517. *Studies in the Sterol Group. Part LV.* Ultra-violet Absorption Spectra of Ethylenic Centres.*

By PETER BLADON, H. B. HENBEST, and GEOFFREY W. WOOD.

The ultra-violet light absorptions of various unsaturated steroids have been determined spectrophotometrically in the 2050—2250-Å region. Isolated ethylenic bonds show appreciable absorption in this region, the intensity of absorption depending primarily on the degree of substitution of the double bond, but also on various environmental factors. It thus becomes possible to determine the degree of substitution (and sometimes the actual position) of a steroid double bond. For instance, the presence of either of the two tetrasubstituted bond types ($\Delta^{8(9)}$ and $\Delta^{8(14)}$) can be readily determined. For this reason, and also because hydroxyl, acetoxyl, and carbonyl groups exhibit only low-intensity absorption, measurements in this region provide a valuable adjunct to infra-red techniques. As an example of the use of the method in structural problems, Westphalen's diol is shown to contain a $\Delta^{9(10)}$-bond, and not a $\Delta^{8(9)}$-bond as is more commonly accepted.

The accuracy of the measurements is discussed with particular reference to the intrusion of " false energy " in this shorter-wave region.

THE main absorption bands of isolated ethylenic linkages are situated in the ultra-violet in the 1600—2000-Å region (cf. Platt and Klevens, *Rev. Mod. Physics*, 1944, **16**, 182; Carr, *Chem. Reviews*, 1947, **41**, 293). It is also now well established that the position and intensity of maximal absorption is dependent on the degree of substitution of the double bond, increasing substitution moving the absorption band to longer wave-lengths as in conjugated dienes (Carr and Walker, *J. Chem. Physics*, 1936, **4**, 751; Price and Tutte, *Proc. Roy. Soc.*, 1940, **174**, 207). Vapour-phase measurements below 2000 Å have been

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* Part LIV, *J.*, 1952, 2380.

made by vacuum spectrographic techniques, but more recently solutions of olefins have been employed, since many (chemically) saturated solvents (*e.g.*, *n*-hexane) transmit sufficient light down to at least 1800 Å when carefully purified (Platt and Klevens, *loc. cit.*).

In the 2100—2300-Å region, measurements with a photographically recording small quartz spectrograph have shown that olefins (such as *cyclo*hexene) exhibit appreciable " end absorption " (Bateman and Koch, *J.*, 1944, 600). The shift of this absorption tail towards longer wave-lengths with increasing substitution of the double bond was also observed, and it was pointed out that measurements performed in this region might be of use in determining the degree of substitution of olefins.

It has now been found that, by using a quartz spectrophotometer, absorption values can be obtained down to about 2050 Å (cf. Doub and Vandenbelt, *J. Amer. Chem. Soc.*, 1947, **69**, 2714), and the absorptions of a range of steroids containing isolated ethylenic bonds have now been determined with one of these instruments. Saidel, Goldfarb, and Kalt (*Science*, 1951, **113**, 683) and Vandenbelt, Henrich, and Bash (*ibid.*, 1951, **114**, 576) have shown that false energy (scattered light) causes decreases in absorption intensities in the shorter wave regions—the effect of such factors on the present measurements is discussed below (Table, H, and Discussion of Method).

A brief account of this work has been published previously (*Chem. and Ind.*, 1951, 866).

