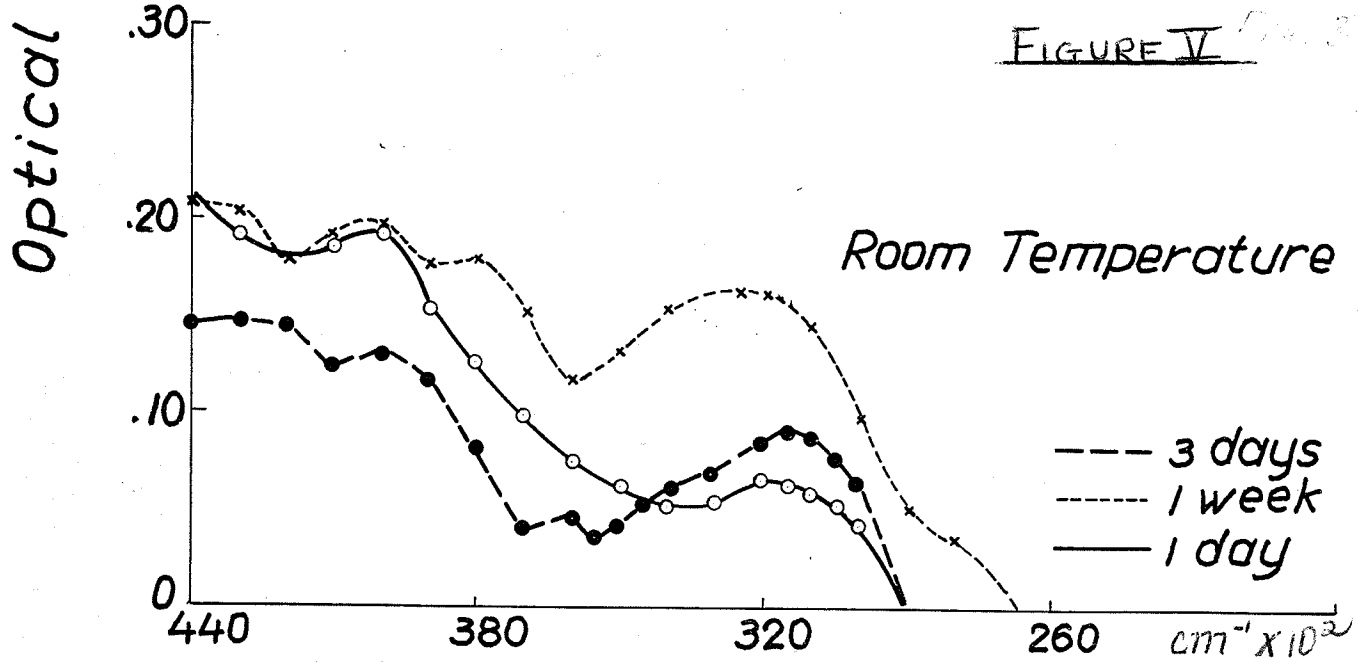


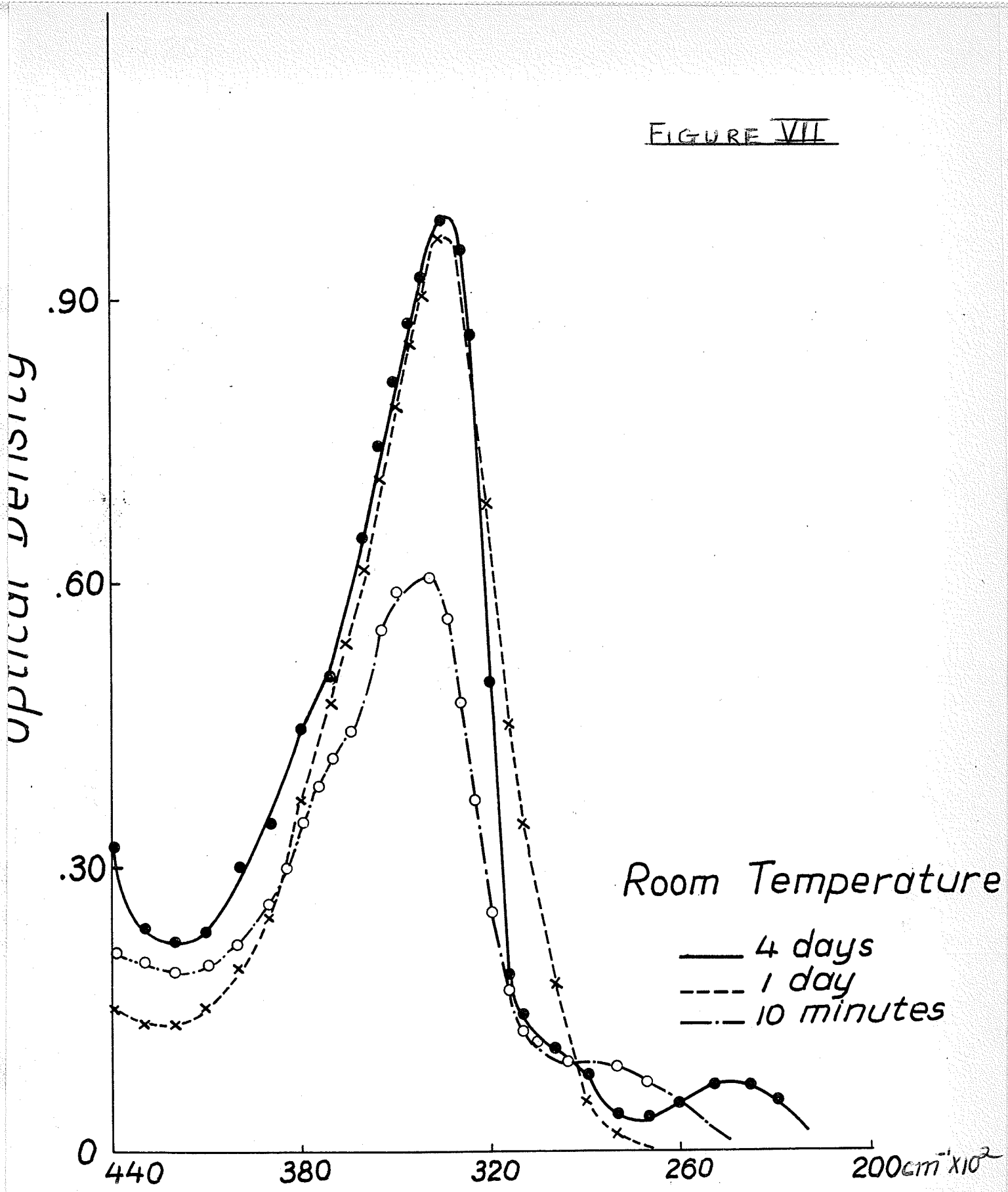
FIGURE V



Optical Density

Pregnanedione 10.1 μgm/cc in Analar

FIGURE VII



Δ^4 Androstene 3, 17 dione 10.3 μgm/cc

Analar

(e) simultaneous addition to both

Since the literature indicates so strongly that protonation occurs, it is unlikely that reaction (b) alone proceeds. Further, since olefins are known to react quickly with sulphuric acid, reaction (a) may be assumed not to proceed alone. This leaves mechanism (c), (d), and (e). On basis of evidence in this work (c) is believed to be the reaction scheme for unsaturated ketones. The saturated ketones are simply protonated.

Table IV indicates the peak positions and the optical densities (15 min. 100°C) calculated for a concentration of 100 µgm/ml. Fig. V and VI, Fig. VII and VIII represent typical spectra of saturated and conjugated ketones.

Considering peak position and intensity only, it is seen that;

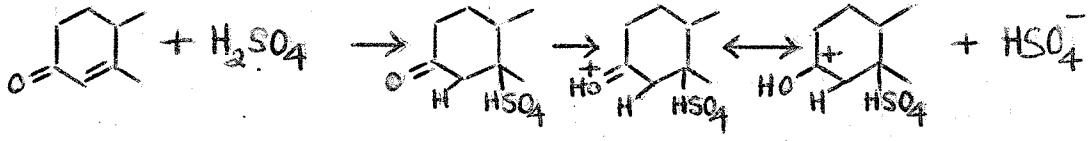
1. A major shift of peak from the alcohol has occurred. This was expected in view of the fact that the absorbing species in now charged, and the K band of the alcohol spectrum vanishes in the new charge resonance spectrum. Braude (7) states that the introduction of a charge from outside will make a radical difference in the observed spectrum. Dewar (19) remarks that solvents of high dielectric constant will cause peaks to shift to lower wavelengths.

