THE ULTRAVIOLET ABSORPTION SPECTRA OF SEVERAL DEHYDRATION AND OXIDATION DERIVATIVES OF CHOLESTEROL[1]

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Abstract

Certain dehydration and oxidation products of cholesterol were prepared for subsequent animal experimentation. The ultraviolet absorption spectra of those derivatives possessing two or more conjugate chromophores were determined spectrophotometrically over the wave length range of 220 mμ to 350 mμ. The relative coincidence of the experimental λ_{max} and ϵ_{max} values of cholestadiene-3, 5 and of the enol acetate of cholesten-4-one-3 is offered as support for a 3, 5-diene structure for the latter steroid in particular and for enol esters of 4,5-unsaturated 3-ketosteroids in general.

Introduction

The ultraviolet absorption spectra of certain dehydration and oxidation derivatives of cholesterol were determined in preparation for fractionation studies on the heated egg yolk and heated cholesterol diets used by Altschul (1) to produce experimental arteriosclerosis and also sclerosis of muscle and nerve fiber in the rabbit. A particular attempt was made to locate maxima and minima in regions of low absorption which might aid in the positive identification of derivatives of cholesterol which might be formed during the heating of the experimental diets. The reliability of Beer's law in the ultraviolet was also investigated in connection with possible quantitative studies. The results of animal experimentation with several of the steroids considered in this investigation have been reported elsewhere (2, 6).

Experimental

Cholesterilene (Cholestadiene-3, 5) and Dicholesteryl Ether

Cholesterol was dehydrated by heating with an equal weight of anhydrous copper sulphate, according to the method of Mauthner and Suida (15), to give the intramolecular dehydration product, cholesterilene (cholestadiene-3, 5) in 66% yield. Repeated recrystallization of the crude material from absolute ethanol yielded colorless needles, m.p. 79–80° C., $[\alpha]_D - 89.7°$, $\epsilon_{max} = 18.450$ at 235 mμ. Two repetitions of this dehydration procedure resulted in yields of cholesterilene equivalent to 62% and 68% of the theoretical. The rest of the starting material was converted in every case to the intramolecular dehydration product, dicholesteryl ether. Purification of the ether by recrystallization from benzene–ethanol gave a colorless product melting from 196° to 199°C. $[\alpha]_D - 39.0°$.

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Cholesterol was also dehydrated using an equal weight of potassium hydrogen sulphate, as suggested by Montignie (18), and gave approximately the same ratio of cholesterilene to dicholesteryl ether as that obtained in the copper sulphate dehydration. However, when the weight of potassium hydrogen sulphate was five times that of cholesterol, the ratio of cholesterilene to dicholesteryl ether was reversed, which confirmed the earlier findings (18).

Cholesteryl Acetate

Cholesteryl acetate was prepared in quantitative yield by treating cholesterol with excess acetic anhydride under reflux. The ester, after repeated recrystallization from absolute ethanol, was obtained as crystalline needles, m.p. 115–116°C., $[\alpha]_D - 43.8°$.

7-Ketocholesteryl Acetate

7-Ketocholesteryl acetate was prepared by chromic acid oxidation of cholesteryl acetate in acetic acid solution at 55°C., according to the procedure of Windaus, Lettre and Schenck (26). The faintly greenish oxidation product, melting from 154° to 156°C., was obtained in a yield of 28% of the theoretical. Several recrystallizations from ethanol – diethyl ether gave long, colorless needles melting at 158–159°C., $[\alpha]_D - 103.2°$. A further small amount of crude product was obtained by extracting the mother liquor with diethyl ether after the addition of sodium chloride solution. Additional oxidation runs carried out as before gave yields of purified 7-ketocholesteryl acetate ranging from 25% to 32% of the theoretical.

Subsequently, it was found that lowering the reaction temperature from 55°C. to 40°C., together with reducing by 50% the quantity of distilled water employed in the original procedure to dissolve the oxidizing agent, raised the yield of 7-ketocholesteryl acetate to 35–40%, an increase of roughly 10% over that reported by Windaus *et al.* This compares with a yield of 33% recently reported by Fieser *et al.* (10) using a strictly anhydrous medium.