EXPERIMENTAL

The results shown in the Table (A—G) below were obtained with a Unicam instrument (model S.P. 500), a hydrogen lamp and a 0·5-cm. absorption cell being used, although more recently a 0·1-cm. cell mounted in a special holder has been employed with advantage. " Clinical absolute alcohol," transmitting sufficient light down to 2050 Å or below, was generally used as the solvent. Moser (*Nature*, 1951, **167**, 656) has recently suggested that even carefully purified ethanol is not suitable for work below 2100 Å, but in our experience this solvent gives more than 50% transmission (relative to air) at 2050 Å in a thickness of 1 mm., and has proved quite satisfactory. Commercial 95% ethanol (2·5 l.), purified by being heated under reflux with zinc dust (50 g.) and potassium hydroxide (50 g.) for 2 hours and then distilled (the first and last 10% of the distillate being rejected), has been found to be as good as, and usually better than, the (unpurified) clinical absolute solvent in optical transparency. It has the added advantage of being less expensive, but it possesses an occasional disadvantage in being a somewhat less powerful solvent than the more anhydrous material for most steroid compounds, especially hydrocarbons. Determinations have also been carried out in *cyclo*hexane, purified by being shaken with concentrated sulphuric acid and then passed through silica gel (cf. Table, G)—essentially similar absorption curves were obtained. Ethanol is preferable as a routine solvent because it dissolves a wider range of steroid compounds; the optical transparency of the 95% ethanol purified by the above simple procedure was usually greater than that of the *cyclo*hexane, although it is probable that this could be reversed by the use of more elaborate methods of purifying the latter (cf. Pestemer, *Angew. Chem.*, 1951, **63**, 118). The concentration of the solution under examination was adjusted so that the highest optical density value (E) lay between 0·3 and 0·5.

With every unsaturated compound examined, a position of maximal absorption was observed (see later), but the readings taken at wave-lengths below the maximum were often not satisfactory, reproducible density values being difficult to obtain owing to the relative insensitivity of the instrument. In order to obtain the best results in the 2050—2250-Å region it was necessary to employ a hydrogen lamp that had not been used for more than 200 hours, and also to ensure that it was focused correctly.

Materials.—Most of the compounds examined were either prepared and purified by recorded methods or obtained by purification of commercial samples to constant m. p. The m. p.s of the compounds described below were determined on a Kofler block. Optical rotations were determined in chloroform solution in a 1-dm. tube at 18—25° unless stated otherwise. The P. Spence alumina (Grade O) used for chromatography had an activity between 2 and 3 on the Brockmann–Schodder scale (*Ber.*, 1941, **74**, 75).

Chol-11-*ene*-3α : 24-*diol*. Methyl-3α-acetoxychol-11-enate {m. p. 100—102°, $[\alpha]_D$ +45° (*c*, 0·715 in methanol); 0·5 g.,} dissolved in dry ether (15 c.c.), was added with stirring to a two-fold excess of lithium aluminium hydride in ether, the solution then being heated under reflux for 1 hour. The product was recrystallised from methanol, to give the *diol* (0·45 g.) as plates, *Light absorption in ethanol* (λ *are in* Å).

A. *Non-ethylenic compounds.*

	ε_{2050}	ε_{2100}
Cholestanol	~200	~150
Cholestanyl acetate	~200	~150
Cholestanone	~350	~200