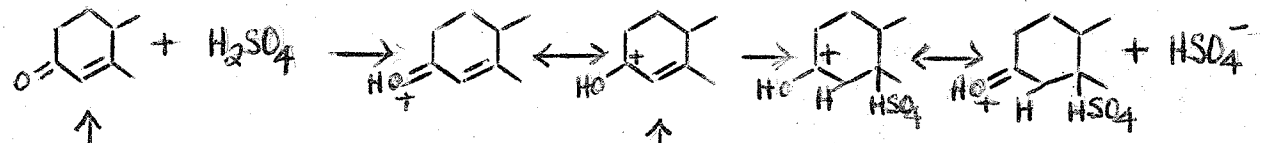
2. The saturated ketones absorb in the sulphuric acid. This is due to the protonated ketonic group. For compounds A and B, the optical density is quite high, whereas for compounds F and G, it is low. Since the

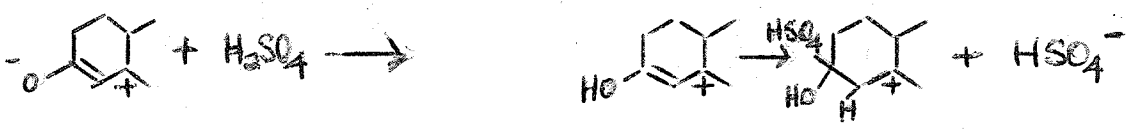
molecule is so large, all the ketonic groups should be able to be protonated. This would mean that G, with three ketonic groups should have the highest optical density. The discrepancies observed must be due to electronic effects, since protonation reactions are not sterically hindered.

It is seen that the saturated ketones absorb at a lower frequency than the conjugated ones. Since the only difference between the two is the ethylenic bond, either as such or opened, i.e. H_2SO_4 having added on, the difference in peak must be attributed to this.

Viewing the structures, these possibilities exist

(i) 

(ii) 

(iii) 

Either (i) or (ii) would cause absorption at a higher frequency because the HSO_4 group at C_5, being polar, would attract

electrons to itself, and hinder resonance. (iii) would be expected to give a substantially defferent absorption curve from either (i), (ii), or that of the saturated ketones, because the absorbing centers are different. Since no such difference is observed, possibilities (i) and (ii) remain.

To distinguish between the two, we examine the spectra of the conjugated ketones. There is no indication that these had an initial peak at 325×10^2 cm^{-1} which gradually shifts to 335×10^2 cm^{-1}. Therefore one must conclude that the reaction occurring at the double bond is very rapid, and that at the ketone group, rate controlling. Thus (i) must be the mechanism.

3. The conjugated ketones, C, D, E, have higher optical densities than the corresponding saturated ones. We postulate that the increase in optical density is a measure of an increased number of absorbing molecules. Since absorption at this frequency is attributed to the protonated keto group, it would seem that the presence of the HSO_4 at C_5 has enhanced the basicity of the C_3 keto. This effect is not random --- the ratio of the optical densities of the compounds C/A and E/F are of the same order of magnitude, respectively 1.48 and 1.34 --- and may be attributed to the electron-withdrawing power of the HSO_4 group.

Within the conjugated series, C, D, and E, differences in optical density occur. These molecules are identical in all respects except the substituent group at C_{17}. Disparities between the compounds are attributed to this. Compounds C and D, with small polar groups at

C_{17} have higher densities than E which has the bigger $COCH_3$ substituent. This difference in density due to the group attached at C_{17} is constant for both saturated, and conjugated ketones --ratio of density $^{A}/_{F} = 1.42$ and $^{C}/_{E} = 1.50$.

This optical density variation for both conjugated and saturated ketones may be explained by two possibilities;

(1) Protonation occurs at all ketonic groups and the steroid nucleus acts as an insulator for the charges. The ratios represent, in this case, the relative extent of the protonation at the C_{17} and C_{20} keto groups. However it would be expected that dehydrocholic acid with 3 keto groups would have a much higher density than it has. The small difference between C and D might be attributed to the greater ease with which the OH is protonatedand is removed as water.

(2) The steroid nucleus is not an insulator, and reactions at given positions exert electronic influence upon reactions at others. If a resonance axis exists through the steroid nucleus, then it is easily seen that (a) HSO_4 group attached at C_5 and outside the resonant system could cause a peak shift to the U.V. side (b) compounds A, B, C, and D, which have the second protonated group in the axis might resonate and distribute the charge over the whole system. Compounds E and F, which have the second protonated group, C_{20}, outside this axis, cannot distribute this charge, and would not be protonated as extensively. Compound G, with three keto groups, might be expected

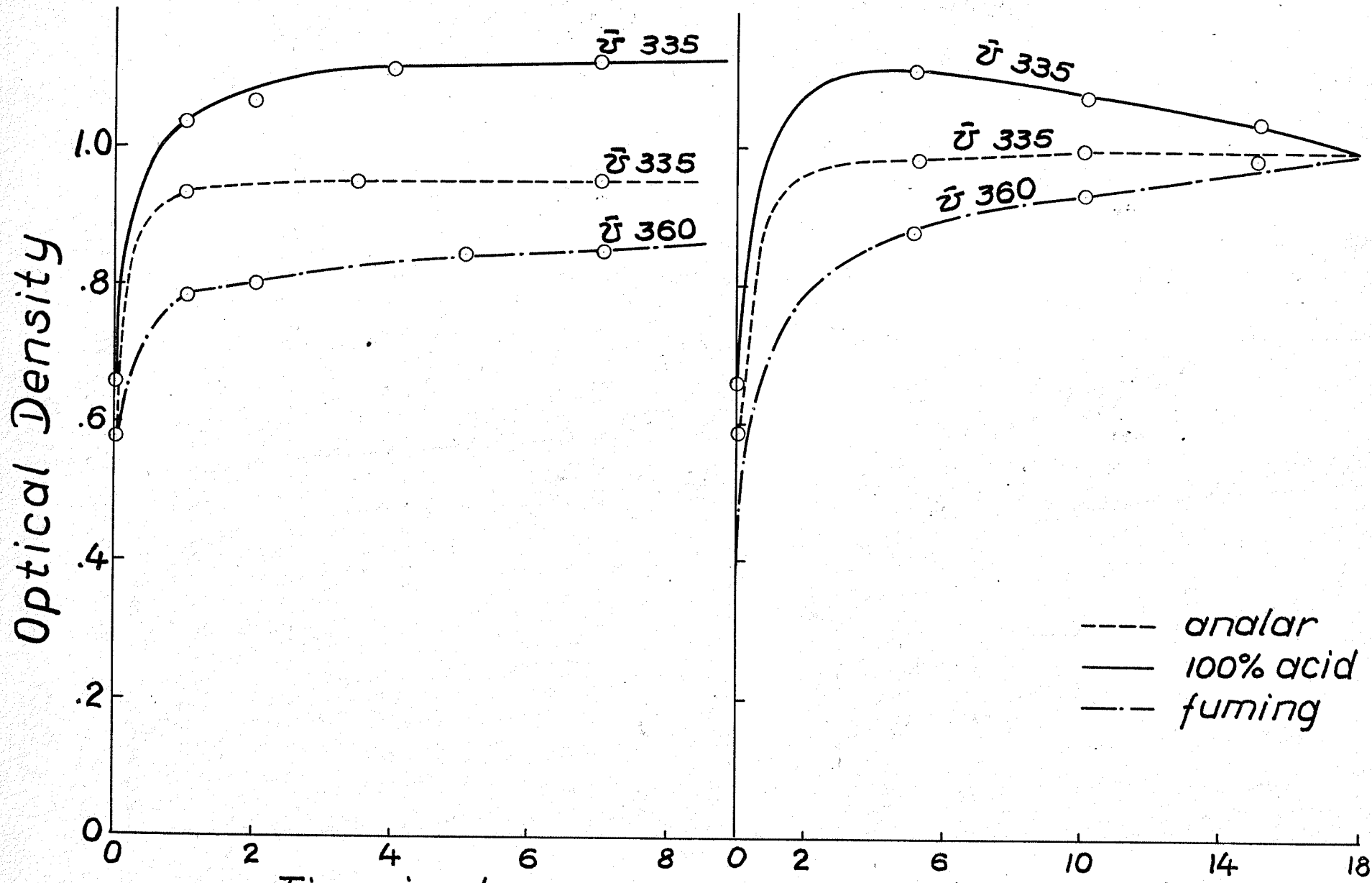


FIGURE VIII Room temperature 100° C

Δ^4 Androstene 3, 17 dione 10.3 μgm/cc

FIGURE IX (a) Room temperature 100° C

to have a very high density if all groups were protonated. However, it is very unlikely that even a resonance axis in as large a molecule as the steroid, could stabilize three positive charges. The observed optical density reflects the extent of this protonation, and the effect of greater proximity between the ketonic groups as compared to compounds with C_3 and C_{17} or C_{20} ketones.