7-Ketocholesterol

7-Ketocholesterol acetate was saponified at room temperature by suspension in 80% methanol containing a slight excess of potassium carbonate for a period of five to six days. The crude 7-ketocholesterol, obtained in approximately quantitative yield, was recrystallized several times from diethyl ether–ethanol and was obtained as colorless needles melting at 169–170°C., $[\alpha]_D - 103.8°$. $\epsilon_{max} = 14.450$ at 236 mμ.

When saponification was carried out more rapidly by refluxing for several hours in 80% methanol containing a slight excess of either potassium carbonate or potassium hydroxide, lower yields of a crude, somewhat brownish product were obtained. Purification of this crude saponification product proved difficult and resulted in a further appreciable loss of material. The smaller yields of a more crude 7-ketocholesterol obtained when more drastic conditions were employed is attributed to the general sensitivity to alkali of an α, β-unsaturated carbonyl system.

7-Ketocholesterilene (7-Ketocholestadiene-3, 5)

7-Ketocholesterilene was prepared from 7-ketocholesteryl acetate according to the method of Stavely and Bergmann (24) in 95% yield. Three recrystallizations of the reaction product from absolute ethanol gave needles melting at 111–112°C., $[\alpha]_D - 314°$, $\epsilon_{max} = 23{,}300$ at 278 mμ.

Epimeric 7-Hydroxycholesterols

7-Ketocholesterol was reduced according to the general method of Meerwein and Schmidt (16) using isopropanol as hydrogen donor and aluminum isopropylate as catalyst. The crude, crystalline reduction product, recovered in 65% of the theoretical yield, melted gradually from 130° to 150°C., a melting point range in agreement with that cited by Buser (5), and gave the expected Lifschutz (14) color reaction. No attempt was made to resolve the crude mixture of α and β epimers. Subsequent ultraviolet absorption studies on this product revealed a small selective absorption in the region of 235 mμ, indicating the presence of some unreduced 7-ketocholesterol. Similar incomplete reduction was subsequently observed by Spencer and Lambert (22) with the Meerwein procedure, but these workers eventually obtained almost quantitative conversion to the diol by employing a lithium aluminum hydride reduction procedure. These observations are in agreement with the recent work of Fieser *et al.* (10), who employed the latter reduction procedure but isolated the reaction product as the dibenzoate.

Cholestenone (Cholesten-4-one-3)

The general method of Oppenauer (19) was followed, but the more readily prepared aluminum isopropylate was substituted for aluminum tertiary butylate as catalyst for the reaction. The yield of almost colorless cholestenone, m.p. 77–79°C., was 76% of the theoretical. Repeated recrystallization from ethanol–diethyl ether gave material melting at 80-81°C., $[\alpha]_D + 88.0°$, $\epsilon_{max} = 15{,}725$ at 240 mμ.

In subsequent oxidations, additional modifications were introduced into the original Oppenauer procedure; in particular, these changes were designed to avoid the troublesome emulsions often encountered in the washing of the benzene extracts and to eliminate the time-consuming problem of bringing about crystallization of the oxidation product. After the oxidation step had been carried out according to the original instructions, the procedure was modified as follows.

Distilled water (slightly more than 3 moles per mole of catalyst) was added to the still warm contents of the reaction vessel to bring about hydrolysis of the aluminum alkoxide catalyst, and stirring was continued for an additional

30 min. The aluminum hydroxide gel so formed was removed from the cold reaction mixture by centrifuging, and the solution of sterol obtained by decantation was concentrated under vacuum (20 mm.) to a yellowish oil. This residue was dissolved in diethyl ether and the ethereal solution was washed with 10% sulphuric acid to remove remaining traces of aluminum hydroxide and then with distilled water until the washings were neutral to litmus. The ethereal solution was then dried over anhydrous sodium sulphate and concentrated under a stream of dry air; gentle rubbing of the oil precipitate of cholestenone as it formed brought about immediate crystallization. When practically all of the diethyl ether had been removed by evaporation, ethanol was added and the mixture was refrigerated for several hours. The almost colorless precipitate was collected by suction filtration and washed thoroughly with ice-cold ethanol to remove residual mesityl oxide. The product was then dried under vacuum to remove final traces of mesityl oxide and was recrystallized as before.