	$\lambda_{\text{max.}}$*	$\varepsilon_{\text{max.}}$	ε_{2100}	ε_{2150}	ε_{2200}	Refs.
B. *Compounds with disubstituted olefinic bonds.*						
Δ^{2}-*Steroid.*						
Cholest-2-ene	2030	600	200	—	—	—
Δ^{6}-*Steroids.*						
Cholest-6-ene-3β : 5α-diol	2040	1300	600	100	—	—
3β-Acetoxycholest-6-en-5α-ol	2040	1700	900	350	200	—
Δ^{11}-*Steroids.*						
Chol-11-ene-3α : 24-diol	2030	2500	800	200	—	1
3α-Hydroxychol-11-enic acid	2050	2400	1000	350	150	2
C. *Compounds with trisubstituted olefinic bonds.*						
Δ^{4}-*Steroid.*						
Cholest-4-ene	2030	4000	3000	1500	850	3
Δ^{5}-*Steroids.*						
Cholest-5-ene	2040	3700	2700	1400	700	—
Cholesterol	2030	3400	1600	700	400	—
Cholesteryl acetate	2030	2800	1500	800	550	—
Cholesteryl chloride	2050	4700	3500	1400	400	—
Cholesteryl bromide	2100	—	5600	5100	3500	—
Chol-5-ene-3β : 24-diol	2030	3000	1800	850	550	1
β-Sitosterol	2030	2800	1600	750	350	—
24(: 28)-Dihydrofucosterol	2040	2800	1800	550	400	—
Dehydro*epi*androsterone	2040	3300	1700	650	300	—
Pregnenolone	2040	3300	1800	600	300	—
7α-Hydroxycholesterol	2050	3300	1600	500	300	—
7β-Hydroxycholesterol	2050	3700	2400	600	200	—
7α-Methoxycholesterol	2060	4100	3000	1200	500	—
Δ^{7}-*Steroids.*						
Ergost-7-ene	2070	4200	4000	2900	1500	3
Ergost-7-en-3β-ol	2070	4500	4300	3000	1500	3
3β-Acetoxyergost-7-ene	2080	4900	4700	3500	1800	3
$\Delta^{9(11)}$-*Steroids.*						
Methyl chol-9-enate	2060	3400	2900	1400	500	3
Chol-9-en-24-ol	2060	3700	2400	1500	500	3
Δ^{14}-*Steroids.*						
Ergost-14-en-3β-ol	2040	4100	2400	800	100	—
3β-Acetoxyergost-14-ene	2040	4100	2800	1000	300	—
Cf. Citronellol	2040	2400	1400	600	250	1

D. *Compounds with tetrasubstituted olefinic bonds.*

	$\lambda_{\text{max.}}$	$\varepsilon_{\text{max.}}$	ε_{2100}	ε_{2150}	ε_{2200}	ε_{2230}	Refs.
$\Delta^{8(9)}$-*Steroids.*							
Cholest-8-ene	2070	4600	4400	3900	3400	2800	3
Cholest-8-en-3β-ol	2070	4700	4500	4000	3500	2900	3
Cholest-8-en-3-one	2070	4700	4400	4000	3500	2800	3
$\Delta^{8(14)}$-*Steroids.*							
Ergost-8(14)-ene	2075	10,400	10,100	8400	5700	3500	3
Ergost-8(14)-en-3β-ol	2080	9800	9500	8100	6000	3400	3
3β-Acetoxyergost-8(14)-ene	2090	10,600	10,500	9200	7000	4400	3
Ergost-8(14)-en-3-one	2080	9700	9500	7500	5300	3000	3
Cholest-8(14)-en-3β-ol	2080	10,000	9900	8300	6000	3600	4
3β-Acetoxycholest-8(14)-ene	2080	10,000	9800	8600	6700	4500	4
Ergosteryl B_3 acetate–maleic anhydride adduct	2100	—	8600	7900	6000	4200	—
$\Delta^{9(10)}$-*Steroids.*							
Westphalen's diol	2050	9300	7700	4700	1900	—	—
,, diacetate	2060	10,000	8400	5100	2000	—	—
,, diketone	2050	7800	6300	5000	4000	—	—

* See below in Discussion of Method.

E. *Unconjugated dienes.*

	$\lambda_{max.}$	$\varepsilon_{max.}$	ε_{2100}	ε_{2150}	ε_{2200}	Refs.
Stigmasterol	2040	3800	1800	600	200	—
Fucosterol	2030	4300	2200	1000	600	—
Zymosterol	2050	6700	5600	4800	4000	—
Cf. Cholest-8-en-3β-ol + citronellol	(at 2050)	(6900)	5900	4600	3750	—
Zymostadienone	2050	6800	6000	4800	3700	—
Cf. Cholest-8-en-3-one + citronellol	(at 2025)	(6900)	5800	4600	3750	—
Ergosta-7 : 22-diene	2045	5400	4900	3500	1700	1
Ergosta-7 : 22-dien-3β-ol	2045	5700	4800	3300	1700	—
3β-Acetoxyergosta-7 : 22-diene	2060	6100	5400	3900	2000	—
Pregna-5 : 17-diene-3β : 21-diol	2050	6000	4800	1600	300	5