Further evidence for the existence of such an axis is found in the rates of the reaction. Fig. VIII, and IX indicate the rates with which equilibrium values of optical densities are established. Since the peak positions at both room temperature and 100°C, are the same, one may conclude that the same reaction is occurring. It is seen, Fig. VIII, that the conjugated ketones establish equilibrium rapidly, the saturated ketones, A and B, Fig. IX (a), a little less rapidly, and compounds F and G, Fig. IX (b), quite slowly. From this one must deduce that when there is present on the steroid nucleus two positions on which a charge may be deposited, and also an electron withdrawing substituent at C_5, the rate is very rapid. When the C_5 substituent is absent, but there are still present the two keto groups at C_3 and C_{17}, the rate is still quite rapid. When neither the C_5, nor the C_{17} are present, the rate is very slow. It is interesting to note the compound F attains equilibrium at approximately the same rate as A.

These arguments indicate that there must exist electronic effects across the steroid nucleus. Dr. J. H. Linford (5) has proposed the existence of an axis through which resonance effects can act.

THE SPECTRA IN FUMING SULPHURIC ACID

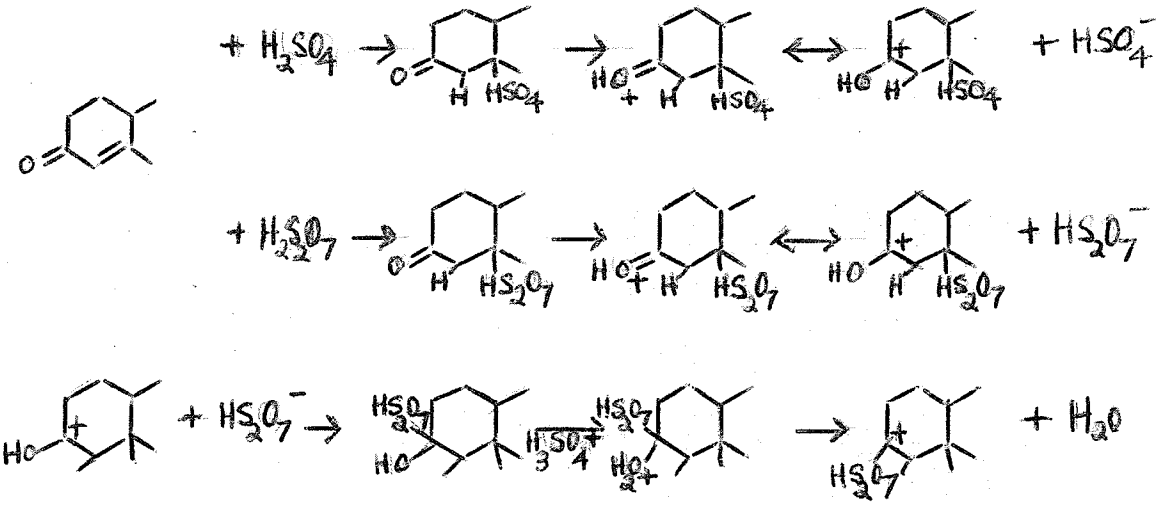
In section A of this work it was indicated that the ionic species present in fuming sulphuric acid are

$$2\ H_2S_2O_7 \rightleftharpoons H_3SO_4^+ + HS_2O_7^-$$

Gillespie (4) has pointed out that $H_2S_2O_7$ in sulphuric acid is a weak acid, its ionization constant

$$Ka = \frac{[H_3SO_4]^+ [HS_2O_7]^-}{[H_2S_2O_7]^2} = .020 \text{ Gm. mol}^2\text{kg}^{-1}$$

Since the concentration of steroid used is approximately 10^{-5} molar, the ionic concentration surrounding it is relatively large. The reactions believed to occur with the ketosteroids are



The initial reaction must be protonation. This is indicated in Fig. X where the peak position, and shape of the curve, measured 10 minutes after solution had been effected at room temperature, was identical with that of Analar. The presence of the initial Analar

FIGURE X

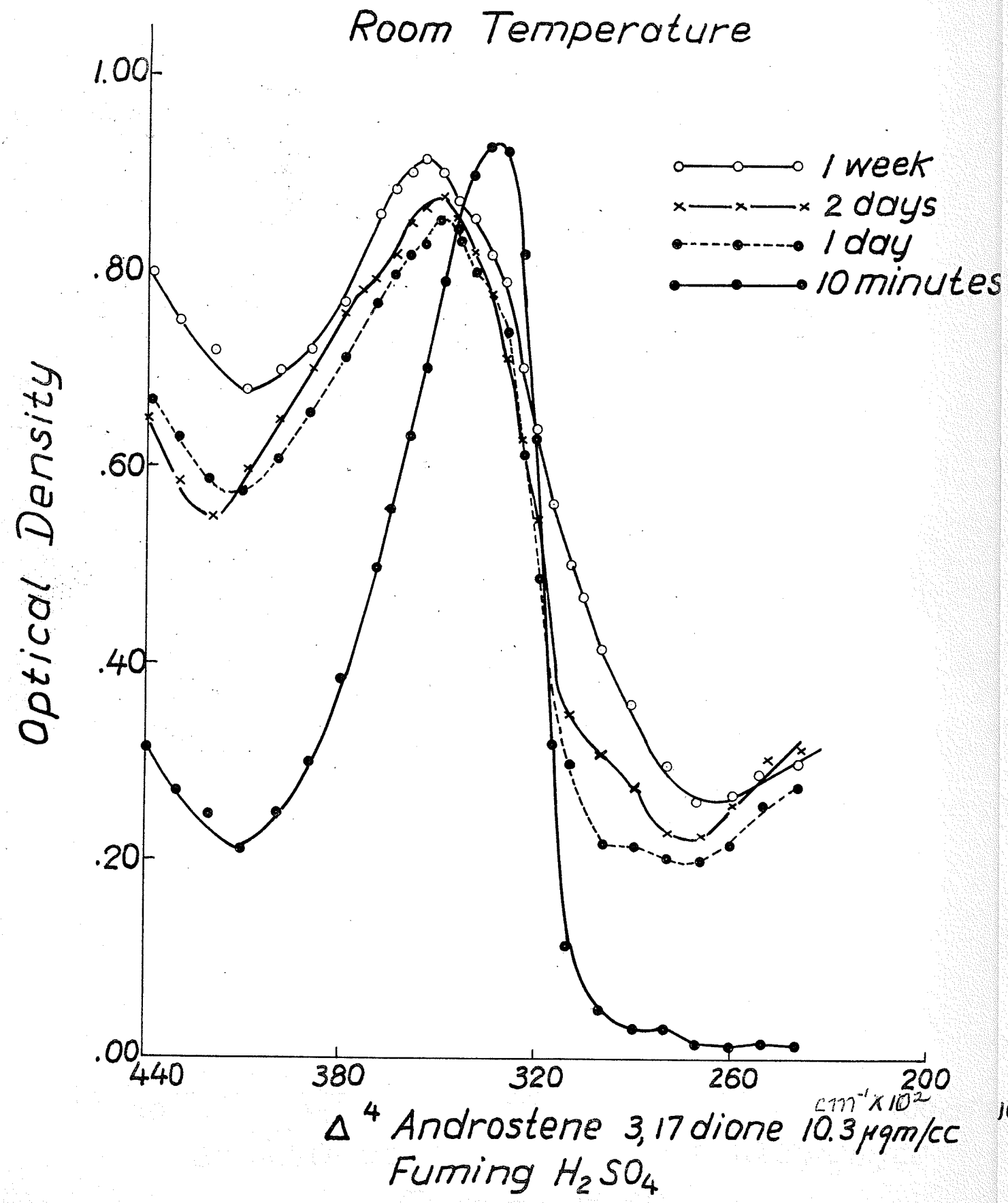

Room Temperature
1.00
.80
.60
.40
.20
.00
Optical Density
1 week
2 days
1 day
10 minutes
440
380
320
260
200
cm⁻¹ x 10²
10²
Δ⁴ Androstene 3,17 dione 10.3 μgm/cc
Fuming H₂SO₄