Cholestenone Enol Acetate

Cholestenone was treated with excess acetyl chloride under reflux for 10 hr. The temperature of reflux assisted in the rapid elimination of hydrogen chloride formed in the reaction and hence prevented the transformation of the enol ester into an enol chloride (20). The brownish needles which precipitated when the reaction solution was cooled were washed with cold ethanol and recrystallized several times from ethanol – diethyl ether to give long, colorless needles melting at 80-81°C., $[\alpha]_D - 92.0$, $\epsilon_{\max} = 19{,}600$ at 235 mμ.

Melting Points

All melting points reported have been corrected against reliable standards.

Optical Rotation Measurements

All specific rotatory values cited for the above cholesterol derivatives were determined at room temperature, which varied from 20°C. to 25°C., with the Rudolph No. 127 precision polarimeter, using a macro-tube 2 dm. in length. Chloroform, purified by refluxing with and distilling from anhydrous calcium chloride, was used as the solvent. The concentrations of the steroids under investigation varied, but were usually of the order of 1–2%. For the sodium "D" line, the wave length used for all rotation measurements, monochromator and slit width settings gave an isolated band width of 9.5 mμ.

Ultraviolet Spectrophotometry

Spectral determinations were made over a wave length range of 220 mμ to 350 mμ with the Beckman DU quartz spectrophotometer. Density readings were never further apart than 4 mμ, while in regions where the optical density was changing rapidly the interval was reduced to 2 mμ, and in the immediate neighborhood of maxima and minima to 1 mμ. The absorption cells were of silica, each having a thickness of 1.001 cm. In every case, concentrations of

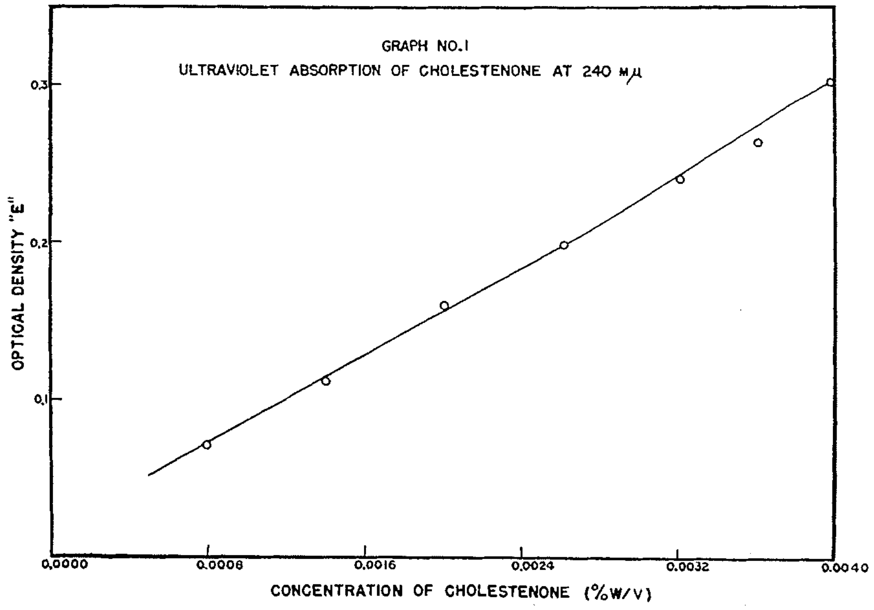
0.1%, 0.01%, and 0.001% of each compound were employed; the stronger concentrations were examined in regions of lower absorption in an attempt to find weak maxima and minima not discernible when concentrations low enough to permit total determination of the curve were used.

A commercial grade of absolute ethanol was found by spectral tests to be satisfactory as a solvent without special purification. In some cases it was necessary, because of the limited solubility in ethanol, to dissolve the sample in a small amount of diethyl ether and then dilute the ether solution to the desired volume with ethanol. The final concentration of ether was never greater than 2% and was of the order of 0.02% in the weakest (0.001%) steroid solutions. In all cases, the reference liquid used as a blank in the spectrophotometer was identical with the solvent of the solution under test, each component being taken from the same batch.

