[Contribution from the Chemical Laboratory, Harvard University]

ABSORPTION SPECTROSCOPY AND THE STRUCTURES OF THE DIOSTEROLS

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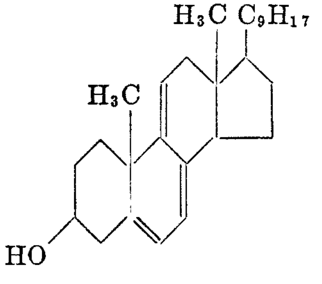
The inferences that we shall present concerning the structures of two isomeric substances derived from cholesterol and characterized by marked reducing properties typical of diosphenols are based largely upon evidence of ultraviolet and infrared spectroscopy. Comparison of data for the ultraviolet region will be made in terms of a scheme developed by two of us for the calculation of the absorption maxima of steroids that constitutes a simplification and extension of those emanating from the studies of Dannenberg (1), Evans and Gillam (2), and Woodward (3). The derivation of this scheme from an analysis of data for steroids of forty-five structural types will be presented in detail in a forthcoming book;[2] the method itself is summarized in Table I.

A heteroannular diene is defined as one in which the two double bonds are distributed between two rings; in a homoannular diene the double bonds are contained in a single ring. In polyene systems, the effects, if any, of a change from one solvent to another or of the introduction of oxygen or halogen substituents can be neglected. The bathochromic effect of an exocyclic double bond (5 mμ) is double if the linkage is exocyclic to two rings. If two chromophoric systems are present, the calculation is based on the one that absorbs at longer wavelength; thus a compound having both homo- and hetero-annular dienic systems is treated as a homoannular diene. The method of calculation is illustrated by the following examples:

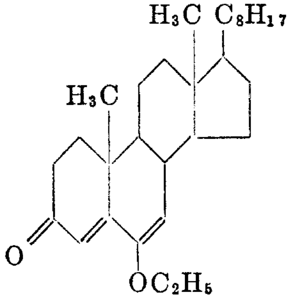
Dehydroergosterol (I), $\lambda_{\text{max}} = 253 + 25$ (alkyl substitution) $+ 15$ (exo bonds) $+ 30$ (double bond) $= 323$ mμ.

Δ^4-Cholestene-3,6-dione enol ether (II), $\lambda_{\text{max}}^{\text{ether}} = 215 + 12$ (β-alkyl) $+ 18$ (γ-OC_2H_5) $+ 18$ (δ-alkyl) $+ 5$ (exo bond) $+ 30$ (double bond) $- 7$ (solvent correction) $= 291$ mμ.



I

$\lambda_{\text{max}}^{\text{ether}}$ 320 mμ



II

$\lambda_{\text{max}}^{\text{ether}}$ 295 mμ

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[2] Fieser and Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, publication in press.

TABLE I

CALCULATION OF ULTRAVIOLET ABSORPTION MAXIMA

A. HETEROANNULAR DIENES

Parent system		214 mμ
Increment for each	Alkyl substituent	5
	Exocyclic >C=	5
		λ_{max} = Total

B. HOMOANNULAR DIENES AND POLYENES

Parent homoannular diene system		253 mμ
Increment for each	Alkyl substituent	5
	Exocyclic >C=	5
	C=C extending the conjugation	30
		λ_{max} = Total

C. α,β-UNSATURATED ENONES AND DIENONES[a]

Parent enone		215 mμ
Increment for each substituent: R, $OCOCH_3$, $OCOC_6H_5$, OR, or Br	α	10
	β	12
	γ	18
	δ	18
Increment for an α-OH group		35
Increment for each exocyclic >C=		5
Increment for C=C extending the conjugation		30
Increment for C=O extending the conjugation		0
		λ^{alc}_{max} = Total