FIGURE XII

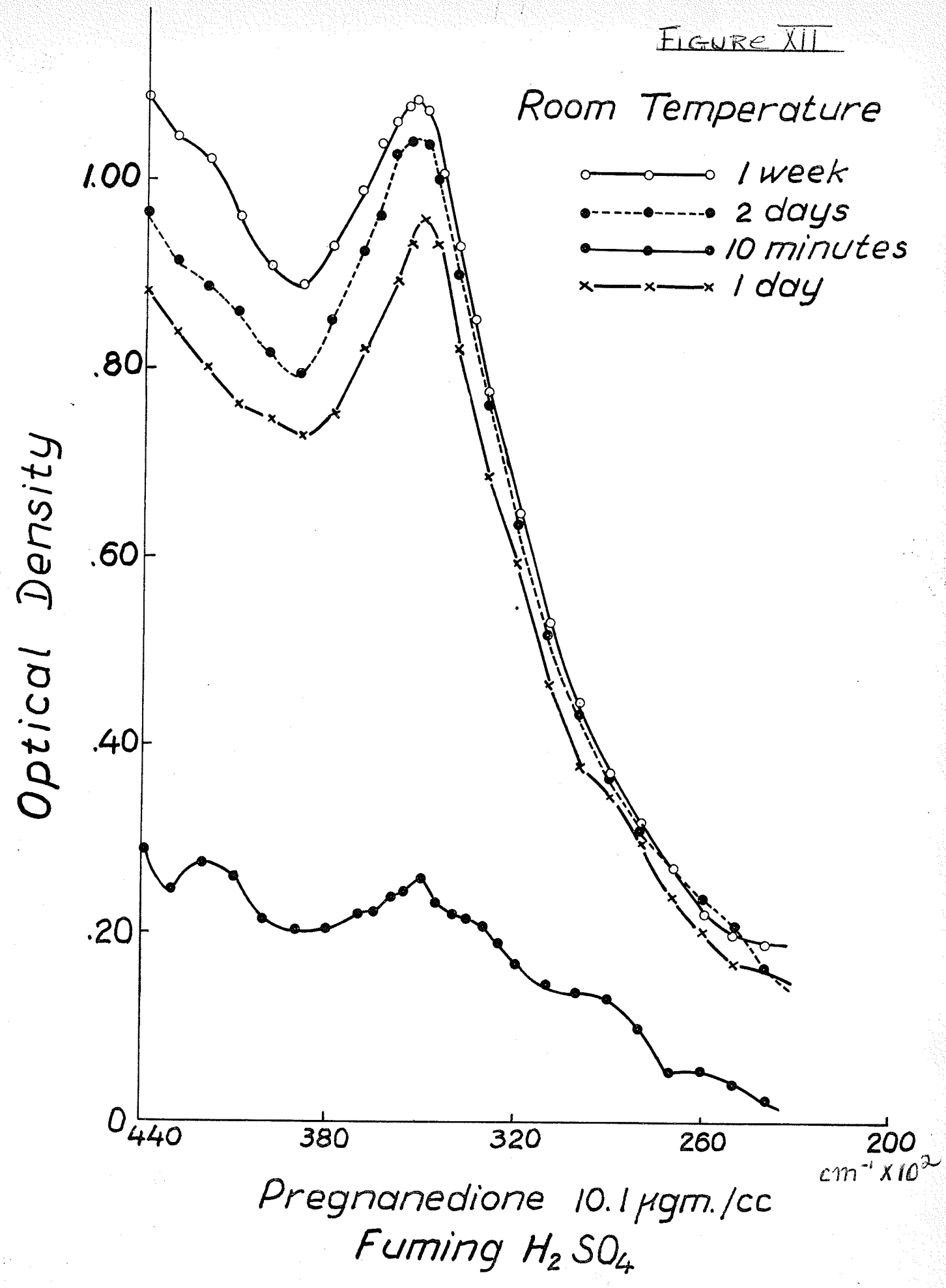
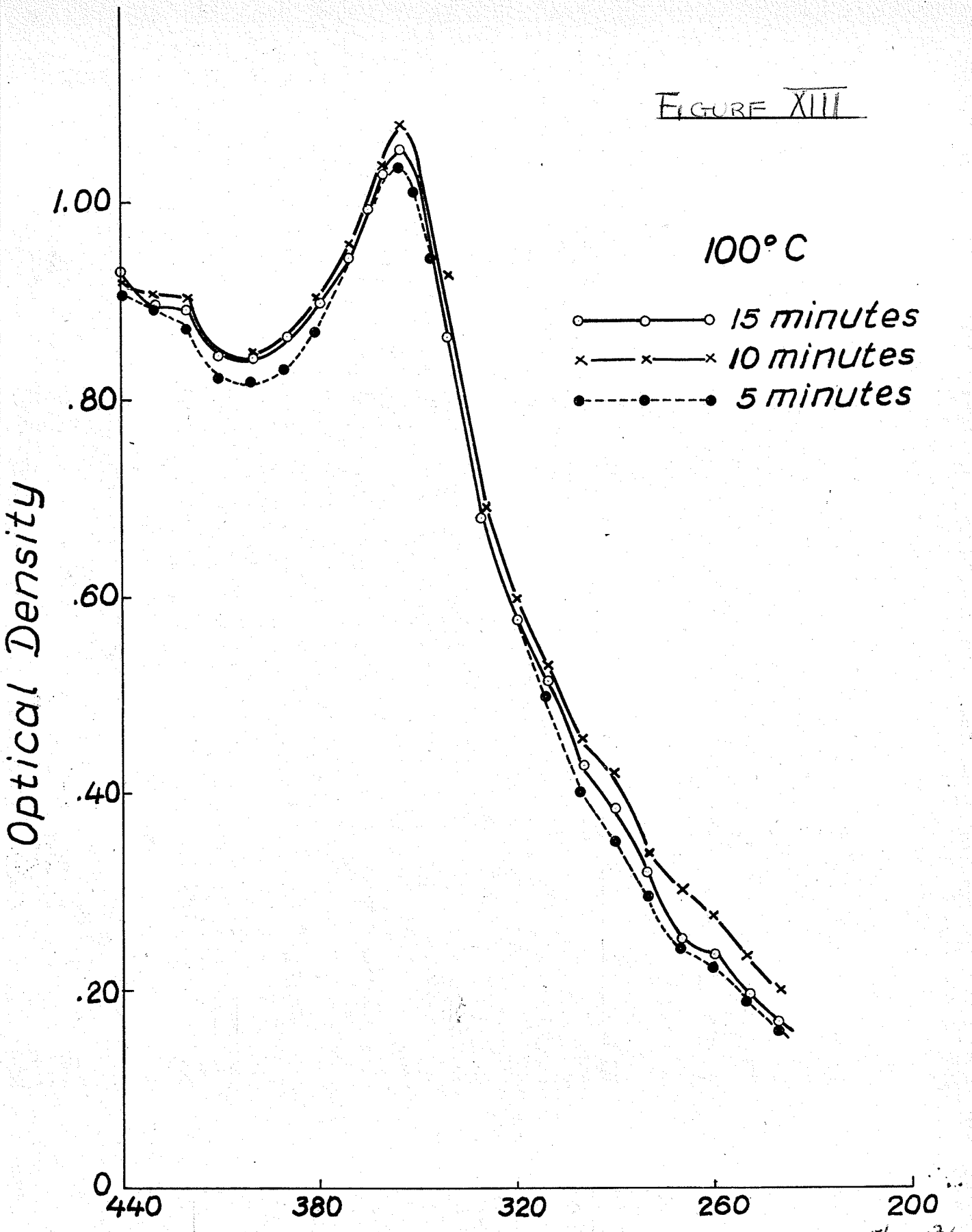

Room Temperature
1 week
2 days
10 minutes
1 day
1.00
.80
.60
.40
.20
0
Optical Density
440
380
320
260
200
cm⁻¹ X 10²
Pregnanedione 10.1 μgm./cc
Fuming H₂SO₄

FIGURE XIII



Pregnanedione 10.1 μgm./cc
Fuming H_2SO_4

spectrum was observed for conjugated ketones only.

As may be expected, the nucleophilic displacement reaction occurring at C_3 is irreversible. This was shown by the slow addition of water to the fuming acid solution. The only effect observed was one of dilution -- the optical density decreased in magnitude with increased quantities of water, but neither the peak position nor the shape of the curve reverted to the Analar.

