RECENT PROGRESS IN
HORMONE RESEARCH

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GREGORY PINCUS

COMMITTEE ON ARRANGEMENTS

R. W. BATES   R. D. H. HEARD

G. PINCUS

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PREFACE

The 1946 Laurentian Hormone Conference took place at St. Adele-on-High during the week of September 16. The Committee on Arrangements is indebted to the speakers for the prompt preparation of manuscripts and of the recorded discussions. The Committee would like to emphasize the role that the discussions have played in the actual course of the meetings. A frank and constructive speaking out was given with enthusiasm under the guidance of the session chairmen. These were Drs. R. D. H. Heard, R. N. Jones, A. White, K. Dobriner, L. T. Samuels, R. Rawson, H. Selye, I. T. Nathanson and A. T. Kenyon. The coordinated activities of these men, the speakers and the discussants are responsible for the meeting of thought and minds that this volume attempts to present.

The expenses of the Conference have been met through contributions from Armour and Co., American Home Products Company, Ayerst, McKenna and Harrison, Breon and Company, Ciba Pharmaceutical Products, Des Bergers-Bismol, Endo Products Company, C. E. Frosst and Company, Glidden Company, Hoffman-LaRoche, W. Horner Ltd., Mallinckrodt Chemical Works, Parke, Davis and Company, Roche-Organon, Schering Corporation, Sharp and Dohme, E. R. Squibb and Sons, Upjohn Company, Winthrop Chemical Company and Wyeth Company.

Their interest has made possible a series of Conferences that gives promise of becoming a vital institution.

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GREGORY PINCUS

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Shrewsbury, Massachusetts

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The Characterization of Sterol Hormones by Ultraviolet and Infrared Spectroscopy

R. NORMAN JONES

*Division of Chemistry, The National Research Council, Ottawa, Canada**

I. INTRODUCTION

One of the major factors in the progress of biological research in recent years has been the continual refinement which has taken place in the methods available for the measurement of the physical properties of the substances composing living organisms. The advent of some new technic in physical measurement is often heralded by its success in solving some particular research problem. Thus we note the characterization of individual proteins which followed the application of the ultracentrifuge and the Tiselius diffusion apparatus to protein chemistry, and to delve back into history we may recall that the elucidation of the true nature of chemical combustion resulted from the application of the balance to the study of chemical reactions by Lavoisier.

In these present times it occasionally happens that after such an initial success, the new technic passes through a series of stages which we may call a *popularity cycle*. Because of its profitable application in some one field of research, the new technic acquires wide publicity and is brought to the attention of investigators occupied with problems of quite a different nature. There ensues a period of enthusiasm—a heyday for the instrument manufacturers and their technical salesmen, during which time the demand for the instrument is very great, and it is applied to a varied range of problems.

Sometimes a feeling of disappointment arises when the technic fails to fulfill its initial promise, or, possibly, in the hands of different investigators it answers the same problem in two different ways. This phase can often be attributed to a failure to appreciate certain inherent limitations of the new technic on the part of those who are attempting to apply it. Only after passing through such a period of disillusionment may the true value of the new method come to be justly appraised.

In this paper we shall discuss some applications of both ultraviolet and infrared absorption spectroscopy to the problem of the elucidation of the structure of sterol compounds, and in doing so, I feel it is worth while to

point out in these introductory remarks that the limitations of any physical method should be emphasized equally with its more positive qualities in an evaluation of its worth.

Ultraviolet spectroscopy has by now passed through this popularity cycle, and sufficient fundamental experience has been accumulated to permit predication of what type of problem can be investigated by this technic with a reasonable prospect of obtaining a decisive answer.

Infrared spectroscopy, at least in regard to its application to bioanalytical and structural problems, is still in the first flush of initial success; its limitations cannot yet be precisely defined, since we have not acquired the necessary background of experience to do so. However, I shall attempt to indicate some of the factors which must limit the applications of infrared spectroscopy in certain directions, and perhaps in this manner I may help to guide it through the depressions of a possible popularity cycle.

The first part of this paper will be concerned with a discussion of the applications of ultraviolet spectroscopy in the field of sterol chemistry. Attention having been drawn to certain of its limitations, we shall later consider the infrared and attempt to evaluate the probability that infrared analysis may solve those questions regarding which the ultraviolet method proves to be inadequate.

In view of the much more elaborate and expensive equipment needed for infrared measurements, I think it can be assumed that where an answer to a given structural problem can be derived from either infrared or ultraviolet measurements, the ultraviolet would most likely be preferred.