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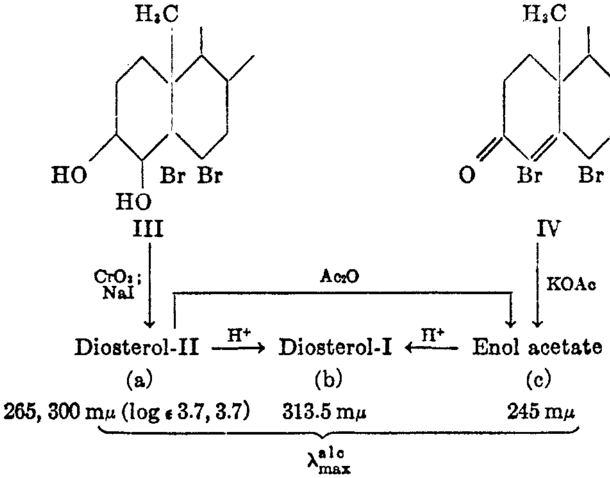
[a] Measurements made in solvents other than ethyl (or methyl) alcohol are corrected to this basis by the addition of the correction factors of Dannenberg (1) as follows: hexane, +11; ether, +7; dioxane, +5; chloroform, +1.

TABLE II

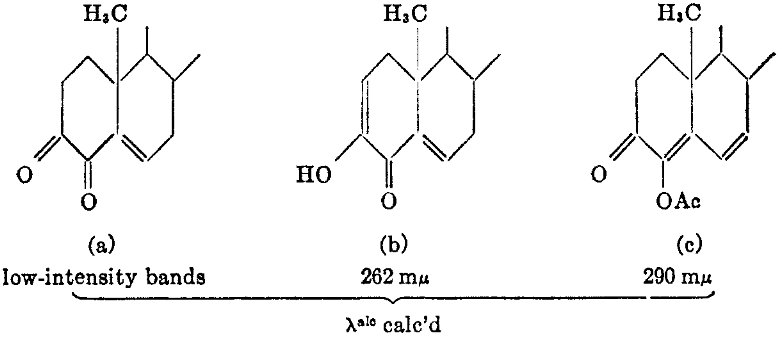
ABSORPTION MAXIMA

COMPOUND	λ^{alc}_{max} (log ε) (mμ)	$\lambda^{CCl_4}_{max.}$ (μ)				
		O—H	C=O		C=C	C—H
			ester	ketone		
Diosterol-I	313.5(3.67)	2.90		5.99	6.15	3.38
Benzoate	232(4.19) 287(4.41)		5.75	5.94	6.15	3.42
Diosterol-II	265(3.71) 300 (3.73)	2.88		5.98	6.15	3.38
Acetate (VIII)	245(4.17)		5.72	5.96	6.14	3.45
Benzoate	234(4.30)		5.77	5.95	6.13	3.44