Table V indicates the peak positions of the ketosteroids, and their optical densities (15 min. 100°C) calculated for 100 µgm/ml fuming H_2SO_4. Fig. X and XI, Fig. XII and XIII, represent the absorption spectra for conjugated and saturated ketones respectively. It is seen that:

(1) There has been a general shift towards the U.V. The new peak position is attributed to the carbonium ion with the HS_2O_7 group attached to it. Fieser (13) has stated that the U.V. absorption positions of steroids are affected by substituent groups near or at the absorbing center. The observed shift was not only expected but its direction could be predicted in view of the Analar results. Here it was found that the presence of an opened double bond, with the HSO_4 group 2 carbons away from the absorbing carbonium ion caused a shift of peak towards the U.V. Hence, in fuming acid where the substituent is directly attached to the carbonium ion there should be an even larger shift.

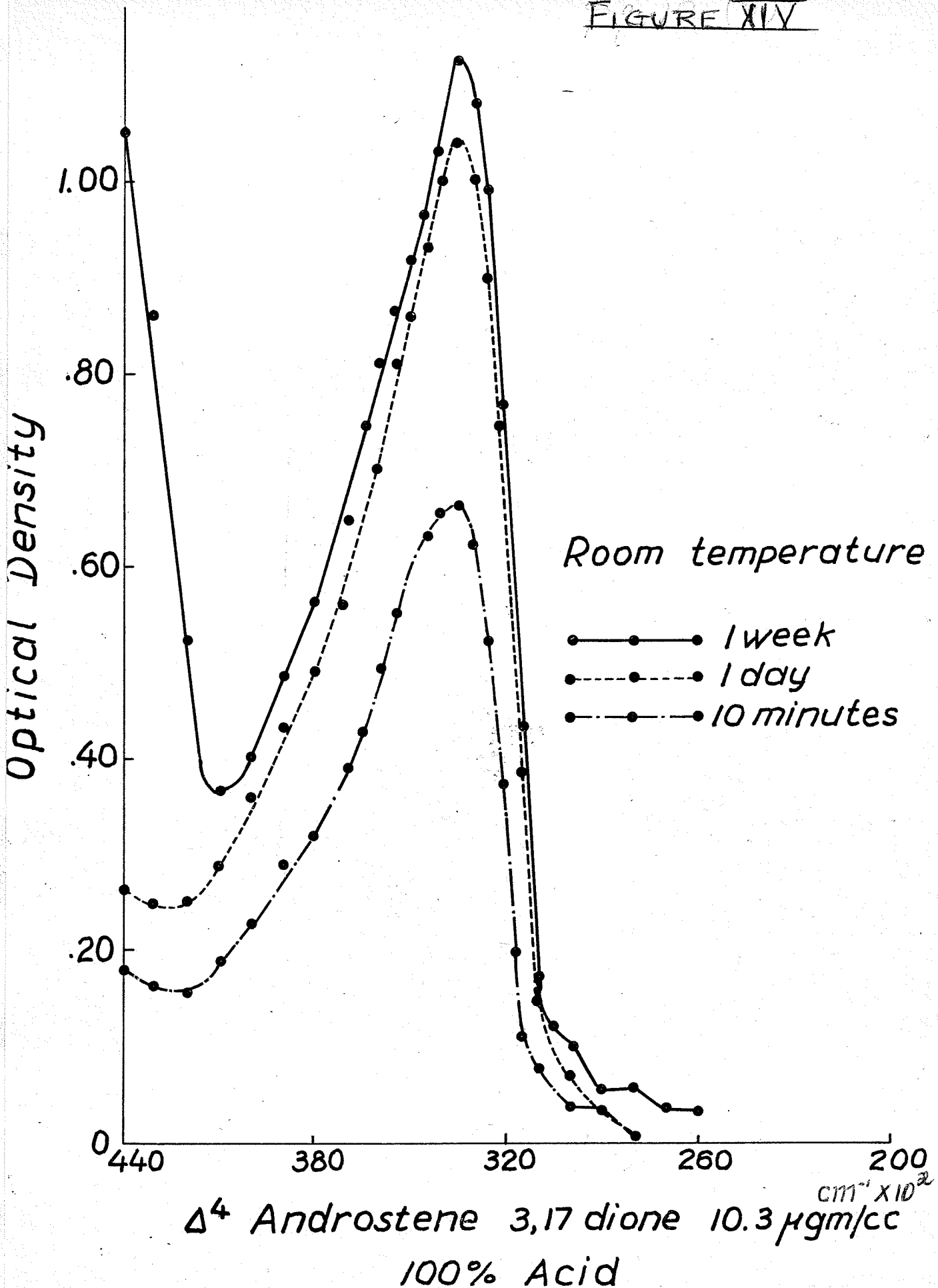
(2) A slight difference in peak position exists between conjugated and saturated double bonds. This is attributed to the opened double bond.

(3) Steric effects are exhibited by the reaction. Compound B, whose ring configuration is such as to hinder the approach of the entering group has a lower density than A. Similarly compounds E and F show that the presence of the substituent group at C_5 hinders the reaction.

(4) The presence of the initial Analar spectrum for the conjugated ketones may be interpreted thus. It was shown that the conjugated ketones attain equilibrium in Analar much more quickly than the saturated ones. The same may be expected in fuming H_2SO_4 . Hence for conjugated ketones the rate controlling process is the nucleophilic attack by HS_2O_7, while for the saturated it is the protonation reaction.

(5) In general the reaction in fuming H_2SO_4 is more complex than in Analar. Since the first part of the reaction is the same as in Analar the electronic factors discussed for this will be applicable here. Superimposed upon these will be the new electronic and steric effects related to the nucleophilic attack. At this time it would be quite difficult to isolate these.