II. Ultraviolet Spectroscopy

The first application of ultraviolet spectroscopy to sterol chemistry was associated with the discovery of vitamin D, and the investigation of the complex series of reactions which take place when a sterol with a $\Delta^{5,7}$-diene system in the B ring, such as ergosterol, is irradiated with ultraviolet light (Fig. 1).

It is of interest to review briefly some of this early spectographic work on the vitamin D problem, with the later stages of which I was fortunate
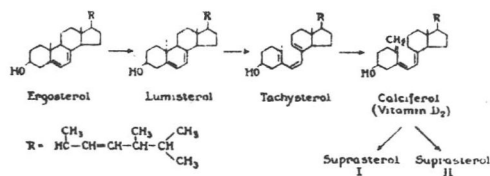

FIG. 1
Products formed by ultraviolet irradiation of ergosterol.

in being associated as a student of Professor Heilbron at Manchester. By 1926 it had been established that the antirachitic activity was associated with the sterol fraction of the diet, and that inactive sterol concentrates from a rachitic diet acquired antirachitic activity on irradiation with ultraviolet light. At that time the complex nature of crystalline sterol concentrates from natural sources was not appreciated and it was assumed initially that such material was essentially pure cholesterol, and that cholesterol was the precursor of the antirachitic vitamin.

In the spectrum of such a cholesterol concentrate Schlutz and Morse (17) in 1925 noted two weak absorption maxima, and the next year Heilbron, Kamm, and Morton (10) subjected large quantities of crude cholesterol to fractional crystallization and examined the ultraviolet spectra of the various fractions. As the recrystallizations proceeded, the main cholesterol fraction became progressively less absorbent, and the absorbing impurity accumulated in the least soluble fraction (Fig. 2). The potency as a precursor of the antirachitic vitamin accompanied the absorbing material (13, 15).

Windaus and Hess (18) and Rosenheim and Webster (16) noted that ergosterol had absorption bands at the same positions as the bands of the active cholesterol impurity (Fig. 2) and found also that on irradiation ergosterol became strongly antirachitic.
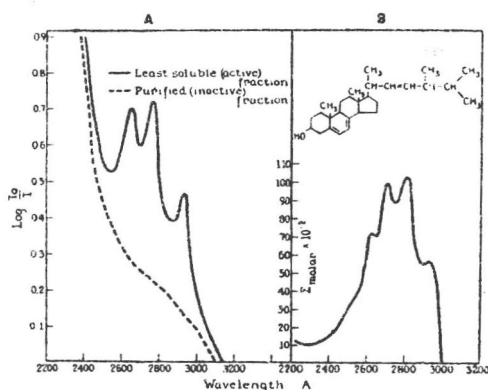
Fig. 2

Comparison of ultraviolet absorption spectra of crude cholesterol and ergosterol.

From these initial observations there followed the work of Heilbron, Windaus, the Hampstead group, and others which led ultimately to the isolation of calciferol and the elucidation of the structures and the interrelationships between the irradiation products shown in Fig. 1.

This series of reactions is of importance in connection with our subject

because it was mainly as a result of studies on model compounds associated with these investigations that the correlations between ultraviolet absorption spectra and sterol structure were first established.

Abandoning from now on the historical approach, we might next summarize some of the empirical findings correlating ultraviolet absorption spectra and chemical structure. Such relations are, of course, of quite general application and not restricted to the sterols, but we shall concentrate attention on those molecular groups which play an important part in the field of the sterol hormones.

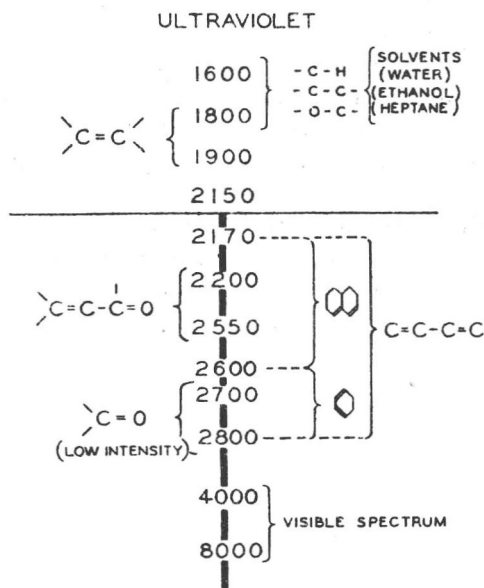The essential information is shown diagrammatically in Fig. 3. The

FIG. 3

Schematic diagram of simple ultraviolet chromophoric groups.

region of the ultraviolet commonly investigated extends from the violet end of the visible spectrum at 4000 Å to about 2150 Å; this short wave limit is set by instrumental considerations.