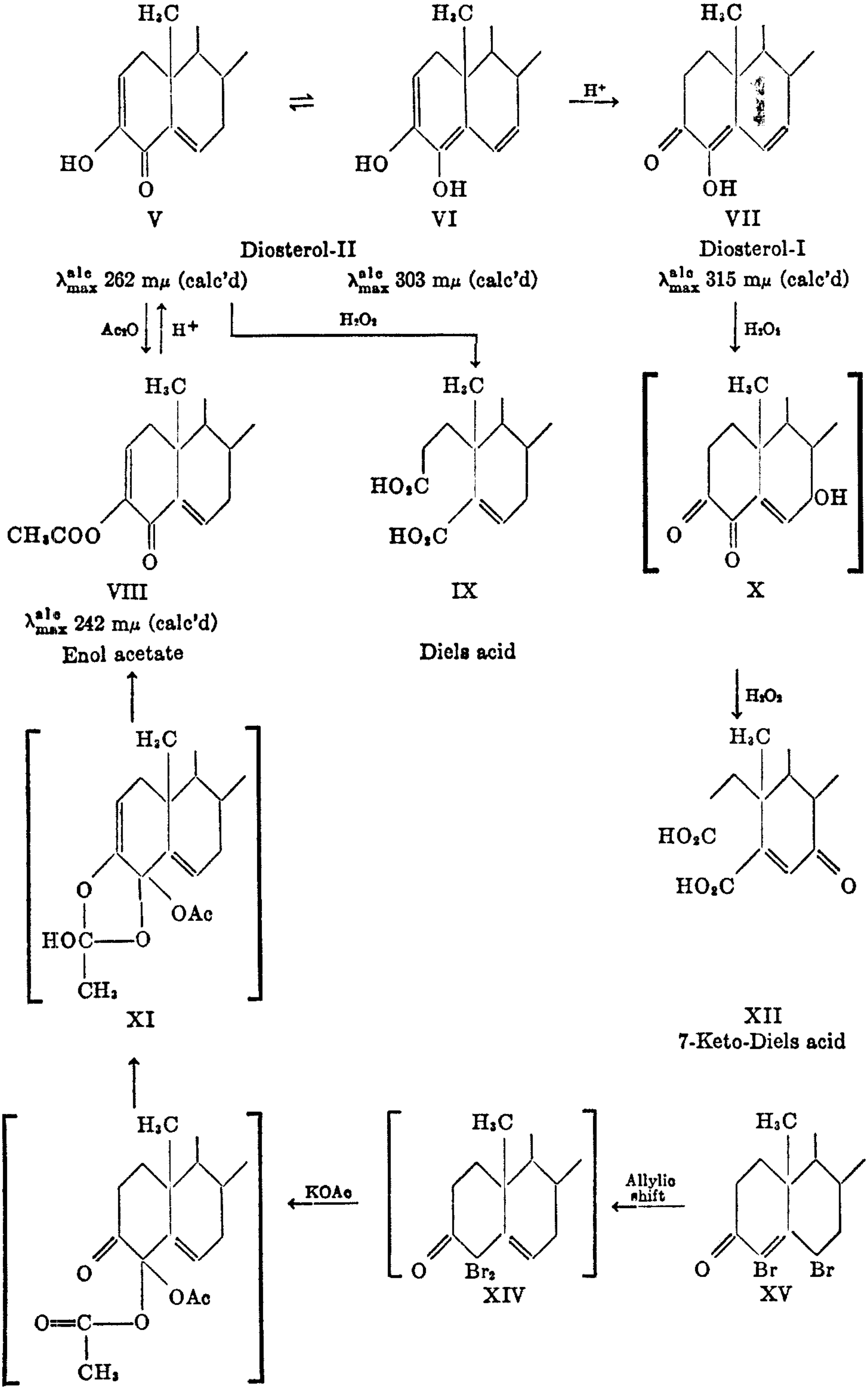
Diosterol-I was prepared by Inhoffen (4) and by Butenandt (5, 6) from cholesterol dibromide by various processes that probably proceed through 4,6-dibromo-Δ^4-cholestenone-3 (IV) and an enol acetate obtainable from it. Petrow and Starling (7) later prepared diosterol-II, a substance that is isomerized by acids to diosterol-I, from the intermediate III. Inhoffen ascribed to diosterol-I and the enol acetate the structures (b) and (c); Petrow and Starling concurred in this view and formulated diosterol-II as in (a). Dannenberg (1), however, pointed out that the absorption maximum at 238 mμ (ether)reported by Inhoffen for the enol acetate is not consistent with formula (c); our value found in alcoholic solution agrees with that of Inhoffen and is 45 mμ lower than the maximum calculated for (c). We may note further that the maximum found for diosterol-I, which we have checked, is much too high for the suggested structure (b). The absorption spectrum of diosterol-II has not previously been reported; a determination has now revealed that formula (a) is also in error, for the substance exhibits strong absorption at 265 mμ and 300 mμ and bears no resemblance to true α-diketones, which have two bands at higher wavelength and of extinction coefficient in the range log ϵ 1.6–2.0 (8, 9).