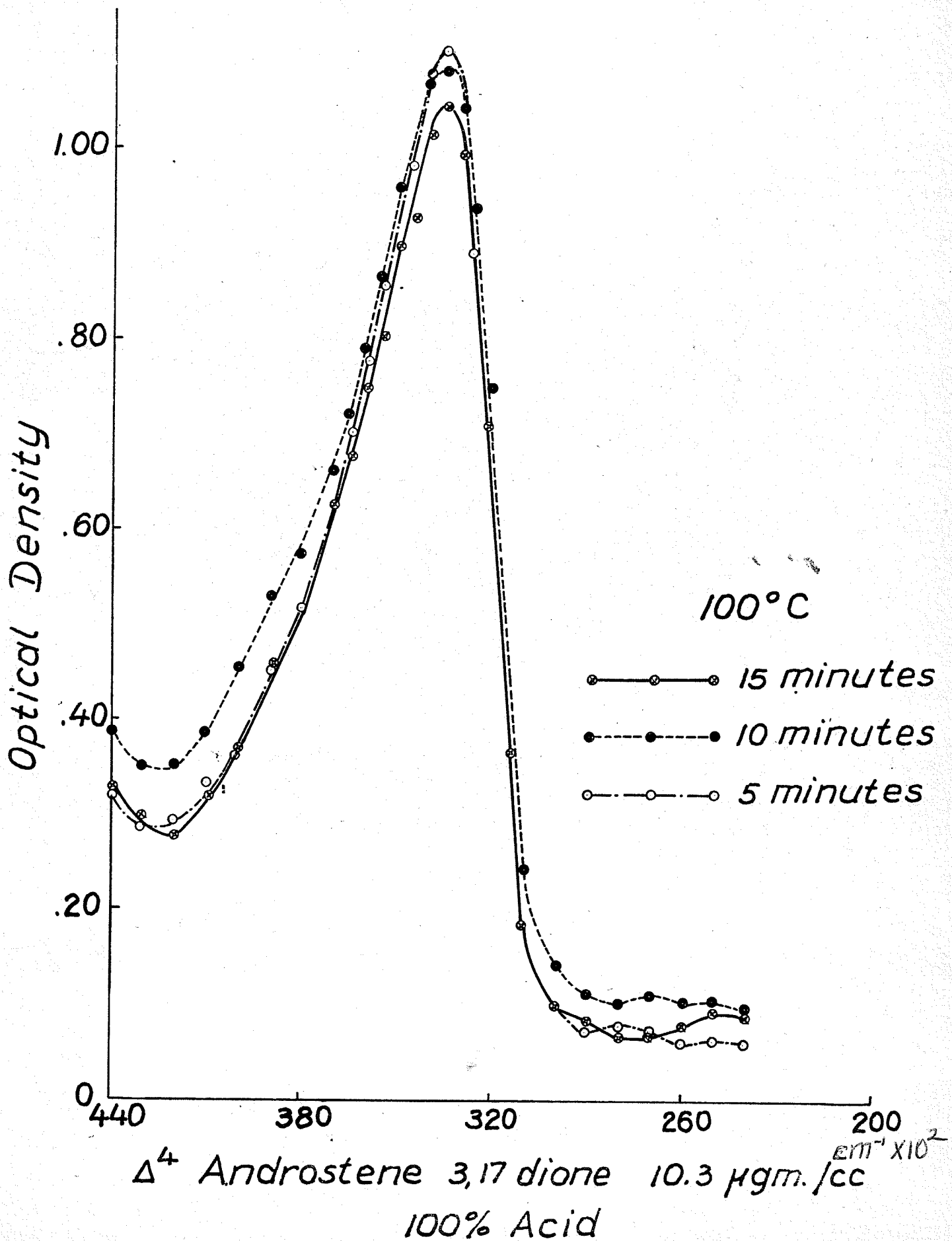
FIGURE XIV



Δ^4 Androstene 3,17 dione 10.3 μgm/cc

100% Acid

FIGURE XV



Δ^4 Androstene 3,17 dione 10.3 μgm./cc

100% Acid

FIGURE XVI

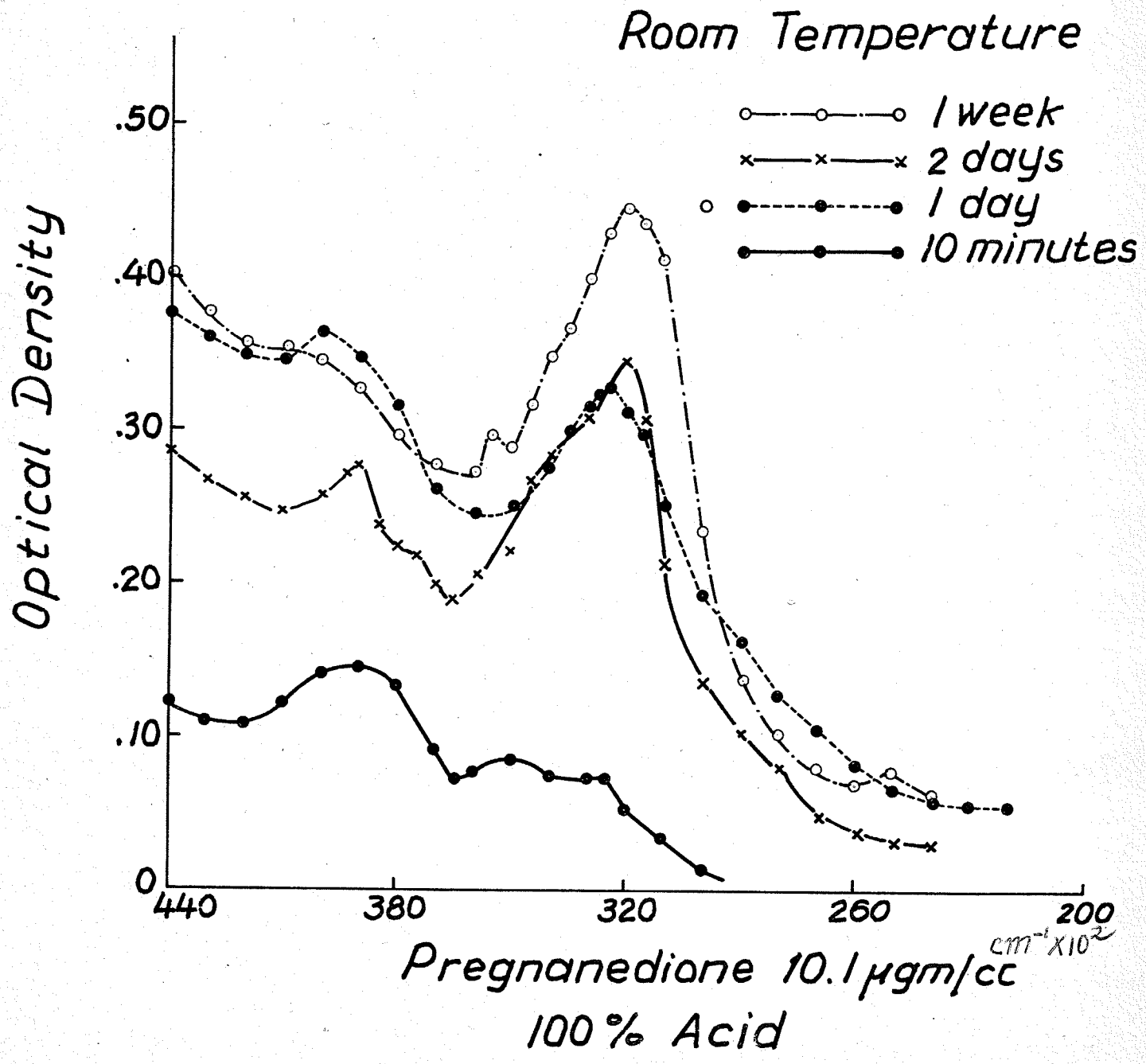
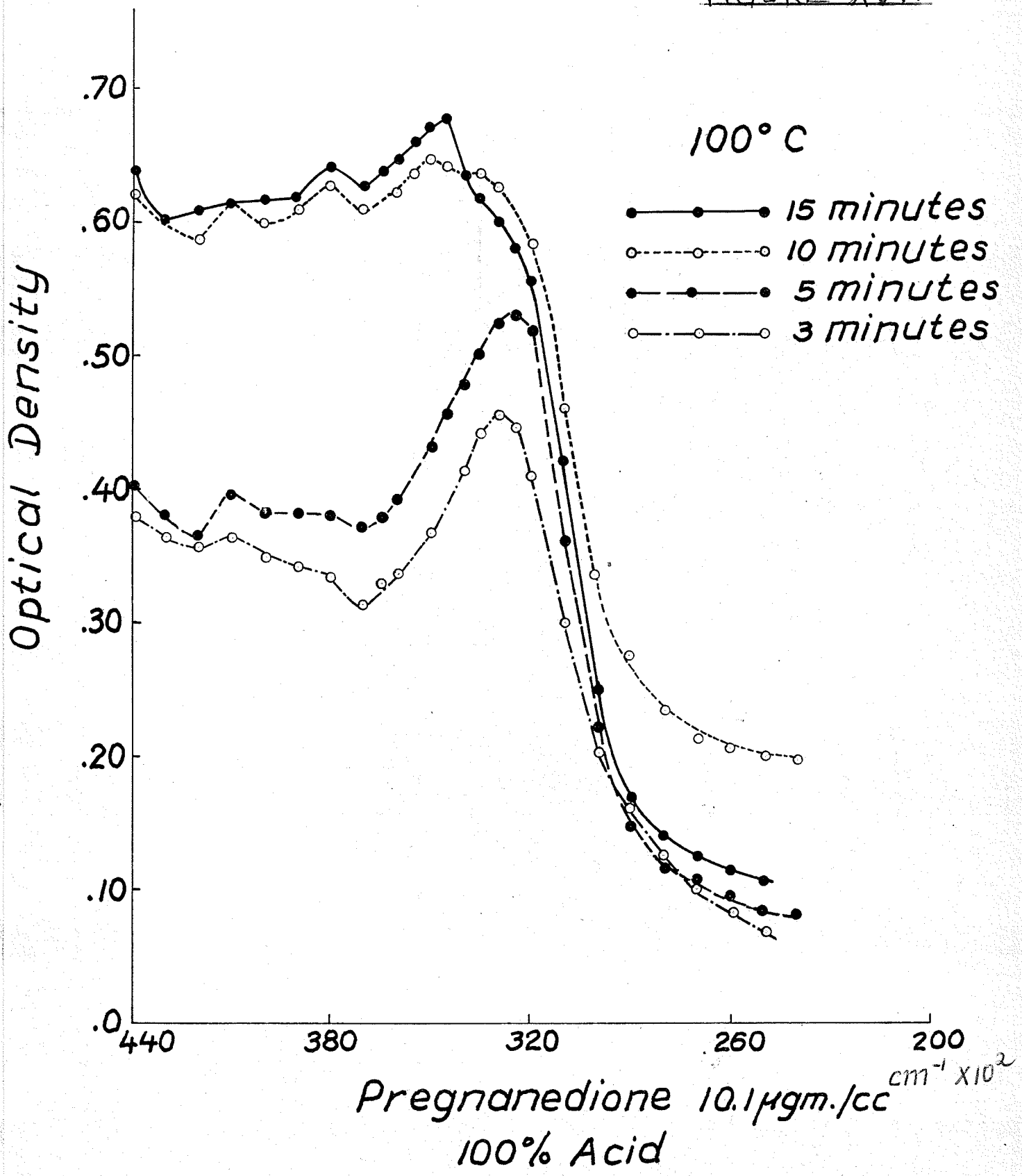