Ultraviolet Absorption Results and Discussion

Validity of Beer's Law

It now appears generally accepted that Beer's law always holds for dilute solutions and that the deviations sometimes encountered are due to a change in the absorbing species with concentration. Graph No. 1 shows that Beer's law is obeyed within the limits of experimental error with ethanolic solutions of cholestenone over the approximate concentration range of 0.001% to 0.005%. (Absorption values were measured at the λ_{max} 240 mμ). However, when the steroid concentration was increased by a further factor of 20 (i.e., to 0.1%), the absorption increased by only a fraction of this factor. This "falling-off" of the ϵ_{max} value which accompanies an increase in concentration was addition-



ally noted during spectral curve determinations, when concentrations of 0.001%, 0.01%, and 0.1% were employed with each steroid investigated. It was also noted that the extrapolated curve failed to pass through the origin.

Spectral Absorption Measurements

Experimental values of molecular extinction coefficients reported for all maxima and minima have been calculated from spectral absorption curves determined for the steroids under investigation. Where the order of magnitude was large ($\epsilon > 1000$), calculations were based upon absorption curves determined using concentrations of 0.001% steroid; on the other hand, where $\epsilon < 1000$, calculations were made from absorption values for solutions of 0.1% concentration. Although this policy introduces the variation of $\epsilon_{\max}$ with concentration change seen experimentally, no adjustment for concentration effect was attempted because of the lesser importance, in a quantitative sense, of maxima and minima in regions of low absorption. The spectral band widths reported for individual maxima and minima were calculated from the observed slit-width and the calibration chart accompanying the spectrophotometer.

For comparison purposes, $\lambda_{\max}$ and $\epsilon_{\max}$ values for the steroids under investigation have also been cited from the literature.

α, β-Unsaturated Ketones

It was early noted (3, 17) that α, β-unsaturated ketones have characteristic spectra. An isolated carbonyl group gives a low intensity band (ϵ = 10–100) near 275 mμ, while an ethylenic group gives a high intensity band ($\epsilon = 10^4$) near 195 mμ. When these two groups are conjugated, producing an α, β-unsaturated carbonyl compound, the two bands, referred to as K and R bands following Burawoy's (4) terminology, occur at longer wave lengths.

Band	Wave length	Intensity
K band	220-260 mμ	10^4
R band	310-330 mμ	10^2

Since the R band is of such low intensity, most attention is focused on the K band.

Cholestenone (Cholesten-4-one-3)

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	$\lambda_{\max}$ (mμ)	$\epsilon_{\max}$		$\lambda_{\max}$ (mμ)	$\epsilon_{\max}$	
Maximum	240	15,725	2.1	242	16,300	(13)
Plateau	312	120	2.9	312	76	(13)
				285	55	(13)

Cholestenone, having an α,β-unsaturated ketonic grouping in ring A was found to have a λ_{max} at 240 mμ (K band) and a plateau on the absorption curve at 312 mμ (suggestive of the R band). Good agreement is seen with available literature values.

7-Ketocholesterol

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	λ_{max} (mμ)	ϵ_{max}		λ_{max} (mμ)	ϵ_{max}	
Maxima	236	14,450	2.0	238	12,500	(27)
	329	50	3.3			
Minimum	284	20	2.4			

7-Ketocholesterol, having an α,β-unsaturated ketonic grouping in ring B, showed a strong selective absorption maximum at 236 mμ (K band) and a weak absorption maximum at 329 mμ (possibly the R band), the latter previously unreported. Fair agreement is seen between the values of λ_{max} and ϵ_{max} determined in this investigation for the K band, and those previously reported.

α,β: γ,δ-Diunsaturated Ketones

Acyclic triply conjugated systems involving two ethylenic and one ether group usually absorb maximally between 240 and 265 mμ, λ_{max} being little affected by the arrangement of the chromophores. The absorption of triple isocyclic systems is similar to that of the corresponding acyclic systems, with bands situated at somewhat higher wave lengths (about 20 mμ) than in analogous acyclic compounds, provided that all three chromophores are not in the same ring (9). Examples of three conjugated isocyclic chromophores are almost wholly confined to the steroid series.