Formulas of Inhoffen, Petrow and Starling:



The strong band shown by diosterol-I at 313.5 mμ seemed to us suggestive of the presence of a dienolone or trienediol system, but either structure would appear inconsistent with Inhoffen's statement that the compound could not be



V

VI

Diosterol-II

λ^{alc}_{max} 262 mμ (calc'd)

λ^{alc}_{max} 303 mμ (calc'd)

VII

Diosterol-I

λ^{alc}_{max} 315 mμ (calc'd)

VIII

λ^{alc}_{max} 242 mμ (calc'd)

Enol acetate

IX

Diels acid

X

XI

XII

7-Keto-Diels acid

XIII

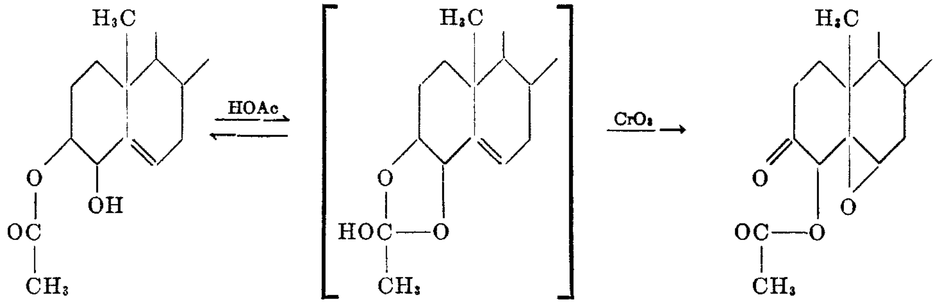
XIV

XV

acetylated. We reinvestigated this point and found that under suitable conditions acetylation apparently can be effected, but even chromatography afforded no crystalline product. Benzoylation proved more successful and yielded a crystalline product characterized as a monobenzoate. Any possibility that the benzoate still contains an unreactive hydroxyl group was eliminated by examination of the infrared spectrum. By this unequivocal method of diagnosis, diosterol-I and diosterol-II were both found to contain one α,β-unsaturated ketonic group and one hydroxyl group; the latter group disappears on benzoylation. On the basis of the combined evidence we now propose the formulas V, VII, and VIII for diosterol-II, diosterol-I, and the enol acetate. The ultraviolet maxima calculated for structures VII and VIII are in good agreement with the values found.[3] The intense band at 265 mμ found for diosterol-II corresponds to that calculated for structure V; the second intense band at 300 mμ may be indicative of another form. The possibility that the substance suffers isomerization to diosterol-I on irradiation in alcoholic solution was eliminated by a test experiment. Since the maximum is close to that expected for the trienediol VI, it is possible that this substance is present in solution in equilibrium with V; the diol may be an intermediate in the acid isomerization of diosterol-II into diosterol-I.

The new formulas suggested account adequately for the formation and reactions of the compounds. The production of the enol acetate (VIII) by the action of potassium acetate on the unsaturated dibromoketone XV in alcoholic solution must proceed by a special mechanism to account for acetylation under nonacetylating conditions, and we suggest the path of allylic shift of bromine (XIV), replacement of the bromine atoms by acetoxyl groups (XIII), and acetyl migration from C_4 to C_3 through the cyclic acetal XI (see analogy below). That diosterol-II on oxidation with hydrogen peroxide yields the Diels acid (7), whereas diosterol-I yields 7-keto-Diels acid (5) is explained by the presence in diosterol-I of a double bond extending to position 7; a likely hypothesis is that hydrogen peroxide adds 1,4 to the $\Delta^{4,6}$-diene system to give the intermediate X.