F. *Bile acid derivatives.*

*apo*Cholic acid	2080	7000	6500	4700	3100	6
Methyl *apo*cholate	2080	9000	8600	6300	3900	6
Dihydroxycholenic acid	2050	3600	2600	1000	300	6
Methyl dihydroxycholenate	2050	3500	2500	900	200	6

G. *Light absorptions in* cyclo*hexane.*

Ergost-7-ene	2090	4400	4300	3400	1700	—
Ergost-7 : 22-diene	2070	4800	4600	3500	1700	—
Ergost-8(14)-ene	2095	8700	8700	8000	5900	—

H. *Light absorptions recorded with various instruments.*

		ε_{2100}	ε_{2150}	ε_{2175}	ε_{2200}
Ergost-8(14)-ene	Hilger small quartz spectrograph	—	10,800	9300	7400
	Manchester Unicam	10,100	8400	7200	5700
	Birmingham Unicam	10,300	8300	7600	6300
	Birmingham Uvispek	6100	8000	6400	5400
Cholest-8(9)-ene	Hilger small quartz spectrograph	5400	5000	4500	3900
	Manchester Unicam	4400	3900	3650	3400
Ergost-7-ene	Hilger small quartz spectrograph	4500	3500	2400	1400
	Manchester Unicam	3500	2900	2200	1500

Refs. 1, This paper. 2, Kindly supplied by Dr. K. Folkers. 3, Bladon, Fabian, Henbest, Koch, and Wood (*J.*, 1951, 2402). 4, Kindly supplied by Dr. D. H. R. Barton. 5, Kindly supplied by Dr. V. Petrow. 6, Kindly supplied by Dr. R. K. Callow. These compounds were recrystallised from methanol and dried at 20° in a vacuum-desiccator; the presence of one molecule of methanol of crystallisation was assumed in calculation of the ε values quoted above.

m. p. 193—194°, $[\alpha]_D$ +42° (*c*, 0·76) (Found: C, 80·45; H, 11·15. $C_{24}H_{40}O_2$ requires C, 79·95; H, 11·2%). Acetylation gave the *diacetate*, crystallising from methanol in needles, m. p. 67—69°, $[\alpha]_D$ +48° (*c*, 0·94) (Found: C, 75·45; H, 10·1. $C_{28}H_{44}O_4$ requires C, 75·6; H, 10·0%).

*Chol-5-ene-*3β : 24-*diol* [with Dr. C. W. GREENHALGH]. A solution of methyl 3β-hydroxychol-5-enate (10 g.) and lithium aluminium hydride (1·5 g.) in dry ether (650 c.c.) was heated under reflux for 3 hours. After cooling, the excess of reagent was decomposed with white dilute sulphuric acid, and the diol (7·0 g.), m. p. 192—194°, isolated by filtration. Concentration of the ethereal layer gave more diol (1·6 g.), m. p. 188—191°. Recrystallisation from methanol afforded the pure *diol* as plates, m. p. 194—195°, $[\alpha]_D$ −47° (*c*, 0·46) (Found: C, 80·1; H, 11·5. $C_{24}H_{40}O_2$ requires C, 79·95; H, 11·2%). It gave the *diacetate*, crystallising from methanol–acetone as flat needles, m. p. 130—130·5°, $[\alpha]_D$ −46° (*c*, 0·76) (Found: C, 75·7; H, 10·1. $C_{28}H_{44}O_4$ requires C, 75·6; H, 10·0%).