7-Ketocholesterilene (7-Ketocholestadiene-3, 5)

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	λ_{max} (mμ)	ϵ_{max}		λ_{max} (mμ)	ϵ_{max}	
Maximum	278	23,300	2.2	280	23,300	(27)
Shoulder	336	150	3.4			

A comparison of the absorption spectrum of 7-ketocholesterilene with that of the α,β-unsaturated ketone 7-ketocholesterol readily illustrates the appreciable increase in λ_{max} and ϵ_{max} which accompanies the introduction of an additional conjugated unsaturation. The shoulder on the absorption curve seen at 336 mμ suggests that this may be the new position of the R band on addition of the third conjugated chromophere. The ϵ_{max} value for 7-ketocholesterilene determined at 278 mμ (K band) is in excellent agreement with the previous value reported in the literature. The fact that our experimental λ_{max} values for 7-ketocholesterilene and for 7-ketocholesterol are 2 mμ lower than those reported by Wintersteiner and Bergstrom (27) suggests a linear difference of 2 mμ in the wave length calibrations of the respective spectrophotometers employed.

Conjugated Dienes

Cyclic conjugated dienes fall into two broad groups ,i.e., homoannular dienes, with the conjugated unsaturations in the same ring, which absorb in the region of 260–285 mμ and heteroannular dienes, with the conjugated unsaturations in different rings, which absorb in the region of 230–250 mμ (9). Both cholesterilene and the enol acetate of cholestenone are considered members of the latter group.

Cholesterilene (Cholestadiene–3, 5)

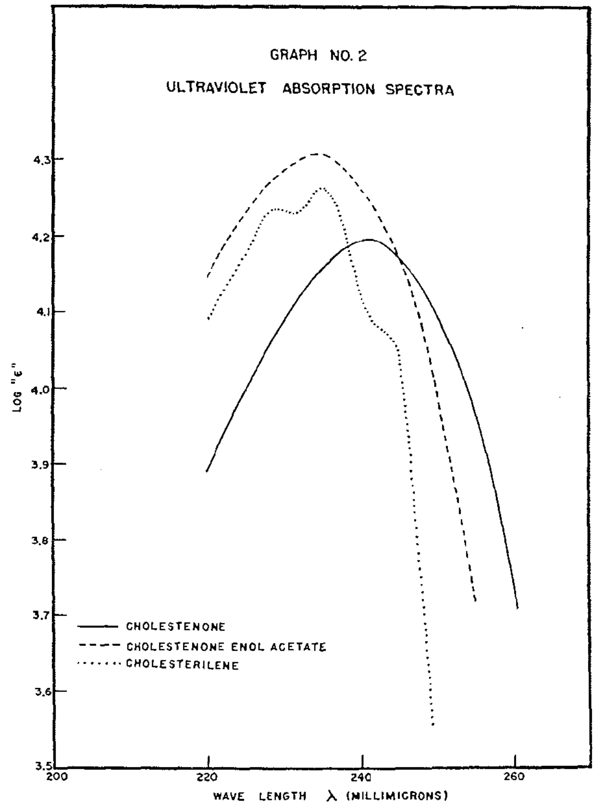
	Experimental values		Spectral band width (mμ)
	λ_{max} (mμ)	ϵ_{max}	
Maxima	235	18,450	2.0
	229	17,300	2.0
Minimum	231	17,000	2.0
Shoulder	243	12,050	2.1

Cholesterilene was originally assigned the structure of cholestadiene–2, 5 by Heilbron (11), but the high absorption of cholesterilene in the ultraviolet is now considered indicative of a conjugated system. The maxima reported at 249, 304, and 312 mμ in its absorption spectrum (12) were later not confirmed; instead, maxima at 229, 235, and 244 mμ (21, 23) and at 235 and 245 mμ (7) have been reported. In the present investigation, maxima were observed at 229 mμ, and at 235 mμ, and a shoulder was found on the curve at 243 mμ (see Graph No. 2); the experimentally determined ϵ_{max} values were of the same order of magnitude as those cited in the literature.