Room Temperature
1 week
2 days
1 day
10 minutes
Optical Density
.50
.40
.30
.20
.10
0
440
380
320
260
200
cm⁻¹ X10²
Pregnanedione 10.1 µgm/cc
100% Acid

fig. 16

FIGURE XVII



THE SPECTRA IN 100% ACID

In 100% acid we have presented the four ionic species resulting from the self dissociation of sulphuric acid.

$$(1) \quad 2H_2SO_4 \rightleftharpoons H_3SO_4^+ + HSO_4^-$$

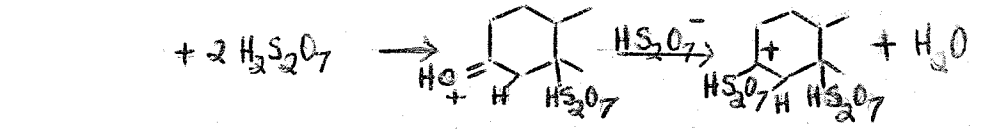
$$(2) \quad 2H_2SO_4 \rightleftharpoons H_3O^+ + HS_2O_7^-$$

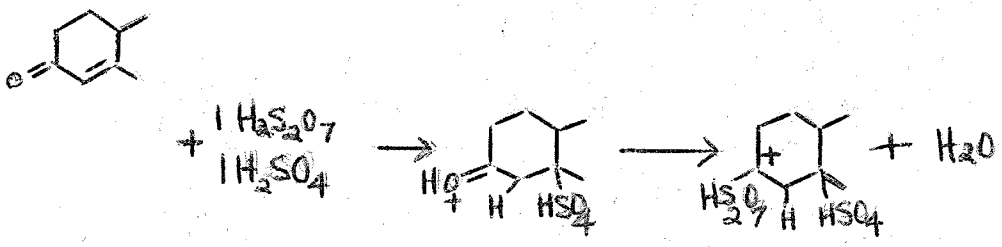
The minimum ionic concentration that can be present in sulphuric acid is .043 molal. The steroid concentrations are well below this, thus they are the limiting factors of the reaction.

Since all the ions present in fuming acid and in Analar are present in 100% acid, both parent reactions may be expected to proceed.

At room temperature, Fig. XIV, XV, and XVI indicate that it is chiefly the Analar reaction which is occurring. However at 100°C the fuming acid reaction must become consequential. This is best seen in Fig. XVII, and in the decreasing magnitude of the 320 cm^{-1} peak of Fig. IX(b). That this is so for compound C of Fig. XV, can be seen by the fact that the 335 cm^{-1} peak value decreases with time. The appearance of a small peak at 360 cm^{-1} could be hidden by the curve.

An attempt to analyze the curves of Fig. XV, and Fig. XVII into the two constituent curves of the Analar reaction and the fuming reaction was unsuccessful. A possible reason for this may be that it is not necessarily the 'pure' constituent curves that exist.

(i) 

(ii) 

Compounds (i) and (ii) would not necessarily have the same ~~either the same~~ probability factor, or even the same absorption peak. Similarly the reason for the increased density may be resolved into two components:

(a) the presence of the four ions and their effect upon protonation.

(b) the extent of the fuming acid reaction and its effect upon the density on the absorption curve.

Fig. IX(b) indicates that in 100% sulphuric acid, the rate of protonation has been increased substantially over the Analar rate. This may be due to the greater proton donating capacity of the $H_3SO_4^+$ ion. The conjugated ketones evidence small differences in rate, showing that (a) they are protonated equally easily by either $H_3SO_4^+$ or H_3O^+ and (b) their reaction is more rapid than that of the non-conjugated system.

SUPPORTING EVIDENCE

I. To support the premise that protonation occurs, another strong protonating reagent, 70% perchloric acid, was used. Fig. XVIII indicates the spectra of a conjugated and non-conjugated ketone, after being dissolved in the acid for 10 minutes at room temperature. The

curve for the saturated ketone is similar in shape and peak position to that in Analar. The curve for the conjugated ketone is similar in shape but it is interesting to note that here the peak position has shifted 10 wave numbers to the U.V. In view of the presence of the double bond, and the probable reaction of the acid with it, a shift in the observed direction may be expected.

The curves in general confirm the chief reaction to be protonation of the ketonic groups. If such were not the case, it is highly unlikely that the spectra would be so similar.

II There exists another possibility -- and this is that oxidation occurs in sulphuric acid, and that the spectra we are measuring are those of the fragments.

Only in the most fortuitous of circumstances would the spectrum of several fragments give an ultraviolet spectrum with a single peak. If oxidation were to occur, one of the fragments would probably be sulphur dioxide. The presence of this compound was investigated through the use of the delicate test first proposed by Steigman, and later modified by Grant (2). If SO_2 is present in solution, a deep red colour develops in the presence of the reagent. Though Grant's work was done chiefly in mildly alkaline or acid solutions, the test is sensitive even in very strong acids.

Fig. XIX shows the U.V. curve of an SO_2 solution in 100% sulphuric acid. The second curve is that of the reagent and a solution of 1.5 μgm/ml. SO_2 in 100% acid. The peak at 285 x $10^2 cm^{-1}$

is due to the reagent itself, that at 182 x 10^2 cm^{-1} to the SO_2 present. It is evident that a concentration one half of that used would produce a detectable peak.

Fig. XX(a) indicates the spectrum of the pure reagent, and that of 100% acid in the reagent.

Fig. XX(b) indicates the spectrum of conjugated and saturated ketones, boiled in sulphuric acid for ten minutes, and then adding the reagent. Obviously there is no SO_2 present. The boiling does not eject any dissolved SO_2, since the spectra were identical whether the steroid was dissolved at room temperature or at 100°C.

The test for SO_2 was performed both hot and cold, in Analar and in fuming sulphuric acid. At no time was there any evidence that SO_2 was present.

The molal extinction coefficient of SO_2 in sulphuric acid, whether Analar or fuming, is of the order of 400, that of the steroids is approximately 1 x 10^4. It is seen that a substantial amount of SO_2 would be required to affect the absorption curve of the steroids.

The concentrations of steroid used were, approximately 3 x 10^{-5} molar. The limit of sensitivity of the SO_2 test is 1 x 10^{-5} molar concentration of SO_2. Thus if any oxidation, or fragmentation of the steroid had occurred, it would have been detected.