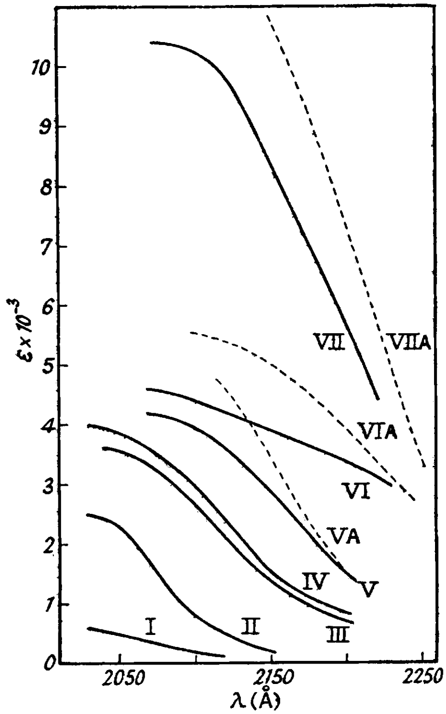
*Cholesta-*8(9) : 24-*dien-*3-*one* (*Zymostadienone*). A solution of aluminium *tert.*-butoxide (6 g.) in dry benzene (100 c.c.) was added to zymosterol (3 g.) in dry acetone (60 c.c.), the mixture being heated under reflux for 6 hours. Most of the mesityl oxide was removed from the steroid product by the addition of xylene (7·5 c.c.), followed by evaporation *in vacuo*. The product was chromatographed on alumina (100 g.); development with benzene gave the *ketone*, which after recrystallisation from methanol formed fine needles (1·7 g.), m. p. 99·5—102°, $[\alpha]_D$ +65° (*c*, 1·40) (Found: C, 84·4; H, 11·15. $C_{27}H_{42}O$ requires C, 84·7; H, 11·1%).

*Ergosta-*7 : 22-*dien-*3-*one*. This ketone was prepared by chromic acid oxidation of α-dihydroergosterol (5 g.) by the general method described in Part LII. Recrystallisation of the chromatographed product from acetone gave the ketone (3 g.) as plates, m. p. 177°, $[\alpha]_D$ +1·5° (*c*, 1·24) (Found: C, 84·6; H, 11·5. Calc. for $C_{28}H_{44}O$: C, 84·8; H, 11·2%). Barton and Cox (*J.*, 1948, 1336) give m. p. 184·5°, $[\alpha]_D$ +2° (*c*, 1·54). Elution of the chromatogram with ether gave unchanged sterol (0·7 g.), m. p. 171—174° (after recrystallisation from ethanol).

*Ergosta-*7 : 22-*diene*. The foregoing ketone (1 g.) was heated with hydrazine hydrate

(7 c.c. of 60% w/v) and a solution of sodium (1·25 g.) in ethanol (25 c.c.) in an autoclave at 190—215° for 5 hours. The steroid was isolated with pentane and chromatographed on alumina (50 g.). Elution with pentane gave ergosta-7 : 22-diene (0·86 g.), which after recrystallisation from ethyl acetate–methanol formed platelets, m. p. 125—127°, $[\alpha]_D$ −17° (*c*, 1·3). Heilbron, Spring, and Webster (*J.*, 1932, 1705) give m. p. 124—125°, $[\alpha]_D$ −10° (*c*, 1·8).

Citronellol. The commercial material contains some geraniol, which was removed by shaking a light petroleum (b. p. 40—60°) solution with manganese dioxide, followed by treating the whole product with semicarbazide acetate. Geraniol was converted by this treatment into citral semicarbazone, and distillation afforded pure citronellol, b. p. 116°/20 mm., n_D^{19} 1·4569.



Disubstituted olefins.
I, Cholest-2-ene.
II, Chol-11-ene-3α : 24-diol.

Trisubstituted olefins.
III, Cholest-4-ene.
IV, Cholest-5-ene.
V and VA, Ergost-7-ene.

Tetrasubstituted olefins.
VI and VIA, Cholest-8(9)-ene..
VII and VIIA, Ergost-8(14)-ene.

Full-line curves are " Unicam " results. Broken curves are " Hilger " small quartz spectrograph results.