Petrow and Starling (7) found a new method for the preparation of diosterol-I consisting in mild oxidation of the 3-monoacetate of Δ^5-cholestene-3,4-diol



XVI

XVII

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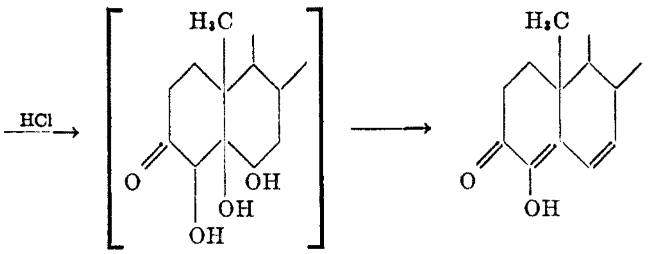
[3] The chromophore of longer-wavelength absorption in VIII is the Δ^5-4-ketonic system; that in V is the Δ^2-4-ketonic system carrying the strongly bathochromic enolic hydroxyl group.



Diosterol-I

(XVI) to a ketoxide, which was then hydrolyzed. We suggest the structure XVII for the ketoxide (the orientation shown for the oxide ring is arbitrary), and interpret its formation as proceeding through a known migration of the acetyl group from the 3- to the 4-position in acetic acid solution (10, 11).

EXPERIMENTAL PART

The enol acetate VIII (m.p. 159°) and diosterol-I (m.p. 160–161°) were prepared according to Inhoffen (4). Diosterol-II (m.p. 134°) was obtained according to Petrow and Starling (7), but the yield was low and the process uncertain.

Diosterol-I benzoate. A mixture of diosterol-I (200 mg.), benzoyl chloride (2 cc.) and pyridine (2 cc.) was warmed on the steam-bath for one hour, poured into water, and the mixture extracted with ether. The ethereal solution was washed free of pyridine and of acid and the neutral product collected and crystallized from alcohol. The substance formed colorless needles, m.p. 160°.

Anal. Calc'd for $C_{34}H_{46}O_3$: C, 81.27; H, 9.16.
Found: C, 81.27; H, 9.26.

Diosterol-I monosemicarbazone was obtained by heating diosterol-I with semicarbazide hydrochloride in pyridine for one hour on the steam-bath. The derivative separated from alcohol as a faintly yellow crystalline powder, m.p. 252–253°, decomp.

Anal. Calc'd for $C_{28}H_{45}N_3O_2$: N, 9.23. Found: N, 9.52.

This result corresponds with that of Petrow and Starling (7), who obtained a mono-dinitrophenylhydrazone; Inhoffen (4) reports the preparation of a dioxime.

Diosterol-II benzoate. A mixture of 4,6-dibromocholestenone (400 mg.), sodium benzoate (1 g.) and alcohol (40 cc.) was refluxed for three hours, diluted to turbidity with water, and cooled. The solid that separated was washed with dilute ammonia, water, and alcohol and the benzoate (200 mg.) crystallized from alcohol. It forms colorless needles, m.p. 176–177°.

Anal. Calc'd for $C_{34}H_{46}O_3$: C, 81.27; H, 9.16.
Found: C, 81.36; H, 9.39.

Absorption spectra. Measurements in the ultraviolet (absolute ethanol) were made with a Beckman spectrophotometer, those in the infrared (2.5% solutions in carbon tetrachloride) with the Baird instrument Model B No. 115 (12). The positions of the infrared bands associated with the vibrations of the α,β-unsaturated carbonyl and ester groups correspond, with allowance for vicinal effects, to bands found for comparable steroids.[4]

The ultraviolet absorption maximum calculated for diosterol-I benzoate is 290 mμ, which agrees with the position of the more intense of the two bands found. The second band at 232 mμ must be associated with the benzoyl group; in the case of diosterol-II benzoate the sole band found was in this region.

SUMMARY

A method for calculation of ultraviolet absorption maxima is presented that is applicable to both heteroannular and homoannular dienes and polyenes, and to substituted or unsubstituted enones and polyenones.

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[4] Data kindly communicated by Drs. R. N. Jones and K. Dobriner (publications in press).

Evidence of ultraviolet and infrared spectroscopy shows that the structures previously assigned to the diosterols and their derivatives are in error. New structures are proposed that account for all of the facts.

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