FIGURE XVIII

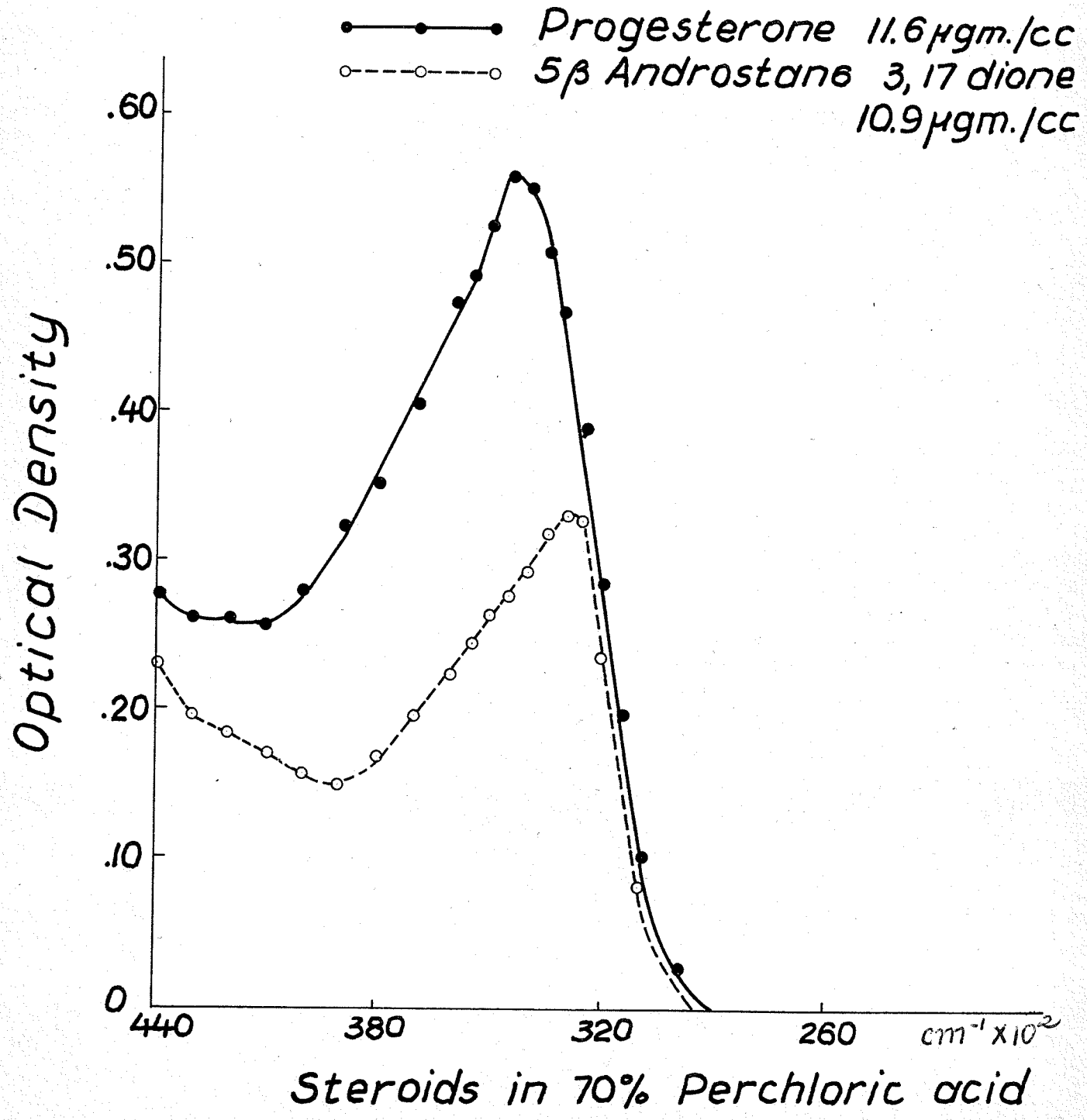
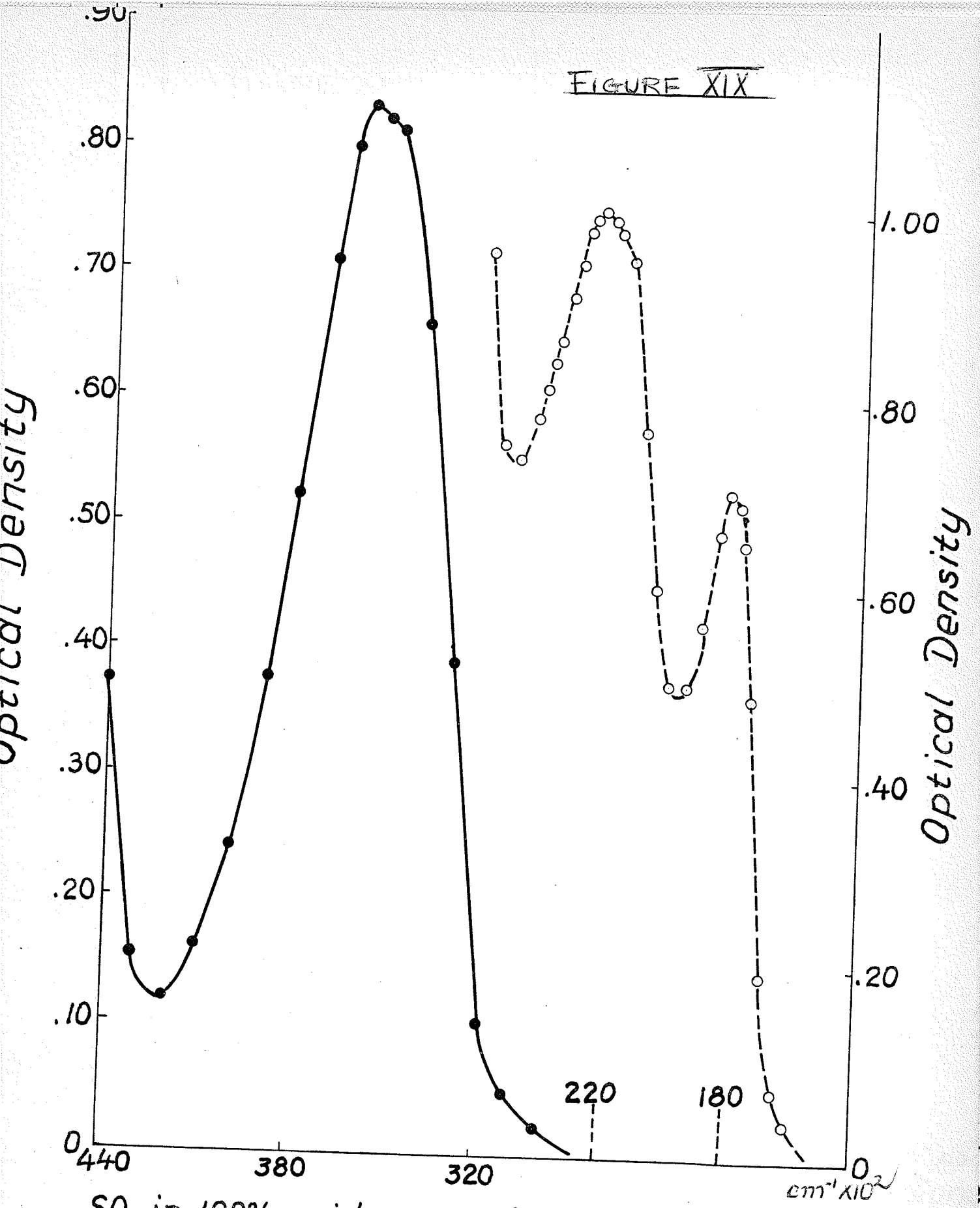


FIGURE XIX



SO_2 in 100% acid
148 μgm./cc

Solution of 1.5 μgm/cc
SO_2 in 100% acid
+Steigman reagent

CONCLUSIONS

(1) The absorption spectra of the keto steroids examined in sulphuric acid of various strengths have been recorded. An explanation for the reactions occurring was advanced. This explanation involves a rate controlling protonation reaction, although in the case of simple molecules, proton transfers from one oxygen to another are usually so rapid that the rate cannot be measured. However, this was the simplest mechanism and was deduced from the following considerations:

(i) The absence of SO_2 indicates that the molecule is not being oxidized. The spectrum with one simple peak suggests that there is a single absorbing species, and no fragmentation has occurred.

(ii) Under the conditions of the experiment, sulphonation of the steroid is not a likely reaction. A saturated system can be sulphonated only under quite drastic conditions.

(iii) Protonation in a strong acid is the most likely reaction. The fact that both perchloric and Analar sulphuric acid give similar spectra supports this. The simplest explanation for the increase in optical density with time and temperature is a time-dependent protonation reaction. There may be other possibilities as well, i.e. a rapid protonation followed by a slow molecular rearrangement reaction giving rise to a more stable carbonium ion, a reaction involving the pentano ring, or a rate controlling loss of water. Clearly a solution to the problem would be the isolation of the products. An attempt was made here but was unsuccessful. However, it showed that the initial reactant cannot be recovered by the simple process of neutralization with brine and

extraction with an organic solvent. This leads to a highly unsaturated compound whose presence was detected by a pungent odour but which could not be isolated. There is no reason to believe that this necessarily is the compound existing in concentrated acid. Gillespie (17) indicates that the reaction between olefins and sulphuric acid though initially simple addition, with time and dilution, becomes complex and leads to highly unsaturated compounds.

(2) Evidence has been advanced supporting the theory that a resonance axis exists in the steroid nucleus. It would be highly desirable to measure the spectra of a large number of ketosteroids with systematically varied structures, and thus detect the points on the nucleus which are most reactive. Such knowledge would be of inestimable value in relating the ketosteroid structure to its biologic activity.

(3) The absorption spectra can be related to the observed cryoscopic behavior. The spectra indicate a single absorbing species. The cryoscopic behavior was attributed to the ability of the steroid to rupture the structure of the solvent. Such rupture would have a marked effect on the infrared spectrum of the solvent, but little or none on the ultraviolet. An investigation of the infrared region would be profitable.

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