DISCUSSION

Method.—It is important to emphasise that the light-absorption maxima observed in the present work are only " apparent maxima," and are not located at the true positions of maximal absorption for the olefins concerned. For instance, cholest-2-ene shows an apparent maximum at 2030 Å, whereas the simpler *cis*-disubstituted olefin, *cyclo*hexene, has $\lambda_{max.}$ at 1820 Å ($\varepsilon = 6600$) (Platt, Klevens, and Price, *J. Chem. Phys.*, 1949, **17**, 466). The decreasing absorption recorded at the shorter wave-lengths is due to the rapid increase of the relative contribution of false-energy (scattered light) factors, dependent mainly on instrument design and the intensity (or age) of the light source. Measurements above the apparent maximum were also affected to an appreciable extent for the same reasons. Confirmation was provided by comparing the Unicam absorptions of ergost-7-ene and cholest-8(9)-ene with those determined with a small quartz spectrograph (for these determinations we are greatly indebted to the late Dr. H. P. Koch), such measurements being known not to be influenced by the intrusion of false energy. Although readings with the quartz spectrograph could only be obtained down to approximately 2120 Å, the appreciably higher ε values obtained above this wave-length (see Figure and Table, H) show that the values obtained from the spectrophotometric instrument in this region are lower than the true ones, the differences diminishing with increasing wave-length. With other instruments, the apparent maximum may be found at different wave-lengths, and has sometimes been thought to be a true absorption maximum. For instance, Bader (*Helv.*

Chim. Acta, 1951, **34**, 1632) has suggested that citronellol exhibits maximal absorption at 2120 Å ($\varepsilon = 950$), whereas with our instrument the maximum was situated at 2040 Å ($\varepsilon = 2400$).

It has been pointed out by Vandenbelt, Henrich, and Bash (*Science*, 1951, **114**, 576) that deviations from Beer's law may occur in the 2200-Å region, which may often be connected with false-energy factors. In the present work, no deviations from Beer's law were observed above 2150 Å, but below this wave-length some dependence of absorption on concentration was observed. For this reason, it is advisable to make absorption measurements for each double-bond position on solutions of similar concentration—as mentioned earlier, this was carried out by keeping the density reading at $\lambda_{\text{max.}}$ within the range 0·3—0·5.

The ε values given in Tables A—G were reproducible to within 5% (usually less) with our particular instrument, but some variation may be expected between two different instruments of the same make in the 2050—2250-Å region depending on the degrees of perfection of the optical systems. Through the courtesy of Professor M. Stacey, the light absorption of ergost-8(14)-ene has been determined with two different spectrophotometers at the Birmingham University Chemistry Department (cf. Table H). The Birmingham Unicam instrument gave intensity values rather similar to those obtained on our instrument. The Uvispek instrument gave slightly lower ε values in the 2150—2200-Å region, and the position of the apparent maximum was located at an appreciably longer wave-length than that recorded with the Unicam instruments.

Despite the fact that the spectrophotometrically recorded intensity values are somewhat lower than the true values in this region, such measurements often provide a convenient empirical method for gaining information regarding double-bond positions, *especially if the absorptions of suitable reference compounds of known structure are recorded before* the absorptions of compounds of unknown constitution are studied. Alternatively, an estimate of the true absorption may be obtained by evaluating the scattered light and calculating suitable corrections (Eglinton, Jones, and Whiting, forthcoming publication).

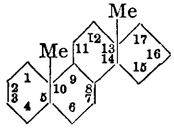
Recent improvements in the design of vacuum spectrographs and hydrogen lamps indicate that it should not be long before instruments for the accurate determination of the light absorption of organic substances in the far ultra-violet region become available, but the techniques described in this paper are sufficiently accurate to afford a useful interim procedure.

Results.—The results show that absorption measurements in the 2050—2250-Å region can usually decide (in the absence of strong absorption at longer wave-lengths) whether olefinic unsaturation is present in a steroid molecule. Of special interest is the fact that ester and carbonyl groups exhibit only low-intensity absorption in this region (Table A), for these groups often render difficult the detection of double bonds by infra-red spectroscopy.