Cholestenone Enol Acetate (Cholesta–3, 5–dien–3yl Acetate)

	Experimental values		Spectral band width (mμ)
	λ_{max} (mμ)	ϵ_{max}	
Maximum	235	20,400	2.0
Plateaux	231	19,600	2.0
	312	130	2.9

In addition to the main absorption maximum at 235 mμ, transient plateaux were observed on the spectral curve for cholestenone enol acetate in the neighborhood of 231 mμ and 312 mμ. The ϵ_{max} value at 235 mμ is in agreement with that determined by Westphal (25), but the corresponding λ_{max} reported by this worker was that of 240 mμ.



Two possible structures have been proposed for 3-enol esters of 4, 5-unsaturated 3-ketosteroids in general, and hence for the enol acetate of cholestenone in particular. These are shown diagrammatically below, together with the structures and certain physical constants of the corresponding known cholestadienes and also of cholestenone itself.

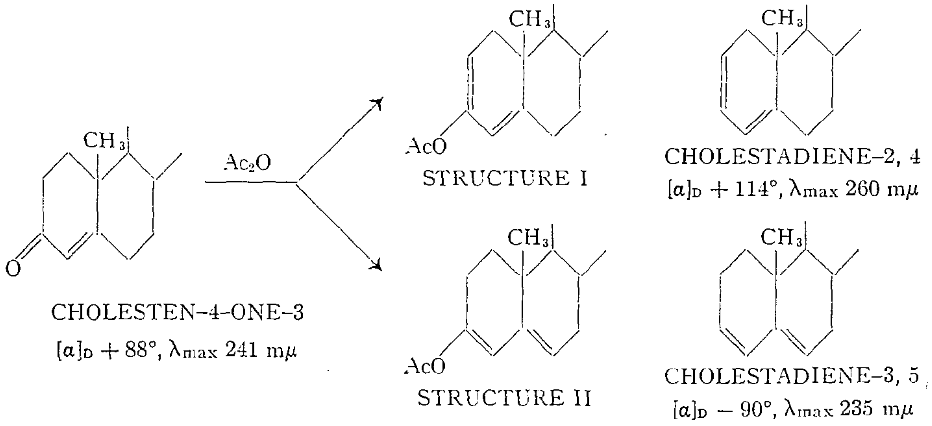


FIG. 1.

Since the specific optical rotation was seen to change from + 88° to − 92° (i.e., to approximately the same negative value as that determined for cholestadiene–3, 5),while the rotation of cholestadiene–2, 4, with a diene structure analogous to that of structure I, is markedly positive, Structure II is suggested for the enol acetate. Similar support has already been offered for Structure II by Westphal (25), based upon optical rotation values differing slightly in magnitude from those reported by us.

Wesphal further supported this postulated shift of the homoannular 2, 4–diene system to the heteroannular 3, 5–diene type by comparing the respective ultraviolet absorption spectra of these steroids. He interpreted the λ_{max} of the enol acetate at 240 $m\mu$ as that characteristic of a 3, 5–diene system, since cholestadiene–3, 5 absorbs maximally at 235 $m\mu$ and cholestadiene–2, 4 at 260 $m\mu$. However, Westphal's λ_{max} at 240 $m\mu$ is somewhat higher than that (about 235 $m\mu$) calculated empirically for this compound by Fieser (9) and others, and in addition is at the same wave length as that of the unesterified parent ketone cholestenone.

In the present investigation, a definite shift in the λ_{max} from 240 $m\mu$ to 235 $m\mu$ (i.e., to the λ_{max} of cholestadiene–3, 5) was observed upon acetylation of cholestenone. The strict coincidence of the λ_{max} value of cholestenone enol acetate with that of cholestadiene–3, 5, and hence with the empirically calculated value, is considered as supporting more strongly Westphal's postulate of a 3, 5–diene structure. (The authors, in the light of their experimental findings, question Woodward's (28) empirical calculations which predicted a λ_{max} for cholestenone enol acetate about 5 $m\mu$ higher than that for cholestadiene–3, 5.) The comparison of the absorption spectra of cholestenone, cholestenone enol acetate, and cholestadiene–3, 5 (cholesterilene) made in Graph No. 2 readily illustrates the shift in λ_{max} on acetylation and the coincidence of the λ_{max} values of the last two steroids. Good agreement is also seen between the λ_{max} values of the last two compounds.

Acknowledgments

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