When ultraviolet light is absorbed by a molecule, its energy is transferred to the bonds which hold the atoms of the molecule together. The simplest of these bonds, the saturated covalent bond, is represented in the conventional organic structural formula by a single line, and is composed of two electrons. In order to excite such a two-electron bond by absorption of light, we must supply a quantum of energy which corresponds to a wave length considerably shorter than 2150 Å, actually around 1800-1600 or less.

Because of this, any molecule which only contains atoms linked by such saturated covalent bonds is transparent over the regions of the ultraviolet normally investigated.

This fact was recognized by the early investigators of ultraviolet absorption spectra, and is of great practical importance, since it provides the spectroscopist with a wide range of transparent solvents. Water, ethanol, dioxane, and petroleum hydrocarbons such as normal heptane and iso-octane are all composed of singly linked atoms only and are valuable solvents for ultraviolet work.

It is only on the introduction of the double bond that absorption maxima appear, and even here an exception has to be made in the case of the simplest double bond, that between two carbon atoms. Reference to Fig. 3 shows that the ethylenic linkage absorbs between 1800-1900 Å* (14). The double bond between carbon and oxygen as it occurs in the ketone group absorbs in the neighborhood of 2700-2800 Å. The position of the absorption maximum of this band in a series of ketones of increasing chemical complexity is shown in Table I, from which we see that, at a first approximation, the absorption is not influenced by the other groups in the molecule. We thus arrive at the important concept of a *chromophore*. A chromophore is a group of atoms which confers on a compound containing it a characteristic type of absorption spectrum.
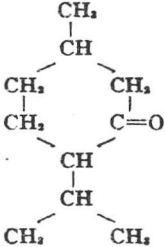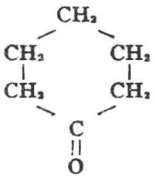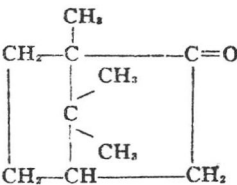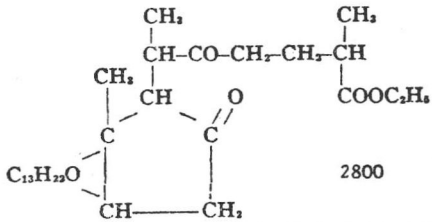
If the double bond, either ethylenic or ketonic, is conjugated with a second double bond, the effect on the spectrum is very great. The spectrum of such an $\alpha\beta$-unsaturated ketone is shown in Fig. 4. Particularly to be noted is the great change in the intensity, the molecular extinction coefficient at the maximum rises from about 20-40 for the nonconjugated ketone to 10,000-20,000.

In the $\alpha\beta$-unsaturated ketones this intense maximum occurs between 2200 and 2600 Å. The spectra of a large number of sterols and other compounds containing this group have been examined, and these data have been reviewed by Dannenberg (3), by Evans and Gillam (6) and by Woodward (19). From a careful analysis of the exact position of the absorption maximum of ethanolic solutions, Woodward has derived certain rules concerning the exact position of the absorption maximum which are of value in the determination of the structure of ketosteroids.

Attached to the ethylene bond of the $\alpha\beta$-unsaturated ketone are three valence linkages (a, β, β) which may be connected with hydrogen atoms or with substituent carbon atoms forming part of a chain or ring system. The position of the absorption maximum depends upon how many of these

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*This range limit is approximate only.

TABLE I

Position of the Absorption Maximum in the Spectra of Aliphatic Ketones
(Solvent Ethanol)

Compound	Maximum Wave length, Å	Intensity (E_{molar})
Acetone CH_3–CO–CH_3	2785	17
Ditertiarybutyl ketone $(CH_3)_3C.CO.C(CH_3)_3$	2968	21
Carvomenthone	2861	24
Cyclohexanone	2804	19
Camphor	2880	37
Ethyl ester of sarsasapogenoic acid	2800	80

Carvomenthone structure:

CH_3
|
CH
CH_3 CH_3
| |
CH_2 $C=O$
CH
|
CH
CH_3 CH_3

Cyclohexanone structure:

CH_2
CH_2 CH_2
| |
CH_2 CH_2
C
‖
O

Camphor structure:

CH_2
|
CH_2–C————$C=O$
| CH_3
| C
| CH_3
CH_2–CH————CH_2

Ethyl ester of sarsasapogenoic acid structure:

CH_3 CH_3
| |
CH–CO–CH_2–CH_2–CH
CH_3 | |
| CH O $COOC_2H_5$
| C $//$
$C_{13}H_{22}O$ C
| |
CH————CH_2

hydrogen atoms are substituted by heavier atoms. Initially Woodward recognized three classes, as shown in Table II. Later a refinement was introduced (20) to take care of particular cases where the double bond is attached directly to a ring ("exocyclic systems"), and Gillam and West (9) have pointed out that a small correction must also be applied when the
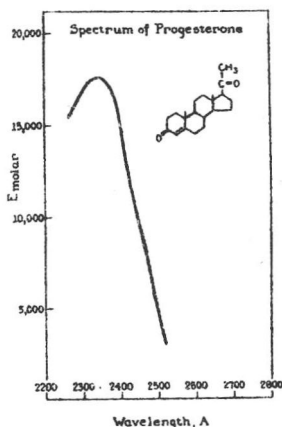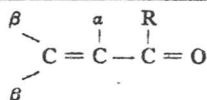
Fig. .4

Ultraviolet absorption spectrum of progesterone (solvent ethanol).

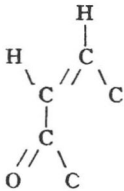TABLE II

Woodward's Rule for Conjugated Ketones

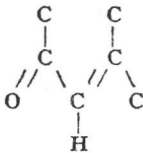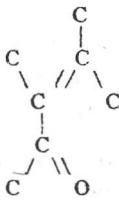$$\overset{\beta}{\underset{\beta}{\diagdown}} C = \overset{a}{\underset{|}{C}} - \overset{R}{\underset{|}{C}} = O$$

		Max.
Type I	a-substituted or β-substituted	$2250 \pm 50\text{Å}$
Type II	$a\beta$-disubstituted or $\beta\beta$-disubstituted	$2390 \pm 50\text{Å}$
Type III	$a\beta\beta$-trisubstituted	$2540 \pm 50\text{Å}$
(Solvent ethanol)		

conjugated ketone is part of a *five*-membered ring system. A few examples illustrative of the application of the rule to some typical ketosteroids are given in Table III.

The conjugation of one ethylenic double bond with a second of the same kind also produces a very intense absorption band which may occur between 2170 and 2800 Å (Fig. 3). Typical examples of steroids containing this chromophoric group are shown in Fig. 5.

As in the case of the conjugated ketone, it has been found possible to reduce this chromophore to a series of subsidiary types. Fieser and Campbell (7) pointed out that when the conjugated pair of double bonds are both in the *same ring*, as in ergosterol, the absorption maximum lies at longer wave lengths than when the double bonds are distributed between two rings, or in an open chain. The examples of the two diene chromophores in Fig. 5 were chosen to illustrate this difference. Woodward has also shown (21) that the linear and bicyclic diene systems can be further

TABLE III
Examples to Illustrate the Application of Woodward's Rule for Conjugated Ketones

Type 1. $(2200\text{-}2300 \text{ A})^1$

	Maximum
Δ^1-Cholestenone-3	2300

Δ^1-Androstanedione-3, 17	2300
Δ^1-Allopregnanedione-3, 20	2300

Type 2. $(2340\text{-}2440)^1$

	Maximum
Δ^4-Cholestenone-3	2420

$\Delta^{4,7}$-Ergostadienone-3	2400
Δ^4-Androstanedione-3, 17	2400
Testosterone	2400
Progesterone	2400

Type 3. $(2490\text{-}2590)^1$

	Maximum
$\Delta^{8,14}$-Ergostenol-5-dione-3, 7.	2540

$\Delta^{3,4}$-Diketocholestane enol acetate	2490

[1] These range limits assume the use of ethanol as solvent.

subclassified on the bases of the degree of substitution in a manner somewhat similar to that described above for the conjugated ketones.

It is thus evident that ultraviolet spectroscopy permits not only the identification of the presence of a conjugated system in the sterol molecule, but also some selection to be made between certain alternative structures which contain the group in question.