The absorption due to a steroid ethylenic bond appears to depend on (*a*) the degree of substitution, (*b*) whether it is in an exocyclic position, (*c*) the absorption of (potential) adjacent olefinic centres, and (*d*) the influence of neighbouring substituents or groups—these factors will be discussed in this order.

From the Figure, it can be seen that the absorption intensity increases with increasing substitution on the double bond. This is probably due to a bathochromic displacement of the absorption band, an increasing area of end absorption being recorded.

The steeper, more intense (at 2100 Å) curves of $\Delta^{8(14)}$-bonds are in marked contrast with the relatively flat curves of $\Delta^{8(9)}$-steroids. The ready detection of the presence of either of these bonds by the present method is a matter of some importance, for they cannot be detected by infra-red spectroscopic methods (Bladon, Fabian, Henbest, Koch, and Wood, *loc. cit.*). The greater absorption of the $\Delta^{8(14)}$-bond is probably connected with its doubly exocyclic position compared with the endocyclic $\Delta^{8(9)}$-bond—conjugated dienes containing one or more of the double bonds in an exocyclic position are well known to exhibit bathochromic shifts of their absorption bands. The exocyclic position of steroid nuclear trisubstituted olefinic bonds is probably the chief reason why



they absorb more strongly than the corresponding non-exocyclic bond in citronellol (Table C).

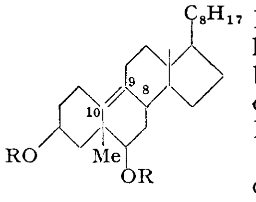
Nuclear trisubstituted bonds exhibit rather similar absorptions, with the exception of Δ^7-compounds, which show appreciably higher absorption intensities. Now, although Δ^5- (say) and Δ^7-bonds appear to be very similar, both being exocyclic to a six-membered ring, they differ in the types of double bonds that can be potentially formed in adjacent positions. Thus, a Δ^5-bond has a trisubstituted (Δ^4) and a disubstituted bond (Δ^6) as immediate neighbours, whereas a Δ^7-bond is adjacent to a tetrasubstituted bond ($\Delta^{8(9)}$ or $\Delta^{8(14)}$) and a disubstituted bond (Δ^6). It is suggested that the absorption due to any ethylenic linkage is influenced by the absorption of the potentially unsaturated centres adjacent to it. Thus a Δ^7-bond possesses some tetrasubstituted olefinic character (including the very strongly absorbing $\Delta^{8(14)}$-bond), which will cause an increase in absorption intensity. Confirmation of these ideas comes from the very similar absorptions of cholest-4- and -5-ene, the bathochromic displacement of the apparent maximum of a $\Delta^{9(11)}$-bond relative to a Δ^5-bond, and the increased absorption of Δ^{11}-steroids compared with a Δ^2-compound. This treatment cannot be applied without reserve to the Δ^{14}-bond, since it is located in a (somewhat strained) five-membered ring.

Introduction of a 3-hydroxyl group into a Δ^5-olefin (cf. cholest-5-ene and cholesterol) significantly lowers the absorption, especially above 2150 Å (Table C). This reduction in intensity is probably connected with some "vicinal electronic effect" between $C_{(3)}$ and $C_{(5)}$—the effect may be compared with the many chemical reactions in which these two groups influence one another. A further noticeable reduction in intensity occurs on acetylation of the 3β-hydroxyl group in a Δ^5-sterol (cf. cholesterol and cholesteryl acetate). However, similar acetylation of sterols with double bonds in positions more remote from the hydroxyl group tends to raise the absorption intensity slightly. Replacement of the hydroxyl group in cholesterol by chlorine increases the absorption, a bromine atom exerting an even larger effect. This is not due simply to an additional contribution of the halogen absorption. Methyl bromide, for example, exhibits only low-intensity absorption ($\varepsilon = 182$ at 2040 Å) in this region (Fink and Goodeve, *Proc. Roy. Soc.*, 1937, *A*, **163**, 595); it is more likely that electronic interaction is taking place possibly somewhat similar in character to that shown by α-phenylalkyl ketones (Kumler, Strait, and Alpen, *J. Amer. Chem. Soc.*, 1950, **72**, 1463, 4558).