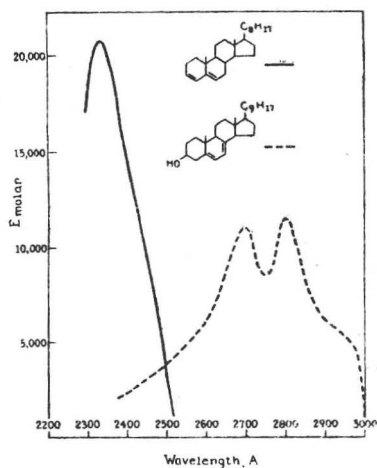If three or more conjugated double bonds are present, the absorption

FIG. 5

Ultraviolet absorption spectra of conjugated dienes (solvent ethanol).
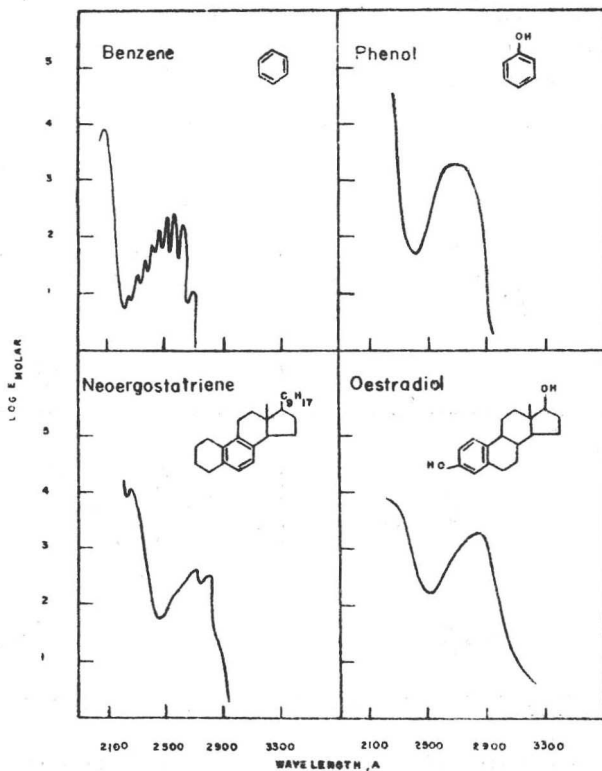

FIG. 6

Ultraviolet absorption spectra of simple and complex benzene derivatives (solvent ethanol).

maxima move further toward the visible region of the spectrum. However, as such systems are rarely encountered among the sterol hormones or their metabolites, we shall not discuss them here, with the exception of the special case of the benzene ring which is of importance in connection with the estrogens.

Aromatic substances have quite different spectra from compounds containing the same number of conjugated double bonds not arranged in benzene rings. In Fig. 6 the spectra of benzene, phenol, and two steroids containing the benzene ring system are illustrated. The spectrum of benzene itself is very beautiful, with a large number of fine sharp maxima present. With the introduction of substituents, particularly the phenolic hydroxyl group, the fine structure disappears and the broad band which remains is of low intensity. Such a band is readily obscured by comparatively small quantities of impurities such as are likely to occur in concentrates from natural sources.

When two or more benzene rings are fused together a class of substances result known as condensed polynuclear aromatic compounds; the simplest of these is naphthalene. Such compounds possess very intense and characteristic absorption spectra with numerous sharp maxima. Four typical examples are shown in Fig. 7.
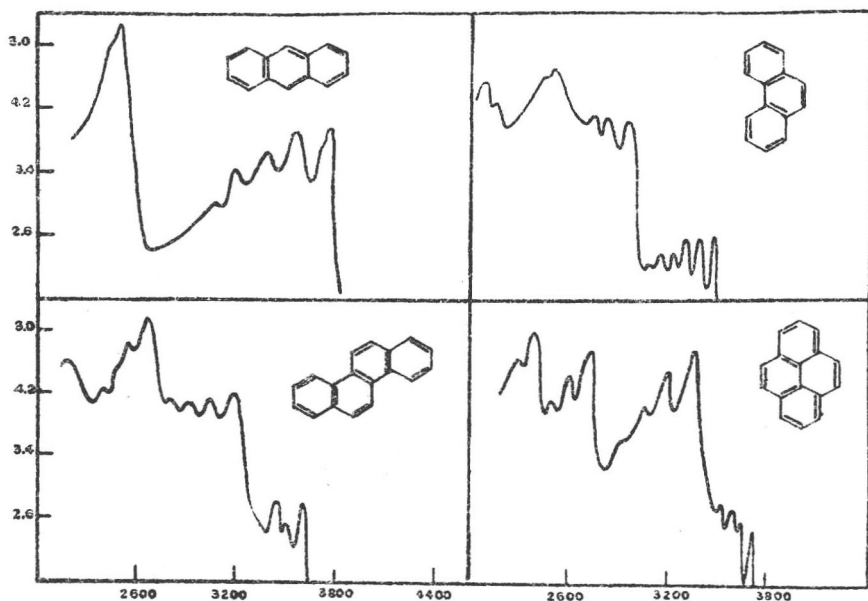

FIG. 7

Ultraviolet absorption spectra of polynuclear aromatic hydrocarbons (solvent ethanol).