No definite conclusions can yet be drawn regarding the effect of groups such as hydroxyl and methoxyl attached at allylic positions, except that, with the 7-hydroxy- and 7-methoxy-Δ^5-compounds examined, the double-bond absorption still lies in the range for trisubstituted olefins.

As expected, the absorption of an unconjugated diene, in which the double bonds are sufficiently separated for interaction to be unlikely, is the sum of the absorptions of the component olefins (cf. zymosterol and zymostadienone, Table E).

Applications.—It has been suggested that the position of the double bond in the compound first prepared by Westphalen (*Ber.*, 1915, **48**, 1064) by acidic dehydration of 3β : 6β-diacetoxycholestan-5α-ol is $\Delta^{9(10)}$ (as inset) (Lettré and Müller, *Ber.*, 1937, **70**, 1947) or, alternatively, $\Delta^{8(9)}$ (Petrow, Rosenheim, and Starling, *J.*, 1938, 677); the latter position has since been assumed by Petrow and his co-workers for a variety of compounds prepared by acid-induced dehydration of 6-acyloxy-5-hydroxy-steroids.



The relatively high-intensity absorptions of Westphalen's diol, diacetate, and diketone (Table, D) definitely show that these compounds contain a tetrasubstituted, doubly exocyclic olefinic bond ($\Delta^{9(10)}$ in this case), the intensities in the 2150—2200-Å region being lowered (relative to the similar $\Delta^{8(14)}$ bond type) by the neighbouring substituents (as in cholesterol and cholesteryl acetate). Further confirmation of the absence of a $\Delta^{8(9)}$-bond was provided by heating the diketone with alcoholic sodium ethoxide, under conditions which have been shown to isomerise a βγ-unsaturated ketone with a similar degree of alkyl substitution to the corresponding αβ-unsaturated ketone (Birch, *J.*, 1950, 367). Most (80%) of the diketone was recovered unchanged from this experiment by a single crystallisation, and spectrographic examination of the remainder of the material showed the absence of any appreciable quantity of αβ-unsaturated ketone(s).

The position of the double bond in *apo*cholic acid and its congeners has aroused some controversy. Through the courtesy of Dr. R. K. Callow, who kindly supplied us with several samples, it has been possible to study their light absorption in the 2050—2200-Å region (Table, F). The high-intensity absorptions of *apo*cholic acid and its methyl ester confirm the presence of an 8(14)-double bond, as suggested by Callow (*J.*, 1936, 462) and by Barton (*J.*, 1946, 1116). The somewhat reduced intensity relative to that of ergost-8(14)-ene is probably due to vicinal effects of the 3α- and 12α-hydroxyl groups (the former being not far from the double bond owing to the *cis*-configuration of rings A/B). The absorptions of dihydroxycholenic acid and its methyl ester are in good agreement with a Δ^{14}-formulation as suggested by Barton (*loc. cit.*) from molecular-rotation evidence.

As further examples of the use of these absorption methods in structural problems may be mentioned the acidic isomerisation of *cyclo*artenone, containing a non-absorbing *cyclo*propane ring, into a compound containing a trisubstituted double bond (Barton, *J.*, 1951, 1444), the detection of endocyclic tetrasubstituted olefinic bonds in the triterpene series (Halsall, *Chem. and Ind.*, 1951, 867), and the assistance in confirming the presence of a Δ^{5}-bond in conessine (Haworth, McKenna, Powell, and Whitfield, *ibid.*, 1952, 215).

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THE UNIVERSITY, MANCHESTER, 